NEURO
PHARMACOLOGY

Blockade of alcohol's amnestic activity in humans by an α5 subtype benzodiazepine receptor inverse agonist

David J. Nutt [a,*], Marie Besson [a], Susan J. Wilson [a],
Gerard R. Dawson [b], Anne R. Lingford-Hughes [b]

[a] *Psychopharmacology Unit, Dorothy Hodgkin Building, University of Bristol, Whitson Street, Bristol, BS1 3NY, UK*
[b] *P1vital LTD., University of Oxford, Warneford Hospital, Department of Psychiatry, Oxford, OX3 7JX, UK*

Abstract

Alcohol produces many subjective and objective effects in man including pleasure, sedation, anxiolysis, plus impaired eye movements and memory. In human volunteers we have used a newly available GABA-A/benzodiazepine receptor inverse agonist that is selective for the α5 subtype (a5IA) to evaluate the role of this subtype in mediating these effects of alcohol on the brain. After pre-treatment with a5IA, we found almost complete blockade of the marked impairment caused by alcohol (mean breath concentration 150 mg/100 ml) of word list learning and partial but non-significant reversal of subjective sedation without effects on other measures such as intoxication, liking, and slowing of eye movements. This action was not due to alterations in alcohol kinetics and so provides the first proof of concept that selectively decreasing GABA-A receptor function at a specific receptor subtype can offset some actions of alcohol in humans. It also supports growing evidence for a key role of the α5 subtype in memory. Inverse agonists at other GABA-A receptor subtypes may prove able to reverse other actions of alcohol, and so offer a new approach to understanding the actions of alcohol in the human brain and in the treatment of alcohol related disorders in humans.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Benzodiazepine; Alcohol; Memory; Inverse agonist; α5 subtype

1. Introduction

The inhibitory effects of natural inhibitory neurotransmitter GABA are positively modulated by agonists such as benzodiazepines and alcohol. Benzodiazepine receptor agonists, such as lorazepam, have been used as treatments for anxiety, insomnia, epilepsy and muscle tension for over 40 years. In addition, they have also been used to induce anterograde amnesia prior to surgical procedures. Alcohol has been used for millennia for similar effects. Most of the GABA$_A$ receptors in the brain contain α, β and γ subunits in a 2:2:1 stoichiometry (Tretter et al., 1997). The BZ binding site occurs at the interface of an α1,

α2, α3 subunit and the γ2 subunit with the α subunit being of particular importance in determining the pharmacology of the benzodiazepine type drugs. Moreover, it has recently been shown that the various effects of benzodiazepine agonists are modulated by different subtypes of the GABA$_A$ receptor depending on which α-subunit it contains (Rudolph and Mohler, 2004). These studies suggest that GABA$_A$ receptors containing an α1 subunit mediate the sedative effects of diazepam whereas α2 or α3 containing receptors mediate its anxiolytic effects. However, the functions of receptors containing an α5 subunit (α5 subtype), which are preferentially expressed in the hippocampus and other limbic regions (Fritschy and Mohler, 1995; Lingford-Hughes et al., 2002) are less well defined although this localisation and evidence from rodent studies suggest that it may play a role in memory (Collinson et al., 2002; Cheng et al., 2006). In addition, the α5 subtype has been

* Corresponding author: Tel.: +44 117 331 3143; fax: +44 117 331 3180.
E-mail address: david.j.nutt@bristol.ac.uk (D.J. Nutt).

implicated in mediating several of the effects of alcohol in rodents, such as liking, motor in-coordination and sedation (June et al., 2001; McKay et al., 2004; Pickering et al., 2007), suggesting that alcohol may act to potentiate the effects of GABA at these receptors. Alcohol has been shown to act as an indirect agonist at the $GABA_A$ receptor complex where it increases the actions of GABA and of other agonist drugs that act on the complex (e.g. benzodiazepines and barbiturates). However alcohol does not bind to either the $GABA_A$ receptor or the benzodiazepine binding site; rather it appears to increase the allosteric coupling between the $GABA_A$ receptor and the chloride ion channel (Wallner et al., 2003).

We have recently demonstrated that the PET tracer, [^{11}C]Ro15-4513, has relative selectivity (10× higher affinity) for the α5- subtype of the $GABA_A$ receptor compared with the other receptor subtypes in both humans and rodents and that the highest density of tracer binding was found in the hippocampus of both species (Lingford-Hughes et al., 2002). Previous work has shown Ro15-4513 to be an inverse agonist at the benzodiazepine receptor which means it has opposite effects to benzodiazepine receptor agonists (e.g. diazepam) in that it decreases, rather than increases the effects of GABA. A number of studies both in vitro and in rodents have shown that Ro15-4513 acts as a functional ''alcohol antagonist'', for it has been shown to significantly block the electrophysiological actions of alcohol and to attenuate both the anxiolytic effects and the behavioural impairment (loss of righting reflex, staggered gait, sedation) produced by alcohol (Suzdak et al., 1986). These actions are presumably mediated via the ability of Ro15-4513 to decrease $GABA_A$-mediated inhibition since they are blocked by the benzodiazepine receptor antagonist flumazenil (Suzdak et al., 1986) and mimicked to some extent by other inverse agonists, e.g. FG 7142 (Nutt and Lister, 1988). However though Ro 15-4513 shows 10-fold selectivity for the α5 subtype (Lingford-Hughes et al., 2002) it is likely that at the doses used in these alcohol reversal experiments it was acting at all the four subtypes (α1, 2, 3, 5) at which it displays inverse agonist actions.

It is not possible to use Ro15-4513 in humans as it has not been through toxicological studies and its inverse agonism at the α2 and α3 subtypes might be predicted to cause anxiety like that found with another non-selective inverse agonist FG 7142 (Horowski and Dorrow, 2002). However recently a compound, a5IA, has been synthesised that shows high affinity but functional selectivity at benzodiazepine receptor subtypes, being an inverse agonist at the α5 subtype and an antagonist at the other subtypes (Sternfeld et al., 2004).

In view of the possible role of the α5 subtype in the amnestic and sedative effects of alcohol and liking for it, we have assessed the ability of this new functionally selective α5 receptor inverse agonist, a5IA, on the effects of alcohol in humans. In this pilot study, a broad range of measures were chosen to detect the major actions of alcohol intoxication such as amnesia, psychomotor function, sedation and pleasure. Specifically we expected that the α5 inverse agonist would reduce the subjective effects of alcohol, particularly those involving pleasure or 'urge to drink'. We also predicted that the α5 inverse

agonist would have less effect on the impairment of objective effects, e.g. saccadic eye movements, due to the low abundance of these α5 receptors in the main brain regions implicated in the control of eye movements.

2. Methods

This was a double-blind placebo-controlled crossover study approved by the research ethics committee and was carried out to Good Clinical Practice guidelines. Twelve male volunteers who regularly drink between 20 and 40 units a week (UK; mean ± SD 27 ± 6 units/week), equivalent to 13−26 standard (US) drinks a week were recruited. All but one scored 8 or more on the Alcohol Use Disorders Identification Test (Saunders et al., 1993), which is the threshold for likelihood of alcohol misuse disorder (11 ± 3). However the mean score on the Severity of Alcohol Dependence questionnaire (Stockwell et al., 1983) was very low (4 ± 4). They were all students undertaking postgraduate degrees (two undergraduate) or post-doctoral researchers with a mean age of 24.6 ± 4.2 years. After informed consent, screening with medical, psychiatric and drinking history, physical examination, routine biochemistry and haematology and urine screen for drugs of abuse was carried out. All the subjects were healthy with average body weight 80 ± 14 kg and had normal physical examination and routine biochemistry and haematology tests. Their urinalysis for drugs of abuse were negative. Subjects completed the Spielberger State and Trait Anxiety Inventory (Spielberger et al., 1970) and Beck Depression Inventory (Beck et al., 1961). They were not anxious or depressed (SSAI 34.8 ± 8; STAI 28 ± 6; BDI 4 ± 4). They then attended our research centre for 2 days, a week apart. Each subject received either placebo or a5IA in a randomised order. They were asked not to consume any alcohol over the 24 hours before the testing days and to have a low fat breakfast on each study day.

