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$GABA_A$ Receptor Subtypes:

Therapeutic Potential in Down Syndrome, Affective Disorders, Schizophrenia, and Autism

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Abstract

The γ-aminobutyric acid (GABA) system plays a pivotal role in orchestrating the synchronicity of local networks and the functional coupling of different brain regions. Here we review the impact of the $GABA_A$ receptor subtypes on cognitive and emotional behavior, paying particular attention to five disease states: cognitive dysfunction and Down syndrome, anxiety disorders, depression, schizophrenia, and autism. Through the bidirectional modulation of tonic inhibition, α_5-subunit-containing $GABA_A$ receptors permit the bidirectional modulation of cognitive processes, and a partial inverse agonist acting at the α_5-subunit-containing $GABA_A$ receptor is in a clinical trial in individuals with Down syndrome. With regard to anxiety disorders, the viability of nonsedative anxiolytics based on the modulation of α_2- and α_3-subunit-containing $GABA_A$ receptors has been established in clinical proof-of-concept trials. Regarding the remaining three disease states, the GABA hypothesis of depression offers new options for antidepressant drug development, cognitive symptoms in schizophrenia are attributed to a cortical GABAergic deficit, and dysfunctional GABAergic inhibition is increasingly understood to contribute to the pathophysiology of autism spectrum disorders.

Keywords

Down syndrome; cognition enhancer; anxiolytics; depression; schizophrenia; autism

GABA AND COGNITIVE DYSFUNCTIONS

Cognition is an umbrella term that covers interrelated mental activities including attention, learning and memory, understanding, reasoning, social interaction, and goal-directed operations such as planning and decision making. Cognitive dysfunctions are highly relevant across psychiatric disorders (**Figure 1**) (1). In cortical operations, a large diversity of cells

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endows the neuronal circuits with the capacity to perform complex processes. In hippocampus, for instance, excitatory pyramidal cells encode spatial and other episodic memories. In these circuits, the relatively uniform pyramidal cells are supported by a rich diversity of GABAergic interneurons that regulate pyramidal cell activity in a domain-specific fashion. The somatic domain, the axon initial segment, and the dendrites each receive distinct GABAergic innervation with independent timing. The interneurons imprint a spatiotemporal conductance matrix on the pyramidal cells that supports synaptic temporal dynamics, network oscillations, and the selection of cell assemblies. These spatiotemporal specializations provide a basis for cognitive behavior (2–4).

$GABA_A$ Receptors and the Temporal Dynamics of Network Operations

The diversity of GABAergic interneurons is paralleled by a diversity of $GABA_A$ receptors that display structural, functional, and positional specifications geared toward the requirement of the respective synapse operation. The $GABA_A$ receptor subtypes are therefore pharmacological targets that provide opportunities for modulating the spatiotemporal pattern of network activity. For instance, one population of GABA interneurons, the fast-spiking parvalbumin (PV)-positive basket cells, release neurotransmitter at somatic pyramidal cell synapses containing $GABA_A$ receptors that are geared toward high-frequency γ-range responses: α_1-subunit-containing $GABA_A$ receptors, also known as α_1 $GABA_A$ receptors or simply α_1 receptors. In contrast, the cholecystokinin (CCK)-containing basket cells---which, like the PV-positive interneurons, target the perisomatic compartment---are not fast spiking and operate with structurally different receptors: α_2- and α_3-subunit-containing $GABA_A$ receptors, also known as α_2 and α_3 $GABA_A$ receptors or simply α_2 and α_3 receptors. Interneurons that preferentially target dendritic sites of pyramidal cells, such as Martinotti cells, influence the synaptic input by affecting input integration, synaptic plasticity, and the generation of calcium spikes. The output of cortical and hippocampal pyramidal cells can be specifically regulated by axo-axonic GABA interneurons (Chandelier neurons) operating largely with α_2-subunit-containing receptors. Sir John Eccles famously wrote, “I always think that inhibition is a sculpturing process. The inhibition, as it were, chisels away at the ... mass of excitatory action and gives a more specific form to the neuronal performance at every stage of synaptic relay” (5, p. 92).

Enhancing γ Oscillations via Extrasynaptic $GABA_A$ Receptors

Extrasynaptic receptors are activated by synaptic spillover and enable neurons to sense low ambient GABA in order to generate tonic inhibition, which is capable of altering oscillatory network behavior and cognitive processes (6, 7). Tonic inhibition of cortical neurons (layer 5) and hippocampal CA3 and CA1 pyramidal cells is mediated predominantly by α_5-subunit-containing $GABA_A$ receptors (also known as α_5 $GABA_A$ receptors or simply α_5 receptors) (**Figure 2**) (8--11). Through their location at the bases of the spines and the adjacent shafts of the dendrites (12) (Table 1), α_5 $GABA_A$ receptors are in a privileged position to modulate the excitatory input arising at the spines via *N*-methyl-D-aspartate (NMDA) receptors (7, 13, 14). In neocortex, in addition to extrasynaptic α_5 receptors, some synaptic α_5 receptors respond to the input from bitufted interneurons on pyramidal cells

(15). α_5 receptors affect the dynamic range of hippocampal γ oscillations (16, 17). Larger-amplitude γ oscillations have been observed in hippocampal slices from $\alpha_5^{-/-}$ mice compared with wild-type mice, with no change in frequency (19, 20). In $\alpha_5^{-/-}$ mice, no compensatory upregulation of other α subunits was apparent apart from a residual tonic inhibition that was attributed to a homeostatic upregulation of δ $GABA_A$ receptors (13). γ oscillation frequency can also be modulated by extrasynaptic δ $GABA_A$ receptors located on GABA interneurons in CA3 (6). Tonic inhibition is mediated by δ-subunit-containing $GABA_A$ receptors (e.g., $\alpha_4\beta\delta$, $\alpha_6\beta\delta$, or $\alpha_1\beta\delta$) in other neuronal cell types such as granule cells in the dentate gyrus and cerebellum, thalamic relay neurons, neocortical pyramidal cells, and striatal medium spiny neurons (Table 1) (7). Thus, tonic inhibition, in particular via α_5 $GABA_A$ receptors, plays a major role in shaping network synchronization (7).

Enhancing Cognitive Behavior by Genetic Targeting of α_5 $GABA_A$ Receptors

A partial knockdown of hippocampal α_5 receptors [in α_5(H105R) mice] improved hippocampus-dependent performance, as shown by trace fear conditioning, appetitive conditioning, and novel object recognition (19, 20, 20a). Similarly, mice with a full deficit of α_5 receptors ($\alpha_5^{-/-}$ mice) showed improved spatial performance (in the Morris water maze) and trace fear memory (14, 21). In contrast, performance in delay fear conditioning, contextual fear conditioning, and two-way avoidance was unaltered (14, 20, 20a, 21), as was hippocampal long-term potentiation (LTP) (20a, 21) except under specific stimulation conditions (14). These results put α_5 $GABA_A$ receptors at center stage as targets for pharmacological modulation of learning and memory.

Restoring Memory Deficits with α_5 $GABA_A$ Receptor Inverse Agonists

In keeping with the results from genetic targeting, partial inverse agonists (also known as negative allosteric modulators or NAMs) acting at the benzodiazepine site of a α_5 $GABA_A$ receptor were expected to improve performance in learning and memory (22). L-655708, a partial inverse agonist with preferential affinity for α_5 $GABA_A$ receptors, reduced tonic inhibition, facilitated LTP, induced γ oscillations in CA3, and enhanced performance in the Morris maze without being proconvulsant (13, 23, 24). It also reduced alcohol consumption in rhesus monkeys (25). However, the anxiogenic activity of L-655708, presumably due to its inverse agonistic activity at $GABA_A$ receptors other than α_5 receptors (see 24 for a review), prevented its use in humans.

Another partial inverse agonist acting at the α_5 $GABA_A$ receptor, α_5IA, displayed preferential efficacy at (but not affinity for) α_5 $GABA_A$ receptors compared with α_1, α_2, and α_3 $GABA_A$ receptors (**Figure 2**). It enhanced spatial memory (encoding and recall in the Morris water maze) without anxiogenic activity (24, 26--28). In healthy volunteers, administration of α_5IA reduced ethanol-induced amnesia (29). Unfortunately, preclinical renal toxicity prevented further clinical development. MRK-016, another inverse agonist acting at the α_5 $GABA_A$ receptor (but with greater intrinsic efficacy than α_5IA), was poorly tolerated in the elderly and was not further developed (reviewed in 24).

The imidazotriazolobenzodiazepine RO4938581 was the first partial inverse agonist with a highly selective preference in both affinity for and efficacy at α_5 $GABA_A$ receptors versus α_1, α_2, and α_3 $GABA_A$ receptors (30). RO4882224 displayed a similar receptor profile with slightly less preferential affinity for α_5 $GABA_A$ receptors (31). Both compounds enhanced hippocampal LTP in vitro (24, 31). Behaviorally, both compounds rescued a scopolamine-induced hippocampus-dependent working memory deficit (delayed matching-to-position task) as well as a diazepam-induced spatial learning deficit (Morris water maze) in rodents (30, 31). Cognitive deficits induced by phencyclidine (object recognition, odor discrimination) were attenuated by RO4938581 (32). Furthermore, in cynomolgus monkeys, the performance of an object retrieval task, which is thought to require executive functions, was enhanced by RO4938581 beyond the physiological performance level (30). Most important, RO4938581 showed no anxiogenic potential (elevated plus maze, social approach avoidance) in rats and no proconvulsive potential (audiogenic seizures), underlining the critical importance of subtype selectivity in binding affinity and functional efficacy. A compound related to RO4938581 was entered into clinical trials in subjects with Down syndrome (see below). Thus, such agents hold the promise of novel treatments for neurological and psychiatric disorders involving cognitive dysfunctions and possibly conditions of hypofrontality such as schizophrenia (33).

Suppressing Memory via α_5 $GABA_A$ Receptors

The modulation of memory performance via α_5 $GABA_A$ receptors is bidirectional. Positive allosteric modulators or an upregulation of α_5 $GABA_A$ receptors impaired cognitive performance (32, 34, 35). Low-dose etomidate increased tonic inhibition, reduced LTP, and impaired memory performance (spatial memory, contextual fear memory) in wild-type but not $\alpha_5^{-/-}$ mice, whereas its sedative/hypnotic properties were retained in $\alpha_5^{-/-}$ mice (34, 36). Etomidate acts at a site independent of the benzodiazepine site of a $GABA_A$ receptor (37). Following etomidate anesthesia, α_5 $GABA_A$ receptors may contribute to postoperative cognitive and memory dysfunction (36).

Increasing the surface expression of α_5 $GABA_A$ receptors is similarly associated with memory loss, as seen in acute systemic inflammation (35). Treatment with the inflammatory cytokine IL-1β or the endotoxin lipopolysaccharide induced memory impairment (contextual fear memory) in wild-type but not in $\alpha_5^{-/-}$ mice, whereas hippocampus-independent cued fear conditioning was unaffected. Interleukin-1β (IL-1β) increased tonic inhibition (through a p38 MAP kinase pathway), reduced LTP in CA1, and induced upregulation of the α_5-subunit protein level in hippocampus, whereas the subunit levels of α_2 and α_1 were unaltered or slightly decreased, respectively (35). In addition to the increased expression of α_5 $GABA_A$ receptors (35), an increase in the extracellular GABA level has also been reported in inflammatory states (reviewed in 35).

Attempts to develop agonists that act selectively at the benzodiazepine site of a α_5 $GABA_A$ receptor have found limited success so far. The seven-acetylene congener of diazepam, QH-II-066, which displays moderately preferential affinity for (11-fold versus α_1, 6-fold versus α_2 and α_3) (38) and partial agonistic activity at α_5 $GABA_A$ receptors (39), impaired cognitive performance (object recognition) in rats at nonsedative doses (32). However,

anticonvulsant activity had been reported earlier in the same dose range in mice (39). The imidazophenylbenzodiazepine SH-053-2′FR-CH3 (also termed SH-053-R-CH3-2′F) is another α_5-preferring partial agonist (40) that attenuates burst activity in a cultured neocortical slice (41) but produces sedation in some species (40). Clearly, more selective tools would be welcome. For studies in humans, a positron emission tomography ligand is available for imaging α_5 receptor occupancy (42).

Potential Adjuvant to Cognitive Behavioral Therapy?

