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α 2-containing GABA_A receptors: A target for the development of novel treatment strategies for CNS disorders

Elif Engin, Jing Liu, and Uwe Rudolph

Laboratory of Genetic Neuropharmacology, McLean Hospital, Belmont, MA 02478, and
Department of Psychiatry, Harvard Medical School, Boston, MA

Abstract

GABA_A receptors have important physiological functions, as revealed by pharmacological studies and experiments involving gene-targeted mouse models, and are the target of widely used drugs such as the benzodiazepines. In this review, we are summarizing current knowledge about the function of α 2-containing GABA_A receptors, a receptor subtype representing approximately 15–20% of all GABA_A receptors. This receptor subtype mediates anxiolytic-like, reward-enhancing, and antihyperalgesic actions of diazepam, and has antidepressant-like properties. Secondary insufficiency of α 2-containing GABA_A receptors has been postulated to play a role in the pathogenesis of schizophrenia, and may be involved in cognitive impairment in other disorders. Moreover, polymorphisms in the *GABRA2* gene encoding the GABA_A receptor α 2 subunit have been found to be linked to chronic alcohol dependence and to polydrug abuse. Thus, α 2-containing GABA_A receptors are involved in the regulation and/or modulation of emotional behaviors and of chronic pain, and appear to be a valid target for novel therapeutic approaches for the treatment of anxiety, depression, schizophrenia and chronic pain.

Keywords

GABA_A receptor; benzodiazepines; anxiety; depression; chronic pain; schizophrenia

1. Introduction

γ -aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS). GABAergic inhibition is essential for temporal modulation and maintenance of the excitation/inhibition balance and for the entrainment of oscillatory activities (Klausberger & Somogyi, 2008). GABA acts at ionotropic GABA_A receptors and at metabotropic GABA_B receptors.

GABA_A receptors are formed as pentameric combinations of homologous subunits. There are 19 genes known encoding GABA_A receptor subunits (α 1–6, β 1–3, γ 1–3, δ , ϵ , θ , π , ρ 1–

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Address for correspondence: Uwe Rudolph, Laboratory of Genetic Neuropharmacology, McLean Hospital, Room MRC123A, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, USA, Phone 001-617-855-2088, Fax 001-617-855-2012, urudolph@mclean.harvard.edu.

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Conflict of Interest Statement:

"In the last three years, UR has provided professional services for Sunovion and for Concert Pharmaceuticals. JL and EE declare that they have no conflict of interest."

3) (Olsen & Sieghart, 2008), with receptors formed by α , β and γ subunits being the most common. The α subunits provide the largest diversity, and for practical purposes GABA_A receptors are most frequently classified by their α subunits.

This diverse set of receptors are the targets of a variety of therapeutically useful CNS-active compounds, e.g. benzodiazepines, zolpidem, zopiclone, barbiturates, etomidate, propofol, and neurosteroids (Rudolph & Antkowiak, 2004; Rudolph & Knoflach, 2011). Out of these compounds, benzodiazepines have been the most extensively studied, largely due to their widespread clinical use in the treatment of anxiety disorders. Benzodiazepines are positive allosteric modulators (PAM) of the GABA_A receptors, with a variety of actions, including sedation, hypnosis, muscle relaxation, and anticonvulsant and amnesic effects, in addition to anxiolysis. This wide array of behavioral effects reduces the therapeutic utility of clinically used benzodiazepines (e.g., diazepam, chlordiazepoxide, midazolam), as even when they are effective in reaching the desired therapeutic targets (e.g., anxiolysis in patients with anxiety disorders), they are associated with a number of unwanted side effects (e.g., sedation and hypnosis). Thus, understanding the molecular substrates for each of these behavioral endpoints has been a major research interest with the hope of producing more therapeutically-specific GABAergic compounds in the future. Differential quantitative and anatomical distribution of GABA_A receptor subunits in the CNS, and differential modulation by benzodiazepines of different GABA_A receptors based on subunit composition provided the first clues in terms of an understanding of benzodiazepine behavioral pharmacology.

Whereas the GABA binding sites on GABA_A receptors are formed by residues on the α and β subunits, the binding sites for benzodiazepines are formed by residues on α and γ subunits (Sigel & Luscher, 2011). Benzodiazepines bind to GABA_A receptors containing the $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$ subunits and a γ subunit (in addition to the obligatory β subunit) (H. Mohler, et al., 2002). Receptors containing the $\alpha 4$ or $\alpha 6$ subunit do not bind these compounds. Whereas the three β subunits $\beta 1$ – $\beta 3$ appear to confer identical properties to the receptor complex with respect to modulation by benzodiazepines (as well as by barbiturates and neurosteroids) (Hadingham, et al., 1993), the γ subunits contribute somewhat differentially to this process (Benke, et al., 1996). Approximately 85% of all γ subunits are $\gamma 2$ subunits (Benke, et al., 1996), and it has been shown that the $\gamma 2$ subunit is required for behavioral actions of benzodiazepines (Gunther, et al., 1995). Thus, GABA_A receptors containing a $\gamma 2$ subunit, in combination with $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits likely mediate all of the above-mentioned behavioral effects of benzodiazepines.

As seen in Fig. 1, approximately 60% of all GABA_A receptors contain the $\alpha 1$ subunit, 15–20% the $\alpha 2$ subunit, 10–15% the $\alpha 3$ subunit, and 5% the $\alpha 5$ subunit (H. Mohler, et al., 2002). In addition to this quantitative difference in their expression levels, the α subunits also show anatomically differential patterns of expression in the CNS. The finding that functionally different neuronal circuits express GABA_A receptors of specific α subunit compositions preferentially, as well as the differential subcellular, sometimes synapse-specific expression of α subunits within individual neurons, suggested that the α subunits may confer different behavioral functions and thus may constitute targets for specific therapeutic endpoints.

