ELSEVIER

European Journal of Pharmacology 426 (2001) 45–54

ejp

www.elsevier.com/locate/ejphar

Influence of benzodiazepine binding site ligands on fear-conditioned contextual memory

Timothy M. DeLorey [a,*], Richard C. Lin [a], Brian McBrady [a], Xiaohui He [b], James M. Cook [b], Jelveh Lameh [a], Gilda H. Loew [a]

[a] *Molecular Research Institute, 2495 Old Middlefield Way, Mountain View, CA 94043, USA*
[b] *Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI, USA*

Received 3 July 2001; accepted 10 July 2001

Abstract

Eight compounds that bind to the benzodiazepine binding site on the γ-amino butyric acid$_A$ (GABA$_A$) receptor were assessed for their influence on contextual memory, an aspect of memory affected in various cognitive disorders including Alzheimer's disease. Using a Pavlovian fear-conditioning paradigm, each ligand was evaluated in C57Bl/6 mice in regards to its direct affect on contextual memory and whether the ligand could attenuate scopolamine-induced contextual memory impairment. Of the eight ligands tested, one impaired contextual memory (agonist), six attenuated scopolamine-induced contextual memory impairment (inverse agonists), and one antagonized the ability of an inverse agonist to attenuate scopolamine-induced contextual memory impairment. Hence, further demonstrating the bi-directional influence benzodiazepine binding site ligands are able to exert on memory modulation. This study serves as an initial starting point in the development of pharmacological tools to be used in deciphering how GABA$_A$ receptors influence contextual memory.

Keywords: GABA$_A$ receptor; Benzodiazepine; Memory, contextual; Fear conditioning

1. Introduction

The striking failure of memory observed in senile dementia of the Alzheimer's type has been attributed to the degeneration of presynaptic neuronal function of the basal forebrain cholinergic system (for review, see Whitehouse, 1998). Pharmacological treatment strategies for the cognitive decline associated with Alzheimer's disease have primarily focused on cholinomimetic mechanisms to address the cholinergic hypofunction. To date, this approach has achieved limited utility (Sarter and Bruno, 1997). These strategies tend to result in direct postsynaptic stimulation causing constant, tonic neuronal activity, which is unfavorable to normal cognitive processing (Sarter and Bruno, 1997) An effective pharmacological treatment for memory dysfunction associated with cholinergic hypofunction is one that would augment *performance-associated* increases in the cortical acetylcholine efflux of the surviving cholinergic neuronal processes, while at the same time preserving the highly complex transmission patterns inherent to cortical cholinergic pathways (Sarter and Bruno, 1997). A potential, and under-appreciated, therapeutic approach to achieving this goal would be to disinhibit γ-amino butyric acid (GABA) mediated regulatory control over the surviving cholinergic neurons, but only when cognitive variables simultaneously activate cortical cholinergic activity, thereby selectively augmenting cognitive functions that are due to cortical acetylcholine efflux (Sarter and Bruno, 1997). Such an approach would likely take advantage of the unique bi-directional properties of benzodiazepine binding site ligands to allosterically affect GABA mediated activation of the γ-amino butyric acid$_A$ (GABA$_A$) receptor chloride ionophore complex, and would consequently have no direct activational effect in the absence of GABA release (Abe et al., 1998; Sarter and Bruno, 1997). Moreover, several benzodiazepine binding site ligands have been clinically evaluated in the treatment of cognitive deficits associated with senile dementia (Robbins et al., 1997; Takada et al., 1996).

Benzodiazepine binding site ligands are structurally diverse compounds that bind to a specific binding site on the GABA$_A$ receptor chloride channel complex. A benzodiazepine binding site ligand can augment (agonism), attenu-

* Corresponding author. Tel.: +1-650-210-0310x209; fax: +1-650-210-0318.
E-mail address: tim@purisima.molres.org (T.M. DeLorey).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01199-2

ate (inverse agonism), or block the effect of an agonist or inverse agonist (antagonism) on GABAergic mediated chloride flux. Consequently, benzodiazepine binding site ligands exhibit bi-directional influences that either diminishes or potentiates multiple behaviors including anxiety, sleep regulation, eating behavior, convulsive states, body temperature regulation, muscle tension, vigilance, and cognitive processing. The anterograde amnesic properties of several of the benzodiazepine binding site ligands and conversely, the enhancement of performance in learning and memory tasks observed with other benzodiazepine binding site ligands has been recognized for some time (Lister, 1985; Venault et al., 1986).

$GABA_A$ receptors are composed of a combination of transmembrane protein subunits, most of which exist in multiple polypeptide isoforms (α_{1-6}, β_{1-4}, γ_{1-4}, δ, ρ_{1-3}, ε, π, θ) (Bonnert et al., 1999; Olsen and DeLorey, 1999). These subunits assemble to form heteropentameric ion channels with chloride ion selectivity and are found ubiquitously throughout the central nervous system (Bonnert et al., 1999; Wisden et al., 1992). The pharmacology of a particular $GABA_A$ receptor pentamer is influenced by the subunit isoforms that comprise it. Interestingly, studies on postmortem brains of Alzheimer's patients reveal $GABA_A$ receptors to be relatively spared throughout the brain, even at terminal stages of the disease, while many other neurotransmitter receptors are clearly reduced (Mizukami et al., 1997). Additionally, positron emission tomography (PET) scans of the brains of living Alzheimer's patients, likewise, provide evidence for the preservation of cortical benzodiazepine binding sites (Meyer et al., 1995). Moreover, inverse agonists acting through the benzodiazepine binding site on $GABA_A$ receptors have been reported to elicit marked cognitive improvement in rodent models of senile dementia of the Alzheimer's type, produced by chemically or electrolytically lesioning the basal forebrain (Sarter and Bruno, 1997). However, the efficacy of benzodiazepine binding site inverse agonists to attenuate lesion induced cognitive impairment is dependent on the extent of the lesion. Animals with almost complete (80–90%) loss of cortical cholinergic fibers do not exhibit much benefit from treatment, while the performance of animals with less pronounced lesions improve with treatment (McGaughy et al., 1996). The loss of cholinergic neurons in aging and dementia is far from complete, commonly in the 40–70% range (Geula and Mesulam, 1996; Lehericy et al., 1993). Hence, the effects of inverse agonists acting through the benzodiazepine site on $GABA_A$ receptors to restore neural transmission in animals with partial loss of cortical cholinergic inputs suggests potential benefit in all but late stage Alzheimer's patients. The cognitive enhancing properties of some benzodiazepine binding site ligands, as outlined above, coupled with the preservation of $GABA_A$ receptors in Alzheimer's patients, warrants further consideration of these ligands in developing alternative therapeutic strategies in Alzheimer's disease.

