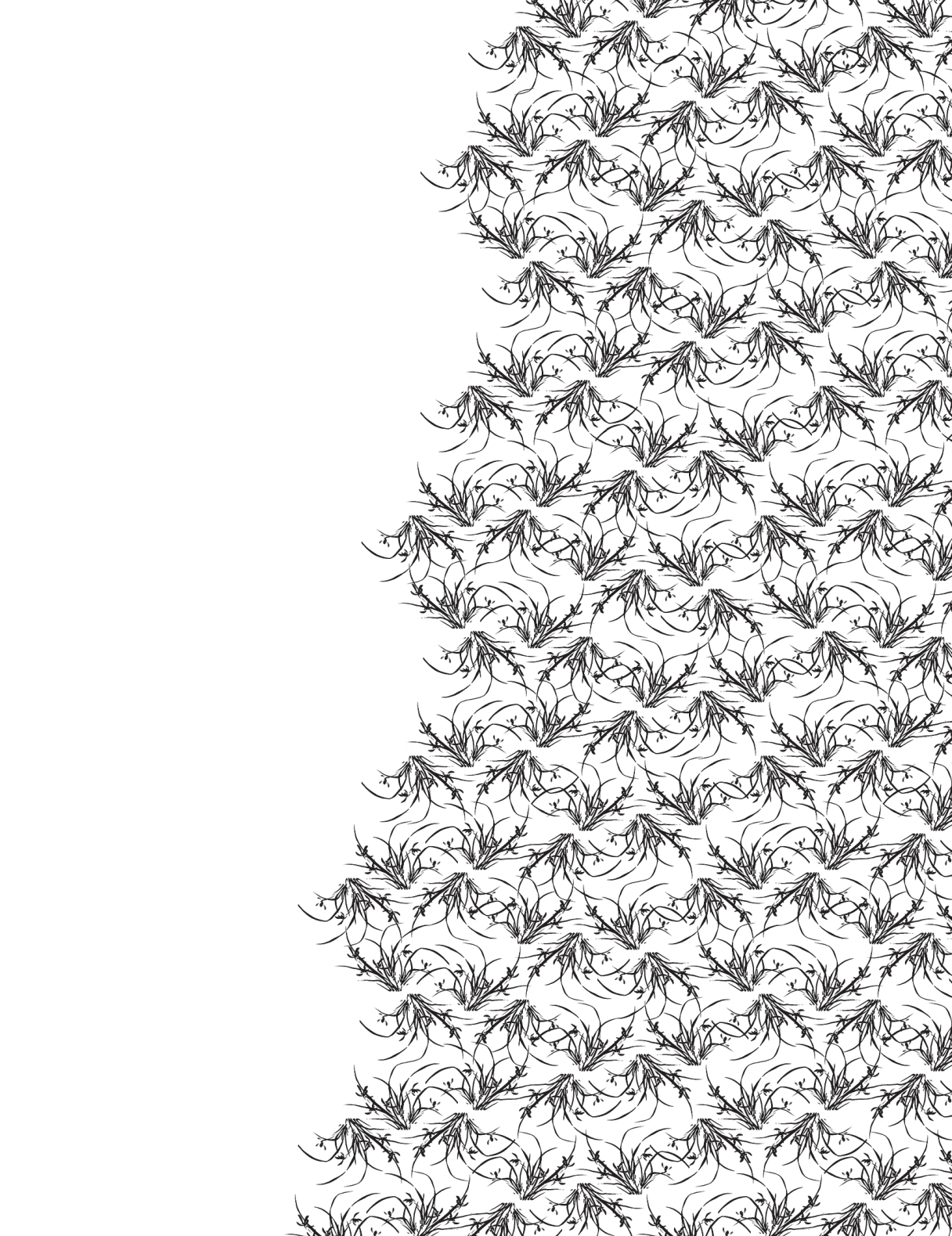
Human pharmacology of current and novel gaba(a)-ergic treatments for anxiety

Chen, X.



ANXIETY: DEFINITION, DIAGNOSIS, EPIDEMIOLOGY, AND CURRENT TREATMENT STATUS

Anxiety is a commonly occurring negative human emotional state and is characterized by subjective feelings of worry and fear. By definition, worry or apprehension refers to thoughts and expectations about future events while fear is an acute reaction to perceived imminent danger. Subjective phenomena are usually accompanied by physical symptoms such as increased heart rate, shakiness, fatigue, and muscle tension, as well as cognitive, and behavioral manifestations. Anxiety can be adaptive that occurs in response to a threat and prepares to cope with the environment. However, anxiety becomes pathological when it causes significant personal distress and impairs everyday functioning. In order to be diagnosed with an anxiety disorder, individuals have to experience a certain number of symptoms that are disproportionate to either actual or imagined environmental threat for at least six months [1,2].

Anxiety disorders are chronic, disabling conditions that impose enormous costs both on individuals and on society [3-6]. These disorders are prevalent in Western countries. According to a recent 3-year multi-method study covering 30 European countries, 14% of the total population (i.e., 514 million people) were suffering from anxiety disorders [4]. In the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (*DSM 5) [1], seven anxiety syndromes are classified, including panic disorder, agoraphobia, social anxiety disorder (SAD), generalized anxiety disorder (GAD), specific phobias, separation anxiety disorder and selective mutism. The etiology of anxiety disorders is multifactorial and includes genetic liability to a certain extent for some syndromes. In addition, drug withdrawal, substance/medication (e.g. alcohol, caffeine, and benzodiazepines) abuse and dependence, occupational exposure to organic solvents, and life stresses have been related to the etiology of anxiety disordersm while psychiatric complications of endocrine disorders like pheochromocytoma and hyperthyroidism have been demonstrated to mimic anxiety disorders. Taken together, the phenomenologically-based diagnostic classification and the multifactorial nature of anxiety disorders are expected to affected efficacy of anxiolytic CNS active drugs that have been discovered in the past decades.

