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Allosteric Modulation of GAB_{AA} Receptor Subtypes: Effects on Visual Recognition and Visuospatial Working Memory in Rhesus Monkeys

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Non-selective positive allosteric modulators (PAMs) of $GABA_A$ receptors ($GABA_A$Rs) are known to impair anterograde memory. The role of the various $GABA_A$R subtypes in the memory-impairing effects of non-selective $GABA_A$R PAMs has not been fully elucidated. The current study assessed, in rhesus monkeys, effects of modulation of α1, α2/3, and α5$GABA_A$Rs on visual recognition and spatial working memory using delayed matching-to-sample (DMTS) and self-ordered spatial search (SOSS) procedures, respectively. The DMTS procedure ($n = 8$) involved selecting a previously presented 'sample' image from a set of multiple images presented after a delay. The SOSS procedure ($n = 6$) involved touching a number of boxes without repeats. The non-selective $GABA_A$R PAM triazolam and the α1$GABA_A$ preferential PAMS zolpidem and zaleplon reduced accuracy in both procedures, whereas the α5$GABA_A$ preferential PAMs SH-053-2′F-R-CH_3 and SH-053-2′F-S-CH_3, and the α2/3$GABA_A$ preferential PAM TPA023B were without effects on accuracy or trial completion. The low-efficacy α5$GABA_A$R negative allosteric modulator (NAM) PWZ-029 slightly increased only DMTS accuracy, whereas the high-efficacy α5$GABA_A$R NAMs RY-23 and RY-24 did not affect accuracy under either procedure. Finally, the slopes of the accuracy dose-effect curves for triazolam, zolpidem, and zaleplon increased with box number in the SOSS procedure, but were equivalent across DMTS delays. The present results suggest that (1) α1$GABA_A$Rs, compared with α2/3 and α5$GABA_A$Rs, are primarily involved in the impairment, by non-selective $GABA_A$R PAMs, of visual recognition and visuospatial working memory in nonhuman primates; and (2) relative cognitive impairment produced by positive modulation of $GABA_A$Rs increases with number of locations to be remembered, but not with the delay for remembering.

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INTRODUCTION

Several clinically important effects of benzodiazepines (BZs), such as the anxiolytic, sedative, and muscle-relaxing effects, occur via positive allosteric modulation of $GABA_A$ receptors ($GABA_A$Rs). BZs also can produce anterograde memory impairment (Haefely *et al*, 1993), which is a useful effect when these drugs are used as adjuncts to surgical anesthesia, but problematic when the drugs are used for other indications (eg, Kleykamp *et al*, 2012; Mintzer and Griffiths, 1999b). The discovery of the heterogeneity of $GABA_A$Rs, particularly those that contain BZ binding sites (ie, α1-, α2-, α3-, and α5-containing $GABA_A$Rs), spurred research into the development of subtype-selective ligands that might produce clinically desired effects without unwanted side effects (Atack, 2011a, b; Ator *et al*, 2010; Clayton *et al*, 2007).

Of the BZ-sensitive $GABA_A$R subtypes, α1-, α2/3-, and α5-containing $GABA_A$Rs have been implicated in cognition and memory. For example, mice with a point mutation in the gene coding for α1$GABA_A$Rs were insensitive to the recall-impairing effects of the non-selective positive allosteric modulator (PAM) diazepam on passive avoidance and punishment of drinking (Rudolph *et al*, 1999). Also in mice, elimination of α5$GABA_A$Rs improved water maze performance (Collinson *et al*, 2002) and mice with a point mutation in α5$GABA_A$Rs displayed greater trace fear conditioning than wild-type mice (Crestani *et al*, 2002). In addition, in mice and rats, investigational drugs that are negative allosteric modulators (NAMs) at α5$GABA_A$Rs improved performance and/or attenuated drug-induced deficits in a variety of preclinical memory assessments (Atack *et al*, 2006; Ballard *et al*, 2009; Chambers *et al*, 2003;

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Collinson *et al*, 2006; Dawson *et al*, 2006; DeLorey *et al*, 2001; Harris *et al*, 2008; Savic *et al*, 2008).