While a few compounds exhibiting relative subtype-selectivity have been developed, no truly subtype-specific compounds have been generated so far. Thus, the traditional pharmacological approach of defining the functions of GABA_A receptor subtypes by synthesizing novel compounds, while extremely valuable for medication development, was met with limited success for clear assignment of functions to receptor subtypes. This limitation stimulated interest in the development of genetic mouse models that would allow

unequivocal elucidation of subunit function. To date, several mouse models involving knockouts or point mutations of specific α subunits have been generated and the studies employing these models have provided invaluable information about the functions of different GABA_A receptor subtypes. For instance, mice carrying histidine to arginine point mutations in one of the four diazepam-sensitive α subunits [α 1(H101R), α 2(H101R), α 3(H126R) and α 5(H105R)] that transform the diazepam-sensitive α subunits into diazepam-insensitive α subunits have been employed extensively to understand the GABA_A receptor subtypes that mediate different behavioral actions of benzodiazepines (Rudolph et al., 1999; McKernan, et al., 2000; Low, et al., 2000; Crestani, et al., 2002; Smith et al., 2012). In these mice, the “missing” diazepam actions in mice carrying the mutated α subunit can then be ascribed to the mutant α subunit. The first studies using these point-mutated mice revealed that while wild type mice are sedated by diazepam, α 1(H10R)mice are not sedated by diazepam, but diazepam still has an anxiolytic-like action in these mice (Rudolph, et al., 1999), suggesting that the sedative effects of diazepam are mediated by the α 1-containing GABA_A receptors. Since these early studies, many other studies employing genetic mouse models revealed specific roles for different α subunits, in addition to refining and re-affirming the earlier findings. In a different line of work, human genetics studies revealed associations between different disorders and personality traits and different α subunits. One subunit, the α 2 subunit, has stood out in both lines of research as being linked to many diverse physiological processes and several disorders of the nervous system, suggesting that this subunit might constitute an extremely fruitful drug target. Here, we summarize findings regarding this subunit in relation to different CNS disorders and their treatment.

In each of the sections below, we first summarize findings from human genetics and/or postmortem human studies, followed by findings from animal research, most commonly from gene-targeted mouse models. In many cases the suggestive evidence from human genetics studies can be corroborated by genetically modified mouse models with mechanistic demonstrations of loss or gain of function in animals following genetic manipulations. A review of the two literatures together also provides an easy way to spot gaps in data in either research approach as a guide for future investigations in this area. We start with the well-replicated studies investigating the role of *GABRA2* in anxiety, followed by findings relating *GABRA2* to alcohol dependence and drug abuse. Emerging evidence linking *GABRA2* to depression is discussed, followed by a section suggesting the role of *GABRA2* in the brain reward system as the potential common ground for the role of α 2-containing GABA_A receptors in substance abuse and depression. We finally discuss the role of *GABRA2* in other CNS disorders, such as schizophrenia and Alzheimer’s disease, in the sleep EEG and in pain.

One caveat to note before we begin our discussion is that in many cases other GABA_A receptor subtypes have also been implicated in a single disorder, as these disorders represent complex neuropathology that involve several brain structures and neurotransmitter systems. The main goal of this review is to demonstrate the potential of α 2-containing GABA_A receptors as valuable drug targets for diverse neuropathology rather than a full discussion of GABAergic mechanisms involved in a certain disorder. While we do discuss other GABA_A receptor subtypes briefly at most points, there are also instances where we leave out the discussion of other subunits in order focus on α 2-containing GABA_A receptors. More extensive discussion of other GABA_A receptor subtypes are available in other recent reviews (Rudolph & Mohler, 2006; Rudolph & Knoflach; 2011).

2. *GABRA2* and Anxiety

The evidence linking the GABA system to anxiety and anxiety disorders is extensive [see (Kalueff & Nutt, 2007; Mohler, 2012 for reviews)]. GABAergic drugs, such as benzodiazepines have been used to treat anxiety disorders for more than half a century. Surprisingly, human genetics studies linking GABA_A receptor subunits and anxiety phenotypes are limited in number and have revealed relatively weak results. A few studies suggested a possible link between the *GABRA2* gene and anxiety. For instance, Nelson, et al. (2009) reported that polymorphisms in *GABRA2* interact with early childhood trauma in increasing the risk for PTSD, and Enoch et al. (2006) reported anxiety as a mediating factor in the link between alcoholism and *GABRA2* (see below). A study of healthy college students revealed a strong correlation between *GABRA2* genotype and amygdalar and insular activation in response to fearful faces (Stein et al., 2006), a phenotype also observed in patients with anxiety disorders [e.g., (Stein et al., 2002; 2007; Cannistraro, et al., 2004)]. In contrast to this suggestive evidence, a study investigating 26 SNPs from four GABA_A receptor genes (*GABRA2*, *GABRA3*, *GABRA6* and *GABRG2*) found that the major allelic variation in none of these genes significantly contributed to liability to anxiety disorders (Pham, et al., 2009).

While the findings from human genetics studies do not provide strong and unequivocal evidence for the involvement of *GABRA2* in anxiety disorders, studies employing gene-targeted mouse models provide strong evidence for the involvement of $\alpha 2$ -containing GABA_A receptors in anxiety, as well as in the anxiolytic effects of benzodiazepines. The first viable GABA_A receptor knockout mice, the $\gamma 2$ heterozygous knockouts, had a developmental anxiety phenotype that could be reversed with very low doses of benzodiazepines (Crestani, et al., 1999; Gunther, et al., 1995; Earnheart, et al., 2007). However, since the $\gamma 2$ subunit is associated with all six α subunits, the question of which GABA_A receptor subtype(s), as defined by the α subunits, mediates this anxiety-related profile was left open until the generation of mice carrying histidine-to-arginine point mutations that render the affected subunit insensitive to benzodiazepines. Studies with these mice reported that diazepam has no anxiolytic-like action in $\alpha 2$ (H101R) mice (Low, et al., 2000). In a recent study (Smith, et al., 2012), we replicated and extended these early findings in mice of a C57BL/6J background, showing that the $\alpha 2$ -containing GABA_A receptors were required for the anxiolytic effects of both diazepam and chlordiazepoxide in tests of unconditioned anxiety (i.e., elevated plus maze; Fig. 2E), as well as conditioned fear (i.e., fear-potentiated startle; Fig. 2A–D), controlling for nonspecific variables such as general locomotor activity. Moreover, these observations were independently confirmed by a study showing that diazepam and pentobarbital are no longer anxiolytic in $\alpha 2$ global knockout mice (Dixon, et al., 2008). Thus, drug-binding to $\alpha 2$ -containing GABA_A receptors is required for the anxiolytic effects of GABAergic drugs.