Current treatment modalities for anxiety disorders can be categorized into psychological treatments (e.g., exposure therapy, cognitive therapy and cognitive behavioral therapy) and pharmacological interventions [2]. The pharmacological interventions can be further divided to chronic or maintenance treatments and short-term treatments inducing acute anxiolysis. Monoamine modulating drugs such as the selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRIs) are considered the first-line drugs for anxiety

disorders. This is mainly due to their 'broad spectrum' anxiolytic efficacy in both short-term and long-term therapy and the relatively good tolerability in terms of side effects and treatment adherence [2]. However, since it is not unusual for treatment response to be reached only after 12 weeks of treatment at a therapeutic dose, the delayed onset of action of SSRIs and SNRIs remains a major disadvantage. In addition, when patients do not respond to or are intolerant of SSRI/SNRI treatment, alternative classes of psychotropic drugs, such as other antidepressant drugs (e.g., tricyclic antidepressants [TCAs], irreversible monoamine oxidase inhibitor [MAOI] phenelzine), anticonvulsant drug pregabalin, antipsychotic drugs (e.g., quetiapine), and anti-histamine drug hydroxyzine, are considered. Nonetheless, even after treatment with multiple anxiolytic drugs, up to 40% of patients with anxiety disorders do not respond to such drugs at all or only respond partially [7]. Given the rapid-onset effectiveness of benzodiazepines (BZDs) in many patients with anxiety disorders, especially in panic disorder, GAD and SAD patients, these drugs are generally reserved for the treatment of patients who have failed to respond to at least three previous treatments (such as after non-response to an SSRI, an SNRI and a psychological intervention). The use of BZDs should however be minimalized and preferably be reserved for short-term treatments to mitigate the risks of troublesome sedation, cognitive impairment and discontinuation symptoms after abrupt withdrawal [8] in both short-term and long-term treatment, and to avoid development of tolerance and dependence with prolonged use. Taken together, an obvious unmet clinical need in the pharmacological treatment of anxiety disorders opens an opportunity for novel pharmacological approaches that demonstrate rapid anxiolytic efficacy that is superior to existing treatments and lacks tolerance induction, abuse liability and withdrawal symptoms.

THE BRAIN CIRCUITRY INVOLVED IN ANXIETY AND THE ROLE OF GAMMA-AMINOBUTYRIC ACID A (GABA) IN THE AMYGDALA

On a neurobiological level, anxiety disorders arise from disruption of the highly interconnected circuits normally serving to process the stream of potentially threatening stimuli detected by the human brain from the outside world. Perturbations anywhere in these circuits cause imbalance in the entire system, resulting in a fundamental misinterpretation of neural sensory information as threatening and leading to the inappropriate emotional- and thereby behavioral-responses seen in anxiety disorders [9].

Briefly speaking, anxiety is linked to compromised interactions between the amygdala and the dorsal and ventral medial prefrontal cortex (MPFC). Tract-tracing studies in rats show that axons originating in the infra-limbic cortex of the MPFC terminate most densely in the ventromedial lateral nucleus, the rostral part of the

accessory basal amygdala, lateral capsular subdivision of the central nucleus and the superficial nuclei (lateral olfactory tract, periamygdaloid cortex and cortical nuclei) [10-12]. Neurons in the more caudal areas of the infra-limbic sub-region also project to the medial and intermediate subdivisions of the central nucleus [11,13]. The pre-limbic cortex of the mPFC is located dorsally adjacent to the infra-limbic sub-region and it has a different pattern of connectivity with the amygdala. Pre-limbic cortex neurons target the basal nucleus of the amygdala (BA), primarily the dorso-medial part [11,14], while caudal pre-limbic cortex neurons concentrate inputs in the medial parvicellular basal nucleus [15].

Fear extinction is defined as a decline in conditioned fear responses following repeated exposure to a feared conditioned stimulus (e.g., a tone in both animals and humans) in the absence of the unconditioned stimulus (usually a footshock in animals) with which it was previously paired [16]. Extincted fear can be recovered with time or change of the experimental context, suggesting that fear extinction reflects a learning process. The fear reduction is associated with inhibition rather than erasure of the original fear memory. Given that fear extinction has a close therapeutic analogue in the form of exposure therapy for patients with anxiety disorders, it has been implicated in many preclinical studies to investigate drugs acting as adjuncts to strengthen extinction and reduce intrusive fear memories in PTSD and specific phobias [17]. The acquisition, consolidation and retrieval of extinction therefore are separable processes that are controlled by different brain regions and neural systems [18].

In both experimental animal and human functional imaging studies, the amygdala and the mPFC has been demonstrated to be associated with the regulation of negative emotion, such as anxiety or worry and apprehension. Neuroimaging studies consistently show that higher levels of anxiety are associated with both attenuated ventral medial prefrontal cortex (vmPFC) activity and exaggerated dorsal medial prefrontal cortex (dmPFC) activity [19,20] in the presence of threatening stimuli. In the absence of threatening stimuli (i.e., at rest) Kim and colleagues [21] reported that the negative connectivity normally seen between the amygdala and the dmPFC at rest was attenuated in high anxious subjects, whereas the positive connectivity normally observed between the amygdala and vmPFC at rest, manifested as negative connectivity in high anxious subjects. Interestingly, the mPFC-amygdala coupling is inversely correlated with self-reported measures of anxiety or anxious temperament, indicating that the mPFC functions to actively regulate the amygdala and impaired connection between the two neural structures may lead to inadequate response to threatening stimuli. On the other hand, the amygdala – nuclei situated in the median temporal lobes – appears to play a crucial role in the regulation of negative affect and therefore anxiety-related symptomatology.

Emerging evidence from functional magnetic resonance imaging supports that amygdala is the key brain region of activity in response to negative emotional stimuli in healthy volunteers [22-24]. Besides, patients with anxiety disorders are prone to amygdala activation in response to a given threatening stimulus more than the non-anxious controls [25]. Moreover, successful treatment of anxiety disorders with cognitive behavioral therapy leads to extinction of this hyperactivation in the amygdala [26]. Taken together, mPFC functions to regulate amygdala function by actively suppressing activity, and so deficiency in the top-down regulation of mPFC and hyperactivation of the amegdela have been implicated in the pathophysiology of anxiety-related disorders.

In the amygdela, two groups of nuclei should be noted, namely the basolateral amygdala complex (BLA) and the centromedial amygdala complex, in particular the central nucleus (CeA) [27,28]. The BLA receives afferent information on potentially negative emotional signals from the thalamus and the sensory association cortex. The BLA activates the CeA either directly through an excitatory glutamatergic pathway or indirectly by activating a relay of inhibitory GABAergic interneurons that lie between the BLA and the CeA and exert an inhibitory influence upon the latter [29,30]. The CeA is the principal efferent pathway from the amygdala. Inhibitory GABAergic neurons project from the CeA to the hypothalamus and brainstem; the activation of these neurons leads to the somatic manifestations of anxiety [31]. Projections to other basal forebrain nuclei such as the ventrotegmental area and the locus ceruleus may be involved in the subjective effects that are related to anxiety, such as apprehension and dysphoria [32]. In addition, neurons from the BLA also activate cells in the adjacent bed nucleus of the stria terminalis, which project to the same areas as the CeA and apparently play a similar role [28,32].

The knowledge about the neurobiology underlining anxiety disorders serves as the basis for the search of novel anxiolytic agents. Compounds that manipulate this potential pathway may provide new options for the treatment of anxiety disorders. Moreover, neuroimaging and neurophysiological measurements that address the corresponding processes may be used to assess human responses to drug-mediated target modulation.