In the present study, the cholinergic antagonist scopolamine was used to disrupt contextual memory, which was generated by Pavlovian fear conditioning, a rapidly acquired form of learning thought to model human explicit memory (Kim and Fanselow, 1992). More specifically, scopolamine suppresses cognitive function with respect to attention and visuo-spatial memory in both normal humans and rats by inducing cholinergic hypofunction (Anagnostaras et al., 1995; Ebert and Kirch, 1998). Using a fear-conditioning paradigm, a reliable method by which to assess contextual memory in mice (Chen et al., 1996a), we have assessed a set of structurally diverse benzodiazepine binding site ligands for their ability to either directly impair contextual memory or attenuate scopolamine-induced impairment of contextual memory.

2. Materials and methods

2.1. Experimental methods

2.1.1. Animals

Male C57Bl/6 mice were obtained from Charles Rivers Laboratories (Holister, CA) at 6 weeks of age. Mice used in fear conditioning were between 7 and 12 weeks of age. Animals were housed eight to a cage in rooms with a normal 12-h light/12-h dark cycle (lights on 700–1900 h) with free access to food and water. Tests were conducted during the light phase between 1300 and 1700 h with a 30-min acclimation period in the testing room prior to drug or vehicle administration. All animal protocols used in this study conform to the guidelines determined by the National Institute of Health (USA) Office for Protection from Research Risks and are approved by the Animal Care and Use Committee of the Palo Alto Veterans Administration Medical Center, Palo Alto, CA (USA).

2.1.2. Drugs

Compounds used in this study include the benzodiazepine binding site ligands Ro15-4513 (ethyl 8-azido-6-dihydro-5-methyl-6-oxo-4 *H*-imidazo[1,5-*a*]-[1,4]benzodiazepine-3-carboxylate), DMCM (methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate), flunitrazepam (1,3-dihydro-5-(*o*-fluorophenyl)-1-methyl-7-nitro-2*H*-1,4-benzodiazepin-2-one) from RBI, Natick, MA, Ro23-1590 (2-(*p*-chloro phenyl)-4-(4-*N*-ethylamide piperazinyl) quinoline), Ro15-1788 (8-fluoro-3-carboxy-5,6-dihydro-5-methyl-6-oxo-414-imidazo[1,5-*a*]1,4 benzodiazepine) from Hoffman-LaRoche, Nutley, NJ; ZK-93426 (ethyl-5-isopropyl-4-methyl-beta-carboline-3-carboxylate) from Schering, Berlin; βCCT (β-carboline-3-carboxylate-*t*-butyl ester), Compound #47 (Ethyl 8-trimethylsilyl-2-accetyl-12, 12*a*-dihydro-9-oxo-9*H*,11*H*-azeto[2,1-*c*]imidazo[1,5-*a*] 1,4 benzodiazepine 1-carboxylate) and RY10 (Ethyl 8-Ethyl-5,6-dihydro-5-methyl-6-oxo-4 *H*-imidazo[1,5*a*][1,4]benzodiazepine-3-carboxylate) were provided by Dr. James Cook

at the Univ. of Wisconsin-Milwaukee. Also used were the cholinergic receptor antagonists (−)-scopolamine hydrobromide (α-(hydroxymethyl)benzeneacetic acid 9-methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]non-7-yl ester hydrobromide and (−)-methylscopolamine bromide (7-(3-hydroxy-1-oxo-2-phenylpropoxy)-9,9-dimethyl-3-oxa-9-azoniatricyclo-[3.3.1.02,4]nonane bromide from RBI, Natick, MA. All drugs were suspended in vehicle (0.9% saline containing 0.2% Tween 80).

2.1.3. Binding studies

Frozen rat whole brain (Pel Freeze, Rogers, AR), approximate weight 1 g, was homogenized with a polytron homogenizer in 20 ml of 50 mM Tris–HCl, pH 7.4 at 4 °C and centrifuged at 20,000 × *g* for 10 min. The supernatant was discarded and the pellet homogenized and centrifuged twice as above. The pellet was resuspended in 5 ml of buffer and frozen at −86 °C overnight. After thawing, the volume of homogenate was restored to the original 20 ml with buffer and washed two more times by centrifugation and rehomogenization. The final membrane pellet was resuspended to a tissue concentration of 100 mg wet weight/ml of buffer and stored in aliquots at −86 °C until used. For binding assays, membranes (30–50 μg/tube) were incubated with 0.3 nM [^{3}H]*N*-methylscopolamine (Amersham Pharmacia Biotech., Piscataway, NJ) and either 10 nM or 10 μM of the unlabeled ligand in a total of 1 ml reaction volume in Tris–HCl, pH 7.4. Incubation was at room temperature for 60 min. The assay was terminated by rapid filtration through Whatman GF/B filters using a FilterMate cell harvester (Packard Instruments, Meriden, CT) followed by three washes, 4 ml each of ice cold Tris–HCl, pH 7.4 buffer. Radioactivity retained on the filters was measured using Microscint O in a TopCount liquid scintillation counter (Packard Instruments, Meriden, CT). All assays were carried out in triplicate.

2.1.4. Spontaneous locomotive activity

Mice were allowed to acclimate to the test room for 30 min prior to drug injection. Thirty minutes after receiving an intraperitoneal (i.p.) injection of drug or vehicle, mice were placed individually into clear plastic monitoring chambers measuring 72 × 32 × 32 cm each. Spontaneous locomotor activity was measured via seven sets of photoelectric sensors evenly spaced along the length of the monitoring chamber, 4 cm above the floor of the chamber (San Diego Instruments, San Diego, CA). Total activity was recorded in arbitrary units reflective of the number of times a mouse interrupts the photoelectric sensors during a 10-min monitoring session. This data was automatically recorded and stored by computer. Compounds that did not significantly affect spontaneous locomotor activity either by reducing activity (agonist) or enhancing activity (inverse agonist), relative to vehicle, at concentrations ≤ 30 mg/kg were also tested for antagonism. Antagonism was determined by assessing spontaneous locomotor activity 30 min after simultaneous i.p. injection of both the putative antagonist and the agonist flunitrazepam (5 mg/kg). Results were compared both to the effects of flunitrazepam alone and the vehicle control. Data were analyzed with one-way analysis of variance (ANOVA) using GraphPad PRISM 2.01 program (GraphPad Software, San Diego, CA). Separate treatment effects between groups were analyzed using the appropriate post hoc comparison.