On the study day they were checked for eligibility with health check, urine drug screen and breathalyser. We measured saccadic eye movements (SEMs) to a target (for method, see Wilson et al., 1993), antisaccades and smooth pursuit eye movements (Holdstock and de Wit, 1999). They completed subjective questionnaires: the Subjective High Assessment Questionnaire (SHAS; (Schuckit, 1980)), the Biphasic Alcohol Effects Scale (BAES; (Martin et al., 1993)) and the Alcohol Urge Questionnaire (Bohn et al., 1995) and visual analogue scales of mood, and performed a specific test of motor coordination and sedation (zig-zag task; Acons et al., 2006). They were then dosed with 4 mg of a5IA or placebo. We chose this dose based on previous studies including one with [^{11}C]flumazenil PET.

Two hours after dosing (peak drug effect based on previous studies) subjects were given alcohol, 0.8 g/kg in the form of vodka, with sparkling orange juice to make up the volume to 400 ml. This is approximately equivalent to 6.5 ounces of 80 proof vodka for a 160 lb man. They consumed the drink in 8 equal aliquots, one every 2 min. After this they were allowed one small measure of their favourite alcoholic drink with some small snacks, e.g. peanuts, potato chips, because we were interested in measures of alcohol liking and urge to drink, and we wanted the latest alcohol to be their preferred drink. We measured breath alcohol, saccadic and smooth pursuit eye movements and subjective ratings regularly for 2 h after drinking finished (see Table 1).

One hour after drinking finished, they were given a printed list of 20 words and told to memorize them and that they would be asked to recall them after 30 min. The zig-zag task was then repeated and the eye movement and questionnaire ratings continued. Thirty minutes after memorizing the list, they were asked to write down as many of the words as they could recall. Lists on the two occasions were matched for length and number of syllables, and contained 10 neutral, 5 positive and 5 negative words with matching emotional ratings.

3. Results

3.1. Effects of a5IA alone

a5IA alone had minimal or no effect on the range of motor and subjective measures when baseline measures were compared with those taken at expected peak a5IA levels at 2 h

Table 1
Timing of observations and measures taken

Observations and measures undertaken	Time from taking medication (time after end of alcohol consumption)																	
	−45 min	−20 min	0	15 min	30 min	1 h	1 h 30 min	1 h 45 min	2 h	2 h 05 min	2 h 45 min (15 min)	3 h (30 min)	3 h 15 min (45 min)	3 h 30 min (60 min)	3 h 45 min (75 min)	4 h (90 min)	4 h 15 min (105 min)	4 h 30 min (120 min)
Breathalyse			Medication taken							Alcohol consumed over next 30 min	✓	✓	✓	✓	✓	✓	✓	✓
Subjective ratings																		
SHAS		✓									✓	✓		✓		✓		✓
BAES		✓						✓			✓	✓		✓		✓		✓
AUQ		✓						✓			✓	✓				✓		✓
Objective measures: SEM																		
Target	✓								✓								✓	✓
Anti-	✓								✓								✓	✓
Smooth pursuit	✓			✓	✓	✓	✓		✓								✓	✓
Cardiovascular: BP, HR	✓			✓	✓	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓	✓
Adverse event enquiry																✓		
Zig-zag task	✓											✓						
Memorize word list		✓										✓						
Recall word list																✓		

(see Tables 2 and 3). Thus all the parameters derived from saccadic eye movements (SEMs) including peak velocity and deceleration, smooth pursuit lead time and number of saccadic intrusions were not affected by a5IA (see Table 3, Fig. 1). Ratings from the 'subjective high assessment scale' (SHAS: total) and 'biphasic alcohol effects scale' (BAES) 'up' and 'down' were not altered by a5IA apart from a slight increase in dizziness and nausea subscales at the expected peak time of the drug's effects (see Table 2). Dizziness was also mentioned when asked about adverse events, but there were no others. There was no effect on the 'urge to drink' (AUQ). Cardiovascular indices, systolic and diastolic blood pressures and heart rate were not significantly altered by a5IA (see Table 3).

3.2. Effects of alcohol alone

Consumption of alcohol increased breath alcohol levels to expected levels (Fig. 1; see Table 2). The first breathalyser reading was taken within 15 min of ingesting alcohol, therefore it is likely that some residual breath alcohol contributed to the reading. As predicted, alcohol alone (i.e. with placebo) affected many subjective measures and motor tasks. We compared the data collected just prior to consumption of alcohol with data from all subsequent time-points. All the parameters derived from SEMs were significantly ($p < 0.05$) impaired by alcohol (see Table 3 and Fig. 2). Alcohol consumption significantly reduced systolic and diastolic blood pressures and there was a trend of increased heart rate (see Table 3). The SHAS 'total' and BAES 'up' but not 'down' ratings were significantly increased by alcohol (see Table 2). The SHAS dizzy and nausea subscales were also not significantly affected however there was a significant increase in 'sleepy'. The 'urge to drink' was significantly increased after consuming alcohol (see Table 2).

In addition performance on the zig-zag task was affected with a significant increase in errors but not in time (see Table 3). Alcohol's effect on memory is evident by the lower number of words recalled (5.33 ± 0.9 words, from a list of 20 words; see Table 3) which is about half of what is found in a normal population (Soo-Ampon et al., 2004).

3.3. Effects of alcohol with a5IA pre-treatment

The active drug, a5IA, had no effect on the breath alcohol time−activity curve which at 30 min after ingestion reached 133 ± 24 mg/ml breath alcohol compared with 138 ± 35 mg/ml breath alcohol with placebo (also see Fig. 2).

After pre-treatment with a5IA, the subjective effects of alcohol consumption were unchanged (see Table 2). Data were analysed comparing pre-alcohol with all the post-alcohol time points as well as analysis of all time points from baseline to the end of the study. Of note, the increase in 'urge to drink' by alcohol was not altered by a5IA pre-treatment (see Table 2). The effects of alcohol significantly increasing SHAS total and BAES 'up' ratings were also not modified by a5IA pre-treatment, including those on dizziness, nausea and sleepy (see Table 2, Fig. 2). In addition the effects of alcohol

