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GABA_A Receptor Subtype Involvement in Addictive Behaviour

DN Stephens¹, SL King¹, JJ Lambert², D. Belelli², and T Duka¹,

¹School of Psychology, University of Sussex, Brighton, UK

and

²Division of Neuroscience, University of Dundee, Dundee, UK

Address for Correspondence:

D N Stephens
School of Psychology
University of Sussex
Falmer
Brighton, BN1 9QG
UK

d.stephens@sussex.ac.uk

Tel +44 1273 678638

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Abstract

GABA_A receptors form the major class of inhibitory neurotransmitter receptors in the mammalian brain. This review sets out to summarise the evidence that variations in genes encoding GABA_A receptor isoforms are associated with aspects of addictive behaviour in humans, while animal models of addictive behaviour also implicate certain subtypes of GABAA receptor. In addition to outlining the evidence for the involvement of specific subtypes in addiction, we summarise the particular contributions of these isoforms in control over the functioning of brain circuits, especially the mesolimbic system, and make a first attempt to bring together evidence from several fields to understanding potential involvement of GABA_A Receptor Subtypes in addictive behaviour. While the weight of the published literature is on alcohol dependency, the underlying principles outlined are relevant across a number of different aspects of addictive behaviour.

Key Words: alcohol; psychostimulant; nucleus accumbens; gene association; gene knockout; animal model; reward; impulsivity; benzodiazepine; conduct disorder; electrophysiology
1 Introduction

Both human and animal studies have implicated GABA_A receptors in addiction processes. This paper sets out to review the evidence for GABA_A receptor involvement in relation to alcoholism and addiction to other drugs of abuse. It will begin with a summary of the structure and function of GABA_A receptors and their subtype diversity, before reviewing the human and animal literature providing genetic and behavioural evidence for the role of GABA_A receptors in addictive behaviour. Finally, we will attempt to integrate these data into our understanding of addiction processes, and their underlying neurobiology. While the review concerns itself broadly with GABAergic systems and addictive behaviour, the weight of the published literature is on alcohol dependency. Nevertheless, the underlying principles outlined are relevant across a number of different aspects of addictive behaviour.

1.1 GABA_A receptors

GABA_A receptors are members of the cys-loop family of ligand gated ion channels that additionally includes acetylcholine nicotinic, 5HT3, and strychnine-sensitive glycine receptors. GABA_A receptors are heteromeric protein complexes, composed of five subunits arranged around a central axis to form an anion-selective ion channel, permeable to chloride and bicarbonate ions (Olsen and Sieghart, 2008, Macpherson et al., 2016). In the central nervous system (CNS), GABA_A receptors are expressed in both neurons and glial cells. The binding of the neurotransmitter, GABA, to specific binding sites on the complex, transiently stabilizes the complex in an open conformation, allowing ionic flux. In most circumstances, the electrochemical gradient (combining the reversal potential for Cl⁻ ions, $E_{Cl}$, and the cell's resting membrane potential, $V_m$) allows for an influx of chloride ions resulting in a hyperpolarisation of the membrane that, in turn, makes the neuron less susceptible to depolarization by other neurotransmitters such as glutamate. Moreover, by decreasing the cell input resistance, GABA acts as a “shunt” for incoming signals. Thus, in most circumstances, GABA acts as an inhibitory neurotransmitter, dampening down the excitability of its target neurons.
In addition to expressing binding sites for GABA, the natural neurotransmitter, GABA<sub>A</sub> receptors also possess a variety of allosteric sites at which a number of other agents act (Fig 1), including the benzodiazepines, certain naturally occurring neurosteroids and a number of structurally diverse <i>i.v.</i> general anaesthetics <i>e.g.</i> propofol, etomidate, barbiturates (Belelli and Lambert, 2005). These compounds act as positive allosteric modulators (PAMs) to enhance the function of GABA by stabilising the open conformation of the channel in the presence of the agonist. Additionally, at greater concentrations than those required for this GABA-enhancing action, the neurosteroids and <i>i.v.</i> anaesthetics can directly gate the receptor-channel complex in the absence of GABA, a so called “GABA-mimetic” effect. Note that benzodiazepines do not share this “agonist” property. As regards GABA<sub>A</sub> receptor inhibitors, picrotoxin acts as a GABA<sub>A</sub> receptor antagonist, by binding to amino acid residues forming the lining of the chloride ion channel pore. Bicuculline and gabazine bind to, or close to, the binding site for GABA, with the former acting as a negative allosteric modulator (NAM) and the latter as a true competitive antagonist. Additionally, there are binding sites for Zn<sup>2+</sup>, which may act as an endogenous modulator of GABA<sub>A</sub>R function, and possibly for alcohol.

**1.2 Subtypes of GABA<sub>A</sub> receptor**

GABA<sub>A</sub> receptors are typically composed of 5 protein subunits arranged around a central pore that forms the ion channel (Fig 1). Each subunit consists of a large N-terminal extracellular domain (at which both the natural ligand, GABA, and pharmaceuticals such as benzodiazepines interact with the complex), four hydrophobic transmembrane domains (TM1-4), of which TM2 is thought to line the pore of the channel, and a large intracellular loop between TM3 and TM4 which may contribute to the ion pore pathway and also may incorporate kinase consensus sequences that influence receptor function and trafficking (Kittler and Moss, 2003). The GABA binding site is located between the α and β subunit interface and that for benzodiazepines in an equivalent location between the α and γ subunit interface. In mammals, nineteen different subunit proteins have been identified, and classified into eight families, based on sequence homology. Several of the families have multiple members: α (1–6), β (1–3), γ (1–3), δ, ε, ρ (1–3), θ and π, and within a family,
subunits have a high sequence similarity (Olsen and Sieghart, 2008). Further, for some GABA<sub>A</sub> subunit isoforms (e.g. γ<sub>2</sub>, α<sub>4</sub> & α<sub>6</sub>), alternative splicing allows a further complexity in the protein product (Bateson et al., 1991, Petrie et al., 2001).

This diversity of subunits fosters the possibility for a very large number of permutations to give a pentameric structure (over a million), but in reality, only a limited number (about 30-40) of GABA<sub>A</sub> receptor subtypes have been estimated to be naturally expressed (Olsen and Sieghart, 2009, Fritschy and Panzanelli, 2014); (see also Section 1.3, GABA<sub>A</sub> receptor subunit chromosomal localisation, for co-ordinated expression of subunits).

Most mammalian receptors appear to consist of 2 members of the α family, 2β, and one γ subunit (Fig 1). Alternatively, in less abundant subtypes, the γ subunit is replaced by a δ or an ε subunit. While for any individual receptor, both α subunits are likely to be of the same isoform, hybrid receptors with two different forms of α subunit also occur (Nusser et al., 1998). Furthermore, although the natural ligand for all combinations known to exist is GABA, some allosteric modulators interact with only a subset of these possible receptor complexes. Thus, for classical benzodiazepines (e.g. diazepam) to interact with the receptor complex, it must contain particular members of the α family (α1, α2, α3, α5) along with members of the β family, and a γ<sub>2</sub> subunit. However, certain atypical benzodiazepines and β-carbolines act at these benzodiazepine-sensitive complexes to negatively modulate the channel in an allosteric fashion and are thus termed “inverse agonists” or negative allosteric modulators (NAMS) (i.e., they reduce the effects of GABA). These substances also bind to α<sub>4</sub> and α<sub>6</sub>/β/γ<sub>2</sub> complexes where they function as positive allosteric modulators (PAMs) (Walker and Semyanov, 2008). A distinct group of receptors that has received recently significant interest is made up of α<sub>4</sub> or α<sub>6</sub> subunits together with β and δ subunits.

**Fig 1 near here**

Different combinations of subunits have differing physiological (e.g. single channel conductance, ion channel kinetics) and pharmacological properties and are heterogeneously distributed within the brain, and within neuronal cellular domains. Thus, receptors
containing $\alpha 1$, 2, 3 or 5 subunits, together with $\beta 1/2/3$ and $\gamma 2$ subunits largely interact with scaffold proteins (such as gephyrin), to cluster at the postsynaptic membrane (Farrant and Nusser, 2005, Fritschy et al., 2012). However, the same isoforms can also localise to extrasynaptic cellular domains (e.g. $\alpha 5\beta 2\gamma 2$ receptor subtype (Fritschy and Panzanelli, 2014)). Alternatively, receptors made up of $\alpha 4$ or $\alpha 6$, in combination with $\beta$ and $\delta$ subunits exhibit an exclusively extrasynaptic localization and may respond to overflow of GABA from the synapse (Belelli et al., 2009, Herd et al., 2013, Walker and Semyanov, 2008). The brain region-selective distribution of the various subunits suggested early on that GABA$_A$ receptor heterogeneity likely subserves differential functional and physiological roles, so that the pharmacological targeting of a particular subunit combination may associate with more subtle behavioural effects than those typically displayed by drugs with a broad spectrum of actions (e.g. classical benzodiazepines and barbiturates), which facilitate transmission at a wide variety of GABA$_A$ receptors located within different neuronal networks (McKernan and Whiting, 1996, Whiting, 2003).

This proposal found substantial support from a series of elegant experiments in mice in which individual members of the $\alpha$ family were mutated to render them insensitive to classical benzodiazepines (e.g. diazepam). Despite their high sequence homology with other members of the $\alpha$ subunit family, the benzodiazepine-insensitive $\alpha 4$ and $\alpha 6$ subunits differ from the other family members by virtue of a crucial arginine (R) residue, which replaces histidine (H) in the homologous position of the benzodiazepine binding domain. Taking advantage of this observation, the groups led by Möhler and McKernan developed a number of mouse lines engineered to harbour a mutated $\alpha$ subunit gene i.e. $\alpha 1$(H101R), $\alpha 2$(H101R), $\alpha 3$(H126R) or $\alpha 5$(H105R) to impart benzodiazepine insensitivity to the receptors incorporating the mutated subunits (Rudolph et al., 1999, McKernan et al., 2000). Behavioural analysis of these mouse lines has allowed the dissection of the pharmacological repertoire normally associated with the administration of classical benzodiazepines. Thus, the sedative properties and anxiolytic properties of benzodiazepines have been shown to be mediated by $\alpha 1$-containing and $\alpha 2$- (and perhaps $\alpha 3$-) subunit containing receptors respectively, while an action at $\alpha 5$-containing receptors makes a significant contribution to the well-characterised amnestic effects of benzodiazepines (Mohler et al., 2002, Collinson et
Taking advantage of the $\beta$-subunit selectivity of etomidate, a similar approach has been also successfully applied to dissect the contribution of distinct $\beta$ subunit-containing isoforms ($\beta$1-3) to the constellation of behavioural effects associated with general anaesthetic usage (e.g. sedation, immobility, hypnosis) (Reynolds et al., 2003b, Kretschmannova et al., 2013). Although acquired in the context of discovering potential selective targets for benzodiazepine-like ligands and general anaesthetics, these data point to quite separate roles for subtypes of GABA$_A$ receptor in different brain circuits, and hence behavioural function.

### 1.3 GABA$_A$ Receptor Subunit Chromosomal Localisation

In humans, genes encoding 14 of the 19 GABA$_A$ receptor subunits are found in four major clusters located on chromosomes 4, 5, 15 and X (Fig 2 (Buckle et al., 1989, Dean et al., 1991, McLean et al., 1995, Bell et al., 1989, Greger et al., 1995, Hicks et al., 1994, Johnson et al., 1992, Russek and Farb, 1994, Sommer et al., 1990, Wilcox et al., 1992). The chromosome 4 cluster (chr 4) contains genes encoding $\gamma_1$, $\alpha_2$, $\alpha_4$ and $\beta_1$ subunits, the chromosome 5 cluster (chr 5) contains genes for $\alpha_1$, $\alpha_6$, $\beta_2$ and $\gamma_2$ subunits, the chromosome 15 cluster $\alpha_5$, $\beta_3$, $\gamma_3$ subunits and the X chromosome $\alpha_3$, $\varepsilon$, and $\theta$ subunits. Syntenic regions for the human clusters occur in the mouse (Fig 2) suggesting a highly conserved organisation of these genes. Thus, the chr 4 cluster of GABA$_A$ receptor genes is also found on mouse chromosome 8 (chr 8; www.ensembl.org), and the human chr 5 cluster on mouse chromosome 11 (chr11) (Garrett et al., 1997). The highly conserved organisation of the genes in clusters raises the question as to whether the expression of genes within each chromosomal cluster may be co-regulated (cis effects) and, additionally, whether there is correlation of gene expression between GABAergic genes that are located in different chromosomes (trans effects). Interestingly, expression of those genes located on human chr 4 (and mouse chr 8) predominate in rodent embryo, but these genes are generally down regulated in the adult rat (Wisden et al., 1992, Laurie et al., 1992), and presumably other mammals, except in particular structures including the cortical areas, thalamus, hippocampus, amygdala, the majority of dopamine (DA) neurons in the substantia nigra and the VTA, where they continue (especially the $\alpha_4$ and $\alpha_2$ genes) to be highly expressed (Laurie et al., 1992, Pirker et al., 2000, Schwarzer et al., 2001).
It is noteworthy that three of the chr 5 cluster genes encode the most frequently expressed \( \text{GABA}_A \alpha 1 \beta 2 \gamma 2 \) receptor, while the chr 4 genes encode the \( \text{GABA}_A \) receptor subunit group \( \alpha 2 \beta 1 \gamma 1 \) that is found almost exclusively in the addiction-related mesolimbic pathways encompassing ventral tegmental area (VTA) and ventral striatal regions, consistent with coordinated transcription within clusters (Enoch et al., 2013). A recent factor analysis by Enoch and colleagues (Enoch et al., 2013) suggested that the \( \text{GABA}_A \) gene clusters show both cis and trans correlations of gene expression. In particular, expression levels of the chr 5 genes were correlated highly, not only with other genes within that cluster, but two chr 15 genes, GABRB3 (encoding subunit \( \beta 3 \)) and GABRG3 (\( \gamma 3 \)) also had high loadings (0.8) on this factor, while the chr 4 gene GABRA4 (\( \alpha 4 \)) and the chr X gene GABRA3 (\( \alpha 3 \)) loaded more modestly (0.7–0.6). A second group of genes, namely the four \( \text{GABA}_A \) subunit genes in the chr 4 cluster, loaded onto a factor that accounted for 0.14 of the total variance in \( \text{GABA}_A \)ergic gene expression: GABRG1 (1.0); GABRA2 (0.8); GABRB1 (0.8) and GABRA4 (0.5), though the authors note that GABRA4 expression correlated better with expression of the chr 5 genes (Enoch et al., 2013). Such correlations of expression levels might suggest that a common mechanism underlies coordinated expression of some subunits, favouring formation of particular receptor assemblies.

1 Evidence for \( \text{GABA}_A \) receptor involvement in addiction

In the remainder of this review we shall discuss evidence from both human and animal studies implicating an important role for \( \text{GABA}_A \) receptors in drug addiction. Alcohol because of its widespread abuse and availability of considerable documented research, will be the main focus of this narrative with additional references to other substances of abuse e.g. cocaine and amphetamine-like psychostimulants where appropriately documented.

2.1 Human gene association studies

2.1.1 Chromosome 4 cluster: \((\gamma 1, \alpha 2, \alpha 4 \text{ and } \beta 1)\)

2.1.1.1 GABRA 2
A number of studies over the past twenty years has suggested a linkage between alcohol dependence and chromosome 4p at the location of the GABA_A gene cluster (Ghosh et al., 2003, Long et al., 1998, Porjesz et al., 2002, Reich et al., 1998, Zinn-Justin and Abel, 1999). More recent studies (using the Collaborative Study on the Genetics of Alcoholism (COGA) cohort) have identified two haplotype blocks extending downstream from intron 3 within GABRA2, with the more common haplotype being a significant risk factor for alcohol dependence (Edenberg et al., 2004). This finding was confirmed in a German population (Soyka et al., 2008). However, subsequent analysis of the COGA dataset revealed that this association held only for those alcoholics who additionally had dependence on other, illicit, drugs (Agrawal et al., 2006), while a further study discovered that the GABRA2 variants were associated with symptoms of childhood conduct disorder in children, though not with alcohol dependence in this population (Dick et al., 2006b). More recently, an association of only a particular SNP (rs9291283) with both alcohol abuse and conduct disorder was found (Melroy et al., 2014). Such evidence might suggest that genetically-associated conduct disorder gives rise to drug, or alcohol misuse as part of the disorder.