In a series of drug-free experiments, Vollenweider et al. (2011) showed that heterozygous $\alpha 2$ knockout mice exhibit heightened anxiety in novelty-suppressed feeding test. Interestingly, in the same study the homozygous $\alpha 2$ knockout mice did not display this phenotype, and the reasons for this are currently unclear. One potential speculation is that in the homozygous $\alpha 2$ knockout mice upregulation of the $\alpha 3$ and $\alpha 4$ subunits (Panzanelli et al., 2011) might be more pronounced than in the heterozygous $\alpha 2$ knockout mice and functionally compensate for the loss of the $\alpha 2$ subunit.

While the involvement of $\alpha 2$ -containing GABA_A receptors in anxiety and anxiolytic drug effects is clear, a few other studies have suggested that the $\alpha 3$ -containing GABA_A receptors may also be involved in modulating anxiety (Dias, et al., 2005; Atack, et al., 2006). However, the evidence for this is still not unequivocal [see Smith & Rudolph, 2012 for a

discussion]. Similarly, we have recently demonstrated that the $\alpha 1$ -containing GABA_A receptors may be involved in the fear-reducing effects of benzodiazepines in models of conditioned fear, while these receptors are not involved in anxiolysis in models of unconditioned anxiety (Smith, et al., 2012). This finding is exciting in terms of reiterating a long-discussed phenomenon that unconditioned anxiety and conditioned fear have separate neuronal mechanisms and animal models of each behavior tap into different functions. This idea is also reflected in terms of the representation of human disorders at the level of animal models. For example, the elevated plus maze has some face and predictive validity for generalized anxiety disorders, while fear-potentiated startle is frequently considered to be a model of post-traumatic stress disorder. The finding that the two tests might involve partially different GABA_A receptor subtypes raises the possibility that GABAergic pharmacological interventions for specific anxiety disorders can be differentially tailored. While interesting in providing a functional dissociation that may have clinical implications, these findings also require further replication before any solid conclusions can be drawn.

2.1. Anxioreselective compounds

Since the introduction of benzodiazepines into clinical practice, it has been a goal to develop a compound with anxiolytic effects comparable to that of diazepam but without sedation. The findings already mentioned above that $\alpha 1$ -containing GABA_A receptors mediate the sedative action of diazepam (Rudolph et al., 1999; McKernan et al., 2000), while $\alpha 2$ -containing GABA_A receptors mediate the anxiolytic-like action of diazepam (Low, et al., 2000) suggest that a compound which is active at $\alpha 2$ -containing GABA_A receptors but not at $\alpha 1$ -containing GABA_A receptors might be a non-sedative anxiolytic. L-838,417, a benzodiazepine site (BZ site) partial agonist at $\alpha 2$ -, $\alpha 3$ -, and $\alpha 5$ -containing GABA_A receptors and an antagonist at $\alpha 1$ -containing GABA_A receptors is indeed anxiolytic-like in the elevated plus maze, but not sedative in rodents (McKernan, et al., 2000). An $\alpha 2/\alpha 3$ BZ site partial agonist, TPA023, also referred to as MK0777, had anxiolytic effects in the elevated plus maze, the fear-potentiated startle paradigm, and in a conditioned suppression of drinking paradigm in rats, as well as reducing conditioned emotional responding in squirrel monkeys (Atack, et al., 2006). It did not have sedative effects in any of the species tested. The $\alpha 2/\alpha 3$ BZ site partial agonist TPA023 has also been tested in Phase III studies in humans, and some evidence has been presented indicating that in humans this compound is anxiolytic but not sedative (Atack, 2010). Unfortunately, due to preclinical toxicity (cataracts), development of this compound has been stopped (Mohler, 2011).

3. GABRA2 and Alcohol Dependence

While it is known that ethanol can potentiate GABA_A receptors *in vitro*, the contribution of this potentiation to the effects of ethanol continues to be controversial. Most synaptic GABA_A receptors show sensitivity to ethanol only at concentrations above the drunk driving limit in many states. One reported exception to this, although not universally accepted (Borghese, et al., 2006) (Yamashita, et al., 2006) are the extrasynaptic $\alpha 4\beta 3\delta$ GABA_A receptors, which are modulated by “social” concentrations of ethanol (Wallner, et al., 2003) and have also been termed the “one glass of wine receptors” (Olsen et al., 2007).

While it is still unknown how ethanol exerts its actions in the CNS, human genetic studies revealed that small nucleotide polymorphisms (SNP) in the *GABRA2* gene encoding the GABA_A receptor $\alpha 2$ subunit are associated with alcohol dependence (Edenberg, et al., 2004). More specifically, *GABRA2* polymorphisms have been linked to the subjective response to ethanol (Pierucci-Lagha, et al., 2005), where the hedonic value of alcohol changes as a factor of the *GABRA2* × breath alcohol concentration interaction (Haughey, et al., 2008). In addition to the subjective response to alcohol, *GABRA2* genotype seems to affect responses to alcohol-related cues. In an fMRI study using a sample of individuals not

undergoing treatment for substance abuse/dependence, subjects homozygous for the high-risk allele at rs2798 displayed an increased response to alcohol-associated cues in medial frontal cortical areas compared to heterozygous subjects. In contrast, the heterozygous subjects displayed an increased response in the ventral tegmental area (Kareken, et al., 2010). Finally, in a family sample enriched for alcoholism, *GABRA2* polymorphisms were found to be linked to increased activation in the insula with reward anticipation, suggesting that *GABRA2* polymorphisms may be linked to not only alcohol reward and alcohol-related cues, but to a general dysfunction of the reward system in alcohol-dependent subjects (Villafuerte et al., 2012).