Table 2
Subjective measures

	Baseline T = 0		Peak a5IA T = 1 h 45 min		Post alcohol consumption								Drug effect[a]	Alcohol effect[b]	Alcohol × drug effect[c]
	P	A	P	A	2 h 45 m (15 min)		3 h 30 min (60 min)		4 h (90 min)		4 h 30 min (120 min)				
					P	A	P	A	P	A	P	A	F, p	F, p	F, p
Breath alcohol (mg/100 ml)	0	0	—	—	—	—	108 ± 37	111 ± 35	83 ± 28	103 ± 40	63 ± 29	89 ± 26			
AUQ	13.9 ± 6	13.3 ± 6	15.2 ± 7	13.0 ± 5.5	27.7 ± 11.5	25.1 ± 11.3	22.2 ± 10.7	18.7 ± 9.5	17.9 ± 5.6	14.4 ± 6.4	14.1 ± 4.9	12.8 ± 3.9	ns	9.5, 0.01	ns
SHAS total	67.5 ± 58.7	76.5 ± 64.1	62.2 ± 74.6	87.5 ± 64.4	273.3 ± 209.4	305.6 ± 212.7	240.3 ± 221.3	235.4 ± 204.1	202.5 ± 186.2	182.7 ± 179.6	165.3 ± 182.1	129.7 ± 142.7	ns	8.6, 0.14	ns
SHAS dizzy	3.4 ± 3.8	2.5 ± 3	3.2 ± 6.5	10.1 ± 11.7	12.7 ± 15	17.3 ± 20.5	12.3 ± 19.6	11.6 ± 13.2	10.8 ± 23.8	10.6 ± 12.9	8.7 ± 18.4	9.3 ± 14.6	8.8, 0.013	ns	ns
SHAS nausea	2.9 ± 3.4	1.4 ± 2.7	2.8 ± 3.7	8.7 ± 11.9	4.8 ± 8	6.8 ± 8.2	3.7 ± 4.8	5.7 ± 6.3	4.3 ± 5.5	3.8 ± 6	4.2 ± 5.2	3.1 ± 5.5	5.9, 0.03	ns	ns
SHAS sleepy	16.0 ± 17.4	13.8 ± 15.7	18.2 ± 20.5	12.4 ± 11.3	10.4 ± 13.6	14.2 ± 22.4	34.2 ± 30.6	26.2 ± 31.6	40.1 ± 33.2	28.1 ± 30.8	33.4 ± 30.2	18.3 ± 25.1	ns	5.83, 0.03	ns
BAES: up	5.4 ± 4.7	5.4 ± 6.1	6.6 ± 8.0	7 ± 6.5	9.2 ± 9.2	13.2 ± 10.4	11.4 ± 9.4	12.5 ± 10.1	13.3 ± 11.7	12.6 ± 11.4	12.5 ± 11.8	11 ± 10.0	ns	27.6, <0.001	ns
BAES: down	5 ± 5.3	5.3 ± 6.1	5.9 ± 9.2	7.2 ± 6.8	9 ± 9.1	12.7 ± 11.4	11.9 ± 9.8	12.7 ± 11.4	14 ± 12.0	12.7 ± 12.2	13.2 ± 12.7	11.3 ± 10.9	ns	ns	ns

P, placebo; A, a5IA.

[a] Comparison between data collected at baseline and at expected peak drug effect (2 h).

[b] Comparison between data collected at 2 h (just prior to alcohol consumption) with all subsequent time points for alcohol + placebo.

[c] Comparison between data collected at 2 h with all subsequent time points.

Table 3
Objective measures

	Baseline T = 0		Peak a5IA T = 2 h		Post alcohol consumption								Drug effect[a]	Alcohol effect[b]	Alcohol × drug effect[c]
					2 h 45 min (15 min)		3 h 30 min (60 m)		4 h (90 m)		4 h 30 min (120 min)				
	P	A	P	A	P	A	P	A	P	A	P	A	F, p	F, p	F, p
Breath alcohol (mg/100 ml)	0	0	—	—	—	—	108 ± 37	111 ± 35	83 ± 28	103 ± 40	63 ± 29	89 ± 26			
Words recalled (correct)									5.3 ± 3.1	8.4 ± 1.9					P < 0.001
Words recalled (incorrect)									1.4 ± 1.1	0.8 ± 1.1					ns
Zig-zag task time (s)	73 ± 31	71 ± 27					67 ± 30	67 ± 29					ns	ns	ns
Zig-zag task no. errors	13.5 ± 6	12 ± 5					15.5 ± 10	12 ± 7					ns	ns	ns
Target saccades peak velocity (deg s^{-1})	546 ± 63	543 ± 101	529 ± 73	552 ± 50	446 ± 56	471 ± 63	450 ± 37	463 ± 60	450 ± 67	473 ± 60	450 ± 64	480 ± 55	ns	9.6, 0.011	ns
Target saccades peak deceleration (deg s^{-2})	−59 ± 14	−61 ± 16	−54 ± 15	−62 ± 12	−39 ± 8	−44 ± 13	−40 ± 6	−43 ± 12	−40 ± 11	−44 ± 12	−40 ± 11	−45 ± 10	ns	6.8, 0.026	ns
Smooth pursuit, lead time (ms)	41 ± 18	40 ± 15	47 ± 26	47 ± 20			50 ± 26	58 ± 30			61 ± 33	53 ± 19	ns	ns	ns
Smooth pursuit, no. saccadic intrusions	25 ± 21	23 ± 18	22 ± 27	27 ± 20			49 ± 20	47 ± 25			43 ± 23	41 ± 27	ns	25.8, 0.001	ns
Systolic blood pressure (mmHg)	132 ± 15	129 ± 15	126 ± 15	131 ± 14	128 ± 18	135 ± 13	123 ± 16	130 ± 14	119 ± 13	124 ± 16	118 ± 12	125 ± 13	ns	5.97, 0.033	ns
Diastolic blood pressure (mmHg)	72 ± 2	70 ± 2	69 ± 9	73 ± 10	69 ± 7	75 ± 9	64 ± 8	66 ± 9	62 ± 6	66 ± 9	66 ± 8	64 ± 9	ns	4.83, 0.05	ns
Heart rate (bpm)	72 ± 11	76 ± 14	58 ± 11	63 ± 15	66 ± 9	71 ± 10	73 ± 13	77 ± 15	73 ± 11	75 ± 10	71 ± 12	76 ± 11	ns	ns	ns

[a] Comparison between data collected at baseline and at expected peak drug effect (2 h).
[b] Comparison between data collected at 2 h (just prior to alcohol consumption) with all subsequent time points for alcohol + placebo.
[c] Comparison between data collected at 2 h with all subsequent time points.
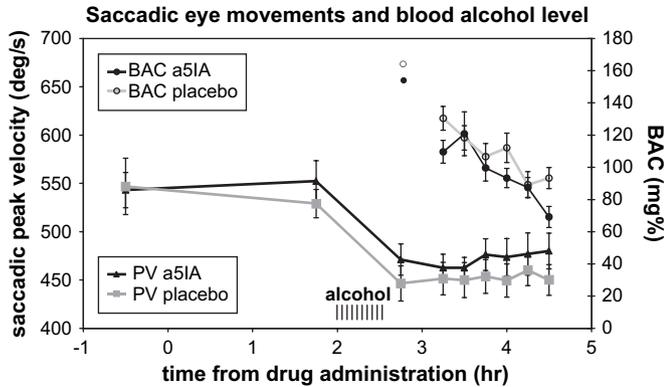
Fig. 1. Effect of alcohol and a5IA on peak velocity target SEM and breath alcohol levels. No effect of a5IA on peak velocity of target saccades, nor does a5IA modify the impairment induced by alcohol. Mean ± SD.

BAES 'down' were not significantly altered by a5IA pre-treatment.

Concerning the objective measures, a5IA pre-treatment did not significantly alter the response to alcohol. All parameters of saccadic eye movements were still significantly impaired after consuming alcohol (see Table 3). The effects of alcohol on systolic and diastolic blood pressures and heart rate were also not modified by a5IA pre-treatment. Similarly the time taken to complete the zig-zag task or the number of errors after drinking alcohol was not significantly altered by a5IA (see Table 3).