While several studies have confirmed a link between GABRA2 variants and alcoholism, other studies have found that the less frequent haplotype is associated with alcohol dependence in Americans of Caucasian origin (Covault et al., 2004), Russians (Lappalainen et al., 2005), and Germans (Fehr et al., 2006). In contrast to the Agrawal report that limits the association to alcoholics with comorbid illicit drug dependence, others (Covault et al., 2004) found the relationship to be most prominent in individuals without comorbid illicit drug dependence. These apparent paradoxes may reflect different populations of alcoholics, those with the more frequent haplotype being associated with alcoholism characterised by high trait anxiety, while low-anxiety alcoholics may be more likely to possess the low-frequency haplotype (Enoch, 2008) (See also discussion below on Type I and Type II alcoholism); however, possession of trait anxiety by itself was not associated with GABRA2 variations (Enoch et al., 2006). In keeping with that interpretation, the COGA alcoholic population which suggested an association of the more frequent haplotype with alcoholism (Edenberg et al., 2004), was shown to possess a high trait anxiety compared to the non-alcoholic group.
Importantly, a post mortem study of brains of alcoholics found lower $\alpha_2$ subunit mRNA in the central amygdala nucleus of alcoholic patients (Cammarota et al., 1995), though, in this case it cannot be determined whether the lowered expression level resulted from long term alcohol abuse rather than being a predisposing influence.

Although a number of studies have failed to find an association between GABRA2 and alcoholism (Covault et al., 2008, Drgon et al., 2006, Matthews et al., 2007), given the heterogeneity of types of alcoholism, this may not be surprising. The association may be strongest in males, while females do not show such an association (Enoch, 2008). From this point of view, the classification of alcohol use disorders is of interest. Type I alcoholism is characterised by late onset and neurotic predisposition, and distinguished from Type II alcoholism with a relatively early onset, and presence of delinquent behaviour (Cloninger et al., 1988). Whereas Type I is not strongly heritable and found more often in females, Type II, almost exclusively seen in males, is strongly heritable. In keeping, a recent study (Strac et al., 2015), which examined associations between three GABRA2 SNPs (rs567926, rs279858 and rs9291283) and alcohol dependence, not only showed an association between rs279858 and alcohol dependence, but the A–C (rs567926 and rs279858) haplotype was more frequently seen in Cloninger Type II alcoholics (presenting with a combination of early onset alcohol abuse and aggression).

A recent meta-analysis found strong associations between GABRA2 and alcohol dependence for SNP rs567926 and rs279858), and between GABRG2 and dependence both on alcohol and heroin for rs211014. A significant association was also observed between GABRA6 rs3219151 and Alcohol Use Disorder (Li et al., 2014). Nevertheless, and perhaps surprisingly, GWAS studies have failed to support the widely replicated GABRA2 finding. In a GWAS of nearly 2,000 alcoholics and 2,000 controls, five GABRA2 SNPs had only a nominal association ($p<0.05$) with Alcohol Use Disorder, with odds ratios of 1.1 – 1.7 (Bierut et al., 2010), perhaps indicating that the direct contribution of GABRA2 genotypic variants to alcoholism genetics may be minor. However, given the heterogeneity of “Alcohol Use Disorder” as a phenotype, and the evidence that alcohol abuse is only one of a number of disorders of behavioural control associated with GABRA2 variants, perhaps it is unsurprising that GWAS finds only weak associations of GABRA2 with alcohol abuse.
2.1.1.2 Other chromosome 4 cluster GABA<sub>A</sub> receptor subunit association with alcohol abuse

There is only limited evidence from gene association studies that other GABA<sub>A</sub> receptor subunit genes located on chr 4p may be involved in addiction risk. The original (Edenberg et al., 2004) study reported a trend for association for two GABRG1 SNPs, while a haplotype block that extends from the intergenic block between GABRA2 and GABRG1 up to GABRG1 intron 3 was identified in two groups of Americans of Caucasian origin, but not those of African descent (Covault et al., 2008). Note that the polygenic markers in the GABRG1 gene are in linkage disequilibrium with markers on the GABRA2 gene, and have been found to contribute to risk of alcohol dependence in an additive manner, with the polymorphic markers in the GABRA2 gene providing the dominant effect (Covault et al., 2008). Nevertheless, other researchers reported that the GABRA2 and GABRG1 genes likely provide independent contributions to alcohol vulnerability (Enoch et al., 2009).

Some studies have reported a robust association between GABRB1 and alcohol dependence comorbid with other substance dependence and psychiatric illnesses (Yang et al., 2012, Kertes et al., 2011), although the strength of the association with alcohol dependence alone is less clear (Parsian and Zhang, 1999, Dick and Foroud, 2003, Song et al., 2003, Reck et al., 2005). Very recently, an association has been identified between the intergenic SNP rs2044081 in GABRB1 and alcohol dependence in a well characterized British/Irish population (Odds Ratio 4.2 [95% Confidence Intervals 1.5-11.5] P<sub>corrected</sub> 3.31x10<sup>2</sup>) (M.Y.Morgan et al (personal communication). Exploiting the IMAGEN database (Schumann et al., 2010), we explored in an adolescent population whether possession of the minor (T) variant of this SNP is associated with performance of tasks measuring aspects of impulsivity, and reward sensitivity that are implicated in drug and alcohol abuse. Allelic variation did not associate with altered performance in either a stop-signal task (SST), measuring one aspect of impulsivity, or a monetary incentive delay (MID) task assessing reward anticipation. However, increased BOLD response in the right hemisphere inferior frontal gyrus, left hemisphere caudate/insula, and left hemisphere inferior temporal gyrus during MID performance was higher in the minor (T) allele group. In contrast, during SST performance, the BOLD response found in the right hemisphere supramarginal gyrus, right hemisphere...
lingual and left hemisphere inferior parietal gyrus indicated reduced responses in the minor genotype. We suggest that β1-containing GABA<sub>A</sub> receptors may play a role in excitability of brain regions important in controlling reward-related behaviour, which may contribute to susceptibility to addictive behaviour (Duka et al., in press).

2.1.2 Chromosome 5 cluster (<i>α1, α6, β2</i> and <i>γ2</i>)

There is considerably less evidence that genes within the chr 5 cluster are associated with addictions. Initial analysis of the COGA database revealed no linkage between chromosome 5 genes and alcoholism (Dick et al., 2005, Song et al., 2003), though a subsequent analysis found an association of <i>GABRA1</i> with individual indications of risk for alcohol dependence; these included a history of blackouts, age at first drunkenness, and level of response to alcohol (Dick et al., 2006c). Other members of the cluster, <i>GABRA6</i> and <i>GABRB2</i>, have been associated with alcohol dependence in both a Finnish, and a Native American population (Radel et al., 2005), and with both alcohol dependence and Korsakoff’s psychosis in a Scottish study (Loh et al., 2000b), while (Loh et al., 2000a) reported an association of antisocial alcoholism with <i>GABRG2</i> in a Japanese population. On the other hand, another study (Sander et al., 1999) found no association of <i>GABRB2</i>, or <i>GABRG2</i> with alcohol dependence in a German population.

A low level of sedation following alcohol is thought to represent a risk factor for developing alcohol dependence, and a non-significant trend for an association between possession of the <i>GABRA6</i> Ser385 allele and a low sedative response to alcohol has been reported (Hu et al., 2005). The Ser385 allele is uncommon, and the study contained no homozygous cases, and only 18 individuals who were heterozygous for the Ser385 allele. Nevertheless, the six men with heterozygous (Pro385/Ser385) genotypes had the lowest sedative response to alcohol, and each had developed alcoholism during follow-up. However, in a previous study, the same allele was associated with lower levels of neuroticism and conspicuously, reduced anxiety, hostility and depression scores, but not with alcohol use (Sen et al., 2004). Curiously, the same substitution is associated with reduced sensitivity to diazepam in children of alcoholics (Iwata et al., 1999), though classic benzodiazepines do not bind to GABA<sub>A</sub> receptors incorporating the α6 subunit. Perhaps the substitution induces a sub-
threshold increase in arousal, a phenotype that could reduce independent influences on sedation.

2.1.3 Chromosome 15 cluster (α5, β3, γ3)

Studies using the COGA dataset have found evidence of haplotype and SNP association between alcohol dependence and GABRG3 (Dick et al., 2004). Interestingly, paternal, but not maternal, transmission of GABRA5 and GABRB3 alleles showed association with alcoholism (Song et al., 2003), consistent with this chromosomal location being subjected to genetic imprinting (Meguro et al., 1997).

The atypical benzodiazepine derivative, Ro15-4513, has been reported to have a relatively higher affinity for the α5 subunit-containing GABA_A receptors (α5-GABA_ARs) over those incorporating α1, α2, α3 subunits, both for in vitro and in vivo studies (Luddens et al., 1994, Wieland and Luddens, 1994, Momosaki et al., 2010), and this observation has been used to exploit [11C]Ro15-4513 binding in PET studies of human brain as a measure of the density of α5-GABA_ARs. Using this method, the expression of α5-GABA_ARs is reduced in the brains of alcoholic patients, particularly in limbic regions, including the nucleus accumbens (NAcc) (Lingford-Hughes et al., 2012). Note that Ro 15-4513 had been suggested previously to target high affinity α4βδ receptors (Hanchar et al., 2006), which have been implicated in alcohol reward (Rewal et al., 2009, Rewal et al., 2012). However, the demonstration that approximately 90% of high affinity Ro 15-4513 binding is lost in the brains of mice rendered insensitive to this atypical BZ ligand by a point mutation in the γ2 subunit (F77I), and, moreover, that Ro 15-4513 binding is prominent in regions devoid of δ subunit expression (Linden et al., 2011) question the relevance of δ-GABA_A receptors as a pharmacological target for Ro 15-4513 actions.

2.1.4 X chromosome (α3, ε, and θ).

We are unaware of any studies indicating associations of these genes with alcohol-related disorders in human populations.
2.1.5 \textit{GABA}_A receptor associations with other addictions

While by far the majority of studies on gene-addiction associations have concentrated on alcohol dependence, a limited number have investigated the association of \textit{GABA}_A receptor subunit genes and addiction to other abused drugs. Human genetic studies suggest \textit{GABA}_A receptors containing the $\alpha_2$ subunit may play a role in nicotine dependence (Agrawal et al., 2008b) and cannabis abuse (Agrawal et al., 2008a). We found an association of \textit{GABRA2} variants with cocaine addiction in a Brazilian population of Caucasian ancestry (Dixon et al., 2010). While our study characterised a different set of SNPs from those reported most frequently for the alcohol studies, one risk SNP from our study, rs279871, is in 100\% linkage disequilibrium with rs279858 widely reported by other groups, suggesting we are studying the same haplotype. This and other intronic SNPs in the vicinity may tag a region that regulates mRNA transcription and stability and thus may alter expression of the \textit{GABRA2} gene.

In a parallel study, African American male patients with lifetime DSM-IV single and comorbid diagnoses of alcohol, cocaine, and heroin dependence were characterised (Enoch et al., 2010). One haplotype predicted heroin addiction, whereas a second haplotype was more common in control subjects and seemed to confer resilience to addiction after exposure to severe childhood trauma. A further SNP, rs11503014, not located in any haplotype block and potentially implicated in exon splicing, was independently associated with heroin addiction; childhood trauma interacted with rs11503014 variation to influence addiction vulnerability, particularly to cocaine. Thus, in some populations, and for some drugs, \textit{GABRA2} variants may contribute to the development of addictions by increasing vulnerability to early-life stress, a factor known to predispose to adult addictive behaviour (Enoch, 2011, Messina et al., 2008, Cadet, 2016).

Subsequent studies have suggested associations of \textit{GABRB2} (Kim et al., 2015), \textit{GABRB3} (Chen et al., 2014), and \textit{GABRG2} (Loh et al., 2007) SNPs with opiate dependence in Han Chinese people. Very recently, further evidence for an association of \textit{GABRB2} and \textit{GABRB3} with opiate or cocaine dependence was reported (Levran et al., 2016), though this association did not survive stringent analysis. Reporter gene assays find the risk allele increases expression of \textit{GABRB3} and this may contribute to its pathogenesis (Chen et al., 2014). Finally, a gender selective association of the novel SNPs rs2279020 and rs4480617 in
the GABRA1 and GABRG2 with methamphetamine abuse have been reported (Lin et al. 2003).

2.2 Animal studies implicating GABA_A receptor subtypes in drug abuse

The human gene association studies linking GABA_A receptor subtypes to drug and alcohol abuse and dependence, are paralleled by functional studies in animals investigating these receptors in drug abuse. Drug (e.g. alcohol) consumption investigated both in animals selectively bred for high alcohol intake and genetically altered rodents, provides a direct measure that appears to model human consumption, but provides relatively little insight into the underlying mechanisms. However, because of the relative ease of performing such experiments they provide much of the data on the consequences of genetic manipulation for alcohol-related behaviour. More sophisticated measures include operant performance to obtain alcohol, as such studies can discriminate between motivational effects and consumption.

In the accompanying sections both types of studies will be reviewed with an emphasis on alcohol but extended to other drugs with abuse and dependence liability as appropriate.

2.2.1 Altered expression of GABAergic genes in animals selectively bred for high drug taking

Several rat lines have been bred for high alcohol consumption. These lines provide certain parallels to human high consumption, and allow direct measurements to be made of receptor expression in discrete brain areas, both in drug-naïve animals, and following drug exposure. However, surprisingly few studies have sought to identify changes in GABA_A receptor gene expression in various alcohol preferring lines. Studies from the Indiana group (McBride et al., 2012, McBride et al., 2013b) are notable in showing a paucity of common gene differences among 5 pairs of rat lines independently bred for high alcohol consumption, and their non-prefering counterparts, in brain areas commonly implicated in
addictive behaviour, e.g., the accumbens shell, central amygdala and ventral tegmental area. None of these differences involved genes encoding GABA_\text{A} receptor subunits. Such findings suggest that high alcohol consumption is under the control of many diverse genes, different combinations of which are selected during selective breeding for high alcohol consumption, and, from the point of view of the current review, such selective breeding does not depend importantly upon varying expression levels of GABA_\text{A} receptor subunit genes (at least in the brain areas studied).

Nevertheless, some studies using microarrays to investigate genome-wide differences in gene expression have reported altered GABA_\text{A} gene expression. Thus, the level of mRNA encoding the $\beta_1$ subunit of the GABA_\text{A} receptor was 1.6-fold higher in iP (Indiana alcohol preferring) than iNP (non-preferring) rats and the level of expression of mRNA for the $\alpha_1$ subunit was higher in the hippocampus of the iP than iNP rats (Homer et al., 2007). P rats also exhibited significantly higher levels of the $\alpha_1$ subunit protein relative to NP rats in the ventral pallidum (Halyburton et al., 2007). These differences in expression may not be primary; the iP rat has a lower GAD expression in the hippocampus, suggesting reduced GABA function, so that the iP rat may produce a compensatory upregulation of the GABA_\text{A} receptor.

$Gabrg2$, encoding the $\gamma_2$ subunit, together with $Gphn$ encoding the associated scaffolding protein, gephrin, required for postsynaptic clustering of GABA_\text{A} receptors, are both up-regulated in P rats (in contrast to their down regulation in human alcoholics and cocaine addicts). P rats also show a significant up-regulation in expression of $Gabrg2$ and $Gabrg3$ gene expression, together with a trend towards a down-regulation of $Gabrb3$ (Enoch et al., 2012). In an independent line of Warsaw alcohol-preferring and non-preferring rats, without exposure to alcohol, a down-regulation of $Gabra4$ was found in hippocampus (Stankiewicz et al., 2015).