THE INVOLVEMENT OF GABA SYSTEM IN THE PATHOPHYSIOLOGY OFANXIETY AND ANXIETY DISORDERS

Mounting evidence has suggested the pathogenesis of human anxiety disorders is related to a dysfunction of central top-down inhibitory mechanisms. By providing the major source of inhibitory neurotransmission in the mPFC and amygdala, GABA exerts a powerful influence on a range of fear- and anxiety-related behaviours,

including fear extinction [33-37]. Temporary inactivation induced by GABA(A) receptor agonists has been implicated to establish necessary contribution of the infralimbic subregion or basolateral amygdala (BLA) (but not prelimbic cortex) to fear extinction [38,39]. Infusions of GABA or GABA receptor agonists into the amygdala were found reducing measures of fear and anxiety (possibly related to effects on memory reconsolidation) in several animal species [40,41]. On the other hand, infusion of the GABA antagonist bicuculline was found to block chlordiazepoxide-induced anxiolytic-like activity in rats, whereas injecting bicuculline methiodide to the anterior basolateral amygdala of rats elicited anxiogenic-like effects in both the social interaction paradigm and the conflict paradigm. Microinjection of bicuculline methiodide into the central nucleus of the amygdala elicited no change in experimental anxiety [42].

In humans, administration of benzodiazepines is translated to anxiolytic effect by attenuating amygdala activation in response to negative emotional stimuli [43,44]. To the contrary, Nutt et al. [45] performed an interesting study, in which they injected the benzodiazepine-antagonist flumazenil to 10 patients with panic disorder and 10 control subjects. Subjective anxiety responses after flumazenil infusion were significantly higher in patients with panic disorder than in the controls, and panic attacks were successfully induced in eight patients with panic disorder but no panic attack occurred in the controls. Although such findings have not been replicated [46], they are regarded as a potential signal for the possible shift of the "receptor set-point" [45]. Nikolaus et al reviewed 14 nuclear neuroimaging (Positron emission tomography [PET] and Single-Photon Emission Computed Tomography [SPECT]) studies conducted in patients with anxiety disorders (160 patients [mostly GAD patients] vs. 172 healthy controls). They identified a widespread decline of GABA(A) receptor binding sites and reduced binding extent in the whole mesolimbocortical system in patients suffering from anxiety disorders, suggesting attenuation of physiological central depression. The disturbances of the downstream dopaminergic and serotonergic neurotransmission are thought to, at least partly, result from the diminished tone of GABAergic neurotransmission [47]. A decrease of cortical GABA neurons and reduction of GABA levels were reported in patients with major depressive disorder (MDD) using proton magnetic resonance spectroscopy [48]. Considering the frequent comorbidity of MDD with anxiety states, a shared underlying pathology that emphasizes the causal contribution of GABAergic deficit is proposed for both anxiety disorders and depression [49-51]. Similar GABA(A) receptors reduction is also seen in patients with panic anxiety or post-trauma stress disorder (PTSD). Noteworthy, the extent of GABA(A) receptor deficit is significantly correlated to the clinical severity of these two disorders [52-56], suggesting an 'exposure'-response relationship and hence reinforcing the contribution of GABAergic deficit to anxiety status.

In summary, all aforementioned research findings suggest GABAergic neurotransmission in the mPFC-amygdala coupling is a promising target for modulation of anxiety-related responses.

GABA(A) RECEPTOR STRUCTURE, FUNCTION, AND ITS IMPLICATION IN THE PHARMACOTHERAPY OF ANXIETY DISORDERS

The discovery of the GABA(A) receptor in the 1970s, originally called benzodiazepine receptor, was essential for elaborating the mechanism of action of benzodiazepines, it was the recognition of benzodiazepine-sensitive GABA(A) receptor subtypes that opened up a new GABA pharmacology [57].

GABA(A) receptors belong to the class of ligand-gated ion channels [58]. The GABA(A) receptors are hetero-pentamers traversing the neuronal membrane. To date, a large number of GABA(A) receptor subtypes have been identified: α 1-6, ß 1-3, γ 1-3, Δ, δ 1-3, θ, π [59]. The majority of GABA(A) receptors in the brain are comprised of two α subunits, two ß subunits, and a γ sub-unit. These subunits construct a cylinder. Activation of the receptor by GABA leads to a conformational change in the protein subunits and results in transient opening of a pore along the axis of the cylinder, allowing the flow of chloride ions from one side of the membrane to another [60]. The pharmacological interaction between benzodiazepines and GABA(A) receptors occurs at a different site independent from the GABA binding site on the GABA(A) receptor. GABA binds within the two interfaces between the α and ß subunits on the GABA(A) receptor. Benzodiazepines bind within the interface between the α and γ sub-units, thereby potentiating GABA-related activation of the chloride conductance through allosteric modulation [61]. Nevertheless, such benzodiazepine recognition site does not exist in all α and γ2 subunit combinations. Therefore, although GABA(A) receptors containing ß, γ2 plus either α_1, α_2, α_3, or α_5 subunits possess a binding site for classical benzodiazepines, analogous receptors containing α4 or α6 subunits do not. The research by Seeburg et al has attributed the benzodiazepine-sensitivity of α_1, α_2, α_3, and α_5 subunits to the histidine residue in a homologous position in their N-terminal extracellular region, which switches to an arginine residue in the benzodiazepine-insensitive α4 and α6 subunits [62].

Given the evolutional preservation of the GABA(A)/Gly receptor-like (GRL) gene sequences in the vertebrates [63], the function of each GABA(A) receptor subunit was initially investigated through a gene knock-out approach. Thanks to the gained experience in gene targeting techniques that enables introduction of specific point mutations, and the recognition that a single amino acid residue in the α subunit determines the sensitivity of a GABA(A) receptor to diazepam, point mutation of the histidine to an arginine in the α_1, α_2, α_3, and α_5 subunits was employed in *in vivo* animal studies to convey the interaction between benzodiazepines and the

$\alpha_{1,2,3,5}$-containing GABA(A) receptors from agonism to inverse agonism [64]. This knock-in approach was used to investigate the underlying pharmacological action of the manipulated receptor subunit.

Based on various experimental knock-in and knock-out mice models, α_1-containing GABA(A) receptors are linked to sedative effect [65-68], while spinal α_2/α_3 GABA(A) receptors are found to mediate analgesia [69-71] and α_5-containing GABA(A) receptors, which relatively specifically express in the hippocampus (the central domain for learning and memory), are associated with cognition [72-77]. The GABA(A) subtype responsible for the anxiolytic effects of benzodiazepines are less clear. The involvement of α_2 GABA(A) receptors in anxiolysis is anticipated given their high expression in human amygdala-prefrontal circuitry [78,79]. Most studies suggest that the α_2 rather than the α_3 subtype is related to the benzodiazepines-induced anxiolysis [80,81], while pharmacological studies using either an α_3-selective inverse agonist [82] or an α_3-selective agonist [83] implicates the α_3 subtype. Despite of the controversies, the affinity and efficacy of current investigational compounds acting at the α_2 and α_3 subtypes are mostly similar at the α_2- and α_3- subunits containing GABA(A) receptors [84].