In contrast to the amount of work conducted on the role of GABA$_A$R subtypes in memory in rodents, relatively little work has been done on the role of GABA$_A$R subtypes in memory in nonhuman or human primates. In one study, an α5GABA$_A$R NAM Ro4938581 improved object retrieval and detour performance (ORD) in cynomolgus monkeys (Ballard *et al*, 2009); in another study, a low-efficacy α2/3GABA$_A$R PAM TPA023 reversed ketamine-induced deficits on a spatial delayed-response task in rhesus monkeys (Castner *et al*, 2010). In rhesus monkeys, ORD deficits produced by the non-selective PAM triazolam and the α1GABA$_A$R-preferring zolpidem were blocked by an α1GABA$_A$-preferring antagonist β-CCT, whereas an α5GABA$_A$R-preferring antagonist XLi-093 only blocked the triazolam-induced deficits (Makaron *et al*, 2013). Finally, in humans, the α1GABA$_A$R-preferring PAM zolpidem has produced an impairment in a variety of recall tasks (Kleykamp *et al*, 2012; Mintzer and Griffiths, 1999a,b).

No study has examined the effects of selective modulation of the various GABA$_A$R subtypes within a single study. The current study investigated the role of selective modulation of α1, α2/3, and α5GABA$_A$Rs on visual recognition and visuospatial working memory in rhesus monkeys. The drugs tested (see Table 1) were a GABA$_A$ PAM with non-selective binding and efficacy (triazolam), two GABA$_A$ PAMs with selective binding affinity at α1GABA$_A$Rs (zolpidem and zaleplon), a GABA$_A$ PAM with selective efficacy at α2, α3, and α5GABA$_A$Rs (TPA023B), two novel PAMs with preferential affinity and/or efficacy at α5GABA$_A$Rs (SH-053-2′F-R-CH$_3$ and SH-053-2′F-S-CH$_3$), and three NAMs with selective binding affinity and varying negative efficacies at α5GABA$_A$Rs (PWZ-029, RY-23, and RY-24).

The behavioral procedures were selected from the Cambridge Neuropsychological Testing Automated Battery (CANTAB), which is a battery of tests that have been used extensively to study cognitive functioning in humans. Due to their non-verbal nature, CANTAB tests have been extended to nonhuman primates (Pearce *et al*, 1998; Roberts *et al*, 1990; Weed *et al*, 1999; Zurcher *et al*, 2010) and thereby allow direct cross-species comparisons. For the present study, a delayed matching-to-sample (DMTS) procedure, designed to measure visual recognition memory, and a self-ordered spatial search (SOSS) procedure, designed to measure visuospatial working memory, were used. DMTS and SOSS performances have been shown to be sensitive to mild cognitive impairment and Alzheimer's disease (Barbeau *et al*, 2004; Lange *et al*, 1995; Riekkinen *et al*, 1998; Sahakian *et al*, 1988), suggesting the clinical relevance of these procedures.

MATERIALS AND METHODS

Subjects

Adult male rhesus monkeys (*Macaca mulatta*, $n = 14$), aged 11.8 ± 4.10 (mean ± SD) years at the start of these experiments,

Table 1 Reported Binding Affinities and Efficacies in Modulating GABA-Induced Ion Flow at GABA$_A$R Subtypes of the Modulators Used in the Current Study