2.2.2 Gene association studies: Drug self-administration
The availability of an extensive number of mouse recombinant inbred strains derived by inbreeding novel inbred strains from the F2 cross between C57BL/6J (B6) and DBA2/J (D2) mice (so-called BXD strains) has been frequently exploited to identify quantitative trait loci (QTLs) of genes associated with complex behavioural traits including responsiveness to several aspects of alcohol- and other drug-related behaviours (Crabbe et al., 1999, Phillips et al., 2002). The availability of related approaches in humans offers the hope of integrating mouse and human studies (Ehlers et al., 2010). While, for practical reasons, such analyses are usually based on simple measures of alcohol consumption, or easily-scored consequences of alcohol administration, or withdrawal, several QTLs associated with alcohol dependence have been identified in chromosomal regions that contain genes that encode GABA\(_A\) receptor subunits (see (Boehm et al., 2004) for a review). Thus, a QTL for alcohol withdrawal spans a region on mouse chromosome 11 that contain the genes encoding \(\alpha 1\), \(\alpha 6\), \(\beta 2\) and \(\gamma 2\) subunits (Buck et al., 1997, Bergeson et al., 2003), but nearby, or overlapping loci are also associated with alcohol tolerance, alcohol-conditioned taste aversion, alcohol-induced hypothermia, and alcohol-induced motor incoordination (Crabbe et al., 1994, Risinger and Cunningham, 1998, Browman and Crabbe, 2000, Kirstein et al., 2002, Phillips et al., 1996). Using the WebQTL (www.webqtl.org) facility (Wang et al., 2003, Chesler et al., 2003), Boehm et al. (2004) related these behavioural observations to levels of gene expression of the respective GABA\(_A\) receptor subunits in either cerebellum or forebrain; significant correlations were found for individual subunits, but these differed to an extent depending on the behaviour, and brain area studied. Similarly, associations with alcohol-related phenotypes were found for mouse chromosome 5 QTLs containing the genes encoding \(\alpha 2\), \(\alpha 6\), \(\beta 2\) and \(\gamma 1\) subunits, and in these cases significant correlations were found only for forebrain expression levels of the \(\alpha 2\) subunit: for chromosome 7 (\(\alpha 5\), \(\beta 3\) and \(\gamma 3\)) with significant correlation with expression of \(\beta 3\), and chromosome 4 (\(\delta\) and \(\rho 1\) and \(\rho 2\)). Nevertheless, it is not clear whether these associations inform on a functional involvement of these subunits in the effects of alcohol, or whether they reflect the involvement of these genes in the level of performance of the respective tasks used to assess alcohol’s effects, allowing alcohol (or any other agent) to exert its effects more readily when performance is already partly compromised by altered level of gene expression. On the other hand, that such studies have not suggested an involvement of chromosomal regions including genes...
for α5 or α6 may indicate a lack of involvement of these genes in alcohol-related behaviours, at least in rodents (Boehm et al., 2004). In this respect, a strikingly more robust signal for the α5 subunit in human vs. rodent NAcc (Lingford-Hughes et al., 2012, Lingford-Hughes et al., 2016) is intriguing and suggests that some receptor isoforms may play a distinct role in human addiction.

A potential weakness of using the BXD mouse lines is that for those chromosomal regions that are identical between the C57BL/6J and DBA2/J lines, there can be no genetic variation, so that BXD analyses will not provide information on the involvement of such genes in behavioural traits. This issue has been recognised, and strategies involving multiple founder lines have been developed (e.g. (Williams et al., 2004)). Using a set of recombinant inbred mouse strains derived from eight different founder lines, no direct evidence for altered GABA\(_A\) receptor subunit expression being associated with alcohol drinking was found (Saba et al., 2011). However, it was possible to identify particular SNP variations (Tabakoff et al., 2009) that interfered with the ability of β2 subunits to interact with AP-2 adaptin protein that is involved in receptor internalisation (Herring et al., 2003). These observations thus suggest that regulation of GABA\(_A\) receptor trafficking may play an important role in the control of alcohol consumption, despite there being little evidence for alcohol drinking relating to expression of GABA\(_A\) receptor subunits.

As far as we are aware, no studies have suggested a QTL for psychostimulant, opiate or cannabinoid effects that implicates GABA\(_A\) receptor subunits.

**2.2.3 Gene deletion and mutation experiments: Drug self-administration**

Complementary to gene-association studies, studies employing direct manipulation of gene expression have also been used extensively to explore the role of GABAergic genes in addictive behaviours. In the following paragraphs, we will examine the impact of specific GABA\(_A\)R genetic manipulations in mice on behaviours relevant to drug dependence with a focus on alcohol. Most of the literature has employed constitutive gene deletion, which may be confounded by compensatory plasticity of GABAergic signalling and of other genes. For
instance, the $\alpha 6$ knockout increases expression of a $K^+$ channel that increases tonic inhibition (as do $\alpha 6$-GABA$_A$Rs in cerebellar granule cells) (Brickley et al., 2001).

2.2.3.1 alpha1.

$\alpha 1$-subunit null mutant mice have been reported to show decreased alcohol and saccharin consumption, increased aversion to alcohol, and a marked stimulation of motor activity after injection of alcohol (Blednov et al., 2003b, Blednov et al., 2003a, Boehm et al., 2004). In keeping, mice lacking the $\alpha 1$ subunit showed marked reductions in both alcohol and sucrose-maintained operant responding, and home-cage alcohol drinking (June et al., 2007). In the June et al study, the null mutants also showed significant increases in locomotor behaviour (confirming Blednov et al’s (2003) findings) after injections of low–moderate alcohol doses (1.75–3.0 g/kg), an effect that could be attenuated by the dopamine (DA) D2R antagonist, eticlopride, and the D1 antagonist, SCH 23390, at doses that themselves did not affect activity. These data suggest that the $\alpha 1$-GABA$_A$R may play an important role in alcohol-motivated behaviour, but also in operant responding for a conventional reinforcer (sucrose). Deleting the $\alpha 1$ subunit also appeared to unmask alcohol's stimulatory effects, by a mechanism related to DA-dependent mechanisms. Notably, deletion of the $\alpha 1$-subunit has been reported to result in a substantial (approximately 38%) compensatory increase in GABA$_A$ receptor $\alpha 2$ and $\alpha 3$ subunit expression (Vicini et al., 2001) and immunoprecipitation (approximately 50%) (Sur et al., 2001), and it is therefore unclear whether the behavioural effects of the deletion may reflect changes in these receptors, rather than being a direct consequence of $\alpha 1$-subunit deletion. Nevertheless, in knock-in mice with Ser270 to histidine and Leu277 to alanine mutations in the $\alpha 1$ subunit (which may alter their response to alcohol, without affecting normal GABAergic transmission), alterations in specific alcohol-induced behavioural effects, but not in alcohol consumption, or place preference conditioning have been reported (Werner et al., 2006).

Experiments attempting to localise these effects of $\alpha 1$ deletion to specific brain areas, employed siRNA to suppress $\alpha 1$ gene expression in ventral pallidum of high-alcohol drinking
rats, and found a marked decrease in binge drinking; importantly, binge sucrose drinking, or water intake were unaffected (Yang et al., 2011).

A recent study examined cocaine self-administration, using Cre-expressing lentiviruses infused during early adolescence into the medial prefrontal cortex of floxed Gabra1 mice to delete α1-expressing genes in this area (Butkovich et al., 2015). Such mice were slower to learn a discriminated nose-poke response to obtain intravenous infusions of cocaine, though following training they achieved similar infusion rates to controls. Following extinction (in which the knockdown resembled controls) the mice were tested for cue-induced reinstatement of responding, a measure which resembles instrumental responding for a conditioned reinforcer (Stephens et al., 2010), but the groups did not differ in this respect.

Butkovich et al., (2015) then asked whether the slower rate of acquisition of cocaine self-administration following Gabra1 knockdown might reflect impaired ability to learn the association between the instrumental response and the cocaine reinforcer. Using a task in which mice were initially trained to perform two different responses to obtain food reinforcers, one of the responses was dissociated from food delivery (which was made random, and thus independent of the instrumental response). While control mice now biased their responding to the contingent response that continued to deliver food, the Gabra1 knockdown mice persisted in performing both responses, suggesting an impairment in action-outcome learning. However, when similar manipulations were carried out in adulthood, the Gabra1 knockdown mice did not differ from controls in distributing their responses to the reinforced response. Thus, a healthy medial prefrontal cortex (PFC) α1-GABA_A receptor tone may be necessary in developing goal-directed decision-making strategies, but not in performing them. A parallel report from the same group (Swanson et al., 2015) suggests that orbitofrontal PFC GABA_A α1 silencing similarly impairs the performance of goal-directed response strategies. Mice with orbitofrontal PFC-targeted GABA_A α1 knockdown were unable to select actions based on their consequences, developing instead habit-like behavioural inflexibility. Thus cortical α1 GABA_A receptors
appear to be important in instrumental learning and test flexibility, though it is unclear whether these aspects may be general to all reinforcers, and not only to drugs of abuse.

2.2.3.2 alpha2

Given the evidence implicating the GABRA2 gene, encoding the α2 subunit, in human addictions, a number of studies have investigated either consumption, or operant self-administration of abused drugs in animals with deletions of this receptor. We found no evidence that α2 knockout mice differed from wild type (WT) mice when lever-pressing to receive increasing concentrations of alcohol on an FR4 schedule of reinforcement (four lever presses required to obtain one reward); both WT and knockout mice self-administered alcohol at similar rates, with no differences in the numbers of reinforcers earned (Dixon et al., 2012). This observation suggests that alcohol reinforcement does not require α2-containing GABA_A receptors. However, the ataxic and sedative effects of alcohol were markedly enhanced in the knockout mice, indicating that α2-GABA_A receptors may play a part in the ability of alcohol to induce ataxia. Curiously, another report (Boehm et al., 2004) indicates that α2-deletion may protect against alcohol-induced ataxia. The reasons for this apparent discrepancy are not understood, but it may be important that although both sets of mice had been derived from the identical founder mutants, those in the Boehm et al study had been backcrossed to C57BL/6J, while those in our study (Dixon et al., 2012) were maintained on a mixed C57BL/6J x Sv129/ev background.

Self-administration of cocaine was also unaltered in α2 KO mice (Dixon et al., 2014); in an extensive dose-response study, WT, KO and heterozygous mice did not differ in their ability to acquire operant intravenous self-administration of cocaine, or in the ability of a priming dose of cocaine, or cocaine-associated stimuli to reinstate self-administration after a period of extinction. These observations suggest strongly that α2- GABA_A receptors are not necessary for the reinforcing properties of either cocaine or alcohol.

On the other hand, Blednov and colleagues (Blednov et al., 2011) did find effects of mutating α2 subunits (serine 270 to histidine and leucine 277 to alanine mutations) that
made them insensitive to potentiation by alcohol yet retained normal GABA sensitivity. These mutant mice showed a complete loss of the motor stimulant effects of alcohol, and also demonstrated complex changes in alcohol intake and preference the direction of which depended upon the exact nature of the test. These studies demonstrate that the effects of alcohol at GABAergic synapses containing the α2 subunit may be important for specific behavioural effects of alcohol putatively relevant to alcoholism.

2.2.3.3 alpha5

Two other studies have investigated the role of GABA$_A$ receptor subunits in alcohol self-administration. α5 knockout mice did not differ from WTs in operant responding for 10% alcohol/10% sucrose, but responded less for 10% sucrose (Stephens et al., 2005). The benzodiazepine site inverse agonist, Ro 15-4513, has high affinity for GABA$_A$ receptors containing the α5 subunit (see Section 2.1.3). This drug dose-dependently reduced lever pressing for alcohol/sucrose in WT mice, but had less effect in α5 knockout mice; lever pressing for sucrose was unaffected (Stephens et al., 2005). These data suggest that α5-GABA$_A$ receptors are not essential for alcohol reward, but the reduction of operant responding for alcohol by Ro 15-4513 may be mediated by α5-GABA$_A$ receptors. In measures of alcohol consumption, α5 knockout mice did not differ from WT at low alcohol concentrations (2-8%), but consumed less alcohol at higher concentrations; these differences were probably not attributable to increased behavioural disruption of the knockout by alcohol, since no differences were seen in sensitivity to alcohol's sedative or ataxic effects. However, the ability of Ro 15-4513 to reduce alcohol consumption was unaffected, suggesting that this effect is not mediated by the α5-GABA$_A$ receptors. In the same study, the ability of a novel α5-efficacy-selective benzodiazepine receptor ligand, alpha5IA-II, that possesses greater inverse agonist activity at α5- than at α1-, α2- or α3-containing GABA$_A$ receptors, to influence operant responding was studied. Alpha5IA-II (0.03-3 mg/kg) dose-dependently decreased lever pressing for 10% alcohol, the minimally effective dose of 1 mg/kg, corresponding to over 90% receptor occupancy, but did not affect lever pressing for 4% sucrose. Although inverse agonists acting at α5-GABA$_A$ receptors reduced alcohol self-administration, the evidence from this study indicates that it is unlikely
that alcohol reinforcement in mice requires activation of $\alpha_5$-GABA$_A$ receptors (Stephens et al., 2005).

A similar conclusion regarding the potential efficacy of inverse agonists in reducing alcohol consumption is provided by (Ruedi-Bettschen et al., 2013). Using rhesus monkeys, this group trained animals to self-administer 6% alcohol sufficient to reach pharmacologically relevant blood alcohol levels ranging from 90 to 160 mg/dl, and which produced changes in behaviour typical of alcohol intoxication. Concentrations of 0.3 to 3% sucrose also reliably maintained self-administration. The $\alpha_5$-GABA$_A$ receptor agonist QH-ii-066 enhanced, and the $\alpha_5$-GABA$_A$ receptor inverse agonist L-655,708 inhibited, alcohol-, but not sucrose-drinking. The changes in alcohol drinking could be reversed with the $\alpha_5$-GABA$_A$ receptor antagonist XLi-093.

### 2.2.3.4 $\alpha_4$ and $\delta$ subunit containing receptors.

A number of studies have used the atypical benzodiazepine, Ro 15-4513, to investigate the function of $\alpha_4$-GABA$_A$Rs in alcohol related behaviours. This drug was initially characterised as an ethanol antagonist, and was thought to achieve this effect via an action at $\alpha_4/6\delta$-GABA$_A$Rs, putatively involved in the actions of ethanol (Hanchar et al., 2006, Wallner et al., 2006); but see (Korpi et al., 2007) for detailed discussion). Some evidence in keeping with this interpretation is provided by data indicating that the ability of Ro 15-4513 to antagonise ethanol-induced ataxia is absent in $\alpha_4$ knockout mice (Iyer et al, 2011), though, curiously, the effect was seen only in male mice. However, approximately 90% of high affinity Ro 15-4513 binding is lost in the brains of mice rendered insensitive to this atypical BZ ligand by a point mutation in the $\gamma_2$ subunit (F77I), and, moreover, Ro 15-4513 binding is prominent in regions devoid of $\delta$ subunit expression (Linden et al., 2011). Thus, the data of Iver et al (2011) may not reflect the involvement of $\alpha_4\beta\delta$ receptors, but instead may possibly involve adenosinergic transmission (Meng and Dar, 1994).

More persuasively, alcohol intake was lowered in mice carrying a constitutive knockout of the $\delta$-subunit, and these animals also showed attenuated withdrawal from chronic alcohol
exposure (Mihalek et al., 2001). It is of interest that these effects could be dissociated from
a normal anxiolytic response to alcohol and a normal hypothermic response to alcohol, and
the knockout mice developed both chronic and acute tolerance.

As mentioned already, constitutive knockout mice provide a blunt instrument for analysing
the contribution of genes to behaviour, as the gene is absent during development
(potentially allowing compensatory changes in expression of other genes), as well as being
effective in all parts of the brain. More recently, techniques have been developed that allow
gene manipulation in adulthood, as well as limiting the manipulation to discrete brain areas,
or to specific neuronal classes. Nie and colleagues used the technique of virally-mediated
RNA interference (RNAi) to reduce expression of the GABA<sub>δ</sub> receptor δ-subunit in adult rats
in localized regions of the NAcc to test the hypothesis that δ-subunit-containing GABA<sub>δ</sub>
receptors in the NAcc are necessary for oral alcohol consumption. They found that
knockdown of the δ-subunit in the medial shell region of the NAcc, but not in the ventral or
the lateral shell, or in the core, reduced alcohol intake. In contrast, δ-subunit knockdown in
the medial shell did not affect intake of a 2% sucrose solution, suggesting that the effects
of GABA<sub>δ</sub> receptor δ-subunit reduction are specific to alcohol (Nie et al., 2011). In a variety
of neurons (e.g. thalamocortical neurons, hippocampal dentate gyrus granule cells) the δ
subunit associates with the α<sub>4</sub> subunit and a β subunit to form extrasynaptic receptors
(Herd et al., 2013, Peden et al., 2008). Indeed, in mouse NAcc medium spiny neurons
(MSNs), using both immunohistochemistry and electrophysiology, we demonstrated the
presence of extrasynaptic α<sub>4</sub>βδ receptors, that are activated by ambient concentrations of
GABA to mediate a tonic inhibitory current that reduces neuronal excitability (Maguire et
al., 2014).

In keeping with this association of alcohol drinking with α4-GABA<sub>δ</sub> receptors, Rewal et al
(2012) further showed that virally-mediated RNAi that transiently reduced expression of the
α4 GABA<sub>δ</sub> subunit, also significantly reduced responding for alcohol after α4 reductions in
the NAcc shell, but not in NAcc core. This reduction was specific to alcohol, as responding
for sucrose was again not altered. Interestingly, responding during reinforced sessions was
not altered during the initial 5 minutes of the session, but decreased after 5 minutes,
following multiple reinforced responses (Rewal et al., 2012), suggesting that the manipulation did not alter initial motivation for alcohol.