NOVEL $\alpha_{2,3}$-SUBTYPE SELECTIVE COMPOUNDS FOR ANXIOLYSIS

In contrast to other areas of pharmacology, in the field of GABAergic receptor modulator, it has been particularly difficult for medicinal chemists to develop subtype-selective ligands [85], mainly because the high flexibility of GABA(A) receptors and the existence of multiple drug-binding sites. In addition, the distinct subunit composition among the GABA(A) receptor subtypes, the contribution of distinct subunit sequences to binding sites of different receptor subtypes, as well as the fact that even subunits not directly connected to a binding site are able to influence affinity and efficacy of drugs, contribute to a unique pharmacology of each GABA(A) receptor subtype [86].

The binding and efficacy profiles of candidate $\alpha_{2,3}$ subtype-selective drugs can be classified to either binding-selectivity or efficacy-selectivity. A compound with binding-selectivity is expected to have higher affinity for α_2 and/or α_3 subtypes in vitro and hence specific receptor occupancy and CNS distribution in vivo. Even though the compound may have comparable efficacy at the four benzodiazepine-sensitive GABA(A) receptor subtypes, its pharmacological selectivity is determined in vivo by preferential occupancy. As for efficacy-selectivity, an ideal compound should have opposite pharmacological interactions at different subtypes. In other words, it should exert agonism at the $\alpha_{2,3}$ subtypes whereas present antagonism or inverse agonsim at the α_1 and α_5 subtypes. Between these two

extreme conditions, there could be multiple permutations, including a compound behaves as a full agonist or a relatively high partial agonist at α_2 and/or α_3 subtypes but has weak or none activity at the α_1 and α_5 subtypes.

Based on these principles, a number of conceptually GABA(A) $\alpha_{2,3}$ subtype-selective compounds have been identified through *in vitro* studies using recombinant human GABA(A) receptors and carried forward into clinical development. Because of their pharmacological selectivity, these compounds are expected to have favorable therapeutic effect with less sedating or cognition impairing effect. Table 1 listed the *in vitro* pharmacological properties of these novel GABAergic compounds.

Table 1 · In vitro pharmacological properties of the GABAergic compounds

	α_1		α_2		α_3		α_5	
Compound	**K_i[1] (nM)**	**Efficacy[2] (%)**	**K_i (nM)**	**Efficacy (%)**	**K_i (nM)**	**Efficacy (%)**	**K_i (nM)**	**Efficacy (%)**
TPA023[3]	0.27	0#	0.31	11	0.19	21	0.41	5
MK-0343[3]	0.22	18	0.40	23	0.21	45	0.23	18
SL65.1498[4]	17	45	73	115	80	83	215	48
Zolpidem	20	75[5]	400 (d)	78[5]	400 (d)	80[5]	5000(d)	9[5]
AZD7325[6]	0.5	0	0.3	18	1.3	15	230	8
AZD6280[7]	0.5	0	21	32	31	34	1680	7
NS11821[8]	1.6	4	9.7	17	3.8	40	2.5	41

1. Ki = constant of receptor-subtype binding / 2. Relative efficacy is defined as the extent of the potentiation of GABA(A) EC_{20}-equivalent current produced by the compound compared to that produced by a nonselective full agonist (chlordiazepoxide/diazepam) / 3. Mean values of 3 experiments in Xenopus oocytes with human recombinant $\alpha\beta3\gamma2$ receptors; efficacy relative to chlordiazepoxide [86,89] / 4. Mean values of 3 experiments in HEK293 cells with recombinant rat receptors $\alpha\beta2\gamma2$; efficacy relative to chlordiazepoxide [97] / 5. Mean values of 3 experiments in Xenopus oocytes with human recombinant $\alpha\beta2\gamma2$ receptor; efficacy relative to diazepam [98,99] / 6. Data adapted from [100] / 7. Data adapted from [101] / 8. Data adapted from [102].

EVALUATION OF HUMAN PHARMACOLOGY

BZDs exert their CNS actions in a concentration-dependent manner [87]. The anxiolytic, hypnotic, muscle relaxant, and amnesic effects of benzodiazepines generally appear concomitantly, and the onset and duration of action correlate closely with the pharmacokinetic profiles of these compounds. Based on nonclinical investigations using *in vitro* assays and animal models of anxiety, the human pharmacology of novel GABAergic agents is approached through clinical pharmacology studies investigating pharmacokinetics, receptor occupancy, and pharmacodynamics (PD) in healthy volunteers. Direct links have been proposed between plasma drug concentration and GABA receptor occupancy [84], as well as between plasma drug concentration and the pharmacodynamic measurements

[88-91]. Such pharmacokinetic/pharmacodynamic (PK/PD) relationships warrant the use of surrogate biomarkers in healthy volunteers treated with single-dose administration of selective novel GABAergic compound(s).

More than 170 pharmacodynamic tests or test variants have been developed to assess the CNS effects of benzodiazepines. De Visser et al. [87] analyzed the inter-study consistency, sensitivity, and pharmacological specificity of the frequently used biomarkers. Saccadic peak velocity (SPV) and visual analogue scale of alertness ($VAS_{alertness}$) were identified as the most sensitive parameters for benzodiazepines. Both measurements showed consistently dose-dependent responses to a variety of benzodiazepines. Based on these finding, the Centre for Human Drug Research (CHDR) has established a selection of computerized CNS-pharmacodynamic tests called the Neurocart battery [92]. The components of this battery target a variety of neurophysiological and/or neuropsychological domains (Table 2).

Table 2 · Component tests of the Neurocart battery and the related CNS domains

Neurocart test	Targeted function	Related CNS domains
Saccadic eye movement	Neurophysiologic function	Superior colliculus, substantia nigra, amygdala
Smooth pursuit	Neurophysiologic function	Midbrain
Adaptive tracking	Visuo-motor coordination	Neocortex, basal nuclei, brain stem, cerebellum
Body sway	Balance	Cerebellum, brain stem
Visual verbal learning test (VVLT)	Memory	Hippocampus
VAS Bond and Lader	Alertness, mood, calmness	Cortex, prefrontal cortex
VAS Bowdle	Feeling high, internal and external perception	Cortex, prefrontal cortex, amygdala

Of this battery, adaptive tracking, saccadic eye movements, and body sway were proven sensitive to the sedating effects of sleep deprivation [93], as well as to the effects of benzodiazepines and other GABAergic hypnotic drugs [89,91]. In the recent years, the Neurocart battery was used in a series of phase I studies to assess CNS pharmacodynamics of partial $\alpha_{2,3}$ subtype selective GABA(A) agonists. Both nonselective and/or selective GABA(A) agonists were administered as single oral dose to healthy volunteers. Clear distinctions were observed between the effect profile of non-subtype-selective full GABA(A) agonist and that of selective partial GABA(A) agonist in these trials [88-90], probably because the subtype specificity of the pharmacodynamic measurements for the pharmacological modulation of GABA(A)-ergic compounds. Unfortunately, none of the novel receptor subtype-selective compounds have reached the market: the development of GABA(A) receptor α_2 and α_3 subunit agonist SL65.1498 [90], was discontinued owing to unexpected amnestic effects, while the phase 2 studies of another compound of this drug class, TPA023, were terminated prematurally due to preclinical toxicity (cataract

formation) in long-term dosing studies [94], despite exhibiting anxioselective activity in GAD; MK-0343 also displayed an anxioselective profile in animal models but produced sedation in humans at low levels of receptor occupancy (<10%) [95].