2.1.5. Pavlovian fear conditioning

Before testing each day, the mice were moved to a holding room and allowed to acclimate for at least 30 min. Each mouse received an i.p. injection of one of the following: vehicle, benzodiazepine binding site ligand (2–30 mg/kg), scopolamine (1 mg/kg), methylscopolamine (1 mg/kg) or scopolamine (1 mg/kg) combined with one of the benzodiazepine binding site ligands (2–30 mg/kg). The dose level chosen for each compound was one that neither elicited convulsions nor impaired locomotion. Twenty minutes after injection, the mice were placed individually in one of four identical experimental chambers (Med Associates, St. Albans, VT) that had been scented with 0.3% ammonium hydroxide solution before testing. Chambers were back-lit with fluorescent light with a white noise generator providing 70 dB of background noise. After 4 min in the chamber, mice were exposed to a loud tone (85 dB, 2.9 kHz) for 32 s with the last 2 s coupled with a 0.5-mA "scrambled footshock". This procedure was repeated for a total of three episodes with a 1-min period separating each episode. One minute after the final footshock, the mice were returned to their home cages. Twenty-four hours later, contextual memory was assessed by placing the mice back into the freshly rescented (0.3% Ammonium hydroxide) conditioning chambers in which they were trained, for a 4-min test period in the absence of footshock. Conditioned fear to the context was assessed by measuring the freezing response according to the methods of Fanselow and Bolles (1979). Freezing was defined as the absence of all visible movements of the body and vibrissae aside from those necessitated by respiration. An observer, blind to the drug(s) used, scored each mouse every 8 s, for a total of 4 min, for presence or absence of freezing. These data were transformed to a percentage of total observations. Data were analyzed by one-way analysis of variance (ANOVA) using GraphPad PRISM 2.01 (GraphPad Software). Separate treatment effects between groups were analyzed post hoc using Dunnett's or Bonferroni's multiple comparisons.

3. Results

3.1. Experimental studies

3.1.1. Spontaneous locomotor activity

Prior to performing the contextual memory studies, each compound was examined for effects on spontaneous

locomotor activity so as not to confound the interpretation of a drug's effect on contextual memory. Each benzodiazepine binding site ligand used in this study (Fig. 1), except DMCM, was tested at increasing doses up to a maximum of 30 mg/kg. DMCM has been reported to have an ED_{50} value for facilitation of clonic convulsion in DBA/2 mice of 4.6 mg/kg (Croucher et al., 1984), therefore, locomotive effect was only determined at a dose of 2 mg/kg. In the spontaneous locomotor activity assay, agonists are defined as being locomotor impairing, inverse agonists are locomotor enhancing, and antagonists block the locomotor impairing effect of the agonist flunitrazepam (5 mg/kg). Overall group effect of treatment with each of the eight benzodiazepine binding site ligands, at various doses (Table 1), and scopolamine were analyzed by one-way ANOVA: [$F(15, 112) = 9.81$, $P < 0.001$]. Post hoc comparisons using Dunnett's multiple comparisons confirmed significant differences between the vehicle and βCCT (30 mg/kg), Ro15-4513 (30 mg/kg), Ro23-1590 (10 mg/kg), RY10 (30 mg/kg), and Flunitrazepam (5 mg/kg) (Table 1).

Benzodiazepine ligands devoid of agonist or inverse agonist activity in the spontaneous locomotor assay were further tested for their ability to antagonize the locomotor impairing effects of flunitrazepam (5 mg/kg). Compounds Ro15-1788 (10 mg/kg), ZK-93426 (10 mg/kg), and compound #47 (10 mg/kg) were each able to significantly antagonize the effects of flunitrazepam (Table 1). Thus, in this small set of eight benzodiazepine binding site ligands all three qualitative behavioral effects at the locomotor endpoint were observed. Additionally, all mice were closely observed during the locomotor studies for any signs of convulsive or absence-like behavior brought on by the administration of the benzodiazepine binding site ligands. At the dose levels used, no indication of any seizure event was noted for the eight benzodiazepine binding site ligands tested. The above results were used to select a dose for each compound to be used in the training phase of the contextual memory task in order to avoid confounding effects associated with locomotor impairment.

3.1.2. Contextual memory

Prior to cognitive assessment, each of the eight structurally diverse benzodiazepine binding site ligands were evaluated in mice for influence on pain sensitivity relative to footshock-elicited flinch-vocalization thresholds as described by Kim et al. (1991). Mice were tested 25 min after receiving an equivalent dose of each benzodiazepine binding site ligand as used in the memory study. No significant difference in pain sensitivity threshold to a mild electric shock was observed between animals receiving one of the benzodiazepine binding site ligands and those receiving vehicle alone (Table 2). Interestingly, mice receiving scopolamine, methylscopolamine or a combination of scopolamine and one of the benzodiazepine ligands, exhibit a significantly higher pain threshold to the electric shock when compared to mice not receiving scopolamine (Table 2). However, there was no significant difference in

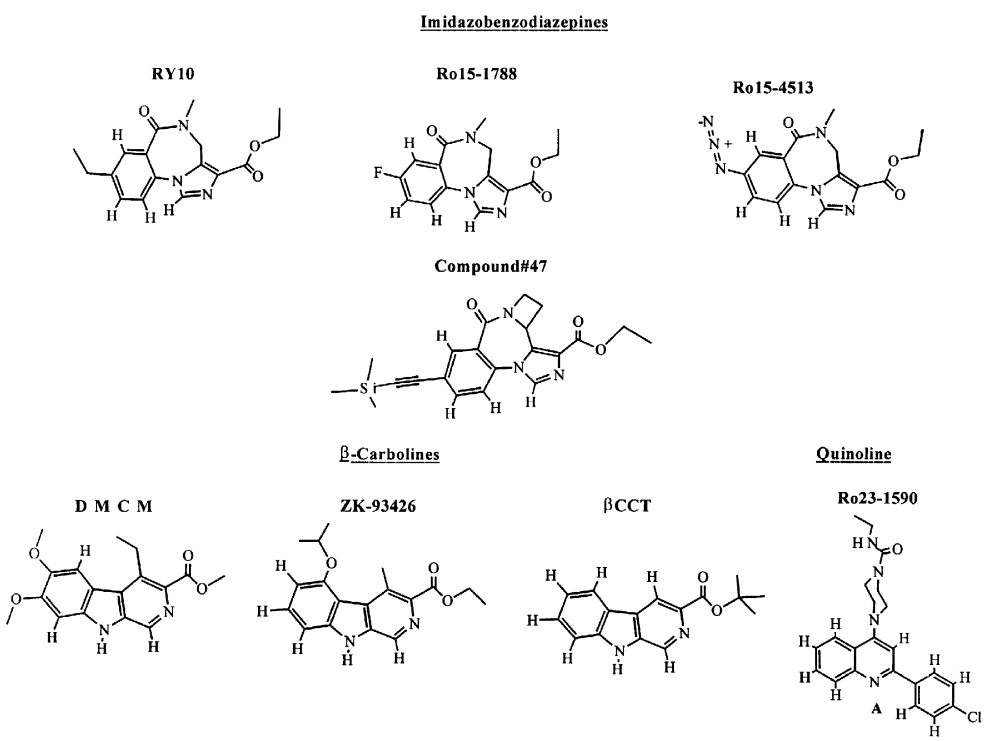

Fig. 1. Chemical structures of the eight benzodiazepine binding site ligands used in the present study.