Enhancing α_5 $GABA_A$ receptor functions in hippocampus may potentially contribute to strategies aimed at erasing previously formed memory in anxiety disorders such as phobias and posttraumatic stress disorder. Memories for fear-associated cues and context are reconsolidated in the amygdala and the hippocampus, respectively (43, 44). In animal models of fear extinction, D-cycloserine, a cognitive enhancer acting as an NMDA receptor coagonist, enhanced fear extinction in a context-, dose-, and time-dependent manner (reviewed in 45). This finding was reproduced in humans, whereby administration of D-cycloserine improved extinction of phobia symptoms in a virtual reality setting (46). It is tempting to speculate that the same behavioral shift may be achieved by a selective reduction of tonic hippocampal inhibition through partial inverse agonists of α_5 $GABA_A$ receptors.

DOWN SYNDROME

Down Syndrome: Cognitive Impairment Due to Excessive Inhibition

Down syndrome, the most common neurogenetic aneuploidy disorder associated with mental retardation, is caused by human trisomy 21 with particular impact from Olg1 and Olg2 genes (47) and occurs in approximately 1 out of 750 births (48--50). The best-characterized Down syndrome model is the Ts65Dn mouse, which contains an extra segment of the ortholog mouse chromosome 16 (51). The neural circuit dysfunctions in Ts65Dn mice include deficits of hippocampal synaptic plasticity [reduced LTP and increased long-term depression (LTD)], a selective decrease in the number of excitatory synapses, an increased number of GABA boutons (% area occupied by GAD65, GAD67, and vesicular GABA transporter), and impaired spatial and recognition memory (Morris water maze, object recognition) (52--56).

On the synaptic level, both $GABA_A$- and $GABA_B$-receptor-mediated components of evoked inhibitory postsynaptic currents were significantly increased largely owing to increased presynaptic GABA release (57). Thus, neuronal plasticity in Ts65Dn mice was thought to be obstructed by excessive GABA-receptor-mediated inhibition. The same authors reported that Gad65 immunofluorescence across the dentate gyrus was not different in wild type and Ts65Dn mice (57), which is in contrast to (56), presumably due to methodological differences, and that the expression of the hippocampal $GABA_A$ receptor subunits (α_1, α_2, α_3, α_5, γ_2), the $GABA_B$ receptor subunits (Gbr 1a, Gbr 1b) was not altered as well (57), in line with presynaptic mechanisms.

Sustained Cognitive Improvement by Pentylenetetrazole

A first pharmacological attempt to overcome the excessive inhibition thought to obstruct synaptic plasticity in Ts65Dn mice was the administration of the $GABA_A$ receptor antagonist pentylenetetrazole (55). As in wild-type animals (58), low-dose administration of the noncompetitive $GABA_A$ receptor antagonist pentylenetetrazole (PTZ) daily for 2 weeks enhanced learning and memory in Ts65Dn mice. Hippocampal LTP, declarative memory (novel object recognition), and alternation score performance (T-maze) were normalized (55), as was spatial cognition (59). Most unexpectedly, hippocampal LTP and improved performance in novel object recognition were maintained in Ts65Dn mice beyond a 17-day oral PTZ regimen and corresponded to wild-type levels even after 1 and 2 months, respectively (55). Due to its potential for seizure induction, PTZ is unsuitable for human trials.

Cognitive Behavior Restored by α_5IA, a Partial Inverse Agonist Acting at α_5 $GABA_A$ Receptors

Importantly, the level of α_5 $GABA_A$ receptors was unaltered in Ts65Dn mice compared with euploid controls, as shown by the expression of α_5 and α_2 subunits in hippocampus (57, 60). Acute treatment of Ts65Dn mice with α_5IA reversed the deficits in spatial reference learning (Morris water maze) and novel object recognition. In the latter test, the performance rose to the level of euploid controls, which, under α_5IA treatment, also showed enhanced novel object recognition (60). Long-term behavioral effects of α_5IA were not investigated.

In the context of the object recognition training (60, 61), the deficit in the activation of hippocampal immediate early genes in Ts65Dn mice (c-fos, arc) was completely rescued by acute α_5IA; control mice also showed an increase in expression of immediate early genes under α_5IA (61). However, in the absence of behavioral stimulation, α_5IA failed to increase Fos protein expression (61), suggesting that α_5IA potentiates evoked neuronal activity and points to a possible state dependency (cognitive stimulation) of the effect of α_5IA. Toxicity prevented the use of α_5IA in humans with Down syndrome.

Cognitive Behavior Restored by RO4938581: Start of a Clinical Trial

Compared with α_5IA, RO4938581 shows superior selectivity as a partial inverse agonist of α_5 $GABA_A$ receptors because it differentiates the α_5 $GABA_A$ receptor from other $GABA_A$ receptor subtypes by both affinity and efficacy (30). Chronic administration of RO4938581 (for 6 weeks) to Ts65Dn mice improved deficits in synaptic plasticity and neurogenesis, as shown by a reversal of hippocampal LTP deficits and a complete restoration of the number of precursor cells in the dentate gyrus. In addition, the enhanced density of hippocampal GABAergic boutons, a hallmark of Ts65Dn pathology, was normalized by RO4938581 (56). Behaviorally, RO4938581 rescued the spatial performance of Ts65Dn mice (Morris water maze) without affecting sensorimotor abilities or motor coordination (rotarod performance test), spontaneous motor activity, seizure induction, or induction of anxiety (open field test). On the contrary, RO4938581 showed anxiolytic properties (plus maze) in Ts65Dn and control mice. In addition, RO4938581 suppressed the hyperactivity found in vehicle-treated Ts65Dn mice in the open field and the plus maze, and it also suppressed defensive behavior

(56). These results provide evidence for the potential therapeutic use of selective partial inverse agonists of α_5 $GABA_A$ receptors to reverse the cognitive deficits in individuals with Down syndrome. RG1662, a compound related to RO4938581, was entered by Hoffmann–La Roche into clinical trials with the aim of counteracting the cognitive disabilities in subjects with Down syndrome (search for RG1662 at http://www.clinicaltrials.gov).

Outlook

A therapeutic effect of RO4938581 would be the first indication that excessive inhibition impairs synaptic plasticity not only in the Ts65Dn mouse model but also in human subjects with Down syndrome (62). Furthermore, aged individuals with Down syndrome show a shared underlying brain pathogenesis with Alzheimer's disease, so RO4938581 may open therapeutic opportunities for cognitive deficits accompanying other disorders (63, 64).

THE QUEST FOR SELECTIVE ANXIOLYTICS

$GABA_A$ Receptor Subtypes for Anxiolysis

Benzodiazepines are effective anxiolytic drugs, but side effects such as sedation and development of withdrawal symptoms limit their long-term use (65). The recognition of $GABA_A$ receptor subtypes (Table 1) provided an incentive to search for novel anxiolytics that retain the rapid and robust antianxiety actions of benzodiazepines while limiting or avoiding their side effects (62, 66, 67).

The functional distinction of benzodiazepine-sensitive $GABA_A$ receptor subtypes (Table 1) was accomplished through the introduction of a histidine-to-arginine point mutation in each of the benzodiazepine-sensitive subunits (α_1, α_2, α_3, and α_5); this mutation rendered the corresponding receptors benzodiazepine insensitive. As a first milestone, drug-induced sedation but not anxiolysis was attributed to α_1 receptors in two studies (68, 69). The latter study (69), however, reported a diazepam-induced locomotor stimulation in α_1(H101R) mice. This aberrant behavior was caused by an unintended undue stress exposure of the animals by performing the anxiolysis test in a novel (instead of a familiar) environment (70).

α_2 $GABA_A$ Receptors Mediate Anxiolysis

Anxiolytic activity was mediated by α_2 $GABA_A$ receptors, as shown in unconditioned tests (elevated plus maze, light/dark test) (71, 72), in a conditioned emotional response paradigm (73), and in a fear-potentiated startle paradigm (72). An additional α_1-receptor-mediated contribution of diazepam was recently described (72) but was seen only in the fear-potentiated startle paradigm.

α_3 $GABA_A$ Receptors: A Potential Backup System for Anxiolysis

As expected, mice with benzodiazepine-insensitive α_3 receptors [i.e., α_3(H126R) mice] retained the anxiolytic activity of diazepam (71, 72). However, TP003, a full agonist with relatively selective efficacy at α_3 $GABA_A$ receptors, also displayed anxiolytic activity (74). Similarly, in mice containing benzodiazepine-insensitive α_2 receptors, L-838417, a partial agonist acting at α_2, α_3, and α_5 receptors, showed anxiolytic activity (fear-potentiated startle; conditioned emotional response) (69, 73). These results pointed to α_3 receptors as a

second anxiolytic target. However, the available data do not provide unambiguous proof of the involvement of α_3 receptors in anxiolysis.

Anxiolytic Distinction Between α_2 and α_3 $GABA_A$ Receptors

The two anxiolytic drug targets (α_2 and α_3 $GABA_A$ receptors) respond at different levels of receptor occupancy. Classical benzodiazepines require a receptor occupancy of 20--25% for anxiolysis (74, 75), which is mediated by α_2 receptors (71, 72). In contrast, TP003, a full agonistic α_3 receptor ligand, needed a 75% receptor occupancy to reach its minimal effective anxiolytic response (74). The α_3 receptor is therefore considered a backup anxiolytic target operating at high receptor occupancy. The low expression of α_3 receptors compared with α_2 receptors in cortical areas and the amygdala may contribute to the differential dependence on occupancy observed for the two receptor types. Recently, α_3 $GABA_A$ receptors were shown to mediate tonic inhibition of principal cells in the basolateral amygdala (BLA) (75a).

Amygdala Fear Circuits and the Vocabulary of $GABA_A$ Receptors

Fear is frequently tested in Pavlovian defensive conditioning paradigms. The lateral amygdala (LA) is responsible for linking a conditioned stimulus (e.g., tone) and unconditioned stimulus (e.g., foot shock) and thus for forming fear memory (**Figure 3**) (76--78). This process requires the formation of LTP of sensory thalamic excitatory synaptic input onto the LA pyramidal neurons.

Acquisition of Fear in the Amygdala: Attenuation via α_2 $GABA_A$ Receptors

The LTP of inputs to LA is under strong GABAergic feed-forward control (**Figure 4**). In the LA and also in the BLA, synaptic inhibition was largely mediated by α_2 and α_1 receptors (79), whereas α_3 receptors contributed to the tonic inhibition of principal cells in the BLA but not in the LA (75a). Thus, drugs acting on α_2 and α_3 receptors are expected to reduce the excitability of LA projection neurons, gate LTP, and limit the fear-inducing impact of conditioned stimuli (reviewed in 81). In the LA, α_1 $GABA_A$ receptors are largely expressed on interneurons. Correspondingly, infusion of an α_1 receptor antagonist reduced auditory fear learning (82).

Fear Expression Through the Amygdala: Suppression via α_2 $GABA_A$ Receptors

The expression of fear-related behavior is governed by the output neurons, located in the medial subdivision of the central amygdala (CEm). These neurons are normally under tight inhibitory control by a population of spontaneously active GABAergic neurons located in the lateral subdivision of the central amygdala (CEl) (**Figure 3**). Diminishing this inhibitory tone through aversive stimuli leads to a disinhibition of the CEm output neurons, triggering the execution of fear and anxiety responses (77, 78). GABAergic inhibition in mouse central amygdala is carried out exclusively by α_2 receptors without a significant contribution form α_1 or α_3 receptors (79, 82). The central amygdala also expresses α_5 receptors (83) (Table 1). Similarly, in postmortem human amygdala, α_2 $GABA_A$ receptors were predominant throughout, in particular in the central amygdala and basal nucleus. In the lateral nucleus, some α_1 receptors are present, and the α_3 receptor is a minor subtype (personal

communication, J. Song & H. Waldvogel, University of Auckland, New Zealand) (**Figure 4**). Thus, dampening the output of the central amygdala via α_2 $GABA_A$ receptors seems to be a major anxiolytic microcircuit.

Fear Extinction and the Role of $GABA_A$ Receptors

Fear extinction, i.e., the repeated presentation of a conditioned stimulus in the absence of an unconditioned stimulus, is an active learning process. During extinction, cortical neurons, in conveying the absence of the aversive unconditioned stimulus, are thought to suppress amygdala output (78, 84). This suppression is achieved largely by activating the GABA neurons of the intercalated cell cluster that surround the amygdala and innervate the basolateral and central nuclei (**Figure 3**). Within hours following fear-extinction training, the transcripts of $GABA_A$ receptor subunits (α_2 and β_2) were upregulated (83). Because in both the LA and the central amygdala, α_2 $GABA_A$ receptors play a major role, testing whether anxiolytics acting on α_2 receptors may facilitate fear extinction would be of interest.