In summary, these reports indicate that the human pharmacodynamic approach with sensitive and CNS-domain specific neuropsychological and neurophysiological measures is useful in predicting the drug's clinical effect on the central nervous system. Inter-species difference is also noted between human and rodents or primates: although a low *in vitro* efficacy at the α_1-containing GABA(A) receptors may not lead to an overtly sedative effect in the experimental animals, it apparently causes sedation in humans at comparable exposure levels. The following questions remain to be answered: 1) is reduction of saccadic peak velocity a promising surrogate marker for clinical anxiolysis? 2) can we also differentiate partial agonism from the full agonism of benzodiazepines via this pharmacodynamic package? 3) is such selective CNS-pharmacodynamic effect profile characteristic for the family of GABA(A) $\alpha_{2,3}$-subtype receptor agonists?

CONCLUSION AND AIM OF THESIS

Anxiety disorders are highly prevalent psychiatric disorders and have high personal and societal costs. The transition from "normal" negative affect or anxiety to an anxiety disorder is implemented by the interplay between psychosocial stressors and a wide array of neurobiological alterations which lead to subjective suffering and functional impairment. Monoamine modulating treatments are widely applied to treat anxiety disorders but are not effective in a large proportion of patients. As the predominant inhibitory neurotransmitter system in the human brain, the GABAergic system in general and its $\alpha_{2,3}$ subunit-containing GABA(A) receptor subtypes in particular, have been implicated in the pathophysiology of anxiety disorders. Novel pharmacological treatments selectively targeting the anxiolysis-mediating GABA(A) receptor subtypes are currently emerging. These range from affinity-selective agents to efficacy-selective agents and represent potentially useful future pharmacological treatments for anxiety disorders [95].

In this thesis, we report several human pharmacology studies that were performed to identify the pharmacologically active doses/exposure levels of several novel compounds with potential anxiolytic effects (Chapter 2, 3, 4). Because of their pharmacological selectivity at the $\alpha_{2,3}$ GABA(A) receptor subtypes, the novel drugs were expected to elicit clinical anxiolysis and less sedating effects. An overview of the performance of the selected and validated pharmacodynamic measurements is composed to summarize the utility of these neurophysiological and neuropsychological biomarkers in early clinical development of novel anxiolytic drugs

(Chapter 5). However, the difficulty of evaluating therapeutic anxiolytic drug effects in healthy volunteers has led to further explorations on the neuroendocrine biomarkers (Chapter 6) and the integration of a stress-challenging procedure into the evaluations (Chapter 7).

REFERENCES

1 American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition: DSM-V®. Arlington, VA: American Psychiatric Association; 2013.

2 Baldwin DS, Anderson IM, Nutt DJ, Allgulander C, Bandelow B, den Boer JA, Christmas DM, Davies S, Fineberg N, Lidbetter N, Malizia A, McCrone P, Nabarro D, O'Neill C, Scott J, van der Wee N, Wittchen HU. Evidence-based pharmacological treatment of anxiety disorders, post-traumatic stress disorder and obsessive-compulsive disorder: a revision of the 2005 guidelines from the British Association for Psychopharmacology. J Psychopharmacol. 2014;28(5):403-39.

3 Kessler, R. C. The global burden of anxiety and mood disorders: putting the European Study of the Epidemiology of Mental Disorders (ESEMED) findings into perspective. J. Clin. Psychiatry 68 (Suppl. 2), 10-19 (2007).

4 Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jönsson B, Olesen J, Allgulander C, Alonso J, Faravelli C, Fratiglioni L, Jennum P, Lieb R, Maercker A, van Os J, Preisig M, Salvador-Carulla L, Simon R, Steinhausen HC.The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur Neuropsychopharmacol. 2011 Sep;21(9):655-79.

5 Kessler RC, Ormel J, Petukhova M, McLaughlin KA, Green JG, Russo LJ, Stein DJ, Zaslavsky AM, Aguilar-Gaxiola S, Alonso J, Andrade L, Benjet C, de Girolamo G, de Graaf R, Demyttenaere K, Fayyad J, Haro JM, Hu Cy, Karam A, Lee S, Lepine JP, Matchsinger H, Mihaescu-Pintia C, Posada-Villa J, Sagar R, Ustün TB. Development of lifetime comorbidity in the World Health Organization world mental health surveys. Arch Gen Psychiatry. 2011 Jan;68(1):90-100.

6 Ormel J, Petukhova M, Chatterji S, Aguilar-Gaxiola S, Alonso J, Angermeyer MC, Bromet EJ, Burger H, Demyttenaere K, de Girolamo G, Haro JM, Hwang I, Karam E, Kawakami N, Lépine JP, Medina-Mora ME, Posada-Villa J, Sampson N, Scott K, Ustün TB, Von Korff M, Williams DR, Zhang M, Kessler RC. Disability and treatment of specific mental and physical disorders across the world. Br J Psychiatry. 2008 May;192(5):368-75.

7 Bystritsky A. Treatment-resistant anxiety disorders. Mol Psychiatry. 2006 Sep;11(9):805-14.

8 Baldwin DS, Montgomery SA, Nil R, Lader M. (2007) Discontinuation symptoms in depression and anxiety disorders. Int J Neuropsycho- pharmacol 10: 73-84.

9 Calhoon GG, Tye KM. Resolving the neural circuits of anxiety. Nat Neurosci. 2015; 18(10): 1394-404.

10 Cassell MD, Wright DJ (1986). Topography of projections from the medial prefrontal cortex to the amygdala in the rat. Brain Res Bull 17: 321-333.

11 McDonald AJ (1998). Cortical pathways to the mammalian amygdala. Prog Neurobiol 55: 257-332.

12 Pinard CR, Mascagni F, McDonald AJ (2012). Medial prefrontal cortical innervation of the intercalated nuclear region of the amygdala. Neuroscience 205: 112-124.

13 Hurley KM, Herbert H, Moga MM, Saper CB (1991). Efferent projections of the infralimbic cortex of the rat. J Comp Neurol 308: 249-276.