The major result of this study was that, by contrast, the pronounced effect of alcohol on memory was attenuated by a5IA (see Table 3). Pre-treatment with a5IA was associated with significantly ($p < 0.01$) more words recalled (8.42 ± 0.5; see Fig. 3a) than with alcohol alone (5.33 ± 0.9). There was a significant ($p < 0.001$) negative correlation between performance on alcohol and placebo and improvement on alcohol and active drug (see Fig. 3b) showing that those most impaired by alcohol improved the most. There was no difference in the
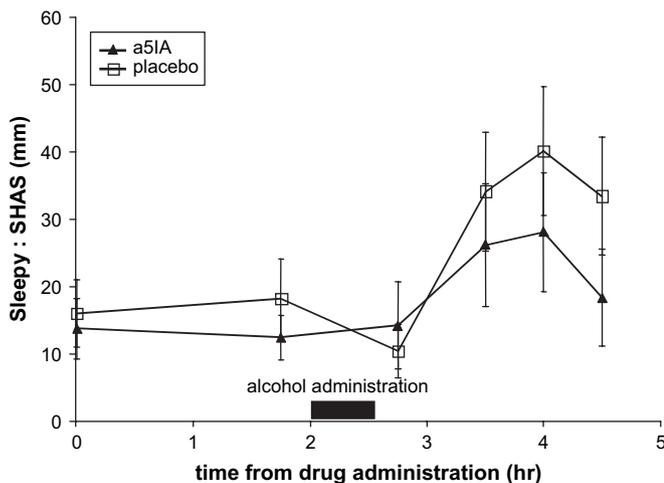
breath alcohol levels between active and placebo days at time of learning the word list (placebo: 138 ± 10 vs a5IA: $133 \pm 7/100$ ml). There was no significant correlation between words recalled and sleepiness rating at the same time point under neither placebo conditions (correlation coefficient: -0.168; $p = 0.603$) or under active a5IA (-0.125; $p = 0.7$). If taken as one group (i.e. if effect was only mediated through sleepiness) a non-significant correlation was also seen (-0.226; $p = 0.289$).

4. Discussion

We have shown that pre-treatment with an inverse agonist at the α5 subtype, a5IA, markedly reduces alcohol's amnestic effect on learning a word list and partially attenuates the sedative actions of alcohol without affecting other functional or subjective impairments. Word list learning involves hippocampal processing (Eichenbaum, 2004) and the attenuation by a5IA of alcohol induced anterograde amnesia is consistent with the very high density of the α5 subtype-containing receptor in this brain region (Fritschy and Mohler, 1995; Lingford-Hughes et al., 2002; Wainwright et al., 2000). Additionally the observation that the antagonism produced by a5IA was proportional to the degree of impairment produced by alcohol suggests a true pharmacological antagonism rather than a simple memory improving action in the opposite direction. This implies that a5IA was not improving memory directly but rather antagonising the actions of alcohol.

Increasing evidence from animal studies suggests that the α5 subtype of the GABA-A receptor is important in learning and memory, though the α1 may also play a role (Rudolph et al., 1999; Mintzer and Griffiths, 1999). For instance mice in which the function of the α5 subtype has been attenuated by knock-out or knock-in strategies, show enhanced learning in tasks involving the hippocampus but not in a non-hippocampal learning or anxiety task (Collinson et al., 2002; Cheng et al., 2006; Crestani et al., 2002). In rodents inverse agonists selective for the α5 subtype have been shown to improve memory and learning (Sternfeld et al., 2004; Dawson et al., 2006). In humans, the α5 subtype has been implicated in changes in cognitive function with ageing and Alzheimer's disease (Rissman et al., 2004). However another study in man showed they were spared (Howell et al., 2000) and a study of aging in rats showed similar preservation of high α5 levels (Yu et al., 2006) suggesting α5 is a potential therapeutic target early in the process of declining cognition. This compound, a5IA, did not improve normal memory function in other experiments (MSD, unpublished observations).

Whilst overall only 5% of the brain's GABA-A receptors contain the α5 subunit, they constitute approximately 25% of the GABA-A receptors in the hippocampus. They are localized primarily to extrasynaptic regions on cell bodies and apical dendrites of glutamatergic pyramidal neurons in the CA1 and CA3 hippocampal regions (Brunig et al., 2002) and mediate tonic inhibitory currents rather than synaptic, transient neurotransmission (Caraiscos et al., 2004). It may be that the α5 subtype is therefore only active in situations when GABA

Fig. 2. Effect of alcohol and a5IA on self-reported sleepiness from SHAS. The sleepiness inducing actions of alcohol are partially attenuated by a5IA. Mean ± SD.
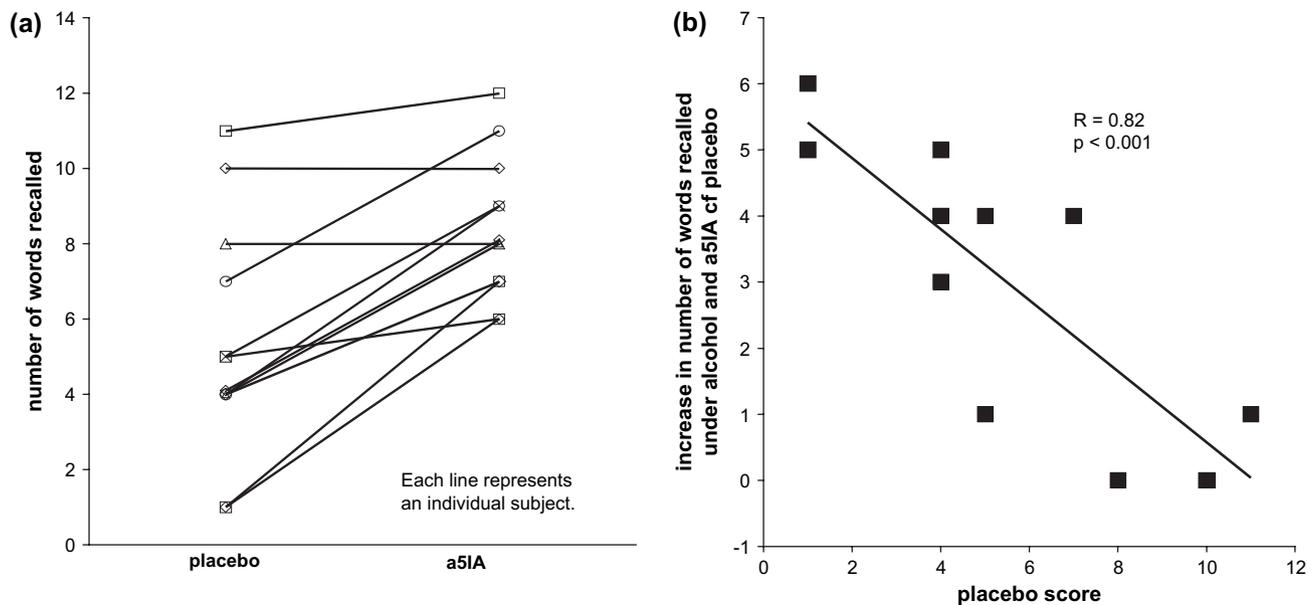
Fig. 3. Effect of alcohol and a5IA on word recall.

concentrations are increased or its function enhanced (e.g. by alcohol). Activity of the glutamatergic pyramidal neurons is strongly linked to long-term potentiation (LTP), a process involved in laying down memories, and alcohol has been shown in pre-clinical models to inhibit LTP (Blitzer et al., 1990). Although alcohol also reduces glutamate release and antagonises NMDA function, evidence suggests that the action of alcohol and benzodiazepines to interfere with LTP occurs pre-synaptically to glutamate release (Shimizu et al., 1998). Therefore activation of these extra-synaptic $\alpha 5$ receptors, e.g. by a benzodiazepine agonist or alcohol may contribute to modulation of LTP. We suggest that a5IA acting as an inverse agonist at $\alpha 5$ receptor allows more LTP and therefore can offset the increase in GABA agonist function produced by alcohol, so restoring memory capability.