Cortical Anxiety Circuit in Conflict Resolution: Role for α_2 $GABA_A$ Receptors

The term anxiety relates to risk assessment of a potential threat and crucially involves uncertainty as to the expectancy of the threat (85). The pregenual anterior cingulate cortex (pACC) is linked to cost-benefit value arbitration and communicates the outcome of the evaluation to other brain areas, including the amygdala, thereby regulating the emotional response triggered by a conflict situation (86). In a macaque version of an approach-avoidance decision task (87), researchers identified neurons in the pACC that represented motivationally positive (P) or negative (N) subjective value (i.e., P for approach, N for avoidance). Their activity patterns flexibly changed depending on the respective offers (aversive air-puff or pleasant syrup) in each task. Electrical microstimulation of N-neurons led to a pessimistic evaluation of future outcome and increased avoidance behavior.

Treatment with diazepam fully blocked the negative biasing of cost-benefit evaluation and stimulus-induced negative decision making (87). In the mouse, conflict situations are attenuated by diazepam via α_2 $GABA_A$ receptors (71), which presumably include α_2 receptors on cortical pyramidal cells of which the receptors in layer 5 of the cerebral cortex are key players in associative processes (88). These results are important as they define a cortical substrate for the ability of α_2 receptor ligands to reverse a negative biasing of behavior. This finding may extend to GABAergic treatment of depression, in which negative biases in cost-benefit evaluations are part of the pathophysiology (62, 89, 90).

Novel GABA Anxiolytics, Validated in Humans

Novel potential anxiolytics developed in recent years targeted the α_2 and α_3 $GABA_A$ receptors (Table 2), as typified by the triazolopyridazine L-838417, which showed anxiolytic activity without causing sedation and displayed only minimal dependence liability (69, 91, 92). Human trials were performed with two analogs of L-838417, TPA023 and MRK-409, with ocinaplon and with the neurosteroid-linked anxiolytic XBD123 (reviewed in 62). Most recently, a Phase 1 clinical trial in healthy volunteers with CTP-354 (formerly C-21191), a new chemical entity based on L-838,417 with selective deuterium integration

and improved pharmacokinetic parameters (Program No. 338.05/C42. 2011 Neuroscience Meeting Planner. Washington, DC, Society for Neurosceience, 2011. Online), was announced(http://www.concertpharma.com/CTP354Phase1Initiation.htm).

TPA023

TPA023, a fluoroanalog of L-838417, was anxiolytic in rodents and primates; did not cause sedation, development of tolerance, or abuse potential (91--95); and acted as a weak partial agonist at α_2 and α_3 receptors (95). In healthy volunteers, TPA023 (0.5 and 1.5 mg), in contrast to lorazepam (2 mg), was inactive in tests of sedation (visual alertness), cognition (word and picture recognition), and posture (body sway) (95). Saccadic eye movement peak velocity was impaired by TPA023, but latency and accuracy were not. In initial clinical trials over 4 weeks, TPA023 (MK-0777) was effective in generalized anxiety disorder (GAD); with an onset of action in the first week, it was associated with a significantly superior reduction in the Hamilton anxiety rating scale (HAM-A) score compared with placebo. Due to problems with toxicity in animals, the clinical development of TPA023 was ultimately discontinued (95).

MRK-409

The triazolopyridazine MRK-409 (also MK-0343) showed only narrow margins of efficacy between α_2 and α_1 receptors [in vitro relative efficacy of 0.18, 0.23, 0.45, and 0.18 for α_1, α_2, α_3, and α_5 receptors, respectively, compared with chlordiazepoxide (1.0)]. MRK-409 was anxiolytic without causing sedation in rodents and nonhuman primates (97). In healthy volunteers, MRK-409 caused reduced alertness, which was attributed to its interaction with α_1 receptors (95, 97a, 98, 99).

Ocinaplon

The pyrazolopyrimidine ocinaplon is a low-potency $GABA_A$ receptor modulator with partial efficacy at all four receptor subtypes [in vitro relative efficacies of 0.8, 0.5, 0.5, and 0.3 for α_1, α_2, α_3, and α_5 receptors, respectively, compared with diazepam (1.0)] (100). However, there is no reason to assume that the anxiolytic activity of ocinaplon (100, 101) is not mediated via α_2 $GABA_A$ receptors. In mice with benzodiazepine-insensitive α_2 $GABA_A$ receptors [i.e., α_2 (H101R) mice], its anxiolytic activity (10 mg/kg p.o.) was absent in the elevated plus maze test (F. Crestani, personal communication). In two trials in GAD patients (100, 102), ocinaplon reduced the HAM-A score significantly, with no evidence of the typical benzodiazepine side effects (101, 102). The 4-week study was terminated early due to elevation of liver enzymes in one patient (101).

Anxiolysis via Neurosteroids

By activating the transport of cholesterol through mitochondrial membranes, agonists of the translocator protein (TSPO; formerly known as peripheral benzodiazepine receptor) stimulate the synthesis of neurosteroids such as allopregnanolone in the brain and thereby enhance GABA transmission (103, 104). Neurosteroids act preferentially on $GABA_A$ receptors containing a δ subunit and, pharmacologically, can induce anxiolytic, hypnotic, and anesthetic effects (105). The TSPO agonist XBD173 was anxiolytic in rodents, an effect

blocked by the antagonist PK11195 (106). In human volunteers, XBD173 suppressed CCK4-induced panic anxiety without causing sedation or withdrawal (104, 106). The lack of clinical efficacy of XBD173 in a Phase II trial in patients with GAD was attributed to the disregard of a frequent TSPO polymorphism that strongly affects ligand affinity (107).

Dependence Liability

Dependence liability of classical benzodiazepines is attributed largely to α_1 $GABA_A$ receptors owing to a disinhibition of ventral tegmental area dopamine neurons. In α_1(H101R) mice, midazolam failed to show oral self-administration (108). In addition, the α_1-preferring ligands zolpidem and zaleplon showed a high dependence liability in self-administration similar to that of classical benzodiazepine (92, 93, 109). In contrast, TPA023, a partial agonist of α_2 and α_3 receptorsthat spares α_1 receptors, lacked self-administration in baboons even at full receptor occupancy (95). Recently, in a mouse model of intracranial self-stimulation (ICSS) that targeted the medial forebrain bundle, zolpidem unexpectedly failed to enhance reward behavior (i.e., failed to result in a reduction of ICSS threshold), indicating that α_1 receptors are not sufficient for reward enhancement. Diazepam (1--4 mg/kg) enhanced reward (110, 111); this effect was blunted in α_1(H101R), α_2(H101R), and α_3(H126R) mice, indicating that it is mediated by α_1, α_2, and α_3 receptors (111). However, the type of reward measured in the ICSS model and its variance with the chronic self-administration models remain to be resolved. Remarkably, eszopiclone, which acts as a partial agonist at all benzodiazepine-sensitive receptors [preferentially at α_2 and α_3 receptors (112)], has no restriction regarding long-term use as hypnotic (113).

DEPRESSION

Depression and related mood disorders are among the greatest public health problems today. The US lifetime prevalence of major depressive disorder (MDD) is 17% (124). Symptoms of depression include depressed mood, a reduced ability to experience reward (anhedonia), feelings of worthlessness or guilt, poor concentration, indecisiveness, thoughts of death, suicidal ideation, appetite/weight changes, sleep disturbances, changes in psychomotor activity, and fatigue (125).

Although the neurobiology of depression has not been fully elucidated, increasing experimental and clinical evidence points to an association between MDD and GABAergic deficits (125a). Patients with MDD have reduced central nervous system (CNS) GABA concentrations (126, 127), which play a prominent role in the neural regulation of stress (125a). Preclinical evidence suggests that clinically used antidepressants exert their actions by ultimately counteracting GABAergic deficits (125a). Additionally, GABAergic neurotransmission plays an important role in hippocampal neurogenesis and neural maturation, both of which have been established as substrates of antidepressant therapies (128). Furthermore, mice with a heterozygous deletion of the γ_2 subunit ($\gamma_2^{+/-}$ mice) exhibit trait anxiety (129) as well as heightened behavioral inhibition in response to behavioral despair, as evidenced by increased immobility in the forced swim test (FST) and in the tail suspension test (TST) (128). Desipramine, but not fluoxetine, normalized these deficits (130). $\gamma_2^{+/-}{}_2$ mice also have hypothalamic-pituitary-adrenal axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic forms of depression (130).

Additionally, adult hippocampal neurogenesis was reduced in $\gamma_2^{+/-}$ mice (128). In addition, the antidepressant effect of BDNF is mediated via $GABA_A$ receptors (130a). These and other studies led to the "GABAergic deficit hypothesis of major depressive disorder" proposed by Lüscher and colleagues (125a).

With the γ_2 subunit contained in approximately 90% of all $GABA_A$ receptors (131), the question arises as to which receptor subtype(s) as defined by the α subunit may be mediating the antidepressant-like actions. α_2 global knockout mice displayed increased immobility in the FST and TST, indicating a depressive-like phenotype (132). A major feature of depression is anhedonia, which can be modeled in rodents using the ICSS paradigm. Mice are implanted with an electrode into the medial forebrain bundle at the level of the lateral hypothalamus and trained to spin a wheel for rewarding stimulation, so that frequency-response curves can be constructed (133). Like cocaine, diazepam shifts these frequency-response curves to the left; i.e., it has a reward-enhancing action (110). In α_2(H101R) and α_3(H126R) mice with diazepam-insensitive α_2 and α_3 $GABA_A$ receptors, respectively, the reward-enhancing effect of diazepam was absent, indicating that α_2 and α_3 $GABA_A$ receptors are required for the reward-enhancing action of diazepam (111). Interestingly, in α_2(H101R) mice, diazepam becomes potentially aversive, as indicated by a shift of the frequency-response curve to the right (111). As α_2 $GABA_A$ receptors may have an important role in antidepressant-like and reward-related behaviors, they may be a target for antidepressant drugs with a novel mechanism of action. The FST and TST models of behavioral despair use short-duration (on the order of minutes) stress in normal rodents, and antidepressants produce a rapid response in these models. In contrast, human depression is thought to develop in genetically susceptible individuals after chronic environmental stress exposure, and only chronic administration of antidepressants produces a response. It is therefore necessary to investigate the role of α_2-containing $GABA_A$ receptors in rodent protocols involving chronic stress, such as the chronic social defeat paradigm, as well as in other depression-related tests (134).

In any case, there is already evidence that modulation of $GABA_A$ receptors can have antidepressant effects. Alprazolam and adinazolam elicit antidepressant responses similar to widely prescribed antidepressants in MDD patients, but within the first two weeks of treatment (135, 136). Eszopiclone, a positive allosteric modulator (PAM) of $GABA_A$ receptors, is used for the treatment of insomnia. In patients comorbid with MDD and insomnia, eszopiclone/fluoxetine cotherapy was associated not only with sleep improvement but also a faster onset of antidepressant response and a greater magnitude of the antidepressant effect (137). Interestingly, eszopiclone also facilitated the antidepressant efficacy of fluoxetine in the chronic social defeat mouse model of depression (138). Further studies---for example, studies using nonsedating α_2/α_3-selective PAMs in animal models of depression---are expected to further validate the GABA hypothesis of depression. Potentially, such α_2/α_3-selective PAMs or other related compounds could be fast-acting antidepressants.

SCHIZOPHRENIA

Several lines of evidence suggest that inhibitory neurotransmission in the CNS plays important roles in modulating circuits in the brain that are involved in the manifestation of symptoms of schizophrenia. A decline in biosynthesis of cortical GABA leads to a downregulation of GABAergic cortical function in schizophrenia and a compensatory (but insufficient) upregulation of $GABA_A$ receptors (139). Furthermore, a deficit in the glutamatergic activation of GABAergic interneurons in the prefrontal cortex, which synapse on pyramidal neurons at the axon initial segment, results in upregulation of the α_2 subunit of the $GABA_A$ receptor in the axon initial segment (140). In rhesus monkeys, ketamine-induced working memory deficits were reduced by TPA023 (MK-0777) (141), which is a partial, α_2/α_3-selective PAM of $GABA_A$ receptors [in vitro relative efficacy of 0.11 at α_2 and 0.21 at α_3 compared with chlordiazepoxide (1.0); see above and Reference 94]. Furthermore, this compound increased frontal gamma band power during a cognitive task in patients with schizophrenia (142). Whereas cognitive improvements were either relatively minor in the study by Lewis et al. (142) or undetectable in a larger study by Buchanan et al. (143), the relative efficacy of MK-0777 at α_2 $GABA_A$ receptors is only 0.11 compared with that of chlordiazepoxide (1.0), a full agonist at the benzodiazepine site (94). A compound with higher efficacy might have greater cognitive effects.