The drug gaboxadol, when used at appropriately low concentrations, acts as a selective agonist of extrasynaptic GABA$_A$ receptors containing the $\delta$ subunit (Belelli et al., 2005). In apparent disagreement with the observations made on the $\alpha_4$ and $\delta$ subunit gene interference studies (Ramaker et al., 2012) found in C57BL/6J mice that gaboxadol dose-dependently decreased alcohol intake in both two-bottle choice, and limited access paradigms, altering both the consumatory and appetitive processes of operant self-administration as well as shifting the drinking patterns in both procedures. In a subsequent study, the same group (Ramaker et al., 2015) found that gaboxadol infused into NAcc shell, decreased consumption of 10% alcohol in a limited access test. Thus, inactivating extrasynaptic tonically-active receptors using viral knockdown reduces alcohol consumption (Nie et al., 2011, Rewal et al., 2012), while facilitating their function with gaboxadol also decreases alcohol intake (Ramaker et al., 2012, Ramaker et al., 2015). Ramaker and colleagues suggest two potential explanations of these apparently incompatible data sets; firstly, gaboxadol at high doses is not specific for $\alpha_4\beta_\delta$ receptors, and it is difficult to estimate local concentrations in experiments using intra-striatal infusions; secondly, dose-response functions for alcohol consumption frequently exhibit an inverted-U shape, so that shifts either to the left or right of the optimal may result in decreased consumption. A further possible account is that in the case of gaboxadol infusions, receptor desensitisation may occur, which would not be the case for the knockdown. We are not aware of data that could throw light on these hypotheses. In contrast to the NAcc injections, administration of gaboxadol into infralimbic cortex was reported to increase alcohol consumption (Fritz and Boehm, 2014).

While this series of studies suggests strongly that extrasynaptic tonically active $\alpha_4\beta_\delta$ GABA$_A$ receptors play a role in regulation of alcohol consumption, it is not clear whether they facilitate or reduce it. We recently investigated the potential role of tonically active GABAergic receptors in mediating alcohol intake, using mice with an N-ethyl-N-nitrosourea-induced point mutation of the $\beta_1$ subunit. This mutation, a leucine-to-arginine exchange
(L285R) in the highly-conserved third transmembrane domain (TM3), near the TM2-TM3 linker, an important area for GABA receptor activation and ion channel gating, resulted in spontaneous GABA<sub>A</sub> receptor ion channel opening (i.e. in the absence of GABA) and increased GABA sensitivity of recombinant GABA<sub>A</sub>Rs, coupled to increased tonic currents in the NAcc (i.e., resembling tonically active receptors). A similar electrophysiological pattern was seen in a spontaneous mutant, Gabrb1<sup>+/P228H</sup>. Both mutants showed an increased consumption of alcohol (but not of sucrose, saccharine, or quinine) in a two-bottle choice experiment. Furthermore, the L285R mutant gave rise to persistent operant responding to obtain alcohol, even to intoxication; in contrast, WT mice reduced their rate of lever pressing, and consumption, as the session progressed (Anstee et al., 2013). The pattern of operant responding suggested to us that the mutation led to a failure to satiate to alcohol, which might correspond to the finding (Rewal et al., 2012) that virally-mediated reduction of α4-containing GABA<sub>A</sub> receptors showed no changes in initial responding for alcohol, but a more rapid decrease in responding following multiple reinforcers (i.e., suggestive of facilitated satiety). Thus, tonically active GABA<sub>A</sub> receptors may play a role in mechanisms underlying satiety to alcohol. In this context, it is of interest that intra-accumbens administration of the GABA<sub>A</sub> agonist, muscimol, reduced operant responding for ethanol reinforcement by terminating responding after a short period of time, an effect that was antagonised by co-administration of the GABA<sub>A</sub> antagonist bicuculline (Hodge et al., 1995). However, we are unaware of evidence suggesting a selective action of muscimol on tonic GABAergic activity.

Thus, in summary, while manipulations of particular subunits of GABA<sub>A</sub>Rs give rise to behavioural alterations, most consistent effects on motivation to obtain alcohol seem to be exerted by modifications of tonic conductance, including by manipulation of α4βδ receptors.
2.3. Influence of abused drugs on GABA<sub>A</sub> receptor subunit expression

In principle, genetically influenced predisposition to drug taking may result from differences in the ability to adapt to drug intake. Thus, for instance, the development of tolerance to drugs increases the likelihood of increasing voluntary drug intake, which may then result in dependence. A factor analysis (Enoch et al., 2013) revealed that distinct groups of genes, notably those involved in GABA synthesis and synaptic transport and members of the chr 4 cluster, previously associated with alcohol and drug dependence in humans, are themselves sensitive to the effects of alcohol/cocaine. In contrast, the two factors on which the chr 15, chr X and most of the chr 5 subunit genes loaded were impervious to alcohol/cocaine effects. However, as this study was limited to mRNA expression, the findings may not necessarily be extrapolated to protein levels.

2.3.1 Chronic drug exposure: alcohol, cocaine and methamphetamine

Many studies have incorporated measures of mRNA or subunit peptide levels following chronic alcohol treatment (see review by (Kumar et al., 2009)). In general, the occurrence, time course and direction of changes in subunit expression varies across brain regions ((Grobin et al., 2000). For instance, decreases in rat α1 and α4 subunit expression assayed by immunoblotting after two weeks of chronic exposure to alcohol have been reported (Papadeas et al., 2001). However, these changes were region specific; in amygdala, expression of both α1 and α4 subunits was decreased by about 20%, while in the accumbens, although there was a decrease of 28% in α4 subunit expression, there was no change in α1 subunit expression; in the VTA, no changes in either α1 or α4 subunit expression were found. Similarly, an impressive study with cynomolgus macaques (Macaca fascicularis), quantified GABA<sub>A</sub> α subunit mRNAs in basolateral amygdala following 18-month-long exposure to alcohol (Floyd et al., 2004). The α2 and α3 subunit mRNA levels were significantly decreased, whereas decreases in the α1 subunit expression only approached statistical significance, with no changes in α4 subunit mRNA levels. As highlighted by the authors, these adaptations in basolateral amygdala GABA<sub>A</sub> receptors are...
similar, but not identical, to those described in rodents after a brief forced alcohol exposure (Papadeas et al., 2001). In agreement with the macaque data, down-regulation of α2 mRNA has also been found post mortem in alcoholic patients (Jin et al., 2014); of 19 GABA<sub>A</sub> receptor subunits investigated in the central nucleus of the amygdala, only the mRNA encoding the α2 subunit was significantly down-regulated in alcoholic patients compared with control subjects, though in the human cohort, it is not possible to deduce whether the lowered expression was present prior to onset of alcohol abuse.

A number of additional studies also document how changes in the expression of a variety of GABA<sub>A</sub> receptor subunits are very much dependent on the brain region investigated (e.g. (Hemby et al., 2006)). Moreover, similar, albeit not identical, scenarios of varied altered mRNA expression pattern have been documented following chronic exposure to cocaine (Suzuki et al., 2000). Of specific relevance and in keeping with a role for α2-containing receptors in addictive behaviour, chronic treatment of rodents with cocaine results in decreased expression of α2 subunits in the NAcc (Chen et al., 2007). In contrast, chronic cocaine treatment in mice resulted in an up-regulation of α4 mRNA specifically in D1-MSNs (Heiman et al., 2008) which may represent a compensatory brake that limits the effect of an excessive DA tone associated with prolonged exposure to the psychostimulant (see also section 3.2.3).

Considerable evidence indicates that neurobiological responses to experimenter-administered cocaine differ from such responses when the cocaine is self-administered by animals (Wolf and Ferrario, 2010), and a recent report (Wydra et al., 2013) indicates that GABAergic transmission in the NAcc shell is differentially affected by self-administered and experimenter-administered cocaine. The patterns of change in receptor function following cocaine changes over time, in keeping with behavioural changes indicating initial intensification of motivation to obtain cocaine (incubation) lasting for several weeks, before becoming stable, and then declining. These behavioural changes are paralleled by changes in the strength of AMPA receptor-mediated synaptic transmission in NAcc mediated by altered calcium permeability (Conrad et al., 2008, Mameli et al., 2009, Loweth et al., 2014). However, the alterations in excitatory transmission do not seem to be paralleled by altered
GABA<sub>A</sub> receptor function (Purgianto et al., 2016); although surface expression of α2-GABA<sub>A</sub> receptor subunits was initially increased (on the second day of cocaine withdrawal), suggestive of increased inhibitory tone, this change was transient and no longer detected on withdrawal day 25 or 48. No changes in surface expression of α1 or α4 subunits were observed at any time. The authors suggest that upregulation of α2-subunit expression may be a protective mechanism against excessive cocaine taking (Purgianto et al., 2016). This proposal is in keeping with Dixon et al’s (2014) observation that while WT mice reduce drug intake over the first few days of self-administration, α2 knockout mice do not.

2.3.2 Intermittent drug exposure: alcohol

Alcohol-dependent individuals often consume alcohol in bouts of excessive drinking, which combine high levels of alcohol during such binge episodes and experience of withdrawal, a pattern which has been shown to be particularly deleterious, resulting in marked changes in brain structure and behavioural competence (Stephens and Duka, 2008, O’Daly et al., 2012, Duka et al., 2003). Exposure of rodents to chronic intermittent alcohol (CIE) replicates some of the features of this pattern of drinking. Using this model, a series of reports combining electrophysiological and immunoblotting approaches have revealed a consistent and coordinated decrease in α1 (synaptic) and δ subunit (extrasynaptic) expression and increases in γ2 and α4 subunits (both synaptic) in hippocampus, accumbens and basolateral amygdala of CIE rats (Cagetti et al., 2003, Olsen and Spigelman, 2012, Lindemeyer et al., 2014, Liang et al., 2014). Interestingly, a similar subunit set, namely α4βδ GABA<sub>A</sub> receptors appeared to be affected by chronic exposure to methamphetamine in rodents, i.e. increased expression (Shen et al 2013). Note, however, that the functional consequences of this alteration, may reverse upon subsequent withdrawal because the polarity of the Cl<sup>-</sup> mediated response switch from inwardly to outwardly directed as a result of alterations in Cl<sup>-</sup> ions transporter expression (see also section 2.3.4 on Functional alterations).

2.3.3 The influence of age (development) on the effects of alcohol

It is widely held that adolescent mammals are particularly sensitive to alcohol exposure, and that this sensitivity is especially severe when exposure is intermittent, such as occurs in
humans with binge drinking (Stephens and Duka, 2008, Smith et al., 2015). Recently, the Indiana group have reported a series of observations on altered gene expression (mRNA) in alcohol-preferring P rats following three weeks consumption of alcohol in a “binge” pattern, reaching blood levels of 100 mg/dL, over a period spanning postnatal days 28 – 49 (McClintick et al., 2015, McBride et al., 2014, McBride et al., 2013a). Striking from this set of data is the diversity of responses in different tissues. Thus, these three studies found no changes in any GABA<sub>A</sub> receptor subunits in accumbens shell, and an up-regulation of only one subunit (β1) in central amygdaloid nucleus. In contrast, rats subjected to adolescent intermittent alcohol (AIE) exposure between postnatal day (PD) 30 and PD46 showed a significant reduction in the δ subunit protein levels in the absence of any changes in mRNA, at both 48 hours and 26 days after the last alcohol exposure (Centanni et al., 2014). The α4 subunit protein levels were significantly reduced, but mRNA levels were increased, 26 days (but not 48 hours) after the last AIE exposure. The α5 subunit protein levels were not changed by AIE, and although α5 subunit mRNA levels were reduced at 48 hours they were normalized 26 days after AIE. In contrast to the effects of AIE, chronic intermittent alcohol (CIE) exposure during adulthood had no effect on expression of any of the GABA<sub>A</sub> receptor subunits examined.

This limited selection of exemplar studies illustrates the complexity of the altered landscape of GABA<sub>A</sub> receptor isoforms within the brain of animals exposed chronically and/or intermittently to drugs of abuse. A common feature across studies is the sensitivity of α1, α4, δ and, to an increasingly documented degree, α2 and γ2 subunits to dynamic changes upon drug exposure.

These observations are intriguing for two reasons. Firstly, the same subunit clusters appear affected, albeit not necessarily in the same direction, by the experience of epileptic events typically recorded in animal models of temporal lobe epilepsy (Scharfman and Brooks-Kayal, 2014, Grabenstatter et al., 2014a). Thus, for example, an increase and decrease in α4- and α1-containing GABA<sub>A</sub>Rs has been consistently reported in the hippocampus dentate gyrus of animal models of temporal lobe epilepsy, appearing specifically prominent in cohorts of animals exhibiting a high frequency of seizures (Gonzalez et al., 2015). Intriguingly, such molecular changes are associated with a reduction in phasic (i.e. synaptic) inhibition, which
has been putatively assigned to an increased contribution by $\alpha_4\beta_2\gamma_2$ over $\alpha_1\beta_2\gamma_2$-containing receptors (Scharfman and Brooks-Kayal, 2014, Gonzalez et al., 2015). Such changes are reminiscent of those described above for animals exposed to chronic intermittent ethanol. Whether this putative commonality of plasticity is independent of the documented relationship between animals withdrawn from alcohol and their sensitivity to epileptic seizures (Finn et al., 1995, Stephens et al., 2001, Becker and Hale, 1993) is not known. However, it raises the prospect that molecular mechanisms akin to those implicated in the neuronal GABA$_A$R-mediated adaptations described for animal models of temporal lobe epilepsy (e.g. BDNF regulation of $\alpha_1$ and $\alpha_4$ subunit expression via inducible cAMP response element repressor (ICER) and early growth response factor (Egr3) pathways respectively, reviewed in (Scharfman and Brooks-Kayal, 2014) may also be relevant to drug addiction and could be targeted to therapeutic advantage (see also paragraph 2.3.4 Alteration in GABA$_A$R function below and Conclusion Section 5). In this regard, the observation that selective early inhibition of STAT3, which mediates BDNF effect upon ICER down-regulation of $\alpha_1$ expression (Grabenstatter et al., 2014b), or increased $\alpha_1$-subunit expression in hippocampus dentate gyrus via a viral-mediated delivery approach (Raol et al., 2006) reduces long-term seizure frequency or the number of animals experiencing spontaneous seizures respectively, in the pilocarpine temporal lobe epilepsy model is intriguing. Secondly, $\alpha_4\beta_6$ GABA$_A$ receptors are known to mediate a sustained form of inhibition known as tonic inhibition, and increased tonic inhibition in the NAcc associates with increased alcohol consumption in two mouse models of increased spontaneous GABA$_A$ receptor channel activity (Anstee et al., 2013, Maguire et al., 2014, Rewal et al., 2012) - see Section 2.2.3.4). Thus, selective manipulation of this specific GABA$_A$ receptor tone may be exploited to therapeutic advantage. In this regard the drug DS2, which is a selective PAM of $\delta$-GABA$_A$ receptors may be of interest (Wafford et al., 2009, Jensen et al., 2013), though its limited solubility raises issues for in vivo experiments.

2.3.4 Alterations in GABA$_A$R function.

In addition to changes in the expression of specific receptor subtypes, a distinct alteration in GABA$_A$R function associated with drug exposure and involving a change in the polarity of the Cl$^-$ flux has been described for GABA$_A$ receptors in the interneurons, but not DA neurons, of
the VTA. Following an initial report documenting the switch of the GABAergic signal from inhibitory to excitatory in the VTA of opioid-dependent rats (Vargas-Perez et al., 2009), Taylor and colleagues recently revealed a potentially important role played by opioid-induced glial release of BDNF, that results in a decreased expression of the K⁺Cl⁻ co-transporter KCC2, thus causing Cl⁻ ion net movement to become outwardly directed upon GABAₐ receptor activation, thereby giving rise to neuronal depolarisation (Taylor et al., 2016). Importantly, the GABAergic signal is not affected in DA neurons, where an inwardly directed Cl⁻ gradient appears to be maintained independent of KCC2. Intriguingly, this type of alteration in GABAₐ receptor function is not unique to drug addiction as a similar switch in the polarity of the GABAₐ receptor-mediated signals has been described for epilepsy and neuropathic pain models (Pathak et al., 2007, Zhou et al., 2012, Barmashenko et al., 2011, Kahle et al., 2016). It is therefore conceivable that similar molecular mechanisms may operate to trigger abnormal GABAₐ receptor function, most notably, phosphorylation of the transporter (Lee et al., 2010, Kaila et al., 2014), and thus offer an opportunity for a selective therapeutic targeting (Friedel et al., 2015, Kahle et al., 2016).