It has been reported that a consistent elevation in the risk for alcohol dependence associated with *GABRA2* is not apparent until the mid-20s and then remains constant throughout adulthood (Dick, et al., 2006b; Kramer, et al., 2008). A gene-environment correlation and a gene-environment interaction associated with *GABRA2*, marital status and alcohol dependence have been reported, suggesting that *GABRA2* interacts with environmental factors and life style in determining the risk for alcohol dependence (Dick, et al., 2006a). Several studies have also noted *GABRA2*×environmental effects×personality traits interactions on alcohol dependence risk. For example, the interaction between parental monitoring as measured at age 11 and *GABRA2* polymorphisms was evaluated in a community-based sample where the output measure was externalizing behaviors (i.e., outward behavior that involves negative interactions with the environment, such as physical aggression, that is seen as a relatively stable personality construct) of the adolescents involved in the study measured longitudinally between 12 to 22 years of age. In this study, two classes of behaviors were distinguished: a stable highly externalizing class, and a moderate externalizing behavior class that showed diminishing externalizing behavior over time. Children exhibiting highly externalizing behavior were more likely to carry the *GABRA2* genotype associated with alcohol dependence, and with high levels of parental monitoring the association of *GABRA2* polymorphism with externalizing behavior diminished (Dick, et al., 2009). *GABRA2* polymorphisms have also been linked to impulsiveness, a trait known to be associated with substance abuse, in samples enriched for alcoholism (Villafuerte, et al., 2012). The haplotype conferring high risk for alcohol dependence was also found to be associated with characteristics of alcohol withdrawal and severity of alcohol dependence (delirium tremens, withdrawal seizures) (Soyka, et al., 2008). Finally, *GABRA2* may also be involved in therapeutic outcomes for alcohol-dependent patients. In a study looking at an association between high-risk and low-risk alleles and responses to three types of psychosocial treatment for alcoholism, it was found that the low-risk allele was associated with greater difference in drinking outcomes between the treatments, compared to the high-risk allele, specifically enhancing the outcomes of twelve-step facilitation (TSF) over Cognitive Behavioral Therapy (CBT) and Motivational Enhancement Therapy (MET) (Bauer, et al., 2007).

The polymorphisms described above do not affect the coding sequence of the *GABRA2* gene, and the question arises whether high-risk and low-risk alleles display different levels of gene expression. In postmortem prefrontal cortical tissue, the *GABRA2* mRNA levels differed significantly between genotypes (AA, AG, and GG at marker rs279858), being lower in heterozygous AG individuals than in homozygous AA and GG individuals. Interestingly, AA and GG individuals reported greater alcohol-induced positive mood and feelings of vigor after an oral alcohol challenge as compared to AG individuals, suggesting that AA and GG individuals may be more sensitive than AG individuals to the rewarding effects of ethanol, and that this may go along with a higher risk of AA and GG individuals compared to AG individuals for developing alcohol dependence (Haughey, et al., 2008). However, while the reduction of *GABRA2* mRNA in the prefrontal cortices of AG individuals was clearly demonstrated, the GABA_A receptor $\alpha 2$ subunit protein level was not

significantly decreased, potentially due to an insufficient number of samples tested (Haughey, et al., 2008).

In summary, *GABRA2* has been linked to the general risk for alcohol dependence and to specific processes involved in alcohol abuse, such as subjective response to alcohol, response to alcohol cues, severity of withdrawal responses, as well as to treatment outcomes. Some studies also suggested interactions between *GABRA2* and other GABA receptor subunit genes with regards to effects on alcohol dependence. For instance, polymorphic markers in the *GABRG1* gene encoding the GABA_A receptor $\gamma 1$ subunit, which is adjacent to the *GABRA2* gene (intergenic distance ~90kb), have been found to be in linkage disequilibrium with markers on the *GABRA2* gene and to contribute to risk of alcohol dependence in an additive manner, whereas the polymorphic markers in the *GABRA2* gene appear to have a dominant effect (Covault et al., 2008). Other researchers reported that the *GABRA2* and *GABRG1* genes likely provide independent contributions to alcohol vulnerability (Enoch, et al., 2009). The function of $\alpha 2$ -containing GABA_A receptors has recently been assessed in $\alpha 2$ (S270H/L277A) knock-in mice in which the $\alpha 2$ -containing GABA_A receptors are insensitive to potentiation by ethanol. Most importantly, these mice did not develop the typical conditioned taste aversion to ethanol, and they displayed a complete loss of the motor stimulant effect of ethanol, and decreased ethanol-induced hypnosis. Interestingly, the anxiolytic-like effect of ethanol was still present in these mice (Blednov, et al., 2011). This report suggests that at least certain effects of ethanol, such as the hypnotic and motor-stimulant effect, are dependent on the potentiation of $\alpha 2$ -containing GABA_A receptors. Considering the convincing evidence that the $\alpha 2$ -containing GABA_A receptors are required for benzodiazepine-induced anxiolysis, the finding that ethanol-induced anxiolysis is still present in these animals is surprising. It is possible that the anxiolytic effects of ethanol are mediated by more than one GABA_A receptor subtype and the potentiation of other subtypes (e.g., $\alpha 4$ -containing GABA_A receptors) is sufficient to generate this effect. These findings start to provide a neurobiological basis for the human genetics studies linking *GABRA2* polymorphisms to altered responses to ethanol in humans, however, further studies are needed to clarify the role of $\alpha 2$ -containing GABA_A receptors in processes such as ethanol reward, ethanol-induced anxiolysis and ethanol withdrawal.

4. *GABRA2* and Substance Abuse

In addition to alcohol dependence, *GABRA2* polymorphisms are also associated with other drug dependence, both in adolescence and adulthood (Enoch, et al., 2010). A study on African-American men with lifetime DSM-IV single and comorbid diagnoses of alcohol, cocaine, and heroin dependence found one unique haplotype predicting heroin addiction, and another one which was more common in controls and appeared to confer resilience to addiction after exposure to severe childhood trauma. An unlinked SNP (rs11503014) was independently associated with heroin addiction, and also interacted with exposure to childhood trauma in predicting cocaine addiction. These findings suggest that *GABRA2* might have at least two independent loci associated with drug addiction (Enoch, et al., 2010). In a separate study, in a sample of mostly Caucasian individuals from Brazil, *GABRA2* polymorphisms were associated with cocaine addiction (Dixon, et al., 2010).

In addition to cocaine and heroin addiction, *GABRA2* polymorphisms were also associated with marijuana and illicit drug dependence, and in one study, the association with ethanol dependence came only from individuals with comorbid illicit drug dependence (Agrawal, et al., 2006); see also (Matthews et al., 2007). These findings suggest that the *GABRA2* gene may be particularly important in cases of polydrug abuse (Drgon, D'Addario, & Uhl, 2006).