The potential involvement of GABAergic inhibition in sensorimotor gating and cognitive function is further supported by the finding that α_3 global knockout mice and mice with reduced expression of α_5 in the hippocampus display deficits in prepulse inhibition of acoustic startle (144, 145). Moreover, the mice with reduced expression of α_5 in the hippocampus also displayed a latent inhibition deficit (146), indicating a role of α_5 $GABA_A$ receptors in cognition. In line with these observations, an α_5-selective PAM, SH-053-2′F-R-CH3, applied systemically or directly into the hippocampus reverses dopaminergic hyperactivation in the methylazoxymethanol acetate (MAM) developmental model of schizophrenia (147).

The findings cited above would be compatible with a drug acting at α_2, α_3, and α_5 $GABA_A$ receptors having both antipsychotic (via α_3 and α_5) and cognitive-enhancing (via α_2) effects, without sedative (via α_1) side effects. In retrospect, it is striking that the partial, nonselective PAM bretazenil, originally developed as a nonsedating anxiolytic, was efficacious as monotherapy in 44% of neuroleptic-free patients with acute episodes of schizophrenia (148).

AUTISM

In a model that has become increasingly popular, Rubenstein & Merzenich (149) have postulated that genetic and environmental factors can lead to an increased excitation/inhibition (E/I) ratio, which may be of pathophysiological relevance for at least some forms of autism. Optogenetic activation of prefrontal cortical pyramidal neurons not only resulted in increased spiking but also caused a substantial reduction in information processing in these cells, a social interaction deficit, and impaired fear conditioning (150). These findings indicate impaired cognition, providing support for the hypothesis that an increased E/I

imbalance is sufficient to elicit neuropsychiatric disease-related symptoms. A meta-analysis revealed that in several mouse models of autism spectrum disorders (ASD), the number of PV-positive GABAergic interneurons was reduced in the neocortex, suggesting that PV-circuit disruption may be relevant in the development of ASD (151). For example, in a *Cntnap2*$^{-/-}$ mouse model of cortical dysplasia-focal epilepsy, a syndromic form of ASD, the number of GABAergic interneurons was reduced, and the network activity was abnormal (152). Evidence that dysfunction of GABAergic neurotransmission is associated with ASD is increasing. For example, loss of MeCP2 from a subset of GABAergic neurons in the forebrain results in a reduced inhibitory quantal size and in the recapitulation of many features of Rett syndrome, indicating that subtle dysfunction of GABAergic neurons potentially contributes to ASD (153). Furthermore, in *Scn1a* ($Na_v1.1$) heterozygous knockout mice (i.e., haploinsufficient mice)---which are a model of Dravet's syndrome, also a syndromic form of ASD---GABAergic neurotransmission is decreased, and mice exhibit hyperactivity, stereotypies, social interaction deficits, and impaired context-dependent spatial memory (154). This phenotype is essentially replicated by a heterozygous knockout of the *Scn1a* gene specifically in GABAergic forebrain neurons, suggesting that dysfunction of GABAergic neurons is the essential deficit in the global *Scn1a*$^{+/-}$ mice and potentially also in patients. Interestingly, a very low dose (0.0625 mg/kg) of clonazepam---a PAM of $GABA_A$ receptors containing the α_1, α_2, α_3, or α_5 subunit---completely rescued abnormal social behaviors and deficits in fear memory (154). At this low dose, clonazepam is not anxiolytic or sedative (154). Dysfunction of GABAergic neurons may result in upregulation and/or sensitization of postsynaptic $GABA_A$ receptors, which would explain why such low doses of clonazepam are effective. Which $GABA_A$ receptor subtypes mediate the phenotypic rescue observed in the *Scn1a*$^{+/-}$ mice is unknown. Given the observation that PV-positive interneurons are reduced in multiple mouse models of ASD, it is tempting to speculate that α_1-containing $GABA_A$ receptors might play a role, as synapses between PV-positive basket cells and principal neurons typically contain α_1 $GABA_A$ receptors. Collectively, the findings cited above support the notion that an elevated E/I imbalance is an essential factor in the pathophysiology of ASD and that correction of this imbalance may be a powerful therapeutic strategy. Given the efficacy of the nonspecific $GABA_A$ receptor modulator clonazepam in *Scn1a*$^{+/-}$ mice, knowledge of the $GABA_A$ receptor subtype(s) that correct the autistic-like phenotype would allow for the development of much more specific compounds with a pharmacological profile different from that of classical benzodiazepines.

Support for the hypothesis that a dysregulated GABA system may play an important role in the pathophysiology of autism comes from postmortem studies, which found reduced expression of $GABA_A$ receptor subunits α_4, α_5, and β_1 as well as and $GABA_B$ receptor subunit β_1 (155). Furthermore, a recent positron emission tomography (PET) pilot study points to lower levels of α_5 $GABA_A$ receptors in the brains of patients with ASD (156), strengthening the emerging connection between deficits in the $GABA_A$ receptor system and ASD. The important role of the E/I imbalance in the pathophysiology of ASD suggests that subtype-selective modulation of $GABA_A$ receptors may be a promising novel therapeutic approach once the functions of individual receptor subtypes in animal models of ASD have been elucidated.

CONCLUSION

$GABA_A$ receptor subtypes offer the promise of a new CNS pharmacology beyond classical benzodiazepines. The most striking impact comes from the recognition that α_5 $GABA_A$ receptors modulate cognitive behavior. Partial inverse agonists (i.e., NAMs of the benzodiazepine site) acting at α_5 receptors overcome deficits in attention, learning, and memory induced either pharmacologically in rodents or nonhuman primates or genetically in a murine Down syndrome model. A clinical trial in individuals with Down syndrome has been initiated. Concerning anxiety circuits, α_2 (and potentially α_3) $GABA_A$ receptors play a key role. Despite promising initial trials, an anxiolytic devoid of sedation is still being awaited. Ligands with selective affinity and efficacy for the α_2 $GABA_A$ receptor subtype might be needed for a breakthrough. This is the more urgent matter, as ligands of this receptor subtype are expected also to ameliorate depressive symptoms and impaired cognitive behavior in schizophrenia. In ASD, attempts to rebalance the increased E/I ratio also points to a role for enhancing the GABA system. Neurosteroids act preferentially at benzodiazepine-insensitive GABA receptors. By stimulating the synthesis of neurosteroids, the drug XBD173 showed antipanic effects in human volunteers. Thus, with an increasingly subtype-specific pharmacology, the GABA system holds expanding therapeutic potential for CNS disorders.

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LITERATURE CITED

1. Millan MJ, Agid Y, Brüne M, Bullmore ET, Carter CS, et al. Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. Nat. Rev. Drug Discov. 2012; 11:141–-68. [PubMed: 22293568]
2. Buzsáki G, Draguhn A. Neuronal oscillations in cortical networks. Science. 2004; 304:1926–-29. [PubMed: 15218136]
3. Klausberger T, Somogyi P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science. 2008; 321:53–-57. [PubMed: 18599766]
4. Buzsáki G, Wang X-J. Mechanisms of gamma oscillations. Annu. Rev. Neurosci. 2012; 35:203–-25. [PubMed: 22443509]
5. Eccles, JC. The Understanding of the Brain. 2nd ed.. McGraw-Hill; New York: 1977.
6. Mann EO, Mody I. Control of hippocampal gamma oscillation frequency by tonic inhibition and excitation of interneurons. Nat. Neurosci. 2010; 13:205–-12. [PubMed: 20023655]
7. Brickley SG, Mody I. Extrasynaptic $GABA_A$ receptors: their function in the CNS and implications for disease. Neuron. 2012; 73:23–-34. [PubMed: 22243744]
8. Glykys J, Mody I. Hippocampal network hyperactivity after selective reduction of tonic inhibition in $GABA_A$ receptor α5 subunit–deficient mice. J. Neurophysiol. 2006; 95:2796–-807. [PubMed: 16452257]
9. Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy JM, Vogt KE. Specific subtypes of $GABA_A$ receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. J. Neurophysiol. 2006; 96:846–-57. [PubMed: 16835366]

10. Yamada J, Furukawa T, Ueno S, Yamamoto S, Fukuda A. Molecular basis for the $GABA_A$ receptor–mediated tonic inhibition in rat somatosensory cortex. Cereb. Cortex. 2007; 17:1782–-87. [PubMed: 16997904]
11. Möhler H. Molecular regulation of cognitive functions and developmental plasticity: impact of $GABA_A$ receptors. J. Neurochem. 2007; 102:1–-12. [PubMed: 17394533]
12. Fritschy JM, Brünig I. Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. Pharmacol. Ther. 2003; 98:299–-323.
13. Glykys J, Mann EO, Mody I. Which $GABA_A$ receptor subunits are necessary for tonic inhibition in the hippocampus? J. Neurosci. 2008; 28:1421–-26. [PubMed: 18256262]
14. Martin LJ, Zurek AA, MacDonald JF, Roder JC, Jackson MF, Orser BA. α5$GABA_A$ receptor activity sets the threshold for long-term potentiation and constrains hippocampus-dependent memory. J. Neurosci. 2010; 30:5269–-82. [PubMed: 20392949]
15. Ali AB, Thomson AM. Synaptic α5 subunit–containing $GABA_A$ receptors mediate IPSPs elicited by dendrite-preferring cells in rat neocortex. Cereb. Cortex. 2008; 18:1260–-71. [PubMed: 17951598]
16. Towers SK, Gloveli T, Traub RD, Driver JE, Engel D, et al. α5 subunit-containing $GABA_A$ receptors affect the dynamic range of mouse hippocampal kainate-induced gamma frequency oscillations in vitro. J. Physiol. 2004; 559:721–-28. [PubMed: 15284346]
17. Glykys J, Mody I. Activation of $GABA_A$ receptors: views from outside the synaptic cleft. Neuron. 2007; 56:763–-70. [PubMed: 18054854]
18. Deleted in proof
19. Yee BK, Hauser J, Dolgov VV, Keist R, Möhler H, et al. GABA receptors containing the α_5 subunit mediate the trace effect in aversive and appetitive conditioning and extinction of conditioned fear. Eur. J. Neurosci. 2004; 20:1928–-36. [PubMed: 15380015]
20. Prut L, Prenosil G, Willadt S, Vogt K, Fritschy JM, Crestani F. A reduction in hippocampal $GABA_A$ receptor α5 subunits disrupts the memory for location of objects in mice. Genes Brain Behav. 2010; 9:478–-88. [PubMed: 20180861]

20a. Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, et al. Trace fear conditioning involves hippocampal α_5 $GABA_A$ receptors. Proc. Natl. Acad. Sci. USA. 2002; 99:8980–-85. [PubMed: 12084936]

21. Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, et al. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the α_5 subunit of the $GABA_A$ receptor. J. Neurosci. 2002; 22:5572–-80. [PubMed: 12097508]
22. Möhler H. The rise of a new GABA pharmacology. Neuropharmacology. 2011; 60:1042–-49. [PubMed: 21035473]
23. Navarro JF, Buron E, Martin-Lopez M. Anxiogenic-like activity of L-655,708, a selective ligand for the benzodiazepine site of $GABA_A$ receptors which contain the alpha-5 subunit, in the elevated plus-maze test. Prog. Neuropsychopharmacol. Biol. Psychiatry. 2002; 26:1389–-92.
24. Atack JR. $GABA_A$ receptor subtype-selective modulators: II. α_5-selective inverse agonists for cognition enhancement. Curr. Top. Med. Chem. 2011; 11:1203–-14. [PubMed: 21050171]
25. Rüedi-Bettschen D, Rowlett JK, Rallapalli S, Clayton T, Cook JM, Platt DM. Modulation of α5 subunit–containing $GABA_A$ receptors alters alcohol drinking by rhesus monkeys. Alcohol Clin. Exp. Res. 2013; 37:624–34. [PubMed: 23126673]
26. Chambers MS, Atack JR, Carling RW, Collinson N, Cook SM, et al. An orally bioavailable, functionally selective inverse agonist at the benzodiazepine site of $GABA_A$ α5 receptors with cognition enhancing properties. J. Med. Chem. 2004; 47:5829–-32. [PubMed: 15537339]
27. Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, et al. An inverse agonist selective for α5 subunit–containing $GABA_A$ receptors enhances cognition. J. Pharmacol. Exp. Ther. 2006; 316:1335–-45. [PubMed: 16326923]
28. Atack JR. Preclinical and clinical pharmacology of the $GABA_A$ receptor α_5 subtype-selective inverse agonist α_5IA. Pharmacol. Ther. 2010; 125:11–-26. [PubMed: 19770002]
29. Nutt DJ, Besson M, Wilson SJ, Dawson GR, Lingford-Hughes AR. Blockade of alcohol's amnestic activity in humans by an α5 subtype benzodiazepine receptor inverse agonist. Neuropharmacology. 2007; 53:810–-20. [PubMed: 17888460]