The receptor subtype at which GABA-A receptor agonists such as benzodiazepines and alcohol act to cause sedation is not fully characterised but the $\alpha 1$ is thought to play a significant part in the actions of the benzodiazepine receptor hypnotics, e.g. zolpidem (Rudolph and Mohler, 2004). Our data suggest that, in part, alcohol-induced sedation in humans may be mediated via the $\alpha 5$ subtype as it seems to be in some but not all rodent models (Cheng et al., 2006; Cook et al., 2005; Stephens et al., 2005). Whether this action is at the level of the hippocampus or elsewhere in the brain is not known. However in this study, a5IA itself did not significantly increase arousal or reduce sleepiness prior to the administration of the alcohol (see Table 2 and Fig. 2).

Our findings do not necessarily prove that alcohol acts to impair memory only via the $\alpha 5$ subtype receptor and it is possible that alcohol acts "upstream" to the brain site at which a5IA is acting, perhaps through an action on $\alpha 4\delta$ subtype receptors (Wei et al., 2004; Wallner et al., 2006) or through augmentation of glutamate function (Carta et al., 2003). Decreasing GABA-A function "downstream" in the hippocampus

by $\alpha 5IA$, probably in the CA3 region where the $\alpha 5$ receptors are most dense could then offset this action (see Fig. 4). If this is true then $\alpha 5IA$ might be able to antagonise the effects of other drugs that impair memory such as anaesthetics and scopolamine, something that could be investigated in future studies.

We cannot eliminate the possibility that the trend effect of a5IA to attenuate sedation was a contributory factor to the effect on memory, although if replicable this is would be of interest itself as a demonstration of the reversal of any effect of alcohol by inverse agonists in humans. Indeed Savic et al. (2007) have shown an $\alpha 5$ agonist to induce some sedation, so the attenuating effects of a5IA would be consistent with this study. However Schuckit et al. (2000) commented that some items, such as sleepiness (and nausea) in the SHAS could be affected by environmental events outside the study (e.g. how well they slept the night before) and therefore introduced large variance. This also happened in our study with one individual describing poor sleep the previous night. No significant effect of alcohol was seen on the 'down' or sedative items of the BAES scale. There was also no correlation between sedation scores and performance on the memory task. In addition other studies with sedating benzodiazepine agents have found no direct relation between sedation and memory impairment (Curran et al., 1995; Curran, 1991; Huron et al., 2002; Buffett-Jerrott et al., 1998).

We also hypothesised that a5IA might reduce alcohol liking since in rodents selective $\alpha 5$ inverse agonists have been shown to reduce alcohol self-administration and reinforcement potential (June et al., 2001; McKay et al., 2004). Furthermore $\alpha 5$ subtype knock-out mice had also been shown to have reduced liking for alcohol, whereas the $\alpha 1$ and $\alpha 2$ subtypes have not been shown to be involved in alcohol preference (Boehm et al., 2004). However we did not find that a5IA modified the subjects' reports about alcohol's pleasurable effects or
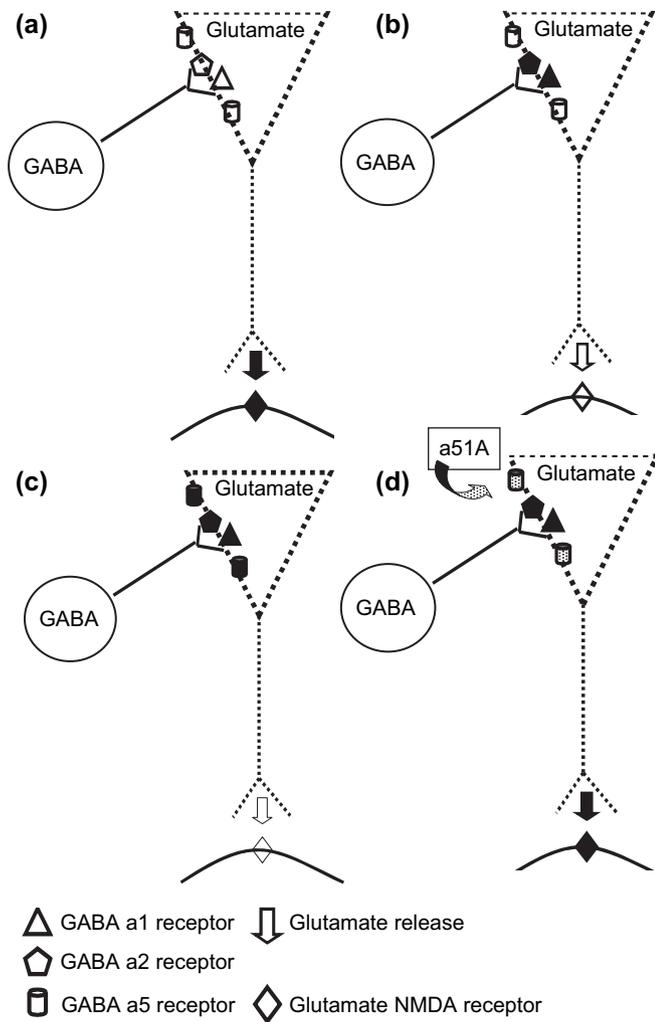
Fig. 4. Relationship between hippocampal glutamatergic and GABA-ergic neurons. (a) Glutamate release activates NMDA receptor. (b) Activation of synaptic $\alpha1$ or a2 receptors on the glutamatergic pyramidal cell reduces glutamate release and NMDA receptor activation. (c) Activation of extra-synaptic a5 receptors by alcohol directly or GABA spill-over further reduces glutamatergic activity. (d) a51A is able to reverse the inhibitory effect of GABA on post-synaptic receptors resulting in greater glutamatergic activity and NMDA receptor activation. Open symbols, resting; solid symbols, active, full agonism; partially filled symbols, active, inverse agonism.

subtype is not essential for ethanol reward but does reduce 'drug seeking' behaviour. In our study, we chose to study heavy experienced social drinkers rather than alcohol dependent patients to avoid the complications of dependence, including brain damage. It would be interesting to determine the effect of a5IA in alcohol dependent subjects.

There are several possible reasons why a5IA's inverse agonism at the $\alpha5$ subtype did not modify any of the other measured effects of alcohol. First, these actions of alcohol are likely to be mediated by interactions at GABA-A receptor subtypes other than $\alpha5$. For instance there is good evidence in rodents that the $\alpha2$ and $\alpha3$ subtypes mediate benzodiazepine anxiolysis (Rudolph and Mohler, 2004). There is accumulating evidence from knock-out mice studies on $\alpha1$, $\alpha2$, $\alpha6$ and $\alpha1$ subunits (Boehm et al., 2004; Blednov et al., 2003; Mihic et al., 1997) and electrophysiological studies on $\alpha4\beta3\delta$ and $\alpha6\beta3\delta$-containing receptors (Wallner et al., 2003) that various subtypes also mediate some of the effects of alcohol. Alternatively, the lack of effect of a5IA on most of the other actions of alcohol could also be because these are mediated by interactions with other neurotransmitters such as glutamate, where alcohol reduces excitatory drive (Carta et al., 2003b).

Whilst inverse agonism at the $\alpha5$ subtype of the GABA-benzodiazepine receptor is the unique aspect of a5IA's pharmacology, it is also an antagonist at the $\alpha1$, $\alpha2$ and $\alpha3$ subtypes (Sternfeld et al., 2004). It seems unlikely that such antagonism contributed to any of the effects seen since flumazenil, the non-selective benzodiazepine receptor antagonist does not generally reverse or oppose the actions of alcohol in animal models (Suzdak et al., 1986; Koob et al., 1986). In humans, flumazenil has been shown not to alter the subjective effects of alcohol intoxication, including sedation, nor reverse psychomotor impairment (Fluckiger et al., 1988; Klotz et al., 1986). Importantly, flumazenil has no effect on explicit memory tasks (Hommer et al., 1993). Although flumazenil has not been directly tested on the effects of alcohol in an explicit memory paradigm in humans it seems unlikely that antagonism at the $\alpha1$, $\alpha2$ and $\alpha3$ subunit-containing receptors rather than inverse agonism at the $\alpha5$ subunit-containing receptor can explain the effects of a5IA on alcohol-induced amnesia, since all the preclinical evidence points to inverse agonism rather than antagonism being necessary to reverse the actions of alcohol.