Finally, there is an association of *GABRA2* polymorphisms (as well as polymorphism in *GABRA4* and *GABRE*) with nicotine dependence (Agrawal, et al., 2008). However, it should be noted that at least one study found that *GABRA2* allelic associations have detectable but only minor effects on alcohol dependence in the general community and no predictive value for nicotine, marijuana and other illicit drug use (Lind, et al., 2008). In summary, there is suggestive evidence from human genetics studies that *GABRA2* may be involved in addiction to heroin, cocaine and nicotine, and in the use of marijuana and illicit drugs. On the other hand, the results of human genetic studies are very sensitive to the characteristics of the samples tested, and as a result, contradictory findings such as the (Lind, et al., 2008) study mentioned above are not uncommon. Thus, the findings from human studies are far from clear and gene-targeted mouse studies to corroborate and clarify these findings are highly needed. Two such studies (Morris, et al., 2008; Dixon, et al., 2010), investigated the role of $\alpha 2$ -containing GABA_A receptors in cocaine-induced behavioral changes. While the $\alpha 2$ knockout mice showed normal behavioral stimulation to acute cocaine and normal conditioned place-preference to cocaine, the ability of cocaine to potentiate responses to conditioned reinforcement and to cause behavioral sensitization following chronic administration was abolished in $\alpha 2$ knockout mice. Moreover, benzodiazepine-potentiation of behavioral sensitization to cocaine was abolished in $\alpha 2$ (H101R) mice, further confirming the role of the $\alpha 2$ -containing GABA_A receptors in behavioral sensitization. These findings suggest that at least some of the effects of cocaine are modulated by $\alpha 2$ -containing GABA_A receptors (presumably in the medium spiny neurons of the nucleus accumbens according to the Dixon et al., 2010 study). Similar studies that investigate the mechanisms of $\alpha 2$ -containing GABA_A receptor involvement in cocaine abuse are needed to clarify the nature of this relationship, and, clearly, an extension of this work to other drugs of abuse is necessary.

5. *GABRA2* and Depression

The neurotransmitter GABA has been implicated to play a role in depression. For instance, GABA levels are reduced in the brains of patients with major depression (MDD) and are normalized following chronic treatment with antidepressants (Sanacora et al., 2002; 2004). Genetic associations have been reported between MDD and the genes encoding several GABA_A receptor subtypes, such as *GABRA1* and *GABRA5* (Oruc, et al., 1997; Horiuchi, et al., 2004), but none of these studies have implicated *GABRA2* so far. This is interesting considering the high comorbidity of anxiety disorders and MDD, and the importance of stress as a vulnerability factor for MDD. The central role of *GABRA2* in fear and anxiety processes, as reviewed above, raises the expectation that *GABRA2* would play a role in depressive symptomatology. While human genetics studies have not provided evidence for this, some recent studies using gene-targeted mouse models point to a relationship in this direction.

The initial evidence that reduction of GABAergic function can result in depressive-like behavior came from the heterozygous $\gamma 2$ knockout mice, which, as described above, also exhibit trait anxiety. In tests of behavioral despair, these mice exhibited a developmental depressive-like phenotype that could be rescued by chronic administration of desipramine, but not fluoxetine (Earnheart, et al., 2007; Shen, et al., 2010). Further studies to elucidate the GABA_A receptor subtypes involved in this effect initially focused on the $\alpha 3$ subunit. Interestingly, $\alpha 3$ knockout mice showed reduced depressive-like behavior in tests of behavioral despair, suggesting that the activation of the $\alpha 3$ -containing GABA_A receptors would have pro-depressant actions (Fiorelli et al., 2008). In contrast, recent studies with $\alpha 2$ knockout mice (Vollenweider et al., 2011) showed that the knockout animals have a depressive-like phenotype in tests of behavioral despair (Fig. 3), similar to heterozygous $\gamma 2$ knockout mice. These results suggest that $\alpha 2$ - and $\alpha 3$ -containing GABA_A receptors may

modulate behavioral despair in opposite directions and that the positive modulation of $\alpha 2$ -containing GABA_A receptors may have antidepressant effects. However, it should be noted that the tests of behavioral despair such as the forced swim test and the tail suspension test, despite having predictive validity for antidepressant drug action, do not have construct validity for depression *per se*. Thus, further studies using other paradigms are needed to substantiate the role of $\alpha 2$ - and $\alpha 3$ -containing GABA_A receptors in mood regulation. As noted above, one possible way that the $\alpha 2$ -containing receptors may be linked to depressive symptomatology is through their involvement in stress-related responses. Neonatal handling and brief maternal separation have been shown to lead to an increase in the expression of the $\alpha 2$ subunit in the dentate gyrus of adult animals, and also to increased activity in response to swim stress (Hsu, et al., 2003). These results show that stress during development induces expression changes in the GABA_A receptor system, which may have functional implications. One interpretation is that the increase in the $\alpha 2$ subunit is a compensatory mechanism, which is however not sufficient to prevent functional consequences.

The above findings have important potential implications for the development of pharmacological therapies for MDD. While the blockade of serotonin and/or norepinephrine reuptake following the administration of selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants is immediate, the therapeutic antidepressant effects may take up to 6 weeks to develop, suggesting that the antidepressant effects of these drugs probably involve long-term changes in other systems than the serotonergic and noradrenergic systems. Chronic SSRI administration increases brain GABA content in the same time frame that the therapeutic effects start to be observed (Sanacora et al., 2002), suggesting that this adaptation may be at least partially responsible for the antidepressant effects. Indeed, some benzodiazepines, specifically alprazolam and adinazolam, have been demonstrated in clinical studies to elicit antidepressant responses similar to widely prescribed antidepressants in MDD patients, but within the first 2 weeks of treatment (Amsterdam et al., 1986; Petty et al., 1995). The fact that the antidepressant actions of benzodiazepines appear in a much shorter time-frame than those of SSRIs raises the possibility that the SSRI-induced increase in GABA levels in the brain (Sanacora et al., 2002) may be crucial for the antidepressant effects of SSRIs. This view is supported by the experimental observation that heterozygous $\gamma 2$ knockout mice do not display the antidepressant-like actions of SSRIs even after chronic treatment (Shen et al., 2010).

It is not known why some benzodiazepines have antidepressant effects while others don't. Potentially, these benzodiazepines display subtle and as yet unknown differences in relative modulation of GABA_A receptor subtypes. Based on the preclinical results presented above, it is tempting to speculate that selective agonism at $\alpha 2$ -containing GABA_A receptors might provide a more reliable antidepressant effect than concomitant positive modulation of $\alpha 2$ - and $\alpha 3$ -containing GABA_A receptors as is the case with clinically used benzodiazepines.