14 Vertes RP (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51: 32-58.

15 Sesack SR, Deutch AY, Roth RH, Bunney BS (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp Neurol 290: 213-242.

16 Chang CH, Knapska E, Orsini CA, Rabinak CA, Zimmerman JM, Maren S. Fear extinction in rodents. Curr Protoc Neurosci. 2009 Apr; Chapter 8: Unit 8.23.

17 Holmes A & Quirk GJ. Pharmacological facilitation of fear extinction and the search for adjunct treatments for anxiety disorders - the case of yohimbine. Trends Pharmacol. Sci. 31, 2-7 (2010).

18 Plendl W, Wotjak CT (2010). Dissociation of within- and between-session extinction of conditioned fear. J Neurosci 30: 4990-4998.

19 Simpson JR, Jr., Drevets WC, Snyder AZ, Gusnard DA, Raichle ME. 2001. Emotion-induced changes in human medial prefrontal cortex: II. During anticipatory anxiety. Proc Natl Acad Sci U S A. 98: 688-693.

20 Straube T, Schmidt S, Weiss T, Mentzel HJ, Miltner WH. 2009. Dynamic activation of the anterior cingulate cortex during anticipatory anxiety. Neuroimage. 44: 975--981.

21 Kim MJ, Gee DG, Loucks RA, Davis FC, Whalen PJ. Anxiety dissociates dorsal and ventral medial prefrontal cortex functional connectivity with the amygdala at rest. Cereb Cortex. 2011 Jul; 21(7): 1667-73.

22 Wager TD, Phan KL, Liberzon I, Taylor SF. Valence, gender, and lateralization of functional brain anatomy in emotion: a meta-analysis of findings from neuroimaging. Neuroimage. 2003;19(3): 513-531.
23 Phan KL, Wager T, Taylor SF, Liberzon I. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and FMRI. Neuroimage. 2002;16(2):331-348.
24 Carlson JM, Greenberg T, Rubin D, Mujica-Parodi LR. Feeling anxious: anticipatory amygdalo-insular response predicts the feeling of anxious anticipation. Soc Cogn Affect Neurosci. 2011;6(1):74-81.
25 Etkin A, Wager TD. Functional neuroimaging of anxiety: a metaanalysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry. 2007;164(10):1476-1488.
26 Straube T, Glauer M, Dilger S, Mentzel HJ, Miltner WH. Effects of cognitive-behavioral therapy on brain activation in specific phobia. Neuroimage. 2006;29(1):125-135.
27 Etkin A. Functional neuroanatomy of anxiety: a neural circuit perspective. In: Stein MB, Steckler T, editors. Behavioral Neurobiology of Anxiety and Its Treatment. Berlin: Springer Verlag; 2009:251-277.
28 Davis M. Neural circuitry of anxiety and stress disorders. In: Davis KL, Charney D, Coyle JT, Nemeroff C, editors. Neuropsychopharmacology: The Fifth Generation of Progress. Philadelphia: Lippincott, Williams, & Wilkins; 2002:729-743.
29 Pitkanen A, Savander V, LeDoux JE. Organization of intraamygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci. 1997;20(11): 517-523.
30 Royer S, Martina M, Pare D. An inhibitory interface gates impulse traffic between the input and output stations of the amygdala. J Neurosci. 1999;19(23):10575-10583.
31 Jongen-Relo AL, Amaral DG. Evidence for a GABAergic projection from the central nucleus of the amygdala to the brainstem of the macaque monkey: a combined retrograde tracing and in situ hybridization study. Eur J Neurosci. 1998;10(9):2924-2933.
32 Forster GL, Novick AM, Scholl JL, Watt MJ. The role of the amygdala in anxiety disorders. In: Ferry B, editor. The Amygdala: A Discrete Multitasking Manager. Rijeka: InTech; 2012:61-102.
33 Ehrlich I, Humeau Y, Grenier F, Ciocchi S, Herry C, Luthi A (2009). Amygdala inhibitory circuits and the control of fear memory. Neuron 62: 757-771.
34 Makkar SR, Zhang SQ, Cranney J (2010). Behavioral and neural analysis of GABA in the acquisition, consolidation, reconsolidation, and extinction of fear memory. Neuropsychopharmacology 35: 1625-1652.
35 Pape HC, Pare D (2010). Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev 90: 419-463.
36 Courtin J, Bienvenu TC, Einarsson EO, Herry C (2013). Medial prefrontal cortex neuronal circuits in fear behavior. Neuroscience 240: 219-242.
37 Domschke K, Zwanzger P. 2008. GABAergic and endocannabinoid dysfunction in anxiety - future therapeutic targets? Curr Pharm Des. 14:3508-3517.
38 Holmes A, Singewald N (2013). Individual differences in recovery from traumatic fear. Trends Neurosci 36: 23-31.
39 Holmes NM, Parkes SL, Killcross AS, Westbrook RF (2013). The basolateral amygdala is critical for learning about neutral stimuli in the presence of danger, and the perirhinal cortex is critical in the absence of danger. J Neurosci 33: 13112-13125.
40 Sanders SK, Shekhar A. Regulation of anxiety by GABA-A receptors in the rat amygdala. Pharmacol Biochem Behav. 1995;52(4):701-706.
41 Barbalho CA, Nunes-de-Souza RL, Canto-de-Souza A. Similar anxiolytic-like effects following intra-amygdala infusions of benzodiazepine receptor agonist and antagonist: evidence for the release of an endogenous benzodiazepine inverse agonist in mice exposed to elevated plus-maze test. Brain Res. 2009;1267:65-76.
42 Sanders SK, Shekhar A. Regulation of anxiety by GABA-A receptors in the rat amygdala. Pharmacol Biochem Behav. 1995; 52: 701-706.
43 Del-Ben CM, Ferreira CA, Sanchez TA, et al. Effects of diazepam on BOLD activation during the processing of aversive faces. J Psychopharmacol. 2012;26(4): 443-451.
44 Paulus MP, Feinstein JS, Castillo G, Simmons AN, Stein MB. Dosedependent decrease of activation in bilateral amygdala and insula by lorazepam during emotion processing. Arch Gen Psychiatry. 2005; 62(3): 282-288.
45 Nutt DJ, Glue P, Lawson C, Wilson S. 1990. Flumazenil provocation of panic attacks. Evidence for altered benzodiazepine receptor sensitivity in panic disorder. Arch Gen Psychiatry. 47: 917-925.
46 Strohle A, Kellner M, Holsboer F, Wiedemann K. 1999. Behavioral, neuroendocrine, and cardiovascular response to flumazenil: no evidence for an altered benzodiazepine receptor sensitivity in panic disorder. Biol Psychiatry. 45:321-326.
47 Nikolaus S, Hautzel H, Müller HW. Focus on GABA(A) receptor function. A comparative analysis of in vivo imaging studies in neuropsychiatric disorders. Nuklearmedizin. 2014; 53(6): 227-37.
48 Hasler G, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC. Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. Arch Gen Psychiatry. 2007 Feb;64(2):193-200.
49 Kalueff AV, Nutt DJ. Role of GABA in anxiety and depression. Depress Anxiety. 2007;24(7):495-517. Review.