It is possible that the more global antagonism of the actions of alcohol in rodents as produced by Ro15-4513 might reflect the inverse agonism of this compound at the other receptor subtypes as it acts as an inverse agonist at $\alpha1$, $\alpha2$, $\alpha3$ and $\alpha5$ subtype receptors—though it acts as an agonist at $\alpha6$ and $\alpha4$ subtypes (Huang et al., 2000; Knoflach et al., 1996) and possibly as an antagonist at the $\alpha4\delta$ subtype (Wallner et al., 2006). It is noteworthy that in the original Ro15-4513 study, Suzdak et al. (1986) used cortical synaptosomes where few $\alpha5$ subtype receptors are present yet still reported large effects of Ro15-4513, suggesting possible $\alpha1$ inverse agonist actions. It is possible that higher doses of a5IA might have led to an action on functions that are impaired by alcohol acting in brain regions in which $\alpha5$ receptor

their urge to drink more alcohol. This study did not aim to reproduce animal models of preference and it may be that the paradigm and questions used are not sufficiently powerful to detect any effect of a5IA or that a more efficacious inverse agonist would be required. A more appropriate test may be to determine if a5IA reduces how much alcohol is drunk by subjects with free access to alcohol as has been shown for haloperidol (Enggasser and de Wit, 2001). Consistent with this proposal is recent pre-clinical evidence that given free access to ethanol, $\alpha5$ subtype knock-out mice consumed less ethanol at high but not low ethanol concentrations, than wild-type mice (Stephens et al., 2005). In addition, a related inverse agonist to the one we have used, a5IA-II was reported to reduce instrumental responding at higher doses for ethanol but not for sucrose (Stephens et al., 2005). They concluded that the $\alpha5$

density is lower, e.g. frontal cortex. Future experiments with a range of doses of a5IA could be helpful in resolving these issues although it would be surprising if all the actions of alcohol could be explained by an action at the α5 subtype. It would therefore be of great interest to determine if inverse agonists selective to other benzodiazepine receptor subtypes would antagonise these other actions of alcohol. Unfortunately compounds with these characteristics have not yet been described but it is to be hoped that our findings will encourage a search for such molecules as they could prove invaluable in understanding the nature of the action of alcohol in the brain. They might also lead to new therapies for alcohol intoxication and possibly alcohol abuse and dependence which could have considerable public health consequences.

Lastly, these findings also have important implications for understanding more about LTP and about hippocampal memory impairment associated with disorders other than alcoholism such Alzheimer's dementia and temporal lobe epilepsy where the α5-subtype is a potential therapeutic target (Howell et al., 2000; Houser and Esclapez, 2003). In addition the effects of anaesthetics on memory have been shown to involve the α5-subtype (Cheng et al., 2006). Indeed a compound such as a5IA may have therapeutic potential as a cognition enhancer as the role of α5-containing GABA-A receptors in the frontal cortex are similar to those in the hippocampus, i.e. they are extrasynaptic and modulate pyramidal glutamatergic neuronal activity (Guidotti et al., 2005).

It needs to be determined whether the a5IA effect is on encoding or retrieval of memory. A recent study of a compound structurally similar to a5IA, a5IA-II, improved performance in a hippocampal task, the Morris water maze, by affecting encoding and recall (Collinson et al., 2006). We are unable to determine this because in our study both drugs were given prior to learning and recalling the word list, although we believe—based on the known effects of alcohol and benzodiazepines—that disruption of acquisition is most likely. Comparable levels of alcohol given prior to an explicit memory task, as in the current study, have been shown to interfere primarily with encoding but also some aspects of recall (Parker et al., 1981; Duka et al., 2001; Weissenborn and Duka, 2000). The number of words recalled in our study, about 5, is similar to that previously reported (Soo-Ampon et al., 2004). Interestingly, in contrast to this anterograde impairment, retrograde facilitation is seen when alcohol is consumed immediately after learning a word list (Parker et al., 1981; Birnbaum et al., 1978; Parker et al., 1980). The effects of benzodiazepine receptor agonists are more complex and, depending on the paradigm, have been shown to impair encoding and have limited effect or facilitate retrieval on explicit memory tasks (Carta et al., 2003; Lister, 1985). Therefore we suggest that a5IA is modifying encoding rather than retrieval in this hippocampal explicit memory task. It would also be of interest to examine whether treatment with a5IA after ingestion of alcohol would reverse alcohol's amnestic actions. Alcohol has been shown to impair other hippocampal tasks, e.g. spatial memory and therefore the effects of a5IA on these need to be explored. Since α5 receptors play an important role in modulating glutamatergic

function in the hippocampus, it is possible that a5IA could antagonise or reverse memory impairment induced by any activity or drug that reduces glutamatergic function in these critical neurons. Therefore alcohol-induced memory impairment may serve as a useful model for investigating the relative roles of glutamate and GABA when used in combination with drugs modifying GABA or glutamate function.

References

Acons, K., Chan, L.S., Drummond, G., Tiplady, B., 2006. Effects of ethanol and promethazine on awareness of errors and judgements of performance. J. Psychopharmacol. 20, 661−669.

Beck, A.T., Ward, C.H., Mendelson, M., Mock, J.E., Erbaugh, J., 1961. An inventory for measuring depression. Arch. Gen. Psychiatry 4, 561−571.

Birnbaum, I.M., Parker, E.S., Hartley, J.T., Noble, E.P., 1978. Alcohol and memory: retrieval processes. J. Verbal Learn. Verbal Behav. 17, 325−335.

Blednov, Y.A., Jung, S., Alva, H., Wallace, D., Rosahl, T., Whiting, P.J., Harris, R.A., 2003. Deletion of the alpha1 or beta2 subunit of GABAA receptors reduces actions of alcohol and other drugs. J. Pharmacol. Exp. Ther. 304, 30−36.

Blitzer, R.D., Gil, O., Landau, E.M., 1990. Long-term potentiation in rat hippocampus is inhibited by low concentrations of ethanol. Brain Res. 537, 203−208.

Boehm, S.L., Ponomarev, I., Jennings, A.W., Whiting, P.J., Rosahl, T.W., Garrett, E.M., Blednov, Y.A., Harris, R.A., 2004. gamma-Aminobutyric acid A receptor subunit mutant mice: new perspectives on alcohol actions. Biochem. Pharmacol. 68, 1581−1602.

Bohn, M.J., Krahn, D.D., Staehler, B.A., 1995. Development and initial validation of a measure of drinking urges in abstinent alcoholics. Alcohol Clin. Exp. Res. 19, 600−606.

Brunig, I., Scotti, E., Sidler, C., Fritschy, J.M., 2002. Intact sorting, targeting, and clustering of gamma-aminobutyric acid A receptor subtypes in hippocampal neurons in vitro. J. Comp. Neurol. 443, 43−55.

Buffett-Jerrott, S.E., Stewart, S.H., Teehan, M.D., 1998. A further examination of the time-dependent effects of oxazepam and lorazepam on implicit and explicit memory. Psychopharmacology (Berlin) 138, 344−353.

Caraiscos, V.B., Elliott, E.M., You, T., Cheng, V.Y., Belelli, D., Newell, J.G., Jackson, M.F., Lambert, J.J., Rosahl, T.W., Wafford, K.A., MacDonald, J.F., Orser, B.A., 2004. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. Proc. Natl. Acad. Sci. U.S.A. 101, 3662−3667.