Table 1
Influence of benzodiazepine binding site ligands on spontaneous locomotor activity

Compound	Dose (mg/kg)	Activity[a]	Antagonism[b]	
			Dose (mg/kg)	Activity[a]
Vehicle	–	185 ± 6	–	–
DMCM	2	171 ± 26	2	93 ± 17 (no effect)
βCCT	10	262 ± 34	–	–
	30	329 ± 30[c] (inverse agonist)	–	–
Ro15-1788	30	246 ± 33	10	157 ± 13[d] (antagonist)
Ro15-4513	10	161 ± 32	–	–
	30	286 ± 19[e] (inverse agonist)	–	–
Ro23-1590	3	162 ± 14	–	–
	10	86 ± 16[e] (agonist)	–	–
RY10	10	156 ± 21	–	–
	30	310 ± 27[c] (inverse agonist)	–	–
Compound #47	30	248 ± 20	10	128 ± 25[f] (antagonist)
ZK-93426	30	161 ± 24	10	173 ± 26[d] (antagonist)
Flunitrazepam	5	56 ± 14[c] (agonist)	–	–
Scopolamine	1	218 ± 24	–	–

[a]Values are expressed in arbitrary units as the mean ± S.E.M. of the total number of times the mice interrupted photoelectric sensors during 10-min monitoring sessions ($n = 8$). Vehicle (0.9% saline + 0.2% Tween 80). Scopolamine and flunitrazepam included reference purposes.

[b]A drug was determined to be an antagonist if it significantly antagonized the locomotor impairing effects of flunitrazepam (5 mg/kg). Differences between treatment groups (vehicle, flunitrazepam, and simultaneous administration of drug with flunitrazepam) were determined by one-way ANOVA with post hoc Bonferroni's multiple comparisons.

[c]$P < 0.01$, significantly different from vehicle control group, one-way ANOVA with post hoc Dunnett's multiple comparisons.

[d]$P < 0.01$, significantly different from flunitrazepam (5 mg/kg) alone and no difference from vehicle group ($P > 0.05$).

[e]$P < 0.05$, significantly different from vehicle control group, one-way ANOVA with post hoc Dunnett's multiple comparisons.

[f]$P < 0.05$, significantly different from flunitrazepam (5 mg/kg) alone and no difference from vehicle group ($P > 0.05$).

shock sensitivity between groups of mice receiving scopolamine alone and ones receiving scopolamine in combination with a benzodiazepine ligand. Furthermore, all mice vocalized at current values ≤ 0.5 mA regardless of whether they received vehicle, one of the benzodiazepine binding site ligands, scopolamine, a combination of benzodiazepine binding site ligand and scopolamine or the scopolamine analog, methylscopolamine (Table 2).

Each of the eight benzodiazepine binding site ligands were evaluated in naive mice, using a Pavlovian fear-conditioning paradigm, for its ability to either directly impair contextual memory or attenuate scopolamine-induced contextual memory impairment. The average "pre-shock" baseline freezing was established in mice treated with vehicle alone during a 4-min monitoring session, with the mean percentage of total observations spent in a freezing posture being 10 ± 4% S.E.M. (data not shown). By contrast, the same vehicle administered mice exhibited 46 ± 2% S.E.M. of a 4-min test session in a freezing posture when tested 24 h after "footshock" training (Fig. 2). Owen et al. (1997) has previously reported similar values for C57BL/6J mice. Mice receiving scopolamine 20-min prior

Table 2
Influence of benzodiazepine on shock sensitivity

Drug	Dose (mg/kg)	Without scopolamine		With scopolamine	
		Flinch[a]	Vocalize[a]	Flinch[a]	Vocalize[a]
Vehicle	–	0.18 ± 0.0	0.34 ± 0.01	0.25 ± 0.02	0.45 ± 0.01
DMCM	2	0.18 ± 0.02	0.30 ± 0.04	0.29 ± 0.07	0.50 ± 0.01
βCCT	30	0.18 ± 0.02	0.35 ± 0.04	0.25 ± 0.01	0.50 ± 0.05
Ro15-1788	10	0.18 ± 0.00	0.34 ± 0.04	0.34 ± 0.04	0.44 ± 0.01
Ro15-4513	10	0.19 ± 0.01	0.29 ± 0.02	0.23 ± 0.02	0.36 ± 0.04
Ro23-1590	3	0.19 ± 0.02	0.30 ± 0.01	0.24 ± 0.02	0.50 ± 0.01
RY10	10	0.15 ± 0.01	0.26 ± 0.04	0.24 ± 0.01	0.48 ± 0.02
Compound #47	30	0.21 ± 0.02	0.29 ± 0.02	0.24 ± 0.03	0.41 ± 0.03
ZK 93426	10	0.22 ± 0.02	0.30 ± 0.01	0.20 ± 0.02	0.41 ± 0.02
Scopolamine	1	0.25 ± 0.02[b]	0.45 ± 0.01[b]	–	–
Methylscopolamine	1	0.21 ± 0.02	0.45 ± 0.05[b]	–	–

[a]Values are expressed in mean ± S.E.M. of the shock intensity milliamperes (mA) required to elicit the measured behavioral response. One-way ANOVA with Dunnet's multiple comparison.

[b]$P < 0.05$, significantly different from vehicle control group, one-way ANOVA with post hoc Dunnett's multiple comparisons.