50 Luscher B1, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. Mol Psychiatry. 2011 Apr;16(4):383-406.

51 Möhler H., The GABA system in anxiety and depression and its therapeutic potential. Neuropharmacology. 2012 Jan; 62(1): 42-53.

52 Bremner JD, Innis RB, Southwick SM, Staib L, Zoghbi S, Charney DS. Decreased benzodiazepine receptor binding in prefrontal cortex in combat-related posttraumatic stress disorder. Am J Psychiatry. 2000 Jul;157(7):1120-6.

53 Malizia AL, Cunningham VJ, Bell CJ, Liddle PF, Jones T, Nutt DJ. Decreased brain GABA(A)-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. Arch Gen Psychiatry. 1998 Aug;55(8):715-20.

54 Hasler G, Nugent AC, Carlson PJ, Carson RE, Geraci M, Drevets WC. Altered cerebral gamma-aminobutyric acid type A-benzodiazepine receptor binding in panic disorder determined by [^{11}C]flumazenil positron emission tomography. Arch Gen Psychiatry. 2008 Oct;65(10):1166-75.

55 Nemeroff CB. The role of GABA in the pathophysiology and treatment of anxiety disorders. Psychopharmacol Bull. 2003;37(4):133-46.

56 Nutt DJ, Malizia AL. Structural and functional brain changes in posttraumatic stress disorder. J Clin Psychiatry. 2004;65 Suppl 1:11-7.

57 Möhler H. The legacy of the benzodiazepine receptor: from flumazenil to enhancing cognition in Down syndrome and social interaction in autism. Adv Pharmacol. 2015;72:1-36.

58 Chebib M, Johnston GA. The 'ABC' of GABA receptors: a brief review. Clin Exp Pharmacol Physiol. 1999 Nov;26(11):937-40.

59 Jacob TC, Moss SJ, Jurd R. 2008. GABA(A)receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nat Rev Neurosci. 9:331-343.

60 Horenstein J, Wagner DA, Czajkowski C, Akabas MH. Protein mobility and GABA-induced conformational changes in GABA(A)receptor pore-lining M2 segment. Nat Neurosci. 2001;4(5):477-485.

61 Sigel E, Luscher BP. A closer look at the high affinity benzodiazepine binding site on GABA-A receptors. Curr Top Med Chem. 2011;11(2): 241-246.

62 Wieland HA, Lüddens H, Seeburg PH. A single histidine in GABA-A receptors is essential for benzodiazepine agonist binding. J Biol Chem. 1992 Jan 25;267(3):1426-9.

63 Tsang SY, Ng SK, Xu Z, Xue H. The evolution of GABA-A receptor-like genes. Mol Biol Evol. 2007 Feb; 24(2): 599-610.

64 McKernan, R. M., Rosahl, T. W., Reynolds, D. S., Sur, C., Wafford, K. A., Atack, J. R., et al. (2000). Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA-A receptor a1 subtype. Nature Neuroscience, 3, 587-592.

65 Mirza NR, Larsen JS, Mathiasen C, Jacobsen TA, Munro G, Erichsen, HK, et al. (2008). NS11394 [30-[5-(1-hydroxy-1-methyl-ethyl)-benzoimidazol-1-yl]- biphenyl-2-carbonitrile], a unique subtype-selective GABA-A receptor positive allosteric modulator: In vitro actions, pharmacokinetic properties and in vivo anxiolytic efficacy. Journal of Pharmacology and Experimental Therapeutics, 327, 954-968.

66 Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR. Different GABA-A receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proc Natl Acad Sci U S A. 2005 Jan 18;102(3):915-20.

67 Rudolph U, Crestani F, Benke D, Brünig I, Benson JA, Fritschy JM, et al. (1999). Benzodiazepine actions mediated by specific g-aminobutyric acid A receptor subtypes. Nature, 401, 796-800.

68 Knabl J, Witschi R, Hösl K, Reinold H, Zeilhofer UB, Ahmadi S, et al. (2008). Reversal of pathological pain through specific spinal GABA-A receptor subtypes. Nature, 451, 330 -334.

69 Knabl, J., Zeilhofer, U. B., Crestani, F., Rudolph, U., & Zeilhofer, H. U. (2009). Genuine antihyperalgesia by systemic diazepam revealed by experiments in GABA-A receptor pointmutated mice. Pain, 141, 233-238.

70 Munro, G., Lopez-Garcia, J. A., Rivera-Arconada, I., Erichsen, H. K., Nielsen, E. Ø., Larsen, J. S., et al. (2008). Comparison of the novel subtype-selective GABA-A receptor-positive allosteric modulator NS11394 [30-[5-(1-hydroxy-1-methyl-ethyl)-benzoimidazol-1-yl]biphenyl-2-carbonitrile] with diazepam, zolpidem, bretazenil, and gaboxadol in rat models of inflammatory and neuropathic pain. Journal of Pharmacology and Experimental Therapeutics, 327, 969-981.

71 Atack, J. R., Bayley, P. J., Seabrook, G. R., Wafford, K. A., McKernan, R. M., & Dawson, G. R. (2006). L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for a5-containing GABA-A receptors. Neuropharmacology, 51, 1023-1029.

72 Ballard, T. M., Knoflach, F., Prinssen, E., Borroni, E., Vivian, J. A., Basile, J., et al. (2009). RO4938581, a novel cognitive enhancer acting at GABA-A a5 subunit-containing receptors. Psychopharmacology, 202, 207-223.

73 Chambers, M. S., Atack, J. R., Broughton, H. B., Collinson, N., Cook, S., Dawson, G. R., et al. (2003). Identification of a novel, selective GABA-A α5 receptor inverse agonist which enhances cognition. Journal of Medicinal Chemistry, 46, 2227-2240.

74 Cheng, V. Y., Martin, L. J., Elliott, E. M., Kim, J. H., Mount, H. T. J., Taverna, F. A., et al. (2006). α5 GABA-A receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. Journal of Neuroscience, 26, 3713-3720.