3. GABAₐ receptors in the neurocircuitry of addiction

A detailed discussion of the neuronal substrates implicated in drug abuse and dependence is beyond the scope of this review and readers are referred to comprehensive reviews on this topic (Silberberg and Bolam, 2015, Everitt and Robbins, 2013, Koob and Volkow, 2010). However, an overview of the neurocircuitry implicated in addictive behaviours will be briefly described to highlight specific areas of relevance to GABAₐ receptor function and pharmacological manipulation.

Addictive drugs share the ability to influence the function of the mesocorticolimbic system implicated in mediating control over motivational aspects of complex behaviours. Within this neuronal circuitry (Fig 3), a central role is played by the NAcc, which receives information about the spatial and temporal availability and value of specific rewards, conveyed in the form of, primarily, glutamatergic inputs originating in specific structures, including the hippocampus, amygdala and cortical areas. This information is integrated in the NAcc with other signals mediated by DA released from neurons projecting from the
midbrain ventral VTA. These DA neurons have been implicated as increasing activity in response to unexpected events with positive valence (rewards), or decreasing activity in response to unexpected events with negative valence (aversive events), and rapidly adapting to provide the corresponding activity to environmental cues predicting such events rather than the positive or negative events themselves (Schultz, 2016). The NAcc encodes an output adjusted to the inputs received, via two classical pathways, the so-called direct striato-nigral and the indirect striato-pallidal routes. Within this complex neuronal assembly, GABA plays a fundamental role as the principal neurotransmitter utilised by approximately 97% of NAcc neurons (both the principal neurons, MSNs, and interneurons), and a variety of interneurons located in the interconnected input and output regions, most notably the VTA, dorsal striatum and substantia nigra (Walsh and Han, 2014, Morello and Partanen, 2015). Accordingly, GABAₐ receptors are expressed at significant locations within these pathways (Fig 3), offering a point of regulation for neuronal function and, thereby, specific behavioural outputs. Nevertheless, until recently, this fundamental role of GABA in motivational pathways had received little attention. In common with other brain circuits, distinct GABAₐ receptor isoforms are expressed within specific neuronal populations and cellular domains, suggesting the possibility of a spatially controlled regulation of GABAₐ receptor function in the motivational neurocircuitry. Amongst the isoforms of particular relevance within the context of drug addiction (see Section 2) are the functionally detected expression of 1) α2βγ2 isoforms within synaptic and extrasynaptic cellular domains of NAcc MSNs and 2) the α4βδ subtype with its exclusively extrasynaptic localization not only in MSNs, but also in specific populations of interneurons in the NAcc (Maguire et al., 2014). α1βγ2 isoforms in NAcc and dorsal striatum MSNs, and in VTA play a separate important role. Amongst additional receptor isoforms inferred by in situ and/or immunohistochemical studies, the α5βγ2 and α2βγ1 subtypes are prominent in the NAcc and amygdala (Kalk et al., 2012, Pirker et al., 2000, Lingford-Hughes et al., 2012, Wisden et al., 1992). It should be noted that α2βγ2 and α1βγ2 isoforms are additionally expressed within neuronal populations of cortical, hippocampal and amygdala areas where they are likely to influence projections from these areas into striatum. A schematic, albeit not exhaustive, representation of the GABAₐ receptor isoforms within circuits of importance in addiction is shown in Fig 3.
3.1 GABA<sub>A</sub> Receptors and Drug Reward

3.1.1 Benzodiazepine self-administration

Many studies of GABA<sub>A</sub> receptor subtypes and reward have been motivated by the need to evaluate abuse potential of novel compounds in development for therapeutic use in humans. In particular, drugs acting at the benzodiazepine binding site of GABA<sub>A</sub> receptors have a reputation for abuse, which potentially may be attributable to an action of such substances at particular subtypes of GABA<sub>A</sub> receptor. The group of James Rowlett has made a particular contribution to this area.

The mechanisms underlying the abuse-related effects of benzodiazepines are not well understood, and may represent a quite different type of drug abuse from psychostimulants, opiates, or alcohol. However, several studies suggest α1 subunit-containing GABA<sub>A</sub> receptors to have a critical role.

The availability of novel compounds that vary with respect to intrinsic efficacy at α2, α3, and α5 subunit-containing GABA<sub>A</sub> receptors but lack efficacy at α1 subunit-containing GABA<sub>A</sub> receptors ('α1-sparing compounds') has allowed investigations of their differential ability to maintain intravenous self-administration (Shinday et al., 2013, Rowlett et al., 2005). These compounds included zolpidem, which shows preferential binding to α1-GABA<sub>A</sub>Rs; L-838,417 (functional selectivity for α2, α3 and α5--GABA<sub>A</sub>Rs), and nonselective conventional benzodiazepines. In a complex series of findings, the group concluded that stimulation of α1-GABA<sub>A</sub>Rs is sufficient, but not necessary, for mediation of the abuse potential of these drugs. In a second experiment, two groups of monkeys were used, one previously trained to self-administer midazolam and the other cocaine. The α1-sparing compounds were self-administered significantly above vehicle levels in monkeys trained under a midazolam baseline, but not under a cocaine baseline over the dose ranges tested. Importantly, TP003 (a compound with some functional selectivity for α3-containing receptors) had significant...
reinforcing effects, albeit at lower levels of self-administration than non-selective benzodiazepine receptor agonists. The authors suggest that α1 subunit-containing GABA_A receptors may have a role in the reinforcing effects of benzodiazepine-type compounds in monkeys with a history of stimulant self-administration, whereas α3 subunit-containing GABA_A receptors may be important mediators of the reinforcing effects of benzodiazepine-type compounds in animals with a history of sedative-anxiolytic/benzodiazepine self-administration. While these studies suggest that different GABA_A receptors (and hence neural systems) may be involved in psychostimulant and benzodiazepine reinforcement, it is worth noting that TP003 may also possess significant activity at α1-GABA_ARs (Peden et al., 2008).

3.2 GABA_A Receptors and processes associated with reward-seeking

Although animal self-administration studies possess considerable “face validity” for human drug abuse, they fail to consider many aspects of behaviour and brain function that may contribute to drug abuse and addictive behaviour in humans. Indeed, although thus far we have treated addiction more or less as a unitary concept, it is clear that many different psychological constructs, and their underlying brain mechanisms contribute to drug abuse, and to drug dependence. Furthermore, addictions to different drugs may involve rather different brain processes, while, as it has already been hinted, addiction to a single drug like alcohol may come about in different ways. Clearly, then, genetic makeup may contribute in many different ways to the development of addiction. A more profitable approach may thus be to consider genetic contributions to different aspects of addiction-related behaviour, only some of which may contribute to any single individual’s addiction.

A very general model of drug abuse mechanisms holds that we need to consider two broad classes of psychological function, one of which concerns motivation to obtain drug (resulting from the intrinsic “rewarding” nature of the drug, memory of past experience with the drug, and current motivational state (e.g. deprived vs. satiated)) while the output of this system is subject to control by higher order functions (low impulsivity, restraint, etc. (see Fig 4 and (Volkow et al., 2004)). Clearly even within such an oversimplified system, gene variations might contribute in a very large number of ways to the behavioural output.
In the next section we will consider different aspects of the overall system, and the extent
to which current knowledge allows us to impute functional roles for GABA_A genes in
particular functions that might contribute to addictive behaviour.

3.2.1 Brain stimulation reward

It has been known since the work of (Olds and Milner, 1954) that mammals will perform
instrumental tasks in order to deliver electrical impulses to certain brain areas (intracranial
self-stimulation; ICSS). By definition, such electrical stimulation is reinforcing (behaviour
preceding the stimulation is strengthened), leading to the easy (but possibly mistaken)
conclusion that such stimulation activates areas of the brain integral to the perception of
“reward”. It has been known for decades that areas within, or interacting with brain DA
pathways, are especially likely to support ICSS (Crow, 1972), and that drugs that facilitate
transmission in DA pathways (amphetamines, cocaine, etc) also facilitate performance for
understood are observations that drugs facilitating transmission at GABA_A receptors (i.e.
benzodiazepine receptor ligands) possess similar properties in enhancing ICSS (Herberg et
al., 1986, Liebman, 1985) with comparable effect sizes (Straub et al., 2010). Diazepam and
cocaine have opposite effects on extracellular DA in the NAcc, with cocaine increasing and
diazepam decreasing DA concentrations (Di Chiara and Imperato, 1988, Finlay et al., 1992,
Invernizzi et al., 1991), indicating that the mechanism of action for the “rewarding effects”
of the two drugs may be different. Note, however, that a recent report indicates that an
inverse agonist at benzodiazepine receptors, Ro 15-4513, is able to antagonise the
facilitatory effects of methamphetamine on ICSS (Tracy et al., 2016).

Rudolph and colleagues postulated that benzodiazepines might achieve their effects on ICSS
by interacting with accumbal GABA_A receptors expressing α2 subunits, and showed that
diazepam-facilitation of ICSS was indeed absent when α2-containing receptors were made
insensitive to benzodiazepines (α2(H101R) mice) (Reynolds et al., 2012). Indeed, reward
thresholds at the highest dose of diazepam in these animals, was increased, which the
authors interpreted to mean that diazepam may even be aversive without the positive
modulation of the α2-GABA_A receptors. However, preventing benzodiazepine sensitivity at
α2-GABA_2 receptors was not unique in altering ICSS reward thresholds, and the corresponding manipulation of α3 also abolished the effects of diazepam, while diazepam’s effects in α1(H101R) mice were reduced. On the basis of previous work showing that α2-GABA_2 receptors makes a significant contribution to GABAergic inhibition in NAcc (Dixon et al., 2010), Rudolph and colleagues attribute the involvement of the α2 subunit to its participation in GABAergic transmission in NAcc, while the action of diazepam at α1-GABA_2 receptors in ventral tegmental area (Tan et al., 2010) is speculated to explain the effectiveness of the α1(H101R) manipulation.

In a subsequent report (Engin et al., 2014), the Rudolph group confirmed that another benzodiazepine, midazolam, also failed to facilitate ICSS in α2(H101R) mice. These animals also consumed less midazolam than WT mice when it was presented with sucrose in a two-bottle choice experiment, consistent with loss of midazolam’s rewarding effects (or its ability to facilitate the rewarding properties of sucrose) in the mutant mice. In an important further step, this latter effect was also found in mice in which α2 had been deleted specifically in accumbens, using virally-mediated knockdown. These findings imply that α2-GABA_2 receptors in the NAcc are involved in at least some reward-related properties of benzodiazepines.

### 3.2.2 Dissecting “reward”

The VTA projections to the NAcc, and associated neural circuits are often referred to as “reward” pathways, though it is frequently unclear what particular function is being ascribed to them. In the context of explanations of drug abuse, the term “reward” is sometimes used as synonymous with subjective pleasure (which itself may have several dimensions such as feelings of euphoria, or of relaxation), sometimes as a synonym for occurrences that, when they follow a particular behaviour, strengthen it (i.e. reinforcers), while for others the term is used loosely to cover a range of more carefully defined psychological constructs such as “incentive” (see (Salamone and Correa, 2012) for a critical review). The mesolimbic DA pathways have been ascribed a central role in signalling these aspects, so that DA is frequently (and uncritically) referred to as the “reward transmitter”.

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Studies of GABA function have slipped easily into this thinking, so that GABAergic influences in addiction are frequently considered in terms of their ability to modulate “reward”.

Two more rigorous considerations of DA function are worth considering in this context. The first holds that a pulse of DA release occurs in the context of unexpected occurrence of biologically valuable events (e.g. unexpectedly obtaining a piece of food), but this pulse soon shifts to environmental stimuli that precede (and thus predict) such events (Schultz et al., 1997). In that context, DA has been referred to as a “teaching signal”, involved in learning associations between otherwise neutral environmental events (e.g. a dinner gong) and biologically valuable events (e.g. dinner), though, in reality the learning of that association must occur somewhere other than within the DA system, but one for which DA release is an important consequence. Secondly, DA release within the NAcc has long been proposed to energise goal-directed behaviours, i.e., to form the biological basis of certain aspects of motivation (see (Salamone and Correa, 2012) for a review). In keeping with that rough distinction, phasic DA release has been suggested to perform the “teaching signal” function, while tonic release may act to energise selected goal-directed behaviour (Hamid et al., 2016, Hart et al., 2014, Cagniard et al., 2006b, Cagniard et al., 2006a). If this is a useful approach to understanding ventral striatal function, and its role in addictions, then it is worth considering how GABAergic systems may be involved.

3.2.3 The role of GABA$_A$ receptors within mesolimbic pathways: a hub for integration of glutamatergic and dopaminergic signals?

Current knowledge suggests that information regarding incentive cues reaches the NAcc via glutamatergic projections from prefrontal cortex, thalamus, hippocampus and amygdala that synapse on the dendrites of the GABAergic MSNs that make up 95% of NAcc neurons and constitute the only output from the nucleus (Tepper et al., 2008, Tepper et al., 2007). Under the influence of dopaminergic inputs from the VTA, these MSNs sort and prioritise the information provided by glutamatergic inputs and project the integrated output to basal ganglia output systems that express motivated behaviour. Understanding the mechanisms underlying this integrative process is fundamental to describing how motivations compete with each other for control over behaviour, yet we know little of its detailed mechanism.
To a considerable extent, striatal MSNs can be differentiated as expressing either the dopamine D1 or D2 receptors (D1R or D2R), and D1R- and D2R-expressing MSNs differ in their output pathways: whereas D1R-MSNs largely project directly to the VTA (direct pathway), synapsing onto GABAergic interneurons that themselves inhibit DA neuron cell bodies projecting to the NAcc, D2R MSNs project in rodents to the ventral pallidum (external pallidum in humans), where they serve to inhibit a further GABAergic output pathway projecting to the VTA (indirect pathway). [Note, this strict dichotomy has been recently challenged by the demonstration that, in mice, outputs from ventral (i.e. NAcc) but not dorsal striatum do not exhibit this strict anatomical segregation of D1R vs D2R MSNs as both D1- and D2-MSNs project from NAcc via the indirect pathway and similarly, both D1- and D2-MSNs directly innervate output neurons of the basal ganglia, thus adding a significant higher level of complexity (Kupchik et al., 2015). Although challenged by some reports in awake rodents (Mahon et al 2006), DA’s ability to modulate the glutamatergic input has been suggested to depend upon the state of polarisation of the MSN dendritic membrane; thus, DA induces further depolarisation via D1 receptors when the local membrane is partly depolarised by glutamatergic inputs (up-state), but has no effect, or may contribute further hyperpolarisation when the membrane is in the hyperpolarised “down-state” (O’Donnell and Grace, 1995, West and Grace, 2002, O’Donnell et al., 1999).

Irrespective, the modulatory effects of DA on the glutamatergic inputs are a function of the receptor activated, D1R and/or D2R, with activation of D1R being usually excitatory and D2R usually inhibitory (Surmeier et al., 2007). DA acting through D1Rs may thus serve to increase the signal-to-noise ratio for glutamatergic cortical, amygdala and hippocampal inputs (Nicola et al., 2004). The integrated sum of such interactions across its dendritic tree will determine the probability of an individual MSN firing an action potential, and thus providing an output contributing to behaviour. Thus, variants within, and manipulation of GABA_A receptors expressed within this circuit (See Section 3 and Fig 5) may contribute in a complex fashion to the function of the basal ganglia.

In principle, GABA_A receptors may influence the functioning of the system in several ways. Firstly, within the striatum, MSNs receive two types of GABAergic inhibitory inputs (Fig 5): 1)
a feed-forward inhibitory pathway from a range of GABAergic interneurons, amongst which the fast-spiking (parvalbumin-expressing) interneurons provide the strongest input with approximately 100 connections targeting the somata of each MSN (Koos and Tepper, 1999); and 2) a lateral inhibitory input largely located on the dendritic tree, through recurrent collaterals from neighbouring MSNs, with individual MSNs thought to receive inputs from about 450 neighbouring MSNs (Tunstall et al., 2002, Wilson, 2007). Of the two inputs, that from the fast-spiking interneurons is considerably stronger, both because of the proximal somatic vs. dendritic location, and because of the high degree of synchronization amongst fast-spiking interneurons (Tunstall et al., 2002). Thus the feed-forward inhibitory pathway may be best suited to influence action potential generation at the soma. On the other hand, lateral inhibition will strongly affect local dendritic processes including plasticity (Tepper et al., 2008).