6. GABRA2 and Reward

In the last three sections we have reviewed evidence suggesting a role for $\alpha 2$ -containing GABA_A receptors in alcohol dependence and other substance abuse, and in depression. One common characteristic of addictive disorders and depression is the dysfunction of the brain reward system. Thus, it is likely that the $\alpha 2$ -containing GABA_A receptors play an important role in the functioning of the brain reward system.

Moreover, benzodiazepine abuse and misuse has been long documented among both patients and poly-drug users, suggesting that the positive modulation of the GABA_A receptors has rewarding effects. The GABA_A receptor subtypes mediating this effect have only recently started to be studied.

In a recent series of studies, we first investigated the rewarding actions of benzodiazepines as compared to those of cocaine, and then the GABA_A receptor subtypes involved in these rewarding actions, using the intracranial self-stimulation (ICSS) paradigm. In this paradigm, animals spin a wheel manipulandum, for which they get rewarded with an electrical stimulation in the medial forebrain bundle at the level of the hypothalamus. In wild type mice, both cocaine and diazepam lead to a left-shift of the dose-reponse curve, i.e. reward enhancement, with comparable effect sizes (Straub, et al., 2010). This is remarkable since benzodiazepines are generally considered to have lower abuse potential than cocaine (see e.g. classification of diazepam as a class 2 drug and of cocaine as a class 4 drug in the US). Moreover, diazepam and cocaine have opposite effects on extracellular dopamine in the nucleus accumbens with cocaine increasing and diazepam decreasing dopamine concentrations (Di Chiara & Imperato, 1988; Finlay, et al., 1992; Invernizzi, Pozzi, & Samanin, 1991), indicating that the mechanism of action for the rewarding effects of the two drugs may be different.

Since the $\alpha 2$ subunit is strongly expressed in the nucleus accumbens, we hypothesized that $\alpha 2$ -containing GABA_A receptors may be mediating the reward-enhancing action of diazepam. Indeed, the above-noted reward-enhancement following diazepam treatment in wild type mice was absent in $\alpha 2$ (H101R) mice (Reynolds, et al., 2012; Fig. 4). This indicates that $\alpha 2$ -containing GABA_A receptors are necessary for the reward-enhancing action of diazepam. Furthermore, there was an increase in reward thresholds at the highest dose of diazepam in these animals, suggesting that diazepam may even be aversive without the positive modulation of the $\alpha 2$ -containing GABA_A receptors. Our studies similarly demonstrated an abolishment of the reward-enhancing diazepam action in $\alpha 3$ (H126R) mice, and a reduction in effect sizes in $\alpha 1$ (H101R) mice, suggesting a complex modulation of the reward circuitry by different GABA_A receptor subtypes, possibly at different points of the system. For example, recent studies have noted the importance of the $\alpha 1$ -containing GABA_A receptors on GABAergic interneurons in the ventral tegmental area in addictive-like properties of benzodiazepines (Tan, et al., 2010), while the above-mentioned Dixon, et al. (2010) study emphasized the importance of $\alpha 2$ -containing GABA_A receptors, potentially those expressed in the nucleus accumbens in behavioral sensitization to cocaine. While the study by Tan, et al.(2010) indicates that positive modulation of $\alpha 1$ -containing GABA_A receptors is required for addictive-like properties of benzodiazepines, so far, there is no experimental evidence on the abuse potential of a highly $\alpha 2$ -specific agonist, which, if present, would limit the therapeutic usefulness of such compounds. A more systematic study of the GABA_A receptors in the brain reward circuitry using gene-targeted models can reveal the exact role of each receptor subtype (i.e., whether they are necessary or sufficient in reward processes) and can provide important insights relevant for the development of novel treatments for psychopathologies such as addiction disorders and MDD.

7. GABRA2 and Sleep EEG

Sleep EEG is altered in several psychiatric and neurodegenerative disorders, suggesting that an altered sleep EEG could be an important biomarker for several disorders, as well as a possible factor that exacerbates the neuropathology [see Petit et al., 2004; Steiger & Kimura, 2010 for reviews]. The patients in one of the studies linking polymorphisms in the *GABRA2* gene to alcohol dependence had the quantitative endophenotype of increased power in the β frequency band (13–28 Hz) of the EEG (Edenberg, et al., 2004), suggesting that changes in sleep EEG may also constitute a part of the neuropathology in alcohol dependence.

Diazepam is known to increase the β power in NREM sleep and waking, to decrease δ activity in NREM sleep and to increase θ activity during REM sleep. The diazepam-induced increase in β power was absent in $\alpha 2$ (H101R) mice, indicating that it is mediated by $\alpha 2$ -

containing GABA_A receptors (Kopp et al., 2004). Thus, alcohol dependence is linked to increased power in the β frequency band in humans and to the gene encoding the $\alpha 2$ subunit of the GABA_A receptor, and in mice positive allosteric modulation of $\alpha 2$ -containing GABA_A receptors results in an increase in β power. It is thus tempting to speculate that the *GABRA2* allele(s) associated with alcohol dependence might result in an increased expression of $\alpha 2$ -containing GABA_A receptors. This would be in line with the finding that the individuals heterozygous (AG) at marker rs279858 were less sensitive to the rewarding effects of alcohol, were considered to be low risk for alcohol dependence and also had lower *GABRA2* mRNA levels compared to the individuals homozygous for either allele in one of the studies reviewed above (Haughey et al., 2008).

In addition, the decrease in δ activity in NREM sleep and the increase in θ activity following diazepam treatment in REM sleep were both attenuated in $\alpha 2$ (H101R) mice compared to wild type controls, suggesting a role for $\alpha 2$ -containing GABA_A receptors in these effects (Kopp, et al., 2004). Moreover, the involvement of $\alpha 2$ -containing GABA_A receptors in θ band activity seems to be state-specific, as the diazepam effects on awake θ activity is unchanged in $\alpha 2$ (H101R) mice.