Drugs	Affinity or % modulation	α1	α2	α3	α5	Reference
Triazolam	Affinity (K_i, nM)	0.41	0.32	1.5	0.42	Smith *et al* (2001)
	% Modulation at 3 μM	132	255	270	165	Sanna *et al* (2002)
Zolpidem	Affinity (K_i, nM)	29.6	160	380	>10,000	Savic *et al* (2010)
	% Modulation at 100 nM	180	132	121	NS	Savic *et al* (2010)
Zaleplon	Affinity (K_i, nM)	66	830	710	1780	Dämgen and Lüddens (1999)
	% Modulation[a]	113	208	362	109	Sanna *et al* (2002)
TPA023B	Affinity (K_i, nM)	0.73	2.0	1.8	1.1	Atack *et al* (2011)
	% Modulation[a]	4	43	67	45	Atack *et al* (2011)
SH-053-R-2′F-CH$_3$	Affinity (K_i, nM)	759.1	948.2	768.8	95.2	Fischer *et al* (2010); Savic *et al* (2010)
	% Modulation at 100 nM	111	124	125	183	Fischer *et al* (2010)
	% Modulation at 1 μM	154	185	220	387	Fischer *et al* (2010)
SH-053-S-2′F-CH$_3$	Affinity (K_i, nM)	468.2	33.3	291.5	19.2	Fischer *et al* (2010); Savic *et al* (2010)
	% Modulation at 100 nM	116	170	138	218	Fischer *et al* (2010)
	% Modulation at 1 μM	164	348	301	389	Fischer *et al* (2010)
PWZ-029	Affinity (K_i, nM)	>300	>300	>300	38.8	Savic *et al* (2008)
	% Modulation at 1 μM	114	105	118	−20	Savic *et al* (2008)
	% Modulation at 10 μM	120	115	145	−20	Savic *et al* (2008)
RY-23	Affinity (K_i, nM)	197	143	255	2.61	Liu *et al* (1996)
	% Modulation[a]	−37	−50		−52	June *et al* (2001)
RY-24	Affinity (K_i, nM)	26.9	26.3	18.7	0.4	Liu *et al* (1996)
	% Modulation at 1 μM	−31	−20.7	0	−40.4	Harris *et al* (2008)

NS = nonsignificant change in current flow.
[a]Concentration not specified.

were singly housed, each in one section of a four-cage housing unit. All caging units were housed in the same temperature- and humidity-controlled vivarium room. Lights were on from 0700 to 02100 hours. Weights ranged between an average of 12.2 kg ± 1.28 at the beginning of the study and 14.5 kg ± 1.72 at the end of the study (~3.8 years). Quantities of primate diet (2050 Teklad Global 20% Protein Primate Diet, Harlan Laboratories) fed once daily (139–208 *g*) were sufficient to permit gradual weight increase over the study. In addition, the monkeys received a piece of fresh fruit or vegetables (eg, ½ orange, ½ apple, and so on) 5 days a week. Daily feeding occurred approximately 2 h after completion of behavioral testing. Water was available at all times.

Apparatus

Experimental sessions were conducted in the home cage using custom-built mobile devices as described previously (Weed *et al,* 2008). Briefly, each held a computer for the control of experimental events (CANTAB software; Lafayette Industries, Lafayette, IN) and two touchscreen monitors (Intellitouch, surface acoustic wave technology, ELO TouchSystems, Menlo Park, CA, USA), which allowed two monkeys to be tested simultaneously. A pellet dispenser (BRS/VLE, Laurel, MD, or Med Associates, St Albans, VT) was used for the delivery of 190-mg food pellets (BioServ, Frenchtown, NJ).

DMTS and SOSS Procedures

Eight monkeys were trained on the DMTS procedure. Each session consisted of 24 trials with 8 trials at each of three delays (2, 30, or 300 s). The delay on each trial was selected randomly without replacement. Each trial began with presentation, on the center of the screen, of a pseudo-randomly selected sample image from a set of 600 different images (Photo Clip Art 150 000 by Hemera Technologies). A touch on the sample image within 30 s turned off the image and initiated the selected delay. After the delay, the original sample image and two other unique, randomly selected images were presented on three corners of the screen. A touch on the image that 'matched' the original sample image produced a food pellet, followed by a 5-s period with the screen darkened. If the monkey did not touch the sample image within 30-s, did not touch one of the three choice images within 30 s, or if the monkey touched one of the two 'non-matching' images, the trial ended without pellet delivery, followed by 10 s with the screen darkened. Sessions usually lasted about 45 min and were conducted Monday–Friday, starting at approximately 1000 or 1100 hours.

Six monkeys were trained on the SOSS procedure. Each session consisted of 54 trials, and each trial involved touchscreen presentation of a configuration of a number of small blue boxes within 16 possible screen locations (screen configured in a 4 by 4 array of possible locations). The number of boxes in the stimulus configuration varied among 2, 3, and 4 boxes (18 trials of each). Each non-repeating touch on any of the boxes produced a food pellet. If the monkey made a repeat touch or failed to make a touch within 30-s of trial onset or from the time of the previous touch, the trial ended and a 9-s period followed, during which the screen remained blank and touching the screen produced no scheduled consequence, which was followed by a new trial. If the monkey touched all the boxes without repetition, the trial ended, was defined as correct, and was followed by 5 s with the screen darkened before the next trial. Sessions generally lasted about 15–20 min and were conducted Monday–Friday, starting at approximately 1000 or 1100 hours.