Importantly for the consequences of GABA_\text{A}_R activation, at rest MSNs exhibit a significantly negative membrane potential (Vm) (approximately -85-90 mV), which is below the reversal potential for Cl^-, meaning the net Cl^- movement through GABA_\text{A}_R-associated channels will be outwards and therefore depolarising (Czubayko and Plenz, 2002, Plenz, 2003, Maguire et al., 2014). Thus, under particular circumstances, a GABAergic signal can be excitatory, and facilitate action potential generation if temporally coupled with an incoming glutamatergic signal within an appropriate time frame (~50-60 ms; (Plenz, 2003, Bracci and Panzeri, 2006). Alternatively, the shunting actions of GABA will give rise to a net inhibitory effect. Transient, synaptic GABAergic inputs are clearly best suited to serve a potentially facilitating excitatory role, whereas sustained activation of an extrasynaptic GABAergic conductance will invariably yield a net shunting inhibitory action (Song et al., 2011). Interestingly, modelling studies support a role for MSNs as input integrators over a time window of 40-60 ms, suggesting that the synaptic GABA_\text{A}_R activation may fine tune the modulatory action of DA to increase or decrease, via D1R or D2R activation respectively, the temporal integration window of glutamatergic cortical and hippocampal inputs (Bracci and Panzeri, 2006, Moyer et al., 2007). Furthermore, the overall GABAergic effect may be dependent on the firing pattern of DA neurons, as phasic tonic firing would give rise to low nanomolar concentrations of DA and target primarily extrasynaptic sites, whereas burst firing would associate with micromolar concentrations of DA and would be restricted largely to synaptic
locations (Phillips and Wightman, 2004, Floresco et al., 2003, Arbuthnott and Wickens, 2007). Lastly, the presence of both D1R and D2R on the presynaptic varicosities of MSNs (Wong et al., 1999) engenders both DA receptor types with the ability to strategically regulate GABAergic transmission within striatal (both dorsal and ventral) circuits (see Fig 5).

Although it is established that a variety of GABA$_A$R subunits is expressed in striatum, not much is known regarding the receptor stoichiometry of GABA$_A$ receptors responsive to the feed-forward inhibitory and lateral inhibitory projections, and whether/how they may differ from each other. However, our electrophysiological studies suggest that perhaps 30% of synaptic inhibition of MSNs is mediated by $\alpha_2$- GABA$_A$ receptors (Dixon et al., 2010). Extrasynaptic inhibition mediated by $\alpha_4\beta\delta$ GABA$_A$ receptors is also prominent in the soma of NAcc MSNs as both the selective $\delta$-GABA$_A$ receptor agonist gaboxadol, and DS2, a selective positive allosteric modulator, greatly increased the tonic current of all MSNs from WT, but not from $\delta$ knockout or $\alpha_4$ knockout mice (Maguire et al., 2014). Coupling DA and tonic inhibition, the acute activation of D1 receptors (by a selective D1 agonist or indirectly by amphetamine) greatly enhanced GABAergic tonic inhibition in D1R-MSNs but not D2R-MSNs (Maguire et al., 2014), suggesting a potential mechanism whereby DA acting at D1 receptors may under some circumstances (e.g. during burst firing mode resulting in high level of DA), acts to brake an excessive excitatory tone at somatic or, possibly, dendritic locations. Although as yet lacking experimental evidence, it is tempting to speculate that similar mechanisms to those described here for somatic regulation of MSNs, may also apply to their dendritic regions where high levels of $\alpha_2$ but also $\alpha_4$ and $\delta$ subunits, are also detected (Maguire et al., 2014). Thus, GABA and DA may interact in a dynamic and reciprocal fashion to provide the filtering mechanism, canonically attributed to DA, which supports stronger inputs at the expense of the weaker ones. A detailed understanding of the conditions and modalities under which such interaction will be physiologically and pathologically relevant will require additional experimental evidence.

A second means whereby GABA may influence the mesolimbic DA pathway, is the GABAergic regulation of the VTA cell bodies of DA neurons projecting to ventral striatum (Fig 3). In this case, $\alpha_1$-GABA$_A$Rs may play a predominant role (Tan et al., 2010) in
hyperpolarising GABAergic interneurons that inhibit the DA cells. Thus, facilitation of GABAergic transmission at α1-GABA\textsubscript{A}Rs may serve to facilitate DA transmission by indirect disinhibition (Lees et al., 2012). Although it is generally observed that drugs such as benzodiazepines that facilitate GABAergic inhibition decrease DA turnover in accumbens (Di Chiara and Imperato, 1988, Finlay et al., 1992, Invernizzi et al., 1991), fast-scan cyclic voltametry studies have shown that activation of GABA\textsubscript{A}Rs by direct administration of the GABA\textsubscript{A}R agonist muscimol into the VTA significantly increased DA release in the NAcc (Xi and Stein, 1998). However, this interpretation is subject to further complexity as under specific circumstances, GABA\textsubscript{A} receptors may contribute to depolarisation at presynaptic locations (Trigo et al., 2008). In keeping with this possibility, muscimol acting at α4βδ receptor isoforms presynaptically increased the GABAergic input to the VTA, thus potentially reducing DA input to the NAcc (Xiao et al. 2007). Thus, the net effect of facilitating GABAergic inputs to VTA may be function of the combined or selective activation of different receptor isoforms. The outcome of such complex interactions for DA projections to NAcc, and for behaviour, will thus be difficult to predict.

Lastly, GABA release from VTA synapses in the striatum will tonically inactivate interneurons (TANs), that employ acetylcholine as their major transmitter (but also co-release glutamate). Although few in number, the extensive connections of TANS may allow them considerable influence over MSN activity, so that control of their activity by GABAergic inputs may play an important role in determining final outputs (Brown et al., 2012).

A major task is now to integrate this emergent understanding of GABAergic function in the striatum into our understanding of the role of GABA\textsubscript{A} receptors in addictive processes.

### 3.2.4 Striatal GABA\textsubscript{A} receptors and conditioned reward

The location of several subtypes of GABA\textsubscript{A} receptors in pathways known to play a role in motivational learning raises the possibility that they may play separate roles, potentially allowing specific manipulation of different aspects of motivated (and addictive) behaviour. We consider these possibilities in the next section.
3.2.4.1 alpha1

Only a limited number of studies appear to have investigated the role of α1 subunits in reward-related behaviour.

As outlined above, deficits in action-outcome association learning (i.e., the ability to learn which particular responses are required to obtain specific rewards) are found in mice with various discrete depletions of the α1 subunit in prefrontal cortex, consistent with known effects of lesions in these areas (Butkovich et al., 2015, Swanson et al., 2015). We are unaware of studies investigating the role of other GABA<sub>A</sub> receptor subtypes on reward and motivation in similar models, though these would be extremely valuable.

In a less-well defined measure of reward learning, the ability of cocaine to support conditioned place preference (CPP) was unaltered in the α1 knockout mice (Reynolds et al., 2003a) despite the psychostimulants cocaine and d-amphetamine failing to induce hyperlocomotion in α1 knockout mice. Intriguingly, using a rat CPP model, Jiao and colleagues have revealed a selective transient down regulation of the α1 (but not α2) subunit in the dorsal striatum (but not PFC, amygdala or hippocampus) of rats place-conditioned to methamphetamine; control rats in which drug administration was not conditioned to the place did not show such changes. These findings suggest a role for α1-GABA<sub>A</sub> receptors in the formation of drug reward memory and, in agreement, intra-(dorsal) striatum administration of the selective α1- GABA<sub>A</sub>R PAM, zolpidem before pairing was able to prevent methamphetamine-induced CPP formation (Jiao et al., 2016). Collectively, the study highlights an important role for α1-GABA<sub>A</sub> receptors in the dorsal striatum in the formation of reward-environment associations. Yet, it remains to be addressed how a history of exposure to different type of addictive drugs e.g. opiates vs. cocaine, may interact with subsequent pharmacological manipulations of selective GABA<sub>A</sub>R isoforms (see Shinday et al., 2013)).
3.2.4.2 alpha2

Since DA projections to ventral striatum exert their influence on behaviour by interacting with the major GABAergic projection neurons, it is worth considering what aspects of those functions ascribed to DA may be affected by modulating striatal GABAergic receptors.

That animals readily learn that arbitrary environmental stimuli can be used to predict the forthcoming availability of food, and adapt their behaviour accordingly, is a classic example of Pavlovian conditioning, and forms the basis of the “teaching signal” hypothesis of DA function mentioned above. Thus, if an arbitrary light stimulus (cue) is illuminated shortly before food is delivered into a receptacle, hungry rodents soon approach the receptacle at light onset, without waiting for food delivery (so-called discriminated approach, or goal tracking). In keeping with the teaching signal hypothesis, 6-hydroxydopamine lesions of the NAcc, leading to approximately 80% reductions in tissue DA, profoundly impair discriminated approach regardless of whether the lesion is made prior to, or subsequent to training (Parkinson et al., 2002). In contrast, mice with constitutive knockout of $\alpha_2$ subunits of GABA$_A$ receptors show no impairment in acquiring discriminated approach (Dixon et al., 2010), strongly suggesting that these receptors are not involved in motivational learning.

Once animals have learned the association between the cue and food delivery, they will learn an arbitrary instrumental response that activates the cue (without activating food delivery). Thus the arbitrary cue has acquired reinforcing properties of its own as a consequence having been paired with food availability. This phenomenon is known as conditioned reinforcement, and again, manipulations that interrupt DA function in ventral striatum disrupt the behaviour (Beninger and Phillips, 1980) while drugs that facilitate DA neurotransmission facilitate it (Kelley and Delfs, 1991a, Kelley and Delfs, 1991b). Again in contrast to the effects of DA manipulations, constitutive deletion of $\alpha_2$ receptors has no effect on conditioned reinforcement (Dixon et al., 2010, Duka et al., 2015). Thus, GABA$_A$ receptors incorporating the $\alpha_2$ subunit, despite their important contribution to decreasing MSN excitability, appear not to play a role in the “teaching signal” aspects of DA receptor
function. Furthermore, another measure of “conditioned reward”, CPP to cocaine, also shows no impairment in α2 knockout mice (Dixon et al., 2010). Although interpretation of CPP experiments can be complex (Stephens et al., 2010), in keeping with a lack of effects of the deletion on reward-associated learning, α2 knockout animals do not differ from WT mice in learning an operant response to obtain food (Dixon et al., 2014, Dixon et al., 2012), or cocaine (Dixon et al., 2014), or alcohol (Dixon et al., 2012).

In contrast, α2-GABA\(_{\text{A}}\) receptors seems to play an important role in the energising aspects of DA; as already mentioned, psychostimulant drugs facilitate instrumental responding to obtain conditioned reinforcers, but both cocaine (Dixon et al., 2010) and methylphenidate (Duka et al., 2015) fail to increase lever pressing, or nose-poking by α2 knockout mice for conditioned reinforcers. Perhaps related to this, while psychostimulants show a normal ability to increase locomotor activity in α2 knockout mice, they do not give rise to behavioural sensitisation to repeated administration of the drug (Dixon et al., 2010, Morris et al., 2008). Both psychostimulant-facilitation of conditioned reinforcement and behavioural sensitisation may reflect an interaction of psychostimulants with conditioned behaviours, and both phenomena are hypothesized to contribute to drug abuse by facilitating incentive learning (Parkinson et al., 1999, Robinson and Berridge, 1993, Le Merrer and Stephens, 2006).

In keeping with a role for α2 subunits in behavioural sensitisation, selective activation of α2-GABA\(_{\text{A}}\) receptors, achieved using Ro 15-4513’s agonist properties in α2(H101R) mice, facilitated the stimulant effects of cocaine, and induced behavioural sensitisation in its own right, such sensitisation showing cross-sensitisation to cocaine (Morris et al., 2008). Importantly, the stimulant effects of Ro 15-4513 in the α2(H101R) mice were also seen when the drug was infused into the ventral striatum, suggesting strongly that these effects were attributable to α2-GABA\(_{\text{A}}\) receptors in this region. While the experiments in constitutive knockout mice do not inform us regarding the precise area in which α2-GABA\(_{\text{A}}\) receptors are important, the effectiveness of drug injection into the ventral striatum suggests that not only are α2-GABA\(_{\text{A}}\) receptors appropriately located in ventral striatum to
influence addiction-related behaviours, their manipulation influences the motivational consequences of cocaine. Thus, $\alpha_2$-GABA$_A$ receptors may be involved in the facilitation of conditioned drug-seeking by psychostimulant drugs although at present it is not known whether they may serve distinct functions in D1 vs D2 MSNs as appears to be the case for $\alpha_4$-GABA$_A$ receptors (see section 3.2.4.3 below).

3.2.4.3 alpha4

Similar to the constitutive $\alpha_2$ knockout mice, $\alpha_4$ knockout mice do not differ from their WT littermates in learning to approach the food chamber following the onset of a predictive cue (Macpherson et al., 2016). Similarly, both $\alpha_4$ knockout and WT mice accurately learned to elicit presentation of the cues via nose-poke responding, thus demonstrating robust conditioned reinforcement. However, in comparison to their WT counterparts (and in contrast to $\alpha_2$ knockout mice), $\alpha_4$-knockout mice displayed higher levels of instrumental responding, suggesting they were more highly motivated to obtain the conditioned reinforcer. Administration of cocaine dose-dependently potentiated instrumental responding for the conditioned reinforcer equally across both genotypes, the heightened level of responding in the $\alpha_4$ knockout mice being maintained across the cocaine doses (Macpherson et al., 2016), suggesting that tonic GABAergic inhibition of MSNs may normally serve to inhibit motivation to obtain conditioned reinforcement. However, when $\alpha_4\beta\delta$ receptors of WT mice were activated by local infusion of the $\alpha_4\beta\delta$-preferring agonist, gaboxadol, into the NAcc, no effect on responding for the conditioned reinforcer was seen, though this treatment reduced cocaine potentiation of responding for conditioned reinforcers in WT (but not $\alpha_4$ knockout- mice).

That these observations resulted from $\alpha_4$ deletion specifically in ventral striatum was shown by infusion of an Ad-sh$\alpha_4$ adenovirus, but not an adenovirus carrying a scrambled sequence, directly into ventral striatum (Macpherson et al., 2016); as with constitutive knockout mice, control and $\alpha_4$-subunit viral knockdown mice demonstrated an equal ability to learn the food-predictive properties of the CS+. Similarly, the $\alpha_4$-subunit viral knockdown mice showed increased instrumental responding for the conditioned reinforcers relative to
controls, providing further evidence that α4-GABA_A receptors specifically within the NAcc are involved in mediating instrumental responding for reward-conditioned cues.

Finally, specific deletion of the α4-subunit from DA D2R-expressing cells (α4_D2 knockout mice), but not from D1R-expressing cells (α4_D1 knockout mice), mimicked the phenotype of the constitutive knockout, potentiating CRf responding and blocking intra-accumbal gadoxadol attenuation of cocaine-potentiated CRf responding. These data demonstrate that α4-GABA_A receptor-mediated inhibition of D2R-expressing neurons reduces instrumental responding for a conditioned reinforcer and its potentiation by cocaine. Importantly, impairing transmission at α4-GABAARs in constitutive α4 knockout mice (Macpherson et al., 2016) or by viral knockdown in rats (Rewal et al., 2012) did not affect responding for conventional reinforcers, while responding for alcohol was decreased following virally-mediated knockdown (Rewal et al., 2012).

These data suggest some similarities, and some differences in the functions of α4 and α2-GABA_A receptors. Deletion of neither subtype had consequences for the animal’s ability to learn the association between the CS and food reward, suggesting that α4, like α2, is not involved in the prediction error/teaching signal function of the striatum. However, unlike α2 subunit deletion, suppression of α4 subunit expression had no consequences for the ability of cocaine to increase responding; instead, the deletion was itself sufficient to facilitate responding for the conditioned reinforcer. Since similar effects were found when the deletion was limited to D2R-expressing neurons, it appears that α4-GABA_A receptor-mediated inhibition of D1-MSNs was not involved in determining the strength of response for the conditioned reinforcer.

Like α2 knockout mice, constitutive α4 knockout mice did not differ from WT in their expression of cocaine-CPP (Maguire et al., 2014). However α4_D1 knockout mice showed facilitated cocaine-CPP (Maguire et al., 2014). Furthermore, the α4βδ agonist, gadoxadol, administered systemically, or directly into the NAcc of WT, but not α4 knockout or α4_D1 knockout mice, blocked the ability of cocaine, administered during the test phase, to enhance CPP. In contrast, α4_D2 knockout mice exhibited normal CPP, but no cocaine
enhancement (Maguire et al., 2014). These findings are complex, particularly in the light of the recent report demonstrating the segregation of D1R and D2R MSNs to the direct and indirect output pathways to be no longer valid for the mouse NAcc (Kupchik et al., 2015). Nevertheless, collectively, these observations suggest that α4-GABA<sub>A</sub> receptor-mediated tonic inhibition of D1R-MSNs serves to weaken the ability of cocaine to facilitate motivated behaviour, while similar inhibition of D2R-MSNs has little effect.