As mentioned previously, $\alpha 1$ -containing GABA_A receptors have been found to be necessary for the sedative action of diazepam (Rudolph et al., 1999; Rudolph & Antkowiak, 2004), and they are also likely sufficient, since the potentiation of $\alpha 1$ -containing GABA_A receptors by the $\alpha 1$ -preferring agonist zolpidem is sufficient to produce sedation (Crestani et al., 2000). The initial expectation was therefore that $\alpha 1$ -containing GABA_A receptors would also mediate key effects of diazepam on the sleep EEG. However, diazepam's effects on sleep latency, REM sleep inhibition, and effects on the EEG were all present in the $\alpha 1$ (H101R) mice (Tobler et al., 2001). Interestingly, the decrease in δ frequencies was even more pronounced in the $\alpha 1$ (H101R) mice compared to wild type, as was the number of brief awakenings. From these EEG findings it appears that $\alpha 1$ -containing GABA_A receptors do not necessarily play a major role in the sleep-promoting action of diazepam, and that the $\alpha 2$ -containing GABA_A receptors may at least partially mediate this action.

8. *GABRA2* and Schizophrenia

GABA abnormalities in the cortex of schizophrenic patients has been a consistent finding of postmortem studies (see Stan & Lewis, 2012 for a review). Human genetics studies have associated several GABA_A receptor subunit genes, such as *GABRA1*, *GABRA6* and *GABRB2*, with schizophrenia (Petryshen et al., 2005). While no study has linked *GABRA2* polymorphisms to schizophrenia so far, there is evidence from postmortem studies of schizophrenia brains, from pharmacological studies and from gene-targeted mouse studies that suggests an important role for the cortical $\alpha 2$ -containing GABA_A receptors in schizophrenia-related cognitive impairments. According to the glutamatergic hypothesis of schizophrenia (see Tsai and Coyle, 2002 for a review), hypofunction of NMDA receptors on parvalbumin-positive GABAergic interneurons in the prefrontal cortex is an essential component of the pathophysiology of schizophrenia (Lisman et al., 2008). Knocking out the NMDA receptor NR1 subunit in GABAergic interneurons in mice has indeed revealed a schizophrenia-like phenotype (Belforte et al., 2010). Postmortem analysis revealed that the glutamate transporter 1 (GAT1) is downregulated and that the GABA_A receptor $\alpha 2$ subunit is upregulated in the axon initial segments, the projection zone of a subset of parvalbumin-positive interneurons, in schizophrenic brains (Lewis, et al., 2005). Both observations are consistent with compensatory changes counteracting the decreased activation of the GABAergic parvalbumin-positive interneurons. It has been suggested that the upregulation of the $\alpha 2$ subunit in the axon initial segment might not be sufficient, and that a positive

allosteric modulation of $\alpha 2$ -containing GABA_A receptors might have a beneficial effect on the cognitive deficits in schizophrenic patients.

A first clinical study with 15 patients using TPA023 (i.e., MK-0777), an $\alpha 2/\alpha 3$ -selective partial agonist at the benzodiazepine site, showed some improvements in cognitive tests and an increased frontal γ power band (Lewis et al., 2008). However, in a second, independent study with 60 patients no cognitive improvements were seen (Buchanan, et al., 2011). It should be noted that the relative efficacy of TPA023 compared to chlordiazepoxide is only 11% at $\alpha 2$ -containing GABA_A receptors (Atack et al., 2006). Thus, it is possible that the efficacy of TPA023 was too low, and that a compound with higher efficacy at $\alpha 2$ -containing GABA_A receptors might provide unequivocal cognitive improvements. It is noteworthy that TPA023 reversed ketamine-induced impairments in spatial working memory in rhesus monkeys, but not ketamine-induced hallucinatory-like behaviors, which indicates the functional specificity of positive allosteric modulation of $\alpha 2$ - (and possibly $\alpha 3$ -) containing GABA_A receptors (Castner et al., 2010).

While studied so far only in the context of schizophrenia, these findings may have implications for other disorders that involve cognitive impairments, such as neurodegenerative disorders. Indeed, abnormal GABAergic function and changes in the GABA_A receptor subunit mRNA levels have been noted in Alzheimer's disease (AD) and Parkinson's disease (Luchetti, et al., 2011), and shifted excitation/inhibition balance has been suggested as a major contributor to cognitive decline in Alzheimer's disease (Rissman & Mobley, 2011). A recent study found an age-related reduction of GABA currents in AD brains, a faster rate of desensitization, and a reduced sensitivity for GABA. Furthermore, an increase in the transcripts for the $\alpha 2$ subunit was one of the changes observed AD brains, along with increases or decreases in the transcripts for other subunits (Limon, et al., 2012). Benzodiazepine binding sites have been shown to have a stable decline with aging in the rat brain (Hoekzema, et al., 2012) and there is some evidence that the $\alpha 2$ mRNA also diminishes in the human prefrontal cortex with age, at least during the time from the neonatal period all the way into adulthood (Fillman et al., 2010). It is possible that digressions from the normal rate of $\alpha 2$ -expression decline can underlie cognitive impairments in different aging-related diseases. Finally, a reduction of the $\alpha 2$ subunit has also been reported in brains from autistic patients (Fatemi et al., 2009), showing a possible linkage of this subunit in cognitive deficits unrelated to aging.

9. GABRA2 and Pain

While diazepam is not usually considered to be an analgesic agent, intrathecal administration of diazepam in rodents which does not cause motor sedation has an antihyperalgesic effect, e.g. in paradigms for neuropathic and inflammatory pain, where it increases the paw withdrawal latencies. Studies with mice carrying point-mutated diazepam-insensitive α subunits indicated that diazepam-induced analgesia is mediated by $\alpha 2$ - and $\alpha 3$ - containing GABA_A receptors in an animal model of inflammatory pain and by $\alpha 2$ -, $\alpha 3$ -, and $\alpha 5$ -containing GABA_A receptors in an animal model of neuropathic pain and in a model of tonic nociception. In these models, the contributions of $\alpha 2$ -containing GABA_A receptors appear to be strongest (Knabl, et al., 2008; 2009). In line with this finding, diazepam-induced potentiation of GABAergic membrane currents in dorsal root ganglion neurons were reduced in $\alpha 2$ (H101R) mice, while the same parameter was attenuated in intrinsic dorsal horn neurons in both $\alpha 2$ (H101R) mice and in $\alpha 3$ (H126R) mice (Knabl, et al., 2008). L-838,417, a BZ site partial antagonist at $\alpha 2$ -, $\alpha 3$ -, and $\alpha 5$ - containing GABA_A receptors but an antagonist at $\alpha 1$ -containing GABA_A receptors induces analgesia in rats, and there is no tolerance to this analgesic action during a 10 day period (Knabl, et al., 2008). fMRI studies revealed that L-838,417 also reduced the activity of brain areas related to the

associate-emotional components of pain (Knabl, et al., 2008). Other partial allosteric modulators of $\alpha 2$ -, $\alpha 3$ -, and/or $\alpha 5$ -containing GABA_A receptors, including TPA023 and NS11934 have also been shown to have analgesic actions in models of inflammatory and neuropathic pain; interestingly, diazepam was ineffective in these models for reasons which are not understood, demonstrating that subtype-selective compounds can have actions different from those of classical benzodiazepines (Munro, et al., 2008; 2011).