30. Ballard TM, Knoflach F, Prinssen E, Borroni E, Vivian JA, et al. RO4938581, a novel cognitive enhancer acting at $GABA_A$ α5 subunit-containing receptors. Psychopharmacology. 2009; 202:207–-23. [PubMed: 18936916]
31. Knust H, Achermann G, Ballard T, Buettelmann B, Gasser R, et al. The discovery and unique pharmacological profile of RO4938581 and RO4882224 as potent and selective $GABA_A$ α5 inverse agonists for the treatment of cognitive dysfunction. Bioorg. Med. Chem. Lett. 2009; 19:5940–-44. [PubMed: 19762240]
32. Redrobe JP, Elster L, Frederiksen K, Bundgaard C, de Jong IEM, et al. Negative modulation of $GABA_A$ α5 receptors by RO4938581 attenuates discrete subchronic and early postnatal phencyclidine (PCP)-induced cognition deficits in rats. Psychopharmacology. 2012; 221:451–-68. [PubMed: 22124672]
33. Ferrarelli F, Massimini M, Peterson MJ, Riedner BA, Lazar M, et al. Reduced evoked gamma oscillations in the frontal cortex in schizophrenia patients: a TMS/EEG study. Am. J. Psychiatry. 2008; 165:996–-1005. [PubMed: 18483133]
34. Martin LJ, Oh GH, Orser BA. Etomidate targets α5 gamma-aminobutyric acid subtype A receptors to regulate synaptic plasticity and memory blockade. Anaesthesiology. 2009; 111:1025–-35.
35. Wang D-S, Zurek AA, Lecker I, Yu J, Abramian AM, et al. Memory deficits induced by inflammation are regulated by α5-subunit-containing $GABA_A$ receptors. Cell Rep. 2012; 2:488–-96. [PubMed: 22999935]
36. Cheng VY, Martin LJ, Elliott EM, Kim JH, Mount HT, et al. α5 $GABA_A$ receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. J. Neurosci. 2006; 26:3713–-20. [PubMed: 16597725]
37. Rudolph U, Antkowiak B. Molecular and neuronal substrates for general anesthetics. Nat. Rev. Neurosci. 2004; 5:709–-20. [PubMed: 15322529]
38. Huang Q, He X, Ma C, Liu R, Yu S, et al. Pharmacophore/receptor models for $GABA_A$/BzR subtypes (α1β3γ2, α5β3γ2, and α6β3γ2) via a comprehensive ligand-mapping approach. J. Med. Chem. 2000; 43:71–-95. [PubMed: 10633039]
39. Skolnick P, Hu RJ, Cook CM, Hurt SD, Trometer JD, et al. [^{3}H]RY 80: a high-affinity, selective ligand for γ-aminobutyric $acid_A$ receptors containing alpha-5 subunits. JPET. 1997; 283:488–-93.
40. Fischer BD, Licata SC, Edwankar RV, Wang ZJ, Hung S, et al. Anxiolytic-like effects of 8-acetylene imidazobenzodiazepines in a rhesus monkey conflict procedure. Neuropharmacology. 2010; 59:612–-18. [PubMed: 20727364]
41. Drexler B, Zinser S, Huang S, Poe MM, Rudolph U, et al. Enhancing the function of alpha-5-subunit-containing $GABA_A$ receptors promotes action potential firing of neocortical neurons during up-states. Eur. J. Pharmacol. 2013; 703:18–24. [PubMed: 23380687]
42. Lingford-Hughes A, Hume SP, Feeney A, Hirani E, Osman S, et al. Imaging the GABA-benzodiazepine receptor subtype containing the α5-subunit in vivo with [^{11}C]Ro15 4513 positron emission tomography. J. Cereb. Blood Flow Metab. 2002; 22:878–-89. [PubMed: 12142573]
43. Lee JL. Memory reconsolidation mediates the updating of hippocampus memory content. Front. Behav. Neurosci. 2010; 4:168. [PubMed: 21120142]
44. McKenzie S, Eichenbaum H. Consolidation and reconsolidation: two lives of memories? Neuron. 2011; 71:224–-33. [PubMed: 21791282]
45. Kaplan GB, Moore KA. The use of cognitive enhancers in animal models of fear extinction. Pharmacol. Biochem. Behav. 2011; 99:217–-28. [PubMed: 21256147]
46. Davis M, Myers KM, Chhatwal J, Ressler KJ. Pharmacological treatments that facilitate extinction of fear: relevance to psychotherapy. NeuroRX. 2006; 3:82–-96. [PubMed: 16490415]
47. Chakrabarti L, Best TK, Cramer NP, Carney RS, Isaac JT, et al. Olig1 and Olig2 triplication causes developmental brain defects in Down syndrome. Nat. Neurosci. 2010; 13:927–-34. [PubMed: 20639873]
48. Parker SE, Mai CT, Canfield MA, Rickard R, Wang Y, et al. Updated National Birth Prevalence estimates for selected birth defects in the United States, 2004--2006. Birth Defects Res. A Clin. Mol. Teratol. 2010; 88:1008–-16. [PubMed: 20878909]

49. Gardiner K, Herault Y, Lott IT, Antonarakis SE, Reeves RH, Dierssen M. Down syndrome: from understanding the neurobiology to therapy. J. Neurosci. 2010; 30:14943–-45. [PubMed: 21068296]
50. Lott IT, Dierssen M. Cognitive deficits and associated neurological complications in individuals with Down's syndrome. Lancet Neurol. 2010; 9:623–33. [PubMed: 20494326]
51. Liu C, Belichenko PV, Zhang L, Fu D, Kleschevnikov AM, et al. Mouse models for Down syndrome–associated developmental cognitive disabilities. Dev. Neurosci. 2011; 33:404–-13. [PubMed: 21865664]
52. Siarey RJ, Stoll J, Rapoport SI, Galdzicki Z. Altered long-term potentiation in the young and old Ts65Dn mouse, a model for Down Syndrome. Neuropharmacology. 1997; 36:1549–-54. [PubMed: 9517425]
53. Siarey RJ, Carlson EJ, Epstein CJ, Balbo A, Rapoport SI, Galdzicki J. Increased synaptic depression in the Ts65Dn mouse, a model for mental retardation in Down syndrome. Neuropharmacology. 1999; 38:1917–-20. [PubMed: 10608287]
54. Kleschevnikov AM, Belichenko PV, Villar AJ, Epstein CJ, Malenka RC, Mobley WC. Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. J. Neurosci. 2004; 24:8153–-60. [PubMed: 15371516]
55. Fernandez F, Morishita W, Zuniga E, Nguyen J, Blank M, et al. Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. Nat. Neurosci. 2007; 10:411–-13. [PubMed: 17322876]
56. Martinez-Cue C, Martinez P, Rueda N, Vidal R, Garcia S, et al. Reducing $GABA_A$ α5 receptor-mediated inhibition rescues functional and neuromorphological deficits in a mouse model of Down syndrome. J. Neurosci. 2013; 33:953–-66.
57. Kleschevnikov AM, Belichenko BV, Gall J, George L, Nosheny R, et al. Increased efficiency of the $GABA_A$ and $GABA_B$ receptor-mediated neurotransmission in the Ts65Dn mouse model of Down syndrome. Neurobiol. Dis. 2012; 45:683–-91. [PubMed: 22062771]
58. McGaugh JL. Time-dependent processes in memory storage. Science. 1966; 153:1351–-58. [PubMed: 5917768]
59. Rueda N, Florez J, Martinez-Cue C. Chronic pentylenetetrazole but not donepezil treatment rescues spatial cognition in Ts65Dn mice, a model for Down syndrome. Neurosci. Lett. 2008; 433:22–-27. [PubMed: 18226451]
60. Braudeau J, Delatour B, Duchon A, Lopes Pereira P, Dauphinot L, et al. Specific targeting of the $GABA_A$ receptor α_5 subtype by a selective inverse agonist restores cognitive deficits in Down syndrome mice. J. Psychopharmacol. 2011; 25:1030–-42. [PubMed: 21693554]
61. Braudeau J, Dauphinot L, Duchon A, Loistron A, Dodd RH, et al. Chronic treatment with a promnesiant $GABA_A$ α_5-selective inverse agonist increases immediate early genes expression during memory processing in mice and rectifies their expression levels in a Down syndrome mouse model. Adv. Pharmacol. Sci. 2011; 2011:153218. [PubMed: 22028705]
62. Möhler H. Cognitive enhancement by pharmacological and behavioral interventions: the murine Down syndrome model. Biochem. Pharmacol. 2012; 84:994–-99. [PubMed: 22898099]
63. Mégarbané A, Ravel A, Mircher C, Sturtz F, Grattan Y, et al. The 50th anniversary of the discovery of trisomy 21: the past, present, and future of research and treatment of Down syndrome. Genet. Med. 2009; 11:611–-16. [PubMed: 19636252]
64. Kleschevnikov AM, Belichenko PV, Salehi A, Wu C. Discoveries in Down syndrome: moving basic science to clinical care. Prog. Brain Res. 2012; 197:199–-221. [PubMed: 22541294]
65. Baldwin DS, Anderson IM, Nutt DJ, Bandelow B, Bond A, et al. Evidence-based guidelines for the pharmacological treatment of anxiety disorders: recommendations from the British Association for Psychopharmacology. J. Psychopharmacol. 2005; 19:567–-96. [PubMed: 16272179]
66. Rudolph U, Möhler H. GABA-based therapeutic approaches: $GABA_A$ receptor subtype functions. Curr. Opin. Pharmacol. 2006; 6:18–-23. [PubMed: 16376150]
67. Rudolph U, Knoflach F. Beyond classical benzodiazepines: novel therapeutic potential of $GABA_A$ receptor subtypes. Nat. Rev. Drug Discov. 2011; 10:685–-97. [PubMed: 21799515]

68. Rudolph U, Crestani F, Benke D, Brünig I, Benson J, et al. Benzodiazepine actions mediated by specific γ-aminobutyric acid$_A$ receptor subtypes. Nature. 1999; 401:796–800. [PubMed: 10548105]
69. McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, et al. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA$_A$ receptor α$_1$ subtype. Nat. Neurosci. 2000; 3:587–92. [PubMed: 10816315]
70. Crestani F, Martin JR, Möhler H, Rudolph U. Resolving differences in GABA$_A$ receptor mutant mouse studies. Nat. Neurosci. 2000; 3:1059. [PubMed: 11036253]
71. Löw K, Crestani F, Keist R, Benke D, Brünig I, et al. Molecular and neuronal substrate for the selective attenuation of anxiety. Science. 2000; 290:131–34.
72. Smith KS, Engin E, Meloni EG, Rudolph U. Benzodiazepine-induced anxiolysis and reduction of conditioned fear are mediated by distinct GABA$_A$ receptor subtypes in mice. Neuropharmacology. 2012; 63:250–58. [PubMed: 22465203]
73. Morris HV, Dawson GR, Reynolds DS, Atack JR, Stephens DN. Both α2 and α3 GABA$_A$ receptor subtypes mediate the anxiolytic properties of benzodiazepine site ligands in the conditioned emotional response paradigm. Eur. J. Neurosci. 2006; 23:2495–504. [PubMed: 16706856]
74. Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, et al. Evidence for a significant role of α3-containing GABA$_A$ receptors in mediating the anxiolytic effects of benzodiazepines. J. Neurosci. 2005; 25:10682–88. [PubMed: 16291941]
75. Facklam M, Schoch P, Bonetti EP, Jenck F, Martin JR, et al. Relationship between benzodiazepine receptor occupancy and functional effects in vivo of four ligands of differing intrinsic efficacies. J. Pharm. Exp. Ther. 1992; 261:1113–21.
75a. Marowsky A, Rudolph U, Fritschy JM, Arand M. Tonic inhibition in principal cells of the amygdala: a central role for α3 subunit-containing GABA$_A$ receptors. J. Neurosci. 2012; 32:8611–19. [PubMed: 22723702]
76. LeDoux JE. The amygdala. Curr. Biol. 2007; 17:R868–74. [PubMed: 17956742]
77. Haubensak W, Kunwar PS, Cai H, Ciocchi S, Wall NR, et al. Genetic dissection of an amygdala microcircuit that gates conditioned fear. Nature. 2010; 468:270–76. [PubMed: 21068836]
78. Johansen JP, Cain CK, Ostroff LE, LeDoux JE. Molecular mechanisms of fear learning and memory. Cell. 2011; 147:509–24. [PubMed: 22036561]
79. Marowsky A, Fritschy JM, Vogt KE. Functional mapping of GABA$_A$ receptor subtypes in the amygdala. Eur. J. Neurosci. 2004; 20:1281–89. [PubMed: 15341600]
80. Deleted in proof
81. Ehrlich I, Humeau Y, Grenier F, Ciocchi S, Herry C, Lüthi A. Amygdala inhibitory circuits and the control of fear memory. Neuron. 2009; 62:757–71. [PubMed: 19555645]
82. Wiltgen BJ, Godsil BP, Peng Z, Saab F, June HL, et al. The α1 subunit of the GABA$_A$ receptor modulates fear learning and plasticity in the lateral amygdala. Front. Behav. Neurosci. 2009; 3:37. [PubMed: 19876409]
83. Heldt SA, Ressler KJ. Training-induced changes in the expression of GABA$_A$-associated genes in the amygdala after the acquisition and extinction of Pavlovian fear. Eur. J. Neurosci. 2007; 26:3631–44. [PubMed: 18088283]
84. Herry C, Ferraguti F, Singewald N, Letzkus JJ, Ehrlich I, Lüthi A. Neuronal circuits of fear extinction. Eur. J. Neurosci. 2010; 31:599–612. [PubMed: 20384807]
85. Bishop SJ. Neurocognitive mechanism of anxiety: an integrative account. Trends Cogn. Sci. 2007; 11:307–16. [PubMed: 17553730]
86. Rangel A, Hare T. Neural computations associated with goal-directed choice. Curr. Opin. Neurobiol. 2010; 20:262–70. [PubMed: 20338744]
87. Amemori K-I, Graybiel AM. Localized microstimulation of primate pregenual cingulate cortex induces negative decision-making. Nat. Neurosci. 2012; 15:776–85. [PubMed: 22484571]
88. Larkum ME. The yin and yang of cortical layer 1. Nat. Neurosci. 2013; 16:114–15. [PubMed: 23354381]