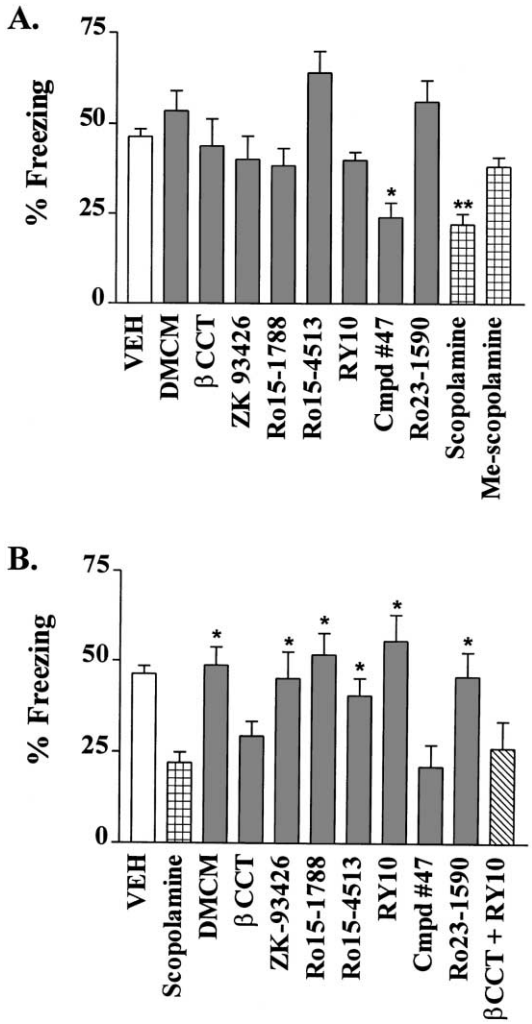

Fig. 2. Assessment of contextual memory using a Pavlovian fear conditioning paradigm 24 h after training. Graphs represent percentage of total observations that mice spent in a freezing posture during the 4-min exposure to the test context (see Materials and methods). (A) Mice injected with vehicle ($n = 12$), one of the benzodiazepine binding site ligands in vehicle ($n = 10$ per group, doses listed below in (B)), scopolamine ($n = 12$, 1 mg/kg), or methylscopolamine ($n = 12$, 1 mg/kg) 20 min prior to training. (B) Mice injected with benzodiazepine binding site ligands simultaneously with scopolamine ($n = 10$ per group, 1 mg/kg) 20 min prior to training. In both (A) and (B), Ro15-1788, Ro15-4513, RY10 and ZK-93426 were administered at 10 mg/kg. Compound #47 and βCCT were administered at 30 mg/kg. DMCM and Ro23-1590 were administered at 2 and 3 mg/kg, respectively. βCCT (30 mg/kg) was also administered simultaneously with both RY10 (10 mg/kg) and scopolamine (1 mg/kg). Histograms of freezing scores are expressed as the mean ± S.E.M. of the percentage of total observations within a group. Differences in treatment groups in histogram A (vehicle and drug) were assessed by one-way ANOVA with post hoc analysis using Dunnett's multiple comparisons. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from vehicle control group. Differences between treatment groups in histogram B (vehicle, scopolamine (1 mg/kg), and simultaneous administration of drug with scopolamine) were determined by one-way ANOVA with post hoc Bonferroni's multiple comparisons. $^{*}P < 0.05$, significantly different from group administered scopolamine alone and not significantly different from vehicle group ($P > 0.05$). Me-scopolamine = methylscopolamine.

to the training session exhibited a significant reduction ($P < 0.01$) in percent freezing to the context when tested 24 h later, 22 ± 3%, compared to vehicle treated mice, 46 ± 2% (Fig. 2). A similar reduction in percent freezing due to scopolamine has been previously reported in rats (Anagnostaras et al., 1995). Methylscopolamine, an analog of scopolamine that does not cross the blood brain barrier, was used as a peripheral control to confirm that the reversal of the scopolamine-induced contextual memory impairment (Fig. 2A) did not contain a peripherally based element as has been observed in other cognitive tasks (Buxton et al., 1994; Evans-Martin et al., 2000).

Fig. 2A demonstrates the extent to which each of the eight benzodiazepine binding site ligands, administered 20-min prior to the training session, were able to influence the percent freezing scores when tested 24 h later. One-way ANOVA revealed overall group differences between mice groups administered vehicle, scopolamine, methylscopolamine or one of the eight benzodiazepine binding site ligands in regards to their freezing response [$F(10,105) = 7.18$, $P < 0.001$]. Post hoc analysis using Dunnett's multiple comparisons confirmed there to be significantly less freezing in the groups administered compound #47 ($P < 0.05$) or scopolamine ($P < 0.01$) as compared to the vehicle control group (Fig. 2A). One-way ANOVA likewise revealed an overall group difference between the three groups of mice administered vehicle, scopolamine or methylscopolamine [$F(2,33) = 23.31$, $p < 0.001$]. Post hoc analysis using Bonferroni's multiple comparisons between vehicle, scopolamine, and methylscopolamine treated mice revealed scopolamine treated mice to freeze significantly less than mice administered either vehicle ($p < 0.01$) or methylscopolamine ($p < 0.01$) while no significant difference was exhibited between mice administered vehicle or methylscopolamine ($p > 0.05$).

Fig. 2B demonstrates the extent to which each of the eight benzodiazepine binding site ligands, administered simultaneously with scopolamine (1 mg/kg) 20 min prior to the training session, were able to attenuate the scopolamine-induced suppression of freezing to the context when tested 24 h later. One-way ANOVA revealed overall group differences between mice groups (vehicle, scopolamine alone, and scopolamine + drug) in their freezing response [$F(10,103) = 5.12$, $P < 0.001$]. Post hoc analysis using Bonferroni's multiple comparisons confirmed the presence of significantly more freezing ($P < 0.05$) in groups administered DMCM, ZK-93426, Ro15-1788, Ro15-4513, RY10, or Ro23-1590 simultaneously with scopolamine as compared to scopolamine administered alone (Fig. 2B). Furthermore, each of these groups exhibited freezing responses similar to those observed in the vehicle control group ($P > 0.05$) suggesting a reversal of the effects of scopolamine on contextual memory (Fig. 2B). As expected, the group administered compound #47 in combination with scopolamine did not differ significantly from the group administered scopolamine alone (Fig. 2B). βCCT

in combination with scopolamine was also found not to differ significantly in freezing response when compared to scopolamine alone ($p > 0.05$) but was significantly different from the vehicle control group ($P < 0.05$) (Fig. 2B). To test whether βCCT (30 mg/kg) exhibited antagonistic properties at this endpoint, it was also administered simultaneously with both scopolamine (1 mg/kg) and RY10 (10 mg/kg), a compound that robustly attenuates the influence scopolamine has on contextual memory. The freezing scores of mice administered βCCT, RY10 and scopolamine simultaneously were significantly different from the vehicle control ($P < 0.05$) but not significantly different from scores obtained when scopolamine was administered alone ($P > 0.05$) (Fig. 2B). Taken together, this suggests that βCCT was able to antagonize RY10's ability to attenuate scopolamine-induced contextual memory impairment. The maximal dose tested for any compound in the contextual memory study was 30 mg/kg (Table 3). Additionally, Table 3 compares the qualitative behavioral results obtained from each of the eight compounds at both the locomotor and contextual memory endpoints.

3.1.3. Cholinergic binding

Benzodiazepine binding site ligands that were able to attenuate the impairing effects of scopolamine on contextual memory were further evaluated for binding at muscarinic acetylcholine receptors in order to determine if their effect was due to a direct effect on scopolamine binding. To this end, the six compounds that were able to attenuate scopolamine's effect on contextual memory were assessed for their ability to directly inhibit in vitro [^{3}H]*N*-methylscopolamine (an analog of scopolamine) binding. As presented in Table 4, the benzodiazepine binding site ligands failed to show robust inhibition of [^{3}H]*N*-methylscopolamine binding at 10 nM, a concentration that one would expect to see strong inhibition if a compound was active at the scopolamine binding site. Furthermore, these compounds also failed to inhibit greater than 50% of the [^{3}H]*N*-methylscopolamine binding even when tested at the relatively high concentration of 10 μM.

Table 3
Comparison of activity at two different behavioral endpoints

Drug	Locomotor activity		Contextual memory	
	M.E.D.[a]	Behavior	M.E.D.[a]	Behavior
DMCM	2	No effect	2	Inverse agonist
Ro15-1788	10	Antagonist	10	Inverse agonist
Ro15-4513	30	Inverse agonist	10	Inverse agonist
Ro23-1590	10	Agonist	3	Inverse agonist
βCCT	30	Inverse agonist	30	Antagonist
RY10	30	Inverse agonist	10	Inverse agonist
Compound #47	10	Antagonist	30	Agonist
ZK-93426	10	Antagonist	10	Inverse Agonist

[a] M.E.D., minimal effective dose (mg/kg) that was able to elicit a statistically verifiable effect on spontaneous locomotor activity or contextual memory impairment (see Materials and methods). In each case, 30 mg/kg was the highest dose tested. DMCM was not tested above 2 mg/kg due to convulsive potential at higher doses.