Assessment of Drug Effects

Drug test sessions usually occurred on Tuesdays and Fridays if subjects had completed at least 7 of 8 trials at each delay (DMTS group) or 16 of 18 trials at each number of boxes (SOSS group) in the preceding session. Drug vehicle administration usually occurred on Thursdays. Baseline (no treatment) sessions occurred on Mondays and Wednesdays. In the DMTS group, the order of testing was RY-23 i.m. (intramuscular), triazolam i.m., PWZ-029 i.m., SH-053-2′F-R-CH3 i.m., RY-24 i.m., zolpidem i.m., RY-23 p.o. (per os), PWZ-029 p.o., SH-053-2′F-S-CH3 p.o., TPA023B p.o., and zaleplon p.o. In the SOSS group, the order of testing was RY-23 i.m., triazolam i.m., PWZ-029 i.m., RY-24 i.m., zolpidem, RY-24 p.o. RY-23 p.o., zaleplon p.o., PWZ-029 p.o., and TPA023B p.o. Doses were studied in a pseudo-random order for each compound with the restriction that the highest two doses were studied after the other doses. Triazolam, zolpidem, SH-053-2′F-R-CH_3, PWZ-029, RY-23, and RY-24 were administered via i.m. injection in the thigh at a volume of 0.2–1.5 ml. PWZ-029, RY-23, and RY-24 also were tested orally (p.o.) to extend the dose range beyond that which was feasible via the i.m. route. Zaleplon, TPA023B, and SH-053-2′F-S-CH_3 only were administered p.o. due to solubility limitations. Pretreatment times were 30 min for i.m. administration of triazolam, zolpidem, PWZ-029, RY-23, RY-24, and SH-053-2′F-R-CH_3 based on preliminary data collected in a subset of monkeys. Oral administration of zaleplon, TPA023B, SH-053-2′F-S-CH_3, PWZ-029, RY-23, and RY-24 occurred 60 min before session start.

Drugs

PWZ-029 (methyl(8-chloro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-yl) methyl ester; Zhang *et al*, 1995), RY-23 (tert-Butyl 8-[(trimethylsilyl)ethynyl]-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4] benzodiazepine-3-carboxylate; Liu *et al*, 1996), RY-24 (tert-Butyl 8-ethynyl-5,6-dihydro-5methyl-6-oxo-4H-imidazo[1,5-a[1,4]benzodiazepine-3-carboxylate; Liu *et al*, 1996), and SH-053-2′F-R-CH_3 ((S)-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4H-2,5,10b-triaz-benzo[3]azulene-3-carboxylic acid ethyl ester; Cook *et al*, 2009), and SH-053-2′F-S-CH_3 ((S)-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4H-2,5,10b-triaz-benzo[3]azulene-3-carboxylic acid ethyl ester; Cook *et al*, 2009) were synthesized at the Department of Chemistry and Biochemistry, University of Wisconsin, Milwaukee, WI. For i.m. injection, PWZ-029, RY-23, RY-24, and SH-053-2′F-R-CH_3 were dissolved in 95% w/v ethanol, which was diluted with a 60:40 mixture of propylene glycol and 0.9% saline to a final concentration of 10–20% ethanol and 90–80%

propylene glycol/saline mixture (prepared fresh each day), depending on doses to be tested. Triazolam (Upjohn Pharmaceuticals, Kalamazoo, MI) was dissolved in propylene glycol at 2 mg/ml (stored for up to 2 months) and then diluted with sterile water on test days to achieve the desired concentration for injection (minimum dilution of 50% sterile water). Zolpidem tartrate (Research Biochemicals International, Natick, MA) was dissolved in 0.9% saline (solutions were stored for up to 2 weeks). Zaleplon (Wyeth-Ayerst Research, Princeton, NJ) and TPA023B (6,2′-difluoro-50-[3-(1-hydroxy-1-methylethyl)imidazo[1,2-b][1,2,4]triazin-7-yl]biphenyl-2-carbonitrile; Merck, Sharp, & Dohme, Harlow, UK) only were administered orally due to solubility limitations.