Carta, M., Ariwodola, O.J., Weiner, J.L., Valenzuela, C.F., 2003. Alcohol potently inhibits the kainate receptor-dependent excitatory drive of hippocampal interneurons. Proc. Natl. Acad. Sci. U.S.A. 100, 6813−6818.

Cheng, V.Y., Martin, L.J., Elliott, E.M., Kim, J.H., Mount, H.T., Taverna, F.A., Roder, J.C., MacDonald, J.F., Bhambri, A., Collinson, N., Wafford, K.A., Orser, B.A., 2006. Alpha5GABAA receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. J. Neurosci. 26, 3713−3720.

Collinson, N., Kuenzi, F.M., Jarolimek, W., Maubach, K.A., Cothliff, R., Sur, C., Smith, A., Otu, F.M., Howell, O., Atack, J.R., McKernan, R.M., Seabrook, G.R., Dawson, G.R., Whiting, P.J., Rosahl, T.W., 2002. Enhanced learning and memory and altered GABAergic synaptic

transmission in mice lacking the alpha 5 subunit of the GABAA receptor. J. Neurosci. 22, 5572−5580.

Collinson, N., Atack, J.R., Laughton, P., Dawson, G.R., Stephens, D.N., 2006. An inverse agonist selective for alpha5 subunit-containing GABA(A) receptors improves encoding and recall but not consolidation in the Morris water maze. Psychopharmacology (Berlin) 188, 619−628.

Cook, J.B., Foster, K.L., Eiler, W.J., McKay, P.F., Woods, J., Harvey, S.C., Garcia, M., Grey, C., McCane, S., Mason, D., Cummings, R., Li, X., Cook, J.M., June, H.L., 2005. Selective GABAA alpha5 benzodiazepine inverse agonist antagonizes the neurobehavioral actions of alcohol. Alcohol Clin. Exp. Res. 29, 1390−1401.

Crestani, F., Keist, R., Fritschy, J.M., Benke, D., Vogt, K., Prut, L., Bluthmann, H., Mohler, H., Rudolph, U., 2002. Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. Proc. Natl. Acad. Sci. U.S.A. 99, 8980−8985.

Curran, H.V., 1991. Benzodiazepines, memory and mood: a review. Psychopharmacology (Berlin) 105, 1−8.

Curran, H.V., Barrow, S., Weingartner, H., Lader, M., Bernik, M., 1995. Encoding, remembering and awareness in lorazepam-induced amnesia. Psychopharmacology (Berlin) 122, 187−193.

Dawson, G.R., Maubach, K.A., Collinson, N., Cobain, M., Everitt, B.J., Macleod, A.M., Choudhury, H.I., McDonald, L.M., Pillai, G., Rycroft, W., Smith, A.J., Sternfeld, F., Tattersall, F.D., Wafford, K.A., Reynolds, D.S., Seabrook, G.R., Atack, J.R., 2006. An inverse agonist selective for alpha5 subunit-containing GABAA receptors enhances cognition. J. Pharmacol. Exp. Ther. 316, 1335−1345.

Duka, T., Weissenborn, R., Dienes, Z., 2001. State-dependent effects of alcohol on recollective experience, familiarity and awareness of memories. Psychopharmacology (Berlin) 153, 295−306.

Eichenbaum, H., 2004. Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 44, 109−120.

Enggasser, J.L., de Wit, H., 2001. Haloperidol reduces stimulant and reinforcing effects of ethanol in social drinkers. Alcohol Clin. Exp. Res. 25, 1448−1456.

Fluckiger, A., Hartmann, D., Leishman, B., Ziegler, W.H., 1988. Lack of effect of the benzodiazepine antagonist flumazenil (Ro 15-1788) on the performance of healthy subjects during experimentally induced ethanol intoxication. Eur. J. Clin. Pharmacol. 34, 273−276.

Fritschy, J.M., Mohler, H., 1995. GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J. Comp. Neurol. 359, 154−194.

Guidotti, A., Auta, J., Davis, J.M., Dong, E., Grayson, D.R., Veldic, M., Zhang, X., Costa, E., 2005. GABAergic dysfunction in schizophrenia: new treatment strategies on the horizon. Psychopharmacology (Berlin) 180, 191−205.

Holdstock, L., de Wit, H., 1999. Ethanol impairs saccadic and smooth pursuit eye movements without producing self-reports of sedation. Alcohol Clin. Exp. Res. 23, 664−672.

Hommer, D., Weingartner, H., Breier, A., 1993. Dissociation of benzodiazepine-induced amnesia from sedation by flumazenil pretreatment. Psychopharmacology (Berlin) 112, 455−460.

Horowski, R., Dorrow, R., 2002. Anxiogenic, not psychotogenic, properties of the partial inverse benzodiazepine receptor agonist FG 7142 in man. Psychopharmacology (Berlin) 162, 223−224.

Houser, C.R., Esclapez, M., 2003. Downregulation of the alpha5 subunit of the GABA(A) receptor in the pilocarpine model of temporal lobe epilepsy. Hippocampus 13, 633−645.

Howell, O., Atack, J.R., Dewar, D., McKernan, R.M., Sur, C., 2000. Density and pharmacology of alpha5 subunit-containing GABA(A) receptors are preserved in hippocampus of Alzheimer's disease patients. Neuroscience 98, 669−675.

Huang, Q., He, X., Ma, C., Liu, R., Yu, S., Dayer, C.A., Wenger, G.R., McKernan, R., Cook, J.M., 2000. Pharmacophore/receptor models for GABA(A)/BzR subtypes (alpha1beta3gamma2, alpha5beta3gamma2, and alpha6beta3gamma2) via a comprehensive ligand-mapping approach. J. Med. Chem. 43, 71−95.

Huron, C., Giersch, A., Danion, J.M., 2002. Lorazepam, sedation, and conscious recollection: a dose-response study with healthy volunteers. Int. Clin. Psychopharmacol. 17, 19−26.

June, H.L., Harvey, S.C., Foster, K.L., McKay, P.F., Cummings, R., Garcia, M., Mason, D., Grey, C., McCane, S., Williams, L.S., Johnson, T.B., He, X., Rock, S., Cook, J.M., 2001. GABA(A) receptors containing (alpha)5 subunits in the CA1 and CA3 hippocampal fields regulate ethanol-motivated behaviors: an extended ethanol reward circuitry. J. Neurosci. 21, 2166−2177.

Klotz, U., Ziegler, G., Rosenkranz, B., Mikus, G., 1986. Does the benzodiazepine antagonist Ro 15-1788 antagonize the action of ethanol? Br. J. Clin. Pharmacol. 22, 513−520.

Knoflach, F., Benke, D., Wang, Y., Scheurer, L., Luddens, H., Hamilton, B.J., Carter, D.B., Mohler, H., Benson, J.A., 1996. Pharmacological modulation of the diazepam-insensitive recombinant gamma-aminobutyric acidA receptors alpha 4 beta 2 gamma 2 and alpha 6 beta 2 gamma 2. Mol. Pharmacol. 50, 1253−1261.

Koob, G.F., Braestrup, C., Thatcher, B.K., 1986. The effects of FG 7142 and RO 15-1788 on the release of punished responding produced by chlordiazepoxide and ethanol in the rat. Psychopharmacology (Berlin) 90, 173−178.

Lingford-Hughes, A., Hume, S.P., Feeney, A., Hirani, E., Osman, S., Cunningham, V.J., Pike, V.W., Brooks, D.J., Nutt, D.J., 2002. Imaging the GABA-benzodiazepine receptor subtype containing the alpha5-subunit in vivo with [^{11}C]Ro15 4513 positron emission tomography. J. Cereb. Blood Flow Metab. 22, 878−889.