89. Ressler KJ, Mayberg HS. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. Nat. Neurosci. 2007; 10:1116–-24. [PubMed: 17726478]

90. Lüscher B, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. Mol. Psychiatry. 2010; 16:383–-406. [PubMed: 21079608]

91. Ator NA. Contribution of $GABA_A$ receptor subtype selectivity to abuse liability and dependence potential of pharmacological treatments for anxiety and sleep disorders. CNS Spectrums. 2005; 10:31–-39. [PubMed: 15618945]

92. Licata SC, Rowlett JK. Abuse and dependence liability of benzodiazepine-type drugs: $GABA_A$ receptor modulation and beyond. Pharmacol. Biochem. Behav. 2008; 90:74–-89. [PubMed: 18295321]

93. Ator NA, Atack JR, Hargreaves RJ, Burns HD, Dawson GR. Reducing abuse liability of $GABA_A$/benzodiazepine ligands via selective partial agonist efficacy at α1 and α2/3 subtypes. J. Pharmacol. Exp. Ther. 2010; 332:4–-16. [PubMed: 19789360]

94. Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, et al. TPA023, an agonist selective for α_2- and α_3-containing $GABA_A$ receptors, is a non-sedating anxiolytic in rodents and primates. J. Pharm. Exp. Ther. 2006; 316:410–-22.

95. Atack JR. Subtype-selective $GABA_A$ receptor modulation yields a novel pharmacological profile: the design and development of TPA023. Adv. Pharmacol. 2009; 57:137–-85. [PubMed: 20230761]

96. Deleted in proof

97. Atack JR, Wafford KA, Street LJ, Dawson GR, Tye S, et al. MRK-409 (MK-0343), a $GABA_A$ receptor subtype-selective partial agonist, is a non-sedating anxiolytic in preclinical species but causes sedation in humans. J. Psychopharmacol. 2011; 25:314–-28. [PubMed: 20147571]

97a. Vinkers CH, van Oorschot R, Nielsen EO, Cook JM, Hansen HH, et al. $GABA_A$ receptor α subunits differentially contribute to diazepam tolerance after chronic treatment. PLoS ONE. 2012; 7:e43054. [PubMed: 22912786]

98. de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, et al. Pharmacodynamic and pharmacokinetic effects of MK-0343, a $GABA_A$ $\alpha_{2,3}$ subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J. Psychopharmacol. 2008; 22:24–-32. [PubMed: 18187530]

99. Atack JR, Hallett DJ, Wafford KA, Ryan C, Sanabria-Bohorquez SM, et al. Preclinical and clinical pharmacology of TPA023B, a $GABA_A$ receptor α2/α3 subtype-selective partial agonist. J. Psychopharmacol. 2011; 25:329–-44. [PubMed: 20156926]

100. Lippa A, Czobor P, Stark J, Beer B, Kostakis E, et al. Selective anxiolysis produced by ocinaplon, a $GABA_A$ receptor modulator. Proc. Natl. Acad. Sci. USA. 2005; 102:7380–-85. [PubMed: 15870187]

101. Skolnick P. Anxioselective anxiolytics: on a quest for the holy grail. Trends Pharmacol. Sci. 2012; 33:611–-20. [PubMed: 22981367]

102. Czobor P, Skolnick P, Beer B, Lippa A. A multicenter, placebo-controlled, double-blind, randomized study of efficacy and safety of ocinaplon (DOV 273,547) in generalized anxiety disorder. CNS Neurosci. Ther. 2010; 16:63–-75. [PubMed: 20041911]

103. Belelli D, Lambert JJ. Neurosteroids; endogenous regulators of the $GABA_A$ receptor. Nat. Rev. Neurosci. 2005; 6:565–-75. [PubMed: 15959466]

104. Rupprecht R, Papadopoulos V, Rammes G, Baghai T, Fan J, et al. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. Nat. Rev. Drug Discov. 2010; 9:971–-88. [PubMed: 21119734]

105. Mitchell EA, Herd MB, Gunn BG, Lambert JJ, Belelli D. Neurosteroid modulation of $GABA_A$ receptors: molecular determinants and significance in health and disease. Neurochem. Int. 2008; 52:588–-95. [PubMed: 18055067]

106. Rupprecht R, Rammes G, Eser D, Baghai T, Schüle C, et al. Translocator protein (18 kDa) as target for anxiolytics without benzodiazepine-like side effects. Science. 2009; 325:490–-93. [PubMed: 19541954]

107. Owen DR, Rupprecht R, Nutt DJ. Stratified medicine in psychiatry: a worrying example or new opportunity in the treatment of anxiety? J. Psychopharmacol. 2013; 27:119–-22. [PubMed: 22522972]

108. Tan KR, Brown M, Labouèbe G, Yvon C, Creton C, et al. Neural bases for addictive properties of benzodiazepines. Nature. 2010; 463:769–-74. [PubMed: 20148031]

109. Fischer BD, Teixeira LP, van Linn ML, Namjoshi OA, Cook JM, Rowlett JK. Role of gamma-aminobutyric acid type A ($GABA_A$) receptor subtypes in acute benzodiazepine physical dependence-like effects: evidence from squirrel monkeys responding under a schedule of food presentation. Psychopharmacology. 2013; 227:347–54. [PubMed: 23354533]

110. Straub CJ, Carlezon WA Jr, Rudolph U. Diazepam and cocaine potentiate brain stimulation reward in C57BL/6J mice. Behav. Brain Res. 2010; 206:17–-20. [PubMed: 19716385]

111. Reynolds LM, Engin E, Tantillo G, Lau HM, Muschamp JW, et al. Differential roles of $GABA_A$ receptor subtypes in benzodiazepine-induced enhancement of brain-stimulation reward. Neuropsychopharmacology. 2012; 37:2531–-40. [PubMed: 22763624]

112. Nutt DJ, Stahl SM. Searching for perfect sleep: the continuing evolution of $GABA_A$ receptor modulators as hypnotics. J. Psychopharmacol. 2010; 24:1601–-12. [PubMed: 19942638]

113. Najib, JS. Efficacy and safety of zopiclone and eszopiclone in the treatment of primary and comorbid insomnia.. In: Monti, JM.; Pandi-Perumal, SR.; Möhler, H., editors. GABA and Sleep: Molecular, Functional and Clinical Aspects. Springer Basel AG; Basel, Switz: 2010. p. 413--51.

114. Deleted in proof

115. Deleted in proof

116. Deleted in proof

117. Deleted in proof

118. Deleted in proof

119. Deleted in proof

120. Deleted in proof

121. Deleted in proof

122. Deleted in proof

123. Deleted in proof

124. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch. Gen. Psychiatry. 2005; 62:593–-602. [PubMed: 15939837]

125. Holtzheimer PE, Mayberg HS. Stuck in a rut: rethinking depression and its treatment. Trends Neurosci. 2011; 34:1–-9. [PubMed: 21067824]

125a. Lüscher B, Fuchs T, Kilpatrick CL. $GABA_A$ receptor trafficking-mediated plasticity of inhibitory synapses. Neuron. 2011; 70:385–-409. [PubMed: 21555068]

126. Sanacora G, Mason GF, Rothman DL, Behar KL, Hyder F, et al. Reduced cortical gamma-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. Arch. Gen. Psychiatry. 1999; 56:1043–-47. [PubMed: 10565505]

127. Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, et al. Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. Arch. Gen. Psychiatry. 2004; 61:705–-13. [PubMed: 15237082]

128. Earnheart JC, Schweizer C, Crestani F, Iwasato T, Itohara S, et al. GABAergic control of adult hippocampal neurogenesis in relation to behavior indicative of trait anxiety and depression states. J. Neurosci. 2007; 27:3845–-54. [PubMed: 17409249]

129. Crestani F, Lorez M, Baer K, Essrich C, Benke D, et al. Decreased $GABA_A$-receptor clustering results in enhanced anxiety and a bias for threat cues. Nat. Neurosci. 1999; 2:833–-39. [PubMed: 10461223]

130. Shen Q, Lal R, Luellen BA, Earnheart JC, Andrews AM, Lüscher B. γ-Aminobutyric acid-type A receptor deficits cause hypothalamic-pituitary-adrenal axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic forms of depression. Biol. Psychiatry. 2010; 68:512–20. [PubMed: 20579975]

130a. Vithlani M, Hines RM, Zhong P, Terunuma M, Hines DJ, et al. The ability of BDNF to modify neurogenesis and depressive-like behaviors is dependent upon phosphorylation of tyrosine residues 365/367 in the $GABA_A$-receptor γ2 subunit. J. Neurosci. 2013; 33:15567–77. [PubMed: 24068823]

131. Gunther U, Benson J, Benke D, Fritschy JM, Reyes G, et al. Benzodiazepine-insensitive mice generated by targeted disruption of the γ_2 subunit gene of γ-aminobutyric acid type A receptors. Proc. Natl. Acad. Sci. USA. 1995; 92:7749–-53. [PubMed: 7644489]
132. Vollenweider I, Smith KS, Keist R, Rudolph U. Antidepressant-like properties of α2-containing $GABA_A$ receptors. Behav. Brain Res. 2011; 217:77–-80. [PubMed: 20965216]
133. Carlezon WA Jr, Chartoff EH. Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. Nat. Protoc. 2007; 2:2987–-95. [PubMed: 18007634]
134. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. Nat. Neurosci. 2010; 13:1161–-69. [PubMed: 20877280]
135. Amsterdam JD, Kaplan M, Potter L, Bloom L, Rickels K. Adinazolam, a new triazolobenzodiazepine, and imipramine in the treatment of major depressive disorder. Psychopharmacology. 1986; 88:484–-88. [PubMed: 3085137]
136. Petty F, Trivedi MH, Fulton M, Rush AJ. Benzodiazepines as antidepressants: Does GABA play a role in depression? Biol. Psychiatry. 1995; 38:578–-91. [PubMed: 8573660]
137. Fava M, McCall WV, Krystal A, Wessel T, Rubens R, et al. Eszopiclone co-administered with fluoxetine in patients with insomnia coexisting with major depressive disorder. Biol. Psychiatry. 2006; 59:1052–-60. [PubMed: 16581036]
138. Brown RW, Noel DM, Smith JJ, Smith ML, Huggins KN, et al. Eszopiclone facilitation of the antidepressant efficacy of fluoxetine using a social defeat stress model. Pharmacol. Biochem. Behav. 2011; 99:648–-58. [PubMed: 21699914]
139. Guidotti A, Auta J, Davis JM, Dong E, Grayson DR, et al. GABAergic dysfunction in schizophrenia: new treatment strategies on the horizon. Psychopharmacology. 2005; 180:191–-205. [PubMed: 15864560]
140. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. Nat. Rev. Neurosci. 2005; 6:312–-24. [PubMed: 15803162]
141. Castner SA, Arriza JL, Roberts JC, Mrzljak L, Christian EP, Williams GV. Reversal of ketamine-induced working memory impairments by the $GABA_{Aα2/3}$ agonist TPA023. Biol. Psychiatry. 2010; 67:998–-1001. [PubMed: 20189164]
142. Lewis DA, Cho RY, Carter CS, Eklund K, Forster S, et al. Subunit-selective modulation of GABA type A receptor neurotransmission and cognition in schizophrenia. Am. J. Psychiatry. 2008; 165:1585–-93. [PubMed: 18923067]
143. Buchanan RW, Keefe RS, Lieberman JA, Barch DM, Csernansky JG, et al. A randomized clinical trial of MK-0777 for the treatment of cognitive impairments in people with schizophrenia. Biol. Psychiatry. 2011; 69:442–-49. [PubMed: 21145041]
144. Yee BK, Keist R, von Boehmer L, Studer R, Benke D, et al. A schizophrenia-related sensorimotor deficit links α3-containing $GABA_A$ receptors to a dopamine hyperfunction. Proc. Natl. Acad. Sci. USA. 2005; 102:17154–-59. [PubMed: 16284244]
145. Hauser J, Rudolph U, Keist R, Möhler H, Feldon J, Yee BK. Hippocampal α5 subunit-containing $GABA_A$ receptors modulate the expression of prepulse inhibition. Mol. Psychiatry. 2005; 10:201–-7. [PubMed: 15263904]
146. Gerdjikov TV, Rudolph U, Keist R, Möhler H, Feldon J, Yee BK. Hippocampal α5 subunit-containing $GABA_A$ receptors are involved in the development of the latent inhibition effect. Neurobiol. Learn. Mem. 2008; 89:87–-94. [PubMed: 17638582]
147. Gill KM, Lodge DJ, Cook JM, Aras S, Grace AA. A novel α5$GABA_A$R-positive allosteric modulator reverses hyperactivation of the dopamine system in the MAM model of schizophrenia. Neuropsychopharmacology. 2011; 36:1903–-11. [PubMed: 21562483]
148. Delini-Stula A, Berdah-Tordjman D. Antipsychotic effects of bretazenil, a partial benzodiazepine agonist in acute schizophrenia---a study group report. J. Psychiatr. Res. 1996; 30:239–-50. [PubMed: 8905533]
149. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav. 2003; 2:255–-67. [PubMed: 14606691]
150. Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, et al. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature. 2011; 477:171–-78. [PubMed: 21796121]