75 Collinson, N., Kuenzi, F. M., Jarolimek, W., Maubach, K. A., Cothliff, R., Sur, C., et al. (2002). Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the a5 subunit of the GABA-A receptor. Journal of Neuroscience, 22, 5572-5580.
76 Dawson, G. R., Maubach, K. A., Collinson, N., Cobain, M., Everitt, B. J., MacLeod, A. M., et al. (2006). An inverse agonist selective for a5 subunit-containing GABA-A receptors enhances cognition. Journal of Pharmacology and Experimental Therapeutics, 316, 1335-1345.
77 Harris, D., Clayton, T., Cook, J., Sahbaie, P., Halliwell, R. F., Furtmüller, R., et al. (2008). Selective influence on contextual memory: Physiochemical properties associated with selectivity of benzodiazepine ligands at GABA-A receptors containing the a5 subunit. Journal of Medicinal Chemistry, 51, 3788-3803.
78 Bukalo O, Pinard CR, Holmes A. Mechanisms to medicines: elucidating neural and molecular substrates of fear extinction to identify novel treatments for anxiety disorders. Br J Pharmacol. 2014 Oct;171(20):4690-718.
79 W Sieghart and G Sperk. Subunit Composition, Distribution and Function of GABA-A Receptor Subtypes. Current Topics in Medicinal Chemistry, 2002, 2, 795-816.
80 Löw, K., Crestani, F., Keist, R., Benke, D., Brünig, I., Benson, J. A., et al. (2000). Molecular and neuronal substrate for the selective attenuation of anxiety. Science, 290, 131-134.
81 Yee, B. K., Keist, R., von Boehmer, L., Studer, R., Benke, D., Hagenbuch, N., et al. (2005). A schizophrenia-related sensorimotor deficit links a3-containing GABA-A receptors to a dopamine hyperfunction. Proceedings of the National Academy of Sciences of the United States of America, 102, 17154-17159.
82 Atack, J. R., Hutson, P. H., Collinson, N., Marshall, G., Bentley, G., Moyes, C., et al. (2005). Anxiogenic properties of an inverse agonist selective for a3 subunit-containing GABA-A receptors. British Journal of Pharmacology, 144, 357-366.
83 Dias, R., Sheppard, W. F. A., Fradley, R. L., Garrett, E. M., Stanley, J. L., Tye, S. J., et al. (2005). Evidence for a significant role of a3-containing GABA-A receptors in mediating the anxiolytic effects of benzodiazepines. Journal of Neuroscience, 25, 10682-10688.
84 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.
85 Crestani F, Rudolph U. Behavioral functions of GABA-A receptor subtypes--the Zurich experience. Adv Pharmacol. 2015;72:37-51.
86 Sieghart W. Allosteric modulation of GABA-A receptors via multiple drug-binding sites. Adv Pharmacol. 2015; 72: 53-96.
87 S. J. De Visser, J. P. Van Der Post, P. P. De Waal, F. Cornet, A. F. Cohen, and J. M. A. Van Gerven. Biomarkers for the effects of benzodiazepines in healthy volunteers. British Journal of Clinical Pharmacology, vol. 55, no. 1, pp. 39-50, 2003.
88 S.L. De Haas, S. J. De Visser, J. P. Van Der Post et al., 'Pharmacodynamic and pharmacokinetic effects of TPA023, a GABA-A $\alpha_{2,3}$ subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers,' Journal of Psychopharmacology, vol. 21, no. 4, pp. 374-383, 2007.
89 de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, Murphy MG, de Smet M, Vessey LK, Ramakrishnan R, Xue L, Cohen AF, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of MK-0343, a GABA(A)alpha2,3 subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacol. 2008 Jan;22(1):24-32.
90 S. L. De Haas, K. L. Franson, J. A. J. Schmitt et al., 'The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A 2,3 selective agonist, in comparison with lorazepam in healthy volunteers,' Journal of Psychopharmacology, vol. 23, no. 6, pp. 625-632, 2009.
91 S. L. De Haas, R. C. Schoemaker, J. M. A. Van Gerven, P. Hoever, A. F. Cohen, and J. Dingemanse, 'Pharmacokinetics, pharmacodynamics and the pharmacokinetic/ pharmacodynamic relationship of zolpidem in healthy subjects,' Journal of Psychopharmacology, vol. 24, no. 11, pp. 1619-1629, 2010.
92 Groeneveld GJ, Hay JL, van Gerven JM. Measuring blood-brain barrier penetration using the Neurocart, a CNS test battery. Drug Discov Today Technol. 2016 Jun; 20: 27-34
93 AL Van Steveninck, BNM Van Berckel, RC Schoemaker, DD Breimer, JMA Van Gerven, and AF Cohen, 'The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation,' Journal of Psychopharmacology, vol. 13, no. 1, pp. 10-17, 1999.
94 Mohler, H. The rise of a new GABA pharmacology. Neuropharmacology 60, 1042-1049 (2011).
95 Atack, J. R. GABA-A receptor α2/α3 subtype-selective modulators as potential nonsedating anxiolytics. Curr. Top. Behav. Neurosci. 2, 331-360 (2010).
96 Bandelow B, Baldwin D, Abelli M, Altamura C, Dell'Osso B, Domschke K, Fineberg NA, Grünblatt E, Jarema M, Maron E, Nutt D, Pini S, Vaghi MM, Wichniak A, Zai G, Riederer P. Biological markers for anxiety disorders, OCD and PTSD - a consensus statement. Part I: Neuroimaging and genetics. World J Biol Psychiatry. 2016 Aug;17(5): 321-65.
97 Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Decobert M, Francon D, Avenet P, Depoortere H, Tan S, Oblin A, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B. SL651498: an anxioselective

compound with functional selectivity for alpha2- and alpha3-containing gamma-aminobutyric acid (A) (GABA(A)) receptors. J Pharmacol Exp Ther 2001; 298: 753-768.
98 Sanna E, Busonero F, Talani G, Carta M, Massa F, Peis M, Maciocco E, Biggio G. Comparison of the effects of zaleplon, zolpidem, and triazolam at various GABA(A)receptor subtypes. Eur J Pharmacol 2002; 451: 103-110.
99 Crestani F, Martin JR, Mohler H, Rudolph U. Mechanism of action of the hypnotic zolpidem in vivo. Br J Pharmacol 2000; 131: 1251-1254.
100 Chen X, Jacobs G, de Kam M, Jaeger J, Lappalainen J, Maruff P, Smith MA, Cross AJ, Cohen A, van Gerven J. The central nervous system effects of the partial GABA-A $\alpha_{2,3}$ -selective receptor modulator AZD7325 in comparison with lorazepam in healthy males. Br J Clin Pharmacol. 2014 Dec;78(6):1298-314.
101 Chen X, Jacobs G, de Kam ML, Jaeger J, Lappalainen J, Maruff P, Smith MA, Cross AJ, Cohen A, van Gerven J. AZD6280, a novel partial γ-aminobutyric acid A receptor modulator, demonstrates a pharmacodynamically selective effect profile in healthy male volunteers. J Clin Psychopharmacol. 2015 Feb;35(1):22-33.
102 Zuiker RG, Chen X, Østerberg O, Mirza NR, Muglia P, de Kam M, Klaassen ES, van Gerven JM. NS11821, a partial subtype-selective GABA-A agonist, elicits selective effects on the central nervous system in randomized controlled trial with healthy subjects. J Psychopharmacol. 2016 Mar;30(3):253-62.