In conclusion, interactions between DA and GABAergic tonic inhibition of D1R- and D2R-MSNs provides an intrinsic mechanism to differentially affect their excitability in response to psychostimulants and thereby influence their ability to potentiate conditioned reward.

3.2.5 Integrating GABAergic molecular and reward-related behavioural dysfunctions

Determining the role of GABA<sub>A</sub> receptor-mediated signalling in the striatum has proved challenging because the currently available experimental approaches cannot adequately access the ensembles of the complex striatal network and their response to the cortical inputs. However, because of the knowledge gained from studies of both the anatomical connections, and the individual conductances at the single cell level, an increasing number of attempts have been made to model how these factors may interact at a network level. A very recent investigation, utilising such a modelling approach has resulted in a better understanding of the putative roles of lateral inhibition and feed-forward inhibition (Moyer et al., 2014). From the modelling perspective, the specific task of lateral inhibition would be to confer timing precision in the response of the neuronal ensembles to incoming coordinated inputs, by silencing the activity of those MSNs that are not synchronised. In this way, lateral inhibition could also constrain the gain of the network even when there is a significant increase in inputs. Feed-forward inhibition would aid this task by specifically targeting inhibition of the non-synchronised, over the synchronised MSNs. Intriguingly, feed-forward inhibition would be most effective when fast-spiking interneurons are, themselves, desynchronised, possibly because desynchronised firing would afford a larger window for inhibition. This proposal is consistent with the long-held view that striatal function is competitive in nature, but also reveals the importance of the latency of the
ensembles responses (rather than the size of the ensembles) in establishing the MSN ensemble “winner” of the competition. The cooperation between lateral inhibitory and fast-spiking interneurons would endow the striatal network with a number of advantages from a behavioural perspective. Thus, lateral inhibition would improve learning of a new action and associating the action outcome with its choice representation. On the other hand, feed-forward inhibition would facilitate both selection of the most appropriate action and efficient shift between different actions, while also preventing competing actions from being active simultaneously.

Addictive behaviours might then result from increased probability of “addictive action” ensembles being prioritised. In this framework, a deficit in inhibition from lateral inhibitory inputs (as expected with a loss of α2-GABA_A receptor signals in the dendrites of MSNs), would conceivably alter the gain of the striatal output and bias the output of MSN ensembles. On the other hand, a deficit in α4-GABA_A receptor-mediated tonic inhibition may impact feed-forward inhibitory inputs; however, the net effect of such deletion is difficult to predict as α4-GABA_ARs are expressed on both fast-spiking interneurons and the target MSNs (Maguire et al., 2014). Our comparative findings with mice selectively lacking the α4 signal in D1R- or D2R-MSNs or both neuronal populations, suggest that the α4-GABA_A receptor-mediated effect of feed-forward inhibition may be dependent on the MSN neuronal type, as D1R+ve MSN-mediated tonic inhibition acts to oppose an excessive DA tone, e.g. as associated with a psychostimulant (Maguire et al., 2014). Yet, D2R-MSN α4-mediated inhibition would appear to control motivation to obtain the reinforcing stimuli. It is tempting to speculate that distinct fast-spiking interneuron populations targeting either D1R- or D2R-MSNs may be activated by specific cortical inputs to elicit defined functional and behavioural outcomes. A similar dichotomy albeit with possibly different behavioural consequences may similarly apply to lateral inhibition where the role of D1R vs D2R-MSNs remains to be elucidated. Studies exploring such possibilities and how they may be affected by chronic drug intake are warranted in the near future.

4. Integrating the human genetic and animal studies

4.1. Altered subjective effects of drugs
The neurobiological mechanisms by which variations in non-coding regions of GABRA2 in humans translate into risk for addictions are not understood. Clearly, the rodent studies showing high density of α2-GABA$_A$ receptors in the NAcc (Dixon et al., 2010, Pirker et al., 2000), a brain area associated with reward and motivation and implicated in drug abuse and addiction (Robbins and Everitt, 2007, Robinson and Berridge, 1993) provide a potential clue. As discussed above, deletion of α2-GABA$_A$ receptors in the mouse (α2 knockout mice) not only reduces GABAergic phasic inhibitory currents in accumbal medium spiny neurons (MSNs) by about 30% (Dixon et al., 2010), but also leads to altered function of systems thought to contribute to control over reward seeking (Dixon et al., 2010), and in particular to facilitation of reward-seeking by psychostimulants. Nevertheless, it is unknown whether human gene-variants conferring increased risk for addiction alter expression of α2-GABA$_A$ receptors in the brain, and through this mechanism affect incentive learning.

NAcc activation during reward anticipation is enhanced in human adolescents carrying the GABRA2 risk G allele of rs279858 (Heitzeg et al., 2014), suggesting an effect on incentive processing consistent with reduced inhibition of accumbens MSNs, but direct evidence associating risk variants with changed α2 subunit expression is inconclusive (Haughey et al., 2008). The rs299858 SNP creates a change in the triplet codon for amino acid residue 132, but does not change the protein sequence. This and other intronic SNPs in the vicinity may tag a region that changes the stability / expression of the mRNA and thus alter expression of the GABRA2 gene. Some support for this interpretation comes from a recent report (Lieberman et al., 2015) that α2 mRNA is lower in neural cell cultures derived from rs279858*C allele (risk allele) carriers. If risk variants of GABRA2 reduce expression of α2 subunits, might such risk variants have behavioural effects in humans that parallel the animal findings with genetic deletion of the subunit?

Integrating data from animal and human experiments is often hindered by the use of different experimental measures in the two species (Stephens et al., 2010). However, using a human version of the mouse conditioned reinforcement test, we found similarities in the ability of the psychostimulant, methylphenidate, to facilitate instrumental responding for a conditioned reinforcer in mice with α2 subunit deletion, and humans carrying risk alleles for
cocaine addiction (Duka et al., 2015). In our experiment, we used possession of the G allele of SNP rs279871 in homozygosity as one of the identifiers of a genetic “risk” population. Although this SNP differs from those used in most studies of GABRA2, SNAP analysis of data from the 1000 Genomes Project (Johnson et al., 2008) shows rs279871 to be in 100% linkage disequilibrium with rs279858, the common risk SNP recognised across multiple studies of addicted populations (Covault et al., 2004, Fehr et al., 2006, Lappalainen et al., 2005) and cocaine abuse (Enoch et al., 2010), as well as childhood conduct disorder (Dick et al., 2006b) and impulsivity during reward anticipation (Villafuerte et al., 2012). Thus variations in this genomic area may alone be driving our genotype differences. It is thus of importance that SNP rs279858 is associated with a reduced \( \alpha_2 \) mRNA expression in neuronal cell cultures derived from the corresponding risk allele, consistent with (but not proving) a lowered level of \( \alpha_2 \)-GABAA receptors in rs279858 risk-allele carriers.

Interestingly, in addition to loss of the ability of methylphenidate to facilitate conditioned reinforcement in the “risk” genotype, we also found marked changes in their subjective responses to the drug, specifically in ratings of drug-induced “restlessness”, “stimulated” and “arousal”. Other (Pierucci-Lagha et al., 2005, Roh et al., 2011), but not all (Arias et al., 2014) previous studies found decreased subjective effects of another drug of abuse, alcohol, in measures of positive affect such as stimulation, vigour and happiness, in carriers of the C-allele of rs279858. In keeping, carriers of the minor alleles for SNPs rs279858, rs279844, rs279845, rs279826, rs279828 and rs279836 had lower 'Negative' alcohol effect scores than individuals homozygous for the common allele at each SNP (Uhart et al., 2013).

Furthermore, compared to heterozygous subjects, subjects homozygous for the high-risk allele at rs279871 displayed an increased response to alcohol-associated cues in medial PFC areas in an fMRI study; 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., 2013, Villafuerte et al., 2012).

4.2 Personality effects

Such changes in reward-related behaviours may precede the development of alcohol dependence. An elevation in the risk for alcohol dependence associated with GABRA2 is not consistently apparent until the mid-20s, but then remains constant throughout adulthood (Dick et al., 2006b). Thus associations between GABRA2 risk haplotypes and sensitivity to reward or personality characteristics like impulsivity may interact with environmental factors leading to alcohol dependence at a later stage. One example of such a potential interaction is provided by a study of the relationship between GABRA2 genotype and family status; while both GABRA2 and marital status contributed independently to the development of alcohol dependence, the high-risk GABRA2 variant is associated with a decreased likelihood of marrying, and an increased likelihood of divorce, which appears to be mediated by personality characteristics (Dick et al., 2006a).

Taken together, these studies may suggest that the primary association of GABRA2 variants is with deficits in personality characteristics associated with behavioural control and with sensitivity to reward. Impairments in such control may reveal themselves in a variety of disorders which includes alcohol and drug abuse. Such impairments are sometimes referred to as “externalizing behaviours” and include aggression and rule breaking (e.g., defiance, theft, and vandalism) that are influenced by both genetic and environmental factors. Several studies have shown the association of adolescent externalizing behaviour with negative consequences in adulthood, but also how externalising behaviour in adolescents is influenced by parental monitoring. Recently, the Michigan group, examining GABRA2 variants (rs279827, rs279826 and rs279858) in adolescents were able to show that adolescents with GABRA2 minor allele (GG, rs279827) are most susceptible to parental monitoring, both adverse and adaptive (Trucco et al., 2016a, Trucco et al., 2016b). Although the relationships between GABRA2 variants and parental monitoring influences seem to be primarily for delinquent behaviour rather than alcohol abuse, per se, the same variants are nevertheless associated with the development of problematic alcohol and drug use, if
Delinquent behaviour is present in mid-adolescence (Trucco et al., 2014). Furthermore, persistence of delinquent behaviour from early adolescence to young adulthood (when problematic alcohol use develops) is associated with variants in the GABRA2 SNP (rs279858) (Dick et al., 2009).

Such developmental trajectories may be the reason for apparently contradictory findings regarding GABRA2 polymorphisms and the presence of alcohol dependence. Clinically symptomatic Conduct Disorder, as well as less severe behaviours of delinquency, have been found to show patterns of behaviour persisting from early adolescence to young adulthood, but also declining when measured in a cohort of children followed from 11 years of age to 22 years of age (Dick et al., 2009). Importantly, the individuals showing patterns of persistently high externalising behaviour were more likely to carry the GABRA2 variant (rs279858), that was found previously associated with increased risk for adult alcohol dependence (Edenberg et al., 2004). The association of GABRA2 with persistent externalising behaviour was influenced by parental monitoring; if parental monitoring was present the externalising behaviour was found to be less persistent.

Regarding reward sensitivity as a factor influencing addictive behaviour, and returning to the animal literature, impaired control over reward-seeking may be a common feature, though directly comparable measures have been seldom carried out in both human and animal studies, to allow an integration of findings. Our own studies in GABRA2 “risk” individuals and α2 knockout mice (Duka et al., 2015) point to commonalities in psychostimulant effects on control over reward seeking, but how these relate to human conduct disorder deficits in risk individuals is at present a matter for surmise. Similarly, evidence that the ability of benzodiazepines to promote aggressive behaviour in mice is dependent on α2-containing receptors (Newman et al., 2015) is perhaps suggestive of involvement of such receptors in behavioural control. However, there is much to do to integrate these kinds of observation into a systematic account.
4.2.1 GABA\textsubscript{A} receptor and impulsivity: an addiction-relevant relationship?

Amongst higher order dysfunctions contributing to drug abuse, impulsivity appears to play a significant role. Variations in GABA\textsubscript{A} receptors play a significant role in impulsivity traits related to drug (and especially alcohol) misuse (Dick et al., 2010, Villafuerte et al., 2012, Villafuerte et al., 2013). Although impulsivity is a complex construct encompassing a number of behavioural traits, a significant body of work has linked the GABRA2 gene to a variety of clinical disorders characterised by a lack of impulse control, or general externalising behaviour (Dick et al., 2013). As many as 11 SNPs within the \( \alpha2 \) gene have been associated with impulsivity and of these, four also associate with Lifetime Alcohol Problem Score (Villafuerte et al., 2013). A bootstrap analysis probing for a causal relationship for these 4 SNPs suggests that about 25% of the association is mediated by their association with impulsivity, with the remaining 75% being accounted by other, yet to be identified, behavioural factors. A relationship between \( \alpha2 \) and impulsivity and alcohol consumption has also been suggested in animals; both the increased impulsivity and high alcohol consumption exhibited by adults rats exposed to a paradigm of early-life stress can be reversed by pharmacological manipulation of \( \alpha2 \) GABA\textsubscript{A}Rs in selective neuronal locations (Gondre-Lewis et al., 2016)- see section 4.3).

Moreover, GABRA2 SNPs associated with impulsivity associate with increased neuronal activation in both insula and NAcc (Villafuerte et al., 2012), thus providing some anatomical evidence for overlapping neuronal mechanisms and substrates between impulsivity and drug misuse mediated by variation in the \( \alpha2 \) gene. How these genetic variants may influence GABA\textsubscript{A} receptor function at selective neuronal loci remains an open question though, as highlighted earlier, a recent study indicates a specific SNP, rs279858 to associate with reduced \( \alpha2 \)mRNA in an iPSC culture model (Lieberman et al, 2015). In this respect, the observation that in humans high levels of impulsivity in adolescents associate with a selective reduction in the GABA signal in the anterior cingulate cortex of adult subjects (Silveri et al., 2013) is of interest. Similarly, in animals, a decrease in GABA\textsubscript{A} receptor binding and GABA content in the anterior cingulate cortex and NAcc core respectively, have been reported for highly impulsive rats (Caprioli et al., 2014, Hayes et al., 2014). Future investigations should explore the impact of other identified SNPs on the receptor function.
with the strongest impulsivity-associated SNPs, rs279827 close to a splicing site able to produce a truncated protein (Tang et al. 2005), emerging as a potential candidate. These studies will be instrumental in providing some of the starting points necessary to begin modelling the impact of GABA$_A$ receptor dysfunction at relevant neuronal networks.

4.3 GABA$_A$ receptor gene-environment interactions

As already noted, for certain addictions, variations in GABA$_A$ receptor subunit genes may interact with childhood trauma in predisposing to the development of an addictive phenotype (Enoch et al., 2010). For example, while experience of childhood trauma alone predicts the development of drug dependence (Enoch, 2011, Messina et al., 2008, Cadet, 2016), this relationship is also markedly influenced by GABRA2 gene variants (Enoch et al., 2010). A considerable animal literature implicates $\alpha_2$-GABA$_A$Rs subunits in the anxiolytic actions of benzodiazepines and barbiturates (Low et al., 2000, Morris et al., 2006, Dixon et al., 2008, Dias et al., 2005, Smith et al., 2012), while genetic deletion of the $\alpha_2$ subunit increases the sensitivity of mice to conditioned anxiety (Dixon et al., 2008). In humans, a preliminary report suggests a strong correlation between GABRA2 genotype and amygdalar and insular activation in response to fearful faces (Stein et al., 2006). Thus, one distinct possibility is that variations in GABRA2 may increase risk for the development of addictions by increasing the emotional response to traumatic life events. In keeping with that hypothesis, (Nelson et al., 2009) reported that polymorphisms in GABRA2 interact with early childhood trauma in increasing risk for post-traumatic stress disorder (PTSD). Alternatively, early life exposure to traumatic events may alter GABAergic gene expression, resulting in altered receptor sensitivity, and sensitivity of gene expression to such events may depend upon genetic polymorphisms. Exposure of rat pups to stress permanently diminishes GABAergic responses at hippocampal GABA$_A$ receptors, an effect that has been attributed to diminished transmission through $\alpha_1$GABA$_A$Rs (Hsu et al., 2003). Very recently, we have found that exposure of mice to early life stress during postnatal days p2 to p9 leads to a specific reduction in adulthood of $\alpha_2$ but not $\alpha_1$ subunit expression in ventral striatum, leading to impaired GABAergic inhibition of accumbal medium spiny neurons, and altered responses to cocaine that resemble those in $\alpha_2$ knockout mice (Lambert, 2013).
In keeping, maternal separation in rats has been shown to engender in adult animals an increase in indices of both impulsive and alcohol binge-like drinking behaviours, which associated with a selective increase in the protein levels of the GABA\(_A\)R \(\alpha_2\) subunit in the mPFC and the amygdala. Remarkably, micro-infusion of 3PBC, a ligand that selectively inhibits \(\alpha_2\)-GABA\(_A\) receptors in either locus, reversed the maternal separation-evoked increase in both behavioural measures, an effect also mimicked by local administration into the amygdala of antialarmin, a CRF antagonist (Gondre-Lewis et al., 2016). Collectively, these findings suggest that a coordinated regulation of expression levels of \(\alpha_2\)-GABA\(_A\) receptors at selective but overlapping neuronal loci of the stress and motivational circuits may mediate the effects of early-life stress on the increased risk for excessive drinking.