151. Gogolla N, LeBlanc JL, Quast KB, Südhof TC, Fagiolini M, Hensch TK. Common circuit defect of excitatory-inhibitory balance in mouse models of autism. J. Neurodev. Disord. 2009; 1:172–-181. [PubMed: 20664807]

152. Penagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, et al. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. Cell. 2011; 147:235–-46. [PubMed: 21962519]

153. Chao HT, Chen H, Samaco RC, Xue M, Chahrour M, et al. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature. 2010; 468:263–-69. [PubMed: 21068835]

154. Han S, Tai C, Westenbroek RE, Yu FH, Cheah CS, et al. Autistic-like behaviour in Scn1a+$^{-}$ mice and rescue by enhanced GABA-mediated neurotransmission. Nature. 2012; 489:385–-90. [PubMed: 22914087]

155. Fatemi SH, Reutiman TJ, Folsom TD, Rooney RJ, Patel DH, Thuras PD. mRNA and protein levels for $GABA_A$α4, α5, β1 and $GABA_B$R1 receptors are altered in brains from subjects with autism. J. Autism Dev. Disord. 2010; 40:743–-50. [PubMed: 20066485]

156. Mendez MA, Horder J, Myers J, Coghlan S, Stokes P, et al. The brain GABA-benzodiazepine receptor alpha-5 subtype in autism spectrum disorder: a pilot [^{11}C]Ro15-4513 positron emission tomography study. Neuropharmacology. 2013; 68:195–-201. [PubMed: 22546616]

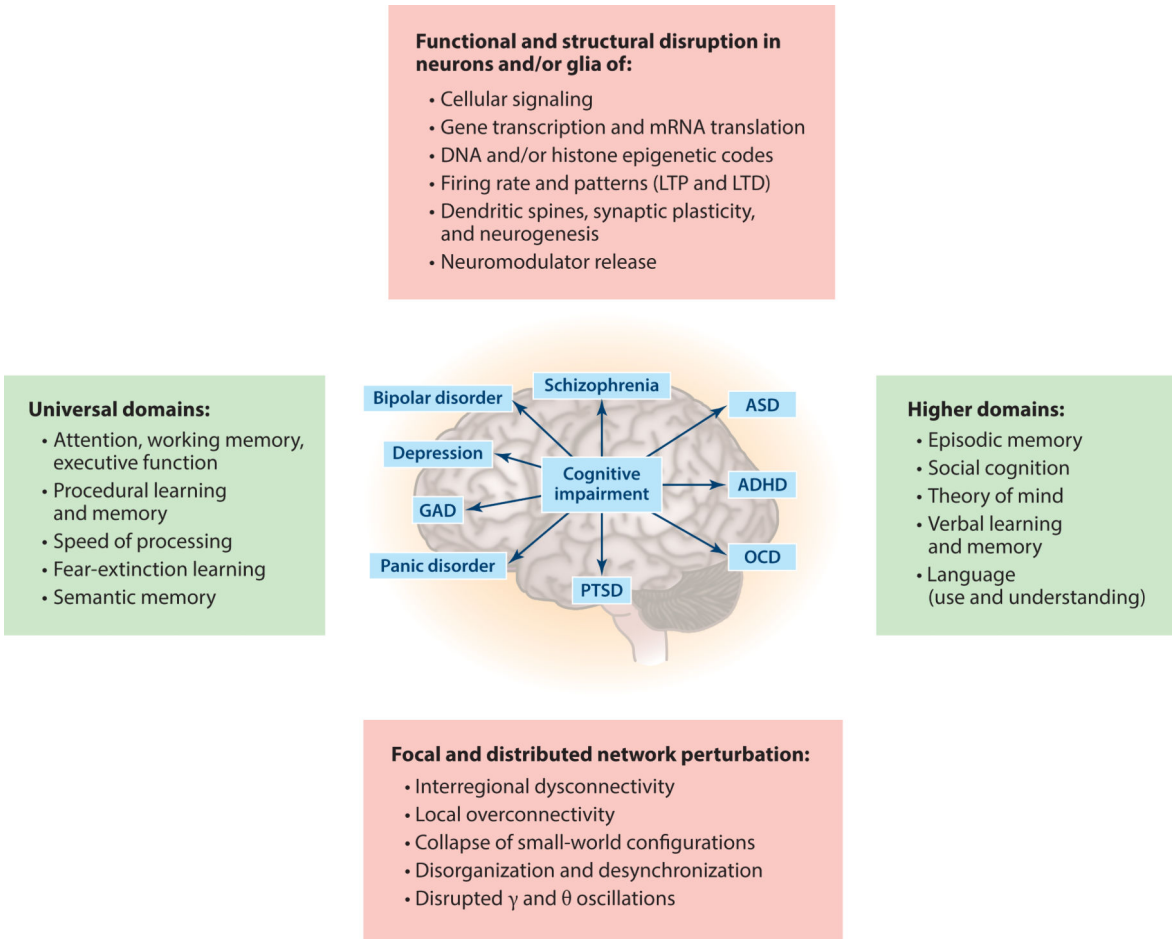

Figure 1.

Cognition in psychiatric disorders. A global view of cognition and its disruption in psychiatric disorders. Psychiatric disorders are associated with complex and disease-specific patterns of cognitive impairment. Abbreviations: ADHD, attention-deficit hyperactivity disorder; ASD, autism spectrum disorders; LTD, long-term depression; LTP, long-term potentiation; OCD, obsessive-compulsive disorder; GAD, generalized anxiety disorder; PTSD, posttraumatic stress disorder. Reprinted from Reference 1 with permission from Macmillan Publishers Ltd.

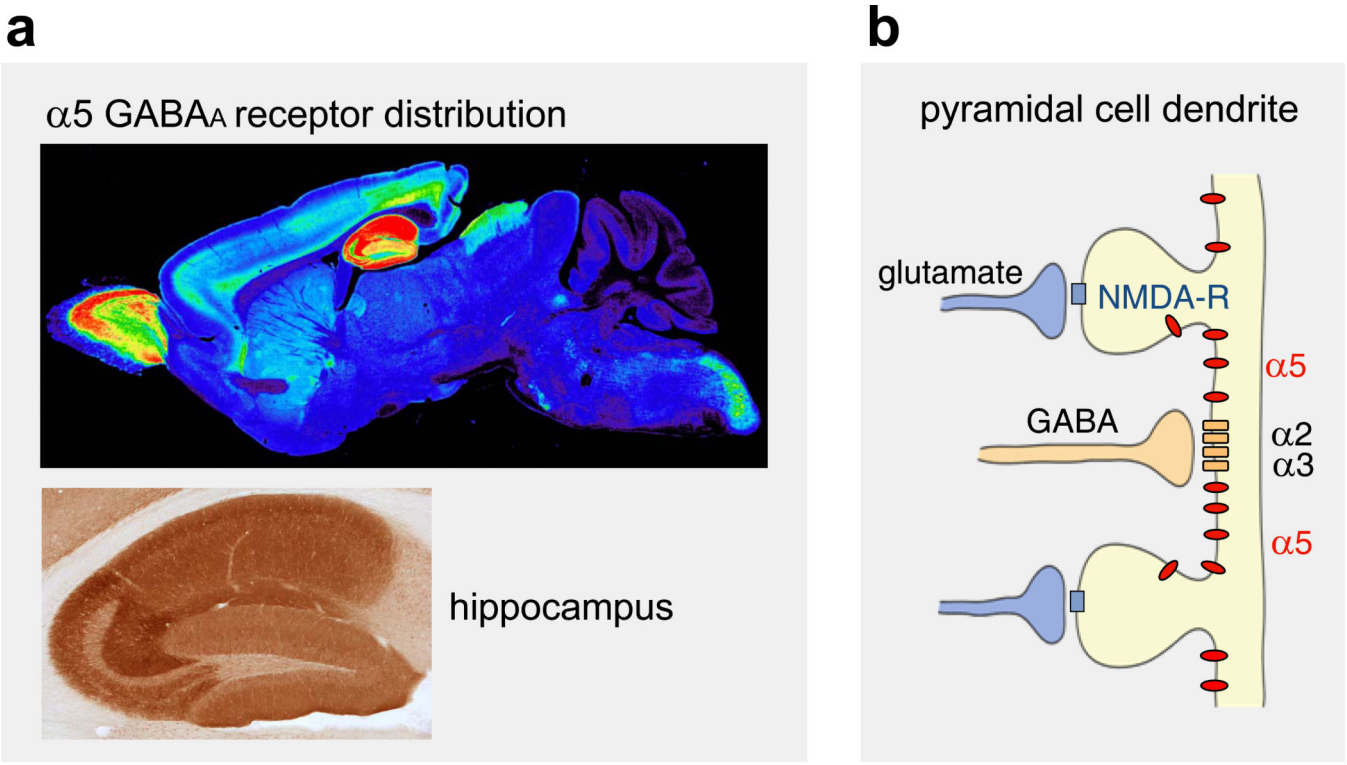

Figure 2.

Distribution of α_5 subunit-containing $GABA_A$ receptors. (***a***)False colour immunohistochemical distribution of the α_5 $GABA_A$ receptor in parasagittal sections of adult mice; the enlargement of the hippocampal formation immunohistochemical staining shows the prominent dendritic localization of the α_5 subunit (reprinted from Reference 18, Copyright (2002): National Academy of Sciences, USA). (*b*) Schematic distribution of $GABA_A$ receptor subtypes at pyramidal cell dendrites (beige). Phasic inhibition is mediated via synaptic α_2 and α_3-subunit-containing $GABA_A$ receptors, whereas α_5 subunit-containing receptors, located at the bases of dendritic spines and the adjacent dendritic shaft, provide tonic inhibition. Abbreviations: GABA, γ-aminobutyric acid; NMDA-R, *N*-methyl-D-aspartate receptor.