Table 4
Inhibition of in vitro [^{3}H]*N*-methylscopolamine binding by benzodiazepine binding site ligands

Ligand	% Inhibition of control[a]	
	10 nM	10 μM
DMCM	9 ± 1	40 ± 2
Ro15-1788	14 ± 6	10 ± 2
Ro15-4513	22 ± 4	19 ± 8
Ro23-1590	6 ± 4	26 ± 2
RY10	14 ± 2	0 ± 2
ZK-93426	0 ± 2	26 ± 1
Scopolamine	80 ± 1	91 ± 1

[a] Values are the mean ± S.D. of triplicate values from one experiment. The experiment was carried out twice with similar results. Scopolamine is included for reference purposes.

4. Discussion

The aim of this study was to assess the ability of a small set of structurally diverse benzodiazepine binding site ligands to influence contextual memory, an aspect of memory affected in various cognitive disorders (McEwen, 1997). For this evaluation to be meaningful, in regards to addressing conditions of cognitive dysfunction, it must employ a model system that fulfills the following criteria: (i) the model exhibits clinical aspects of the symptoms associated with the dysfunction being studied (face validity); (ii) the model is based on theoretical and conceptual rationale associated with the targeted dysfunction (construct validity); and ultimately, (iii) the model provides some indication as to how well a drug would perform in a human (predictive validity) (for review, see Sarter et al., 1992; Willner, 1986). In the present study, contextual memory was assessed in groups of mice receiving either the test benzodiazepine binding site ligand alone or simultaneously with scopolamine, a compound that induces cholinergic hypofunction resulting in contextual memory impairment (Anagnostaras et al., 1995). A limitation of using scopolamine in animals to model cognitive disorders such as senile dementia of the Alzheimer's type is that the pharmacological impairment caused by scopolamine is likely induced by the post-synaptic blockade of muscarinic M1 acetylcholine receptors, while in Alzheimer's disease cognitive dysfunction is likely due to destruction of presynaptic cholinergic neurons (Sarter et al., 1992). Furthermore, scopolamine antagonizes muscarinic M1 acetylcholine receptors throughout the brain and periphery in contrast to the anatomically selective deficits associated with Alzheimer's disease. Despite these limitations, both the construct and face validity of using scopolamine to mimic key features of Alzheimer's disease is still consid-

ered to be high (see Sarter et al., 1992). Additionally, electroencephalography (EEG) studies in which scopolamine is administered to young healthy humans or to rats causes an increase in delta frequency bands and a reduction in alpha frequency bands similar to what has been observed in the EEGs of individuals with senile dementia of the Alzheimer's type (Buzsaki et al., 1988; Sannita et al., 1987). Unfortunately, to date, there is no true gold standard by which to assess the predictive value of an animal model of Alzheimer's disease when trying to extrapolate the results to humans. However, as seen in Fig. 2, ZK-93426 was able to significantly attenuate the contextual memory impairing effects of scopolamine in mice consistent with its ability to partially antagonize most of the effects of scopolamine on memory and attention when tested in normal humans (Duka et al., 1996). An in-depth assessment of the predictive validity of models such as this awaits the discovery of therapeutic agents that exhibit more robust treatment profiles in cognitive disorders, such as Alzheimer's disease, than are currently available.

Previous investigations reveal that under conditions of cognitive deficit, as induced by scopolamine or by lesioning of the basal forebrain, the benzodiazepine binding site ligands, DMCM, ZK-93426, Ro15-1788, and Ro15-4513 were able to reverse learning or retention deficits (Jensen et al., 1987; McNamara and Skelton, 1992; Prather et al., 1992; Sarter and Steckler, 1989). In the present study, the impairing effects of scopolamine on contextual memory was significantly attenuated by six of the eight benzodiazepine binding site ligands tested, these include DMCM, ZK-93426, Ro15-1788, Ro15-4513, RY10, and Ro23-1590 (Fig. 2B). As expected, this attenuation was not simply due to the benzodiazepine binding site ligands interfering with scopolamine binding. None of the six benzodiazepine binding site ligands that attenuated scopolamine's influence on contextual memory were able to inhibit more than 50% [^{3}H]*N*-methylscopolamine binding at either 10 nM or at the relatively high concentration of 10 μM (Table 4). The two remaining benzodiazepine binding site ligands, compound #47 and βCCT, were unable to attenuate scopolamine-induced contextual memory impairment. However, compound #47 administered in the absence of scopolamine impaired contextual memory directly, thereby suggesting it to have agonistic properties (memory impairing) at this endpoint separate from scopolamine's action (Fig. 2A; Table 3). Thereby, its inability to attenuate scopolamine's influence on contextual memory was of no surprise (Fig. 2B). βCCT, on the other hand, failed to exhibit a significant effect on freezing behavior associated with contextual memory whether administered alone or in combination with scopolamine (Fig. 2A and B). However, when βCCT was tested in the presence of scopolamine and RY10, an inverse agonist at this endpoint, it blocked RY10's ability to attenuate scopolamine's influence on contextual memory (Fig. 2B). These results suggest that βCCT behaves as an antagonist at this memory endpoint.

It could be argued that a reduction in shock sensitivity caused by scopolamine (Table 2) could result in the observed decrease in freezing behavior as opposed to a direct effect on contextual memory. However, the following three lines of evidence argue against this interpretation: (i) The intensity of the electric shock (0.5 mA, 2 s) was sufficiently noxious to cause vocalization in all mice tested regardless of what drug they were administered (Table 2); (ii) benzodiazepine ligands that were able to attenuate scopolamine-induced contextual memory impairment did not significantly alter scopolamine's effect on shock sensitivity (Table 2); and (iii) methylscopolamine, an analog of scopolamine that does not cross the blood brain barrier, also reduced shock sensitivity but did not significantly reduce freezing behavior in the contextual memory assay when compared to vehicle (Fig. 2A). Consequently, the change in pain sensitivity observed with both scopolamine and the peripherally specific analog, methylscopolamine, suggests that this effect is peripherally based (Table 2).

The failure of methylscopolamine to significantly effect the contextual memory assay while causing a reduction in the electric shock sensitivity (Fig. 2A and Table 2) also argues against there being an appreciable contribution from the peripheral muscarinic M1 acetylcholine receptors in scopolamine-induced contextual memory impairment. An alternative possibility is that benzodiazepine ligands acting on $GABA_A$ receptors found in the periphery elicit some change that is conveyed to the CNS and subsequently influence scopolamine's effect on memory. However, this is considered unlikely as the prevalence of binding sites for central-type benzodiazepine binding in the periphery is rather low compared to those found in the brain (Anholt et al., 1985).