For oral administration, monkeys were first habituated gradually, across days, to drinking a bitter solution consisting of increasing concentrations of quinine (up to 0.32 mg/ml) in 60 ml of Tang orange-drink solution off the tip of a 60-ml syringe. Consuming the 60 ml was followed immediately by the opportunity to drink 40 ml of unadulterated Tang (adapted from Turkkan *et al*, 1989). Once the consumption of the 0.32 mg/ml quinine solution was reliable, drug doses were suspended, fresh each test day, in 60 ml of a matrix of 1 g/l of Bio-Serv Agent K (Frenchtown, NJ), prepared in a blender in water that was flavored with the Tang powder.

Data Analysis

For the DMTS procedure, the percentage of correct trials in the session was calculated for individual monkeys by dividing the total number of correct trials at each delay by the total number of trials completed at that delay. The percentage of trials completed at each delay was calculated by dividing number of trials completed by total trials possible. For the SOSS procedure, the percentage of correct trials for each configuration (2, 3, and 4 boxes) was calculated for individual monkeys for each session by dividing the number of trials correct (ie, all boxes touched) divided by the number of trials in which the monkey touched at least one box. The percentage of trials completed for each number of boxes was calculated by dividing the number of trials in which the monkey touched at least one box by the number of trials possible. If a monkey did not complete three or more trials at any particular delay (DMTS) or number of boxes (SOSS) in a session, the percentage of correct trials was not calculated for that monkey. Group averages for percentage of correct trials were calculated only if three or more monkeys met the individual trial completion criterion.

Two-way repeated-measures analysis of variance (ANOVA) was used to identify statistically significant effects of drug dose and task parameter (DMTS: delay; SOSS: box number). Percentages of correct trials and trials completed were converted to proportions and arcsine square root transformed to increase normality for statistical analysis (McDonald, 2009). Post-hoc comparisons using the Holm-Sidak method were conducted to compare performance in vehicle sessions with performance following drug administration.

For drugs that affected accuracy, accuracy values for individual subjects at each dose were calculated as a percentage of the average vehicle accuracy at each parameter value for each of the two procedures. A linear model was fitted to the descending portion of the dose-response curve using log dose and the pooled individual subject data (Graphpad Prism version 5, Graphpad Software, San Diego, CA). Minimal effective doses (MED) for reducing accuracy were calculated from the linear model as the dose necessary to reduce accuracy by 15% of vehicle control. This value was selected because the drugs that significantly reduced accuracy all did so by 15% or more at each parameter. In order to facilitate comparison of potencies to reduce accuracy and trial completion, the same method was used to predict the dose required to reduce trial completion by 15%. Because trial completion did not vary across delay or number of boxes, normalization was unnecessary.

RESULTS

Control Sessions

DMTS accuracy after vehicle administration was highest after delays of 2 and 30 s and lowest after delays of 300 s (Figure 1, top panels, points above V). The percentage of trials completed did not differ as a function of delay (Figure 1, bottom panels, points above V). Similarly, SOSS accuracy following vehicle administration declined as the number of boxes in the stimulus configuration increased from 2 to 4 (Figure 2, top panels, points above V), whereas the percentage of SOSS trials completed did not differ as a function of the number of boxes (Figure 2, bottom panels, points above V).