Lister, R.G., 1985. The amnesic action of benzodiazepines in man. Neurosci. Biobehav. Rev. 9, 87−94.

Martin, C.S., Earleywine, M., Musty, R.E., Perrine, M.W., Swift, R.M., 1993. Development and validation of the Biphasic Alcohol Effects Scale. Alcohol Clin. Exp. Res. 17, 140−146.

McKay, P.F., Foster, K.L., Mason, D., Cummings, R., Garcia, M., Williams, L.S., Grey, C., McCane, S., He, X., Cook, J.M., June, H.L., 2004. A high affinity ligand for GABAA-receptor containing alpha5 subunit antagonizes ethanol's neurobehavioral effects in Long-Evans rats. Psychopharmacology (Berlin) 172, 455−462.

Mihic, S.J., Ye, Q., Wick, M.J., Koltchine, V.V., Krasowski, M.D., Finn, S.E., Mascia, M.P., Valenzuela, C.F., Hanson, K.K., Greenblatt, E.P., Harris, R.A., Harrison, N.L., 1997. Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. Nature 389, 385−389.

Mintzer, M.Z., Griffiths, R.R., 1999. Selective effects of zolpidem on human memory functions. J. Psychopharmacol. 13, 18−31.

Nutt, D.J., Lister, R.G., 1988. Strain differences in response to a benzodiazepine receptor inverse agonist (FG 7142) in mice. Psychopharmacology (Berlin) 94, 335−336.

Parker, E.S., Birnbaum, I.M., Weingartner, H., Hartley, J.T., Stillman, R.C., Wyatt, R.J., 1980. Retrograde enhancement of human memory with alcohol. Psychopharmacology (Berlin) 69, 219−222.

Parker, E.S., Morihisa, J.M., Wyatt, R.J., Schwartz, B.L., Weingartner, H., Stillman, R.C., 1981. The alcohol facilitation effect on memory: a dose-response study. Psychopharmacology (Berlin) 74, 88−92.

Pickering, C., Avesson, L., Lindblom, J., Liljequist, S., Schiöth, H.B., 2007. Identification of neurotransmitter receptor genes involved in alcohol self-administration in the rat prefrontal cortex, hippocampus and amygdala. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 53−64.

Rissman, R.A., Bennett, D.A., Armstrong, D.M., 2004. Subregional analysis of GABA(A) receptor subunit mRNAs in the hippocampus of older persons with and without cognitive impairment. J. Chem. Neuroanat. 28, 17−25.

Rudolph, U., Mohler, H., 2004. Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annu. Rev. Pharmacol. Toxicol. 44, 475−498.

Rudolph, U., Crestani, F., Benke, D., Brunig, I., Benson, J.A., Fritschy, J.M., Martin, J.R., Bluethmann, H., Mohler, H., 1999. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 401, 796−800.

Saunders, J.B., Aasland, O.G., Babor, T.F., de la Fuente, J.R., Grant, M., 1993. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption − II. Addiction 88, 791−804.

Savic, M.M., Huang, S., Furtmuller, R., Clayton, T., Huck, S., Obradovic, D.I., Ugrešic, N.D., Sieghart, W., Bokonjic, D.R., Cook, J.M., 2007. Are GABAA receptors containing a5 subunits contributing to the sedative

properties of benzodiazepine site agonists? Neuropsychopharmacology (Epub ahead of print).

Schuckit, M.A., 1980. Self-rating of alcohol intoxication by young men with and without family histories of alcoholism. J. Stud. Alcohol 41, 242–249.

Schuckit, M.A., Smith, T.L., Kalmijn, J., Tsuang, J., Hesselbrock, V., Bucholz, K., 2000. Response to alcohol in daughters of alcoholics: a pilot study and a comparison with sons of alcoholics. Alcohol Alcoholism 35, 242–248.

Shimizu, K., Matsubara, K., Uezono, T., Kimura, K., Shiono, H., 1998. Reduced dorsal hippocampal glutamate release significantly correlates with the spatial memory deficits produced by benzodiazepines and ethanol. Neuroscience 83, 701–706.

Soo-Ampon, S., Wongwitdecha, N., Plasen, S., Hindmarch, I., Boyle, J., 2004. Effects of word frequency on recall memory following lorazepam, alcohol, and lorazepam alcohol interaction in healthy volunteers. Psychopharmacology (Berlin) 176, 420–425.

Spielberger, C., Gorsuch, R.L., Lushene, R.E., 1970. Manual for the State-Trait Anxiety Inventory. Consulting Psychologist Press, Palo Alto, CA.

Stephens, D.N., Pistovcakova, J., Worthing, L., Atack, J.R., Dawson, G.R., 2005. Role of GABAA alpha5-containing receptors in ethanol reward: the effects of targeted gene deletion, and a selective inverse agonist. Eur. J. Pharmacol. 526, 240–250.

Sternfeld, F., Carling, R.W., Jelley, R.A., Ladduwahetty, T., Merchant, K.J., Moore, K.W., Reeve, A.J., Street, L.J., O'Connor, D., Sohal, B., Atack, J.R., Cook, S., Seabrook, G., Wafford, K., Tattersall, F.D., Collinson, N., Dawson, G.R., Castro, J.L., Macleod, A.M., 2004. Selective, orally active gamma-aminobutyric acidA alpha5 receptor inverse agonists as cognition enhancers. J. Med. Chem. 47, 2176–2179.

Stockwell, T., Murphy, D., Hodgson, R., 1983. The severity of alcohol dependence questionnaire: its use, reliability and validity. Br. J. Addict. 78, 145–155.

Suzdak, P.D., Glowa, J.R., Crawley, J.N., Schwartz, R.D., Skolnick, P., Paul, S.M., 1986. A selective imidazobenzodiazepine antagonist of ethanol in the rat. Science 234, 1243–1247.

Tretter, V., Ehya, N., Fuchs, K., Sieghart, W., 1997. Stoichiometry and assembly of a recombinant GABA$_A$ receptor subtype. J. Neurosci. 17, 2728–2737.

Wainwright, A., Sirinathsinghji, D.J., Oliver, K.R., 2000. Expression of GABA(A) receptor alpha5 subunit-like immunoreactivity in human hippocampus. Brain Res. Mol. Brain Res. 80, 228–232.

Wallner, M., Hanchar, H.J., Olsen, R.W., 2003. Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc. Natl. Acad. Sci. U.S.A 100, 15218–15223.

Wallner, M., Hanchar, H.J., Olsen, R.W., 2006. Low-dose alcohol actions on alpha4beta3delta GABAA receptors are reversed by the behavioral alcohol antagonist Ro15-4513. Proc. Natl. Acad. Sci. U.S.A. 103, 8540–8545.

Wei, W., Faria, L.C., Mody, I., 2004. Low ethanol concentrations selectively augment the tonic inhibition mediated by delta subunit-containing GABAA receptors in hippocampal neurons. J. Neurosci. 24, 8379–8382.

Weissenborn, R., Duka, T., 2000. State-dependent effects of alcohol on explicit memory: the role of semantic associations. Psychopharmacology (Berlin) 149, 98–106.

Wilson, S.J., Glue, P.G., Ball, D., Nutt, D.J., 1993. Saccadic eye movement parameters in normal subjects. Electroencephalogr. clin. Neurophysiol. 86, 69–74.

Yu, Z.Y., Wang, W., Fritschy, J.M., Witte, O.W., Redecker, C., 2006. Changes in neocortical and hippocampal GABAA receptor subunit distribution during brain maturation and aging. Brain Res. 1099, 73–81.