Although many benzodiazepine binding site ligands like flunitrazepam and abecarnil are known to exhibit qualitatively similar effects (agonist) over most behavioral endpoints (Davies et al., 1994), there is evidence that others do not. For example, Ro16-6028 (*t*-butyl(*S*)-8-bromo-11,12,13,13 *a*-tetrahydro-9-oxo-9*H*-imidazo[1,5-*a*][1,4] benzodiazepine-1-carboxylate) has been reported to behave as an antagonist at a locomotor endpoint (blocking the locomotor impairing effects of an agonist), but behaves as an agonist at an anxiolytic endpoint (causes a reduction in anxiety) (Chen et al., 1996b). Interestingly, the benzodiazepine binding site ligands, βCCT, Ro23-1590, ZK-93426, Ro15-1788, and compound #47 used in the present study exhibit qualitatively different behavioral effects on locomotion and memory (Table 3). The presence of these qualitative differences supports the notion of separating undesirable effects (i.e. locomotor impairment) from desirable ones (i.e. memory potentiation). The benzodiazepine binding site ligands Ro15-4513, Ro15-1788, ZK-93426, and RY10 lacked locomotor impairing effects at the doses tested but were able to attenuate scopolamine's effects on contextual memory (Table 3, Fig. 2B). This type of profile is desirable when considering potential therapeutic value

without side effects. It is interesting to note that although each of these four compounds are inverse agonists at the cognitive endpoint, both Ro15-4513 and RY10 exhibit inverse agonism at the locomotor endpoint while Ro15-1788 and ZK-93426 behave as antagonists. Each of these compounds has similar binding affinities with respect to recombinantly expressed $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors (1–20 nM) (Huang et al., 1998; ZK-93426 unpublished data), a subunit combination believed to influence spontaneous locomotor activity (Rudolph et al., 1999). This suggests that there is a subtle difference in the way Ro15-1788 and ZK-93426 interact with the $GABA_A$ receptor subtype(s), presumably containing the α_1 subunit, to influence locomotion as compared to Ro15-4513 and RY10.

In conclusion, eight structurally diverse benzodiazepine binding site ligands were assessed for their ability to influence contextual memory in mice. Seven of the eight benzodiazepine binding site ligands tested were without significant effect on contextual memory when administered alone. However, six of the eight ligands were able to significantly attenuate the contextual memory impairment associated with the administration of the muscarinic M1 acetylcholine receptor antagonist, scopolamine (Fig. 2B). Four of these ligands, Ro15-4513, Ro15-1788, ZK-93426, and RY10, gave no indication of locomotor impairment at doses up to 30 mg/kg (Table 3), thereby warranting further investigations into exploiting such compounds for potential therapeutic value in treating conditions that exhibit cholinergic hypofunction and the resulting contextual memory impairment. Sarter and Bruno (1997) attribute the cognitive improving properties of benzodiazepine binding site ligands, like ZK-93426, to their ability to potentiate activated acetylcholine efflux while only modestly increasing resting acetylcholine efflux. The specific mechanism by which benzodiazepine binding site ligands are able to potentiate this acetylcholine efflux and consequently influence memory is still unclear. However, the $GABA_A$ receptor remains a good target for cognitive intervention. Using specific benzodiazepine ligands to disinhibit GABAergic control over cholinergic neurons is likely to augment performance-associated increases in cortical acetylcholine efflux while preserving the highly complex transmission patterns inherent to cortical cholinergic pathways (Sarter and Bruno, 1997). The unique cognitive profile of benzodiazepine binding site ligands coupled with the preservation of $GABA_A$ receptors in brain areas most affected by Alzheimer's disease (Meyer et al., 1995; Mizukami et al., 1997), in contrast to other potential neurotransmitter targets that are not so well preserved, suggests that $GABA_A$ receptors warrant further investigation into their utilization as a therapeutic target in which to modulate memory in conditions of cognitive deficit.

Thus, these results provide an initial starting point in order to identify benzodiazepine binding site ligands that specifically enhance contextual memory under conditions that contribute to cognitive dysfunction while minimizing potential side effects. Ligands exhibiting specific affects on contextual memory can be further utilized in identifying the particular $GABA_A$ receptors subtypes in which this affect is being mediated.

Acknowledgements

Work supported by NIDA Grant DA 06304. Helpful discussions with Dr. Danni L. Harris and Dr. Marta Filizola are gratefully acknowledged.

References

Abe, K., Takeyama, C., Yoshimura, K., 1998. Effects of S-8510, a novel benzodiazepine receptor partial inverse agonist, on basal forebrain lesioning-induced dysfunction in rats. Eur. J. Pharmacol. 347, 145–152.

Anagnostaras, S.G., Maren, S., Fanselow, M.S., 1995. Scopolamine selectively disrupts the acquisition of contextual fear conditioning in rats. Neurobiol. Learn. Mem. 64, 191–194.

Anholt, R.R., Souza, E.B.D., Oster-Granite, M.L., Synder, S.H., 1985. Peripheral-type benzodiazepine receptors: autoradiographic localization in whole-body sections of neonatal rats. J. Pharmacol. Exp. Ther. 233, 517–526.

Bonnert, T.P., McKernan, R.M., Farrar, S., Bourdelles, B.I., Heavens, R.P., Smith, D.W., Hewson, L., Rigby, M.R., Sirinathsinghji, D.J., Brown, N., Wafford, K.A., Whiting, P.J., 1999. Theta, a novel gamma-aminobutyric acid type A receptor subunit. Proc. Natl. Acad. Sci. U. S. A. 96, 9891–9896.

Buxton, A., Callan, O.A., Blatt, E.J., Wong, E.H., Fontana, D.J., 1994. Cholinergic agents and delay-dependent performance in the rat. Pharmacol., Biochem. Behav. 49, 1067–1073.

Buzsaki, G., Bickford, R.G., Ponomareff, G., Thal, L.J., Mandel, R., Gage, F.H., 1988. Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. J. Neurosci. 8, 4007–4026.

Chen, C., Kim, J.J., Thompson, R.F., Tonegawa, S., 1996a. Hippocampal lesions impair cotextual fear conditioning in two strains of mice. Behav. Neurosci. 110, 1177–1180.

Chen, S.W., Chen, H.A., Davies, M.F., Loew, G.H., 1996b. Putative benzodiazepine partial agonists demonstrate receptor heterogeneity. Pharmacol., Biochem. Behav. 53, 87–97.

Croucher, M., Sarro, G.D., Jensen, L., Meldrum, B., 1984. Behavioural and convulsant actions of two methyl esters of beta-carboline-3-carboxylic acid in photosensitive baboons and in DBA/2 mice. Eur. J. Phamacol. 104, 55–60.