Additional GABA\(_A\) receptor isoforms may also be sensitive to environmental influences. Thus, consistent with a specific pattern of altered expression of distinct GABA\(_A\) receptor subtype following exposure to early-life stressor, a selective decrease in \(\alpha_3\) but not \(\alpha_2\) subunit expression in the amygdala and an associated increase in anxious-like behaviour has been reported for a peri-pubertal stress paradigm in rats (Tzanoulinou et al., 2014). Intriguingly, a persistent down regulation of Gabrd in the VTA of rats exposed to chronic physical or emotional stress has been also reported and is accompanied by behavioural abnormalities indicative of a depressive-like behaviour (Warren et al., 2013). A decreased expression of \(\alpha_3\)-containing and/or \(\delta\) receptor isoforms, which mediate tonic inhibition in the amygdala (Marowsky et al., 2012) and other neuronal populations within motivational pathways (Fig 5), may also contribute to addictive behaviour.

4.3.1 Neurosteroids: a role in neurocircuits involved in addiction?

In the context of interactions between early-life stress and GABA\(_A\) receptor function, it is worth noting that the function of such receptors can be endogenously facilitated allosterically by a group of brain-derived neurosteroids (Belelli and Lambert, 2005, Gunn et al., 2015). Therefore, variations in the neuronal levels of neurosteroids can impact on GABA\(_A\) receptor function across the CNS. Our recent electrophysiological and immunohistochemical analysis in the NAcc has revealed that both synaptic and
extrasynaptic GABA<sub>A</sub> receptors in MSNs are sensitive to physiological concentrations of neurosteroids while immunoreactivity for one neurosteroid, allopregnanolone, is evident in MSNs (DARPP-32 positive), but also DARPP-32 negative cells, presumably interneurons or glia cells (Mitchell, Belelli and Lambert, unpublished). Collectively these findings are consistent with previous reports suggesting a role for endogenous neurosteroid in the modulation of NAcc function via GABA<sub>A</sub> receptors (Frau et al., 2013).

Of specific relevance in the contest of substance abuse, it has been suggested that alcohol may mediate some of its actions by modulating the synthesis of these GABA<sub>A</sub> receptor-active neurosteroids (Sanna et al., 2004, Kumar et al., 2009, Morrow et al., 2006). In keeping, a number of publications have found effects of allopregnanolone on ethanol operant self-administration by rats (Janak et al., 1998, Janak and Michael Gill, 2003), while the administration of the synthetic analogue, ganaxalone, reduced limited access ethanol consumption in mice, when given either systemically (Ramaker et al., 2012), or into NAcc (Ramaker et al., 2015). Importantly, knocking out the gene encoding the 5α-reductase type I gene, essential in the synthesis of allopregnanolone, increased ethanol consumption in female mice (Ford et al., 2015). While the relationship between alcohol intake and neurosteroid signalling is complex, the observation that tolerance to the alcohol-induced increase in neurosteroid levels developed in alcohol-dependent animals (Khisti et al., 2005) suggests this adaptation may contribute to the excessive alcohol consumption in such animals (reviewed in Morrow et al. 2006). Detailed investigations into neurosteroid production and its regulation in relevant neuronal loci in rodent lines selectively bred for alcohol consumption are still limited. However, neurosteroid content, sensitivity and synthetic enzyme expression levels (e.g. 5α-reductase) are differentially altered in alcohol-withdrawal seizure-prone and resistant mouse lines (Tanchuck et al., 2009), thus indirectly supporting a dynamic relationship between alcohol, neurosteroids and GABA<sub>A</sub> receptor function. Future targeted studies exploring the role of a range of neurosteroids are necessary to clarify a GABA<sub>A</sub> receptor-mediated role of neurosteroids in alcohol actions (Porcu and Morrow, 2014). In that context it is of interest that that GABRA2 alleles influence the ability of finasteride (5 alpha-reductase inhibitor that decreases levels of GABA<sub>A</sub> receptor active neurosteroids) to decrease the subjective effects of alcohol,
suggesting that certain effects of neurosteroids are mediated by α2-GABA_{A}Rs (Pierucci-Lagha et al., 2005).

4.4 EEG related changes; a potential biomarker for GABRA2 variants?

Electrical activity of the brain measured by EEG and, in particular, β power (brain waves of 15 to 30 Hz) has been proposed as a key endophenotype for alcohol and cocaine dependence (Costa and Bauer, 1997). Further studies have confirmed the increase of beta power in alcoholics (Rangaswamy et al., 2002) but also in individuals with family history of alcoholism (Rangaswamy et al., 2004, Bauer and Hesselbrock, 1993). Variations in GABRA2 have been found to be strongly associated with the β frequency which represents 15–20% of the resting awake EEG in the majority of healthy adults (Edenberg et al., 2004). Furthermore because variations in the GABRA2 gene affect the level of neural excitability, it has been proposed that such increase in neuronal excitability would predispose to alcohol abuse and dependence.

In a cohort of 44 alcohol dependent patients who had been abstinent from alcohol for a minimum of 24 months a significant association between four GABRA2 SNPs (rs548583, rs279871, rs279863, rs279841) and increased EEG β power was found (Lydall et al., 2011). Increased β power has been reported in the EEGs of the offspring of alcohol dependent parents, and in individuals with a family history of alcohol dependence. Recently an association between the GABRA2 gene and beta power was confirmed in a community sample of 4,026 twin adolescents, and parents genotyped for 527,829 SNPs (Malone et al., 2014).

How could an altered GABAergic signal mediated by α2-GABA_{A}R lead to an increase β power EEG? A comparison with our current understanding of the increased power of β oscillations associated with Parkinsonism may be instructive in this respect (Goldberg et al., 2002, Costa et al., 2006). Recent studies in human and in animal models of Parkinson’s disease, combined with computer modelling have suggested that a decrease in lateral inhibition plays a crucial role in driving the increased β power. In these studies, deficits in DA, typically associated with Parkinson’s disease, primarily cause a reduction in lateral inhibition that
appears to provide a crucial drive for the increased power of the $\beta$ oscillation (Damodaran et al., 2014, Damodaran et al., 2015). Thus, it is tempting to speculate that if the effect of the $\alpha_2$ SNPs does indeed translate to a reduced GABA$_{\alpha}$R function, as suggested by recent reports (Lieberman et al., 2015), this could in turn similarly weaken lateral inhibition through the reduced $\alpha_2$-GABA$_{\alpha}$ receptor signal. Such an effect may be particularly evident at synaptic locations along MSN dendrites where GABA would normally facilitate incoming glutamatergic signals to increase MSN firing (see section 3.2.3). Inhibition may also be decreased by the loss of the $\alpha_2$-GABA$_{\alpha}$R signal on the soma of MSNs (which make up an estimated 30% of the synaptic charge) and combined with a reduction in lateral inhibition, these alterations may be sufficient to entrain more MSNs into the $\beta$ frequency range under the strong influence of fast-spiking interneurons firing, as previously demonstrated for both hippocampal and cortical assemblies (Buzsaki and Chrobak, 1995, Gray, 1994). It is also possible that these changes may be accompanied by additional alterations in the MSN intrinsic properties e.g. $K^+$ conductances, which are known to be sensitive to changes in GABA$_{\alpha}$ receptor function (Czubayko and Plenz, 2002, Plenz, 2003) and/or fast-spiking interneurons synchronized firing (Damodaran et al., 2014, Damodaran et al., 2015). Furthermore, a reduction in the $\alpha_2$-GABA$_{\alpha}$ receptor-mediated conductance in interconnected areas e.g. cortex may additionally contribute to the increased power of the $\beta$ oscillation.

5. Conclusions

Investigations of the past decade into the role of GABA$_{\alpha}$ receptors in drug addiction and dependence have begun to uncover the complexity of the modalities whereby different GABA$_{\alpha}$ receptor isoforms orchestrate the integration of dopaminergic and glutamatergic signals within the reward pathway. Although human genetic association studies provide provocative evidence that certain genetic variants are associated with various addictions, the biological link between the genetic variations and addictive behaviour remains largely unexplained. And while animal studies have identified a number of potential behavioural changes following manipulation of specific subtypes of GABA$_{\alpha}$ receptor, integrating those findings into a broader account of addictive behaviour remains a task to be undertaken.
For the moment, we can point to $\alpha_2$-GABA$_A$ receptors as playing an important (albeit unknown) role in striatal inhibition, and to interactions between psychostimulant drugs and reward-conditioned behaviour, while variants in the genes encoding those same receptors appear to be associated in a complex fashion with behavioural traits predisposing (inter alia) to addictive behaviour. A clear answer to the question of whether human $GABRA2$ gene variants have consequences for regulation of $\alpha_2$–GABA$_A$ receptor expression in people is urgently needed, as are tools for understanding the spatial and temporal organisation of the different receptor isoforms within specific neuronal populations and cellular domains, and interrogation of their respective physiological roles within the relevant neuronal networks. Nevertheless, the presently available data are consistent with a role of $\alpha_2$-GABA$_A$ receptors in protecting against factors predisposing to addictive behaviour.

It is a curiosity that while no human genetic association data provide a link between $GABRA4$ polymorphisms and addictions, within animal studies, discrete manipulations of $\alpha_4$-GABA$_A$ receptors have suggested they play an important role in striatal physiology, and that their manipulation has important consequences for motivation to obtain and consume alcohol, but also suggest they play a role in aspects of psychostimulant reward. Similarly, $GABRA1$ polymorphisms have not been identified as associating with human addictions, while animal data suggest that deletion of this subunit decreases alcohol (and sucrose) intake.

Hitherto, the ubiquitous role of GABAergic inhibition across the entire brain, and the non-selective actions of drugs influencing GABAergic transmission has meant that pharmaceutical manipulation of GABAergic systems has been non-specific, both with regard to the neuronal system addressed, and the behavioural consequences of drug treatment. The advances in understanding the particular roles of GABA$_A$ receptor subtype in different aspects of addictive behaviour outlined in this review, now begin to hold promise that it might be worthwhile targeting specific receptor subtypes to influence addictive behaviour. While we are still some way from providing a rational therapeutic approach, it would seem worthwhile to investigate whether selective facilitation of $\alpha_2$-GABA$_A$ receptors might be
useful for controlling addictive behaviours. While such drugs are not yet available clinically, prototypes have been developed (Atack, 2011).

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Figure Legends

Figure 1. The relative expression of GABA_A receptor isoforms in rodent brain and a simplified model of the receptor. GABA_A receptors are made up of five protein subunits, that are arranged to form a central ion channel permeable to chloride and bicarbonate anions. The majority of proteins making up the complex come from 3 related subunit families, named α (1-6), β (1-3) and γ (1-3). Most commonly receptors are made of two members of the α family, two β, and one γ subunit although in some cases the γ subunit may be substituted by a δ subunit. The GABA (G) binding sites are formed by the N-terminal large extracellular domains between the α and β subunit interface and the benzodiazepine (Bz) site is similarly formed by the N-terminal extracellular domains between the α and γ subunit interface. A putative binding site for the neurosteroids (N) is shown within the transmembrane region of the α subunit.

Figure 2. Chromosomal locations of major GABA_A receptor gene clusters. The genes for individual subunits are present in clusters conserved across mammalian genomes. The figure shows the four main gene clusters and their relative locations on human and mouse chromosomes (shown on human and mouse chromosomes in red). The red arrow designates orientation along the chromosome. These clusters are expanded and the arrangement of the genes within them encoding the individual subunits displayed. α subunit genes are shown in red, β subunit genes are blue, γ subunit genes are green. Other GABA_A receptor subunit families are shown in yellow. The subunits are encoded by the following genes: α1, GABRA1; α2, GABRA2; α3, GABRA3; α4 GABRA4; α5, GABRA5; β1, GABRB1; β2, GABRB2; β3, GABRB3; γ1, GABRG1; γ2, GABRG2; γ3, GABRG3; ε, GABRE; θ, GABRQ. Black arrows indicate the direction of transcription for the individual genes.

Figure 3. Synaptic and extra-synaptic GABA_AR isoforms in the reward circuitry. A sagittal section of a rodent brain illustrating the expression profile of both synaptic and extra-synaptic GABA_A receptor isoforms throughout the reward circuitry. Depicted within this circuit are the principal excitatory (green), inhibitory (red) and dopaminergic (blue)
projections. The reported expression of GABA\(_A\)R subtypes is derived from both immunohistochemical and electrophysiological studies (see text).

Note that GABA\(_A\)R isoforms responsible for mediating phasic and tonic inhibition are expressed in a neuron-specific manner throughout the mesolimbic neurocircuitry. For example, in the NAcc, in addition to the \(\alpha4\beta\delta\) subtype, \(\alpha\chi\beta\gamma2\) are expressed extrasynaptically on MSNs. Of this pool, a proportion has been suggested to incorporate the \(\alpha5\) subunit (Lingford-Hughes et al., 2012, Mendez et al., 2013). An extrasynaptic localization of \(\alpha5\)-containing GABA\(_A\)Rs has also been reported for MSNs of the dorsal striatum (Ade et al., 2008), where, however, their developmental expression in rodents has been debated (Janssen et al., 2009, Santhakumar et al., 2010). See also Fig 5 for detail on GABA\(_A\)R isoforms expressed in the NAcc. For simplicity, GABA\(_A\)R isoforms expressed in various regions of the amygdala are not displayed but a discussion can be found in (Marowsky et al., 2012, Herman et al., 2013). Similarly, note that in the VTA \(\alpha1\beta\gamma2\) and \(\alpha3\beta\gamma2\) subtypes are selectively expressed in GABAergic interneurons and dopaminergic neurons respectively (Tan et al., 2010, Okada et al., 2004). A population of extrasynaptic \(\alpha4\beta\delta\) receptors has also been described for VTA GABAergic interneurons (Vashchinkina et al., 2012, Xiao et al., 2007).

**Figure 4. Postulated influence of \(\alpha2\)-GABA\(_A\) receptor activation on control of addiction-related, broad behavioural constructs**

A) Shows normal conditions of non-susceptible brain. \(\alpha2\)-GABA\(_A\)R activation allows control over delinquent behaviour, and potentiates control over motivation to take the drug. The negative sign (-) denotes the general inhibitory effects of GABA in the brain

B) Shows the susceptible brain because of GABRA2 risk variants present which lead to a hypothesised reduction of GABA function. A tendency towards delinquent behaviour is facilitated by weakness in parental monitoring, leading to reduction of control over motivation to take drugs. These behavioural aspects weaken the control over motivation to take the drug

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(Note: We speculatively ascribe a role to GABA<sub>A</sub> receptors in modulating the output between motivation and drug-taking, on the basis of the association of GABRA2 variants with subjective effects of drugs including psychostimulants and alcohol).

Figure 5. Synaptic and extra-synaptic GABA<sub>A</sub>R isoforms expressed in the MSNs and interneurons of the NAcc and the effect of early-life stress and identified SNPs upon their expression and the EEG β power.

NAcc core neurocircuitry and the GABA<sub>A</sub> receptor isoforms expressed in the MSNs and Fast-Spiking Interneurons (FSIs). MSNs receive a powerful inhibitory input on their soma from parvalbumin-containing FSIs, giving rise to feed-forward inhibition (FFI). Additionally, fast-spiking interneurons, which are coupled via gap junctions, send a much weaker input to MSN dendrites. MSNs can inhibit each other via lateral inhibition (LI) and receive powerful glutamatergic inputs from a number of limbic and forebrain regions and dopaminergic inputs from the VTA, largely on their dendritic tree (see Fig 3 for details). Burst DA firing is likely to target synaptic receptors whereas tonic DA firing may affect largely extrasynaptic receptors. DA actions are mediated by D1R and D2R on direct and indirect pathway MSNs respectively although this view has been recently challenges for the NAcc (Kupchik et al., 2015). Changes in lateral inhibition, due to decreased α2 GABA<sub>A</sub>R in rodents (as a result of early life stress, (Lambert, 2013)) or humans (dependent on specific GABRA2 variants e.g. SNP rs279858 (Lieberman et al., 2015)) might be the basis for the increased beta power measured in EEGs (Costa and Bauer, 1997, Rangaswamy et al., 2002, Edenberg et al., 2004, Lydall et al., 2011).
Figure 1
**Figure 4**

**GABRA 2 genetic variants support conditions of risk to drug abuse**

**A Non susceptible brain**

![Diagram of non-susceptible brain](image)

**B Susceptible brain**

![Diagram of susceptible brain](image)
Figure 5