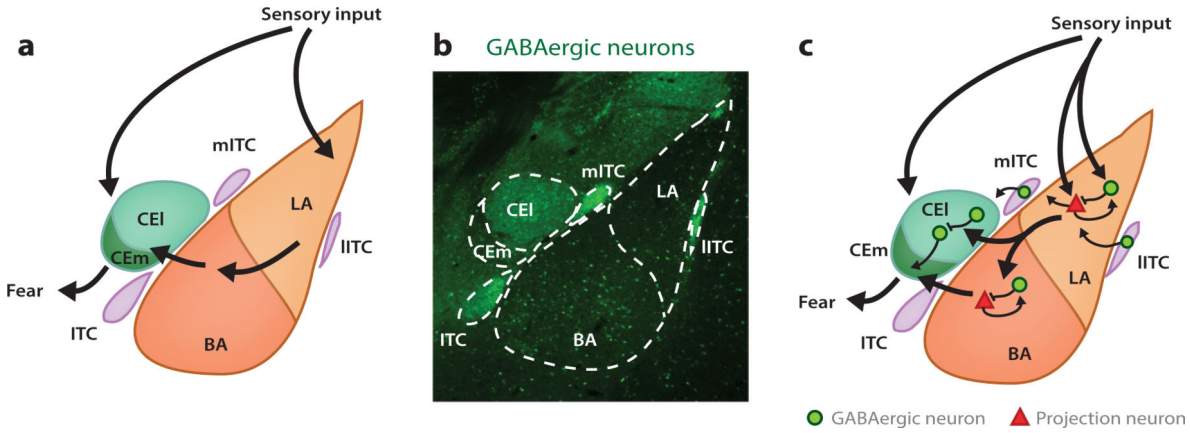

Figure 3.

General organization of amygdala circuitry and GABAergic neurons. (*a*) Scheme of the basic organization and overall flow of information within the amygdaloid complex. (*b*) Coronal brain slice stained for the 67-kDa isoform of the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD67); the distribution of GABAergic neurons across the amygdaloid complex is illustrated. (*c*) Simplified scheme of the organization and function of inhibitory interneurons in amygdaloid nuclei. In the LA and BA, local interneurons are part of feed-forward and feedback circuits, and they control projection neuron output. The lITCs and mITCs relay feed-forward inhibition, which may also participate in controlling CEl output. Abbreviations: BA, basal amygdala; CEl, lateral subdivision of the central amygdala; CEm, medial subdivision of the central amygdala; GABA, γ-aminobutyric acid; LA, lateral amygdala; lITC, lateral intercalated cell cluster; mITC, medial intercalated cell cluster. Reproduced from Reference 81 with permission.

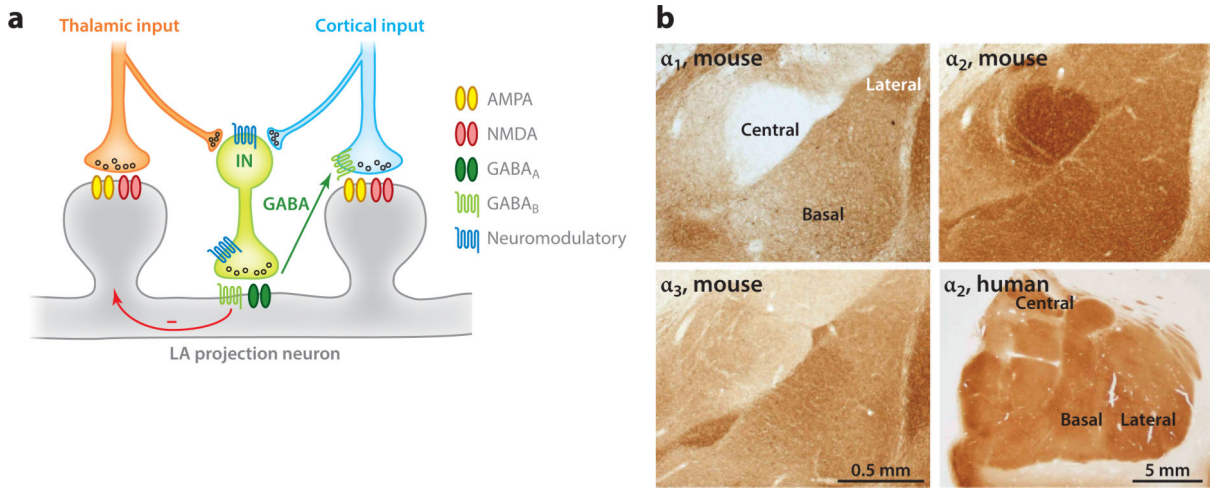

Figure 4.

Inhibitory gating of long-term potentiation (LTP) in the lateral amygdala (LA) in fear acquisition and $GABA_A$ receptor distribution. (*a*) Pyramidal projection neurons in the LA (*gray*) receive converging thalamic and cortical sensory afferents. LTP at thalamic and cortical sensory synapses, induced by fear conditioning, is tightly controlled by GABA released from feed-forward interneurons (IN) (*green*). At thalamic afferents, this control is predominantly postsynaptic via $GABA_A$ receptors. At cortical afferents, this control is presynaptic via $GABA_B$ receptors. The GABA IN are targets of neuromodulators that modify their output activity and thereby gate the induction of LTP by transiently altering the levels of pre- and postsynaptic inhibitory drive. By depressing GABA feed-forward inhibition, dopamine and noradrenaline enhance LTP, whereas 5-hydroxytryptamine (5-HT) reduces LTP. Reproduced from Reference 81 with permission. (*b*) Immunohistochemical distribution of $GABA_A$ receptor subtypes in the amygdala. In the mouse, the α_2-subunit staining is prominent throughout, in particular in the central nucleus where no α_1 subunit immunoreactivity is detectable. Both α_1 and α_2 subunits produce a diffuse labeling of neuropil in the lateral and basolateral nuclei. The α_3 subunit is detected in the basolateral nucleus and, to a lesser extent, in the lateral and central nuclei (75a, 79). Similarly, in human amygdala, α_2-subunit staining is prominent throughout and most prevalent in the central and basal nuclei. α_1-Subunit staining was prominent only in the lateral nucleus. Minimal staining for α_3 subunits was apparent throughout the amygdala. Other Abbreviations: AMPA, 1-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate. Data and image of human amygdala courtesy of J. Song and H. Waldvogel, Center of Brain Research, University of Auckland, New Zealand.

Table 1

Cellular and subcellular localization of $GABA_A$ receptor subtypes*

Main subunit	Proposed subunit repertoire	Benzodiazepine site pharmacology	Major sites of expression	Identified neurons	Subcellular localization
α_1	$\alpha_1\beta_2\gamma_2$	Major subtype (60% of all $GABA_A$ receptors); mediates the sedative, amnestic, and— to a large extent— anticonvulsant action and dependence liability of benzodiazepine site agonists	Cerebral cortex (layers 1-6), hippocampus, amygdala, olfactory bulb, thalamus, basal forebrain, globus pallidus, substantia nigra pars reticulata, inferior colliculus, cerebellum, brainstem	Mitral cells and short-axon cells (olfactory bulb), principal cells and selected interneurons in cerebral cortex and hippocampus, GABAergic neurons in pallidum and substantia nigra, thalamic relay neurons, Purkinje cells, and granule cells	Synaptic in soma and dendrites; extrasynaptic in all neurons with high expression
α_2	$\alpha_2\beta_3\gamma_2$	Minor subtype (15--20%); mediates anxiolytic action of benzodiazepine site agonists	Cerebral cortex (layers 1-4), hippocampal formation, amygdala, striatum, olfactory bulb, hypothalamus, superior colliculus, inferior olive, motor nuclei, spinal cord dorsal horn	Principal cells in hippocampal formation and amygdala, spiny stellate striatal neurons, olfactory bulb granule cells, motor neurons, dorsal root ganglion cells, and intrinsic dorsal horn neurons	Mainly synaptic; perisomatic and enriched in axon initial segment of cortical and hippocampal pyramidal cells
α_3	$\alpha_3\beta_{2,3}\gamma_2$	Minor subtype (10--15%); mediates anxiolytic action of benzodiazepine site agonists, although only at high receptor occupancy	Cerebral cortex (layers 5, 6), amygdala, olfactory bulb, thalamic reticular and intralaminar nuclei, superior colliculus, brainstem, spinal cord, locus coeruleus, raphe, medial septum	Tufted cells (olfactory bulb), reticular thalamic neurons, cerebellar Golgi type II cells, serotonergic and catecholaminergic neurons, basal forebrain cholinergic neurons, principal cells in lateral and basolateral amygdala	Mainly synaptic, including in some axon initial segments; extrasynaptic in principal cells of the amygdala and in inferior olivary neurons
α_4	$\alpha_4\beta_{2,3}\delta$	Less than 5% of all receptors; insensitive to classical benzodiazepines	Dentate gyrus, thalamus	Dentate gyrus granule cells	Extrasynaptic
α_5	$\alpha_5\beta_3\gamma_2$	Less than 5% of all receptors; mediates the memory-enhancing effects of benzodiazepine site partial inverse agonists	Highest in hippocampus, considerably lower in deep cortical layers, amygdala, olfactory bulb, hypothalamus, superior colliculus, superior olivary nucleus, spinal trigeminal nucleus, spinal cord	Pyramidal cells (hippocampus, cerebral cortex), granule cells and periglomerular cells (olfactory bulb), superior olivary neurons, spinal trigeminal neurons	Extrasynaptic in hippocampus, cerebral cortex, and olfactory bulb; synaptic and extrasynaptic in spinal trigeminal nucleus and superior olivary nucleus; synaptic in bitufted cells
α_6	$\alpha_6\beta_{2,3}\gamma_2$; $\alpha_6\beta_{2,3}\delta$	Less than 5% of all receptors; insensitive to classical benzodiazepines	Cerebellum, dorsal cochlear nucleus	Granule cells (cerebellum)	Synaptic in cerebellar glomeruli; extrasynaptic in granule cell dendrites and soma

Adapted from Reference 12.

Table 2

Ligands of GABAa receptor subtypes

Drug	Main activity	Interaction with recombinant $GABA_A$ receptors
Ligands at benzodiazepine sites		
Zolpidem	Hypnotic	Preferential affinity for α_1
Zaleplon	Hypnotic	Preferential affinity for α_1
Indiplon	Hypnotic	Preferential affinity for α_1
L-838417	Anxiolytic, antinociceptive	Comparable affinity for α_1, α_2, α_3, and α_5; partial agonist at α_2, α_3, and α_5 (not α_1)
Ocinaplon	Anxiolytic	Comparable affinity for α_1, α_2, α_3, and α_5; partial agonist at α_2, α_3, and α_5; nearly full agonist at α_1
SL651498	Anxiolytic, antinociceptive	Agonist at α_2 and α_3; partial agonist at α_1 and α_5
TPA023 (MK-0777)	Anxiolytic	Comparable affinity for α_1, α_2, α_3, and α_5; partial agonist at α_2, α_3; no efficacy at α_1 and α_5
TPA023B	Anxiolytic	Comparable affinity for α_1, α_2, α_3, and α_5; partial agonist at α_2, α_3, and α_5
TPA123	Anxiolytic	Partial agonist at α_1, α_2, α_3, and α_5
TP003	Anxiolytic	Comparable affinity for α_1, α_2, α_3, and α_5 nearly full agonist at α_3; acts at high receptor occupancy
ELB 139	Anxiolytic	Selective receptor profile uncertain
NS11394	Anxiolytic, antinociceptive	Comparable affinity for α_1, α_2, α_3, and α_5; partial agonist at α_2 and α_3; nearly full agonist at α_5
α_3IA	Anxiogenic	Weak inverse agonist at α_3
L-655708	Memory-enhancing	Partial inverse agonist with preferential efficacy at α_5
α_5IA	Memory-enhancing	Partial inverse agonist with preferential efficacy at α_5
RO4938581	Memory-enhancing	Partial inverse agonist with preferential affinity for and efficacy at α_5 receptors
MRK-016	Memory-enhancing	Partial inverse agonist at α_5 receptors
PWZ-029	Memory-enhancing	Partial inverse agonist at α_5 receptors
Ligands at modulatory sites other than benzodiazepine sites		
Ethanol	Anxiolytic, sedative	High sensitivity (≥3 mM) at $\alpha_1(\alpha_6)\beta_3\delta$; medium sensitivity (≥30 mM) at $\alpha_4(\alpha_6)\delta$; low sensitivity (≥100 mM) at $\alpha_4(\alpha_6)\beta_3\gamma_2$
Neurosteroids (e.g., 3α,5α-THDOC)	Anxiolytic, sedative, anesthetic	High sensitivity at δ-subunit-containing receptors and at α_1 and α_3 receptors in combination with β_1 subunit
Intravenous anesthetics (etomidate, propofol)	Sedative, anesthetic	Act on receptor subtypes containing β_2 and β_3 (etomidate) or β_1, β_2, and β_3 (propofol); β_2 receptors mediate sedation, whereas β_3 receptors mediate immobility
Ligands at GABA site		
Gaboxadol	Hypnotic, antinociceptive	Partial agonist at α_1 and α_3 subtypes; full agonist at α_5; and superagonist at $\alpha_4\beta_3\delta$ receptors

Abbreviation: GABA, γ-aminobutyric acid; THDOC, tetrahydrodeoxycorticosterone.

For references, see text and Reference 22.