Davies, M.F., Onaivi, E.S., Chen, S.W., Maguire, P.A., Tsai, N.F., Loew, G.H., 1994. Evidence for central benzodiazepine receptor heterogeneity from behavior tests. Pharmacol., Biochem. Behav. 49, 47–56.

Duka, T., Ott, H., Rohloff, A., Voet, B., 1996. The effects of a benzodiazepine receptor antagonist beta-carboline ZK-93426 on scopolamine-induced impairment on attention, memory and psychomotor skills. Psychopharmacology (Berlin) 123, 361–373.

Ebert, U., Kirch, W., 1998. Scopolamine model of dementia: electroencephalogram findings and cognitive performance. Eur. J. Clin. Invest. 28, 944–949.

Evans-Martin, F.F., Terry, A.V., Jackson, W.J., Buccafusco, J.J., 2000. Evaluation of two rodent delayed-response memory tasks. A method with retractable levers versus a method with closing doors. Physiol. Behav. 70, 233–241.

Fanselow, M.S., Bolles, R.C., 1979. Naloxone and shock-elicited freezing in the rat. J. Comp. Physiol. Psychol. 93, 736–744.

Geula, C., Mesulam, M.M., 1996. Systematic regional variations in the

loss of cortical cholinergic fibers in Alzheimerr's disease. Cereb. Cortex 6, 165–177.

Huang, Q., Liu, R., Zhang, P., He, X., McKernan, R., Gan, T., Bennett, D.W., Cook, J.M., 1998. Predictive models for GABAA/benzodiazepine receptor subtypes: studies of quantitative structure–acitivity relationships for imidazobenzodiazepines at five recombinant GABAA/benzodiazepine receptor subtypes [axb3g2 (x = 1–3, 5, and 6)] via comparative molecular field analysis. J. Med. Chem. 41, 4131–4142.

Jensen, L.H., Stephens, D.N., Sarter, M., Petersen, E.N., 1987. Bidirectional effects of beta-carbolines and benzodiazepines on cognitive processes. Brain Res. Bull. 19, 359–364.

Kim, J.J., Fanselow, M.S., 1992. Modality-specific retrogade amnesia of fear. Science 256, 675–677.

Kim, J.J., DeCola, J.P., Landeira-Fernandez, J., Fanselow, M.S., 1991. *N*-methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning. Behav. Neurosci. 105, 126–133.

Lehericy, S., Hirsch, E.C., Cervera-Pierot, P., Hersh, L.B., Bakchine, S., Piette, F., Duyckaerts, C., Hauw, J.J., Javoy-Agid, F., Agid, Y., 1993. Heterogeneity and selectivity of the degeneration of cholinergic neurons in the basal forebrain of patients with Alzheimer's disease. J. Comp. Neurol. 330, 15–31.

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

McEwen, B.S., 1997. Possible mechanism for atrophy of the human hippocampus. Mol. Psychiatry 2, 255–262.

McGaughy, J., Kaiser, T., Sarter, M., 1996. Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical acetylcholineE-positive fiber density. Behav. Neurosci. 110, 247–265.

McNamara, R.K., Skelton, R.W., 1992. Assessment of a cholinergic contribution to chlordiazepoxide-induced deficits of place learning in the Morris water maze. Pharmacol., Biochem. Behav. 41, 529–538.

Meyer, M., Koeppe, R.A., Frey, K.A., Foster, N.L., Kuhl, D.E., 1995. Positron emission tomography measures of benzodiazepine binding in Alzheimer's disease. Arch. Neurol. 52, 314–317.

Mizukami, K., Ikonomovic, M.D., Grayson, D.R., Rubin, R.T., Warde, D., Sheffield, R., Hamilton, R.L., Davies, P., Armstrong, D.M., 1997. Immunohistochemical study of GABA(A) receptor beta2/3 subunits in the hippocampal formation of aged brains with Alzheimer-related neuropathologic changes. Exp. Neurol. 147, 333–345.

Olsen, R.W., DeLorey, T.M., 1999. GABA and glycine. In: Siegel, G.J., Agranoff, B.W., Albers, R.W., Molinoff, P.B. (Eds.), GABA and Glycine. Raven Press, New York, NY, pp. 335–346.

Owen, E.H., Logue, S.F., Rasmussen, D.L., Wehner, J.M., 1997. Assessment of learning by the Morris water task and fear conditioning in inbred mouse strains and F1 hybrids: implications of genetic background for single gene mutations and quantitative trait loci analyses. Neuroscience 80, 1087–1099.

Prather, P.L., Forster, M.J., Lal, H., 1992. Learning and memory-enhancing effects of Ro 15-4513: a comparison with flumazenil. Neuropharmacology 31, 299–306.

Robbins, D.K., Hutcheson, S.L., Miller, T.D., Green, V.I., Bhargava, V.O., Weir, S.J., 1997. Pharmacokinetics of MDL 26,479, a novel benzodiazepine inverse agonist, in normal volunteers. Biopharm. Drug Dispos. 78, 325–334.

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.

Sannita, W.G., Maggi, L., Rosadini, G., 1987. Effects of scopolamine (0.25–0.75 mg i.m.) on the quantitative EEG and the neuropsychological status of healthy volunteers. Neuropsychobiology 17, 199–205.

Sarter, M., Bruno, J.P., 1997. Trans-synaptic stimulation of cortical acetylcholine and enhancement of attentional functions: a rational approach for the development of cognition enhancers. Behav. Brain Res. 83, 7–14.

Sarter, M., Steckler, T., 1989. Spontaneous exploration of a 6-arm radial tunnel maze by basal forebrain lesioned rats: effects of the benzodiazepine receptor antagonist beta-carboline ZK 93426. Psychopharmacology (Berlin) 98, 193–202.

Sarter, M., Hagan, J., Dudchenko, P., 1992. Behavioral screening for cognition enhancers: from indiscriminate to valid testing: Part I. Psychopharmacology (Berlin) 107, 144–159.

Takada, S., Sasatani, T., Chomei, N., Adachi, M., Fujishita, T., Eigyo, M., Murata, S., Kawasaki, K., Matsushita, A., 1996. Synthesis and structure–activity relationships of fused imidazopyridines: a new series of benzodiazepine receptor ligands. J. Med. Chem. 39, 2844–2851.

Venault, P., Chapouthier, G., Carvalho, L.P.d., Simiand, J., Morre, M., Dodd, R.H., Rossier, J., 1986. Benzodiazepine impairs and beta-carboline enhances performance in learning and memory tasks. Nature 321, 864–866.

Whitehouse, P.J., 1998. The cholinergic deficit in Alzheimer's disease. J. Clin. Psychiatry 59 (Suppl. 13), 19–22.

Willner, P., 1986. Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. Prog. Neuro-Psychoparmacol. Biol. Psychiatry 10, 677–690.

Wisden, W., Laurie, D.J., Monyer, H., Seeburg, P.H., 1992. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain: I. Telencephalon, diencephalon, mesencephalon. J. Neurosci. 12, 1040–1062.