10. Conclusion

While the role of $\alpha 2$ -containing GABA_A receptors in benzodiazepine-induced anxiolysis has been known for a long time (Low et al., 2000), more recent studies reveal the involvement of these receptors in processes as diverse as reward and alcohol/drug abuse, schizophrenic symptomatology, depression and chronic pain. Since $\alpha 2$ -containing GABA_A receptors do not mediate sedation (Rudolph, et al., 1999) (McKernan, et al., 2000), it is predicted that $\alpha 2$ -specific agonists would not be compounded by sedation. $\alpha 2$ -specific agonists would be predicted to be therapeutically useful for anxiety disorders and possibly depression, with the involvement of the $\alpha 2$ -subunit in reward processes suggesting that the positive modulation of these receptors may help with the anhedonic responses in major depression. The recent finding that positive modulation of $\alpha 1$ -containing GABA_A receptors is required for the addictive properties of benzodiazepines (Tan, et al., 2010) suggests that $\alpha 2$ -specific agonists may lack significant abuse potential, although this still has to be demonstrated experimentally. The use of $\alpha 2$ -specific agonists in the treatment of chronic pain would also address the increased anxiety that is frequently comorbid with chronic pain. It is our belief that the potential for GABA_A-receptor subtype specific compounds will become more evident as further studies clarify the role of $\alpha 2$ and other GABA_A receptor subtypes in different processes.

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Abbreviations

GABRA2	γ -aminobutyric acid (GABA) A receptor, $\alpha 2$
CNS	central nervous system

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Figure 1.

Approximate abundance, distribution and functions of α2-containing GABA_A receptors.

Left panel: Approximate abundance of major GABA_A receptor subtypes. **Middle panel:** Immunohistochemical distribution of the GABA_A receptor α2 subunit in a parasagittal mouse brain section (expression levels from high to low: white-yellow-red-blue). The expression of the α2 subunit is particularly strong in hippocampus, striatum, and olfactory bulb, as well as in the amygdala (not shown). Reproduced with permission from (H. Mohler, et al., 2002). **Right panel:** Major functions and associations of α2-containing GABA_A receptors. The lower lines in the square represent the effects of positive allosteric modulation of α2-containing GABA_A receptors in different behavioral and physiological domains with the exception of the lowest square, where “SNPs” indicates that single nucleotide polymorphisms have been found to be associated with alcohol dependence. The evidence regarding antidepressant-like actions via α2-containing GABA_A receptors is so far only suggestive, and the improvement of cognitive deficits in schizophrenia by allosteric modulation of α2-containing GABA_A receptors in humans is still controversial and requires further study.

Figure 2.

Panels A–D: Impact of diazepam on conditioned fear in the fear-potentiated startle test in wild type and $\alpha 2(H101R)$ mice. Mean startle amplitude measures in wild type (**A**) and $\alpha 2(H101R)$ (**C**) mice following diazepam (0, 1, 2 mg/kg) show that both genotypes show fear-potentiation of the baseline startle response in tone+startle stimulus trials (a=startle amplitude on Tone+Startle trials significantly different from both Leader and Startle Only trials). Diazepam reduced percent potentiation of the startle response following fear conditioning in wild type (**B**) but not in $\alpha 2(H101R)$ (**D**) mice. **Panel E:** Impact of diazepam on unconditioned anxiety in the elevated plus maze test in wild type and $\alpha 2(H101R)$ mice.

Percentage of time spent in the open arms was increased significantly by diazepam administration in wild type (left) but not in $\alpha 2$ (H101R) (right) animals. *Different from the vehicle group of the animals in the same genotype at $p < 0.05$. **Different from the vehicle group of the animals in the same genotype at $p < 0.01$. N = 9 - 12 per group. Figure modified from (K.S. Smith, et al., 2012).

Figure 3.

Anxiety-like and depressive-like behavior of wild type mice ($\alpha 2^{+/+}$), and mice heterozygous ($\alpha 2^{+/-}$) and homozygous ($\alpha 2^{-/-}$) for the $\alpha 2$ knockout allele. **Panel A:** In the novelty-suppressed feeding test (NSFT), a conflict-based test assessing anxiety-like behavior, $\alpha 2^{+/-}$ mice showed greater latency to consume a food pellet in a novel environment compared to wild type mice, with the $\alpha 2^{-/-}$ mice ranking in between wild type and $\alpha 2^{+/-}$. **Panels B–C:** In two tests of behavioral despair, the forced swim test (FST) and the tail suspension test (TST), $\alpha 2^{-/-}$ mice showed greater immobility compared to wild type mice. **Different from the wild type ($\alpha 2^{+/+}$) group at $p < 0.01$. $N = 12$ per group. Figure modified from (Vollenweider et al., 2011).

Figure 4.

Impact of diazepam on brain stimulation reward (intracranial self-stimulation, ICSS) in wild type and $\alpha 2(H101R)$ mice. **Panel A:** A reduction in reward thresholds (i.e. the lowest frequency of stimulation that the animals find rewarding) is considered to be a reward-enhancing effect and is observed following the administration of drugs of abuse and other rewarding manipulations. Diazepam dose-dependently reduced reward thresholds in wild type mice, but not in $\alpha 2(H101R)$ mice. In $\alpha 2(H101R)$ mice, at the highest dose, diazepam even increased the reward threshold, indicating that it had aversive properties. **Panel B:** Diazepam did not lead to a reduction in maximum response rates in either genotype, suggesting that it was not sedative / motor impairing at the administered doses. The increase in maximum response observed in wild type mice is an artifact of the reward-enhancing effects of diazepam, and is abolished in $\alpha 2(H101R)$ mice. *Different from the vehicle group of the animals in the same genotype at $p < 0.05$. **Different from the vehicle group of the animals in the same genotype at $p < 0.01$. $N = 12$ per group. Figure modified from (Reynolds, et al., 2012).