PAMs: Triazolam, zolpidem, zaleplon, TPA023B, SH-053-2′F-R-CH$_3$, and SH-053-2′F-S-CH$_3$. The non-selective high-efficacy PAM triazolam and the high-efficacy α1GABA$_A$R-preferential PAM zolpidem reduced accuracy of DMTS performance at all delays (Figure 1; $F_{6,18} = 12.8$, $P < 0.001$ and $F_{5,25} = 7.22$, $P < 0.001$, respectively) with MED values of 0.004–0.012 and 0.172–0.18 mg/kg, respectively (Table 2). Zaleplon, another high-efficacy α1GABA$_A$R-preferential PAM also reduced overall DMTS accuracy (Figure 1; $F_{6,24} = 6.20$, $P < 0.001$; main effect of 18 mg/kg) with MED values of ~4–20 mg/kg (Table 2). Triazolam dose dependently reduced the percentage of DMTS trials completed ($F_{6,30} = 10.5$, $P < 0.001$), whereas zolpidem and zaleplon did not (Figure 1). TPA023B, a PAM with non-selective affinity, but selective efficacy at α2/3GABA$_A$Rs, did not affect DMTS accuracy or trial completion (Figure 1). Finally, the two α5GABA$_A$R-preferential PAMs SH-053-2′F-R-CH$_3$ and SH-053-2′F-S-CH$_3$ did not affect DMTS accuracy or trial completion (Table 3).

Triazolam, zolpidem, and zaleplon also dose dependently decreased SOSS accuracy (Figure 2; $F_{7,35} = 18.7$, $P < 0.001$, $F_{5,35} = 7.88$, $P < 0.001$, $F_{6,30} = 5.30$, $P < 0.001$, respectively). The lowest dose of zaleplon increased SOSS accuracy on four-box trials (Figure 2, top rightmost graph). MED values for decreasing SOSS accuracy were 0.002–0.022, 0.098–0.162, and 2.65–14.2 mg/kg for triazolam, zolpidem, and zaleplon, respectively (Table 2). Triazolam decreased SOSS trials completed (Figure 2; $F_{7,35} = 3.18$, $P = 0.01$) on three- and four- but, not on two-box trials (post-hoc comparisons)

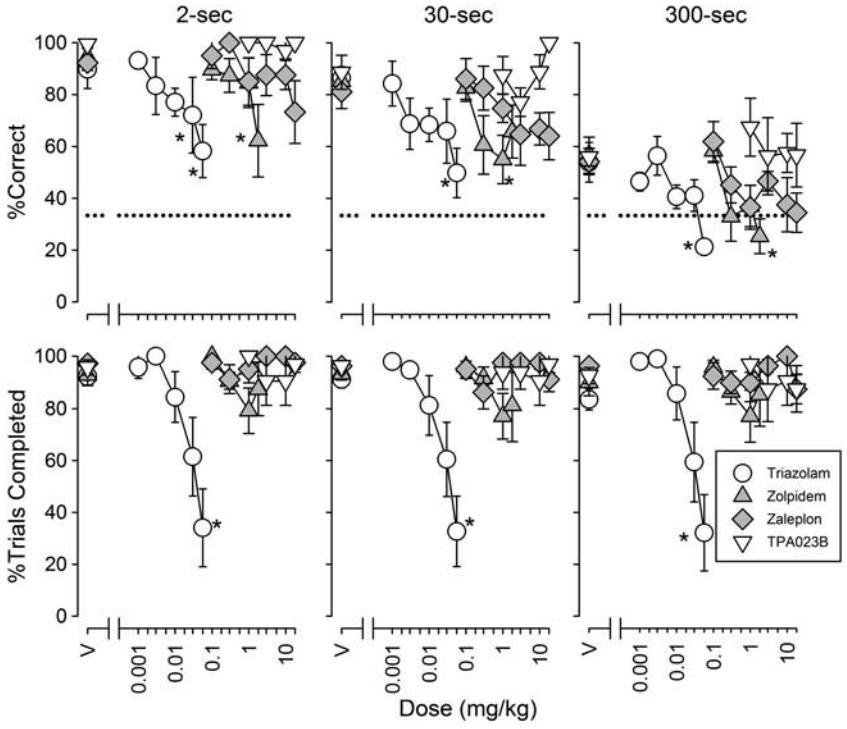

Figure 1 Effects of triazolam ($n = 6$), zolpidem ($n = 6$), zaleplon ($n = 5$), and TPA023B ($n = 4$) on accuracy (top row) and trial completions (bottom row) for trials at each delay value in the delayed matching-to-sample (DMTS) procedure. Points above 'V' represent results following vehicle administration. Each data point represents the mean across monkeys. Error bars represent ± 1 SEM. The horizontal dashed line indicates a chance accuracy (33.3%). Statistically significant post-hoc comparisons relative to vehicle are denoted by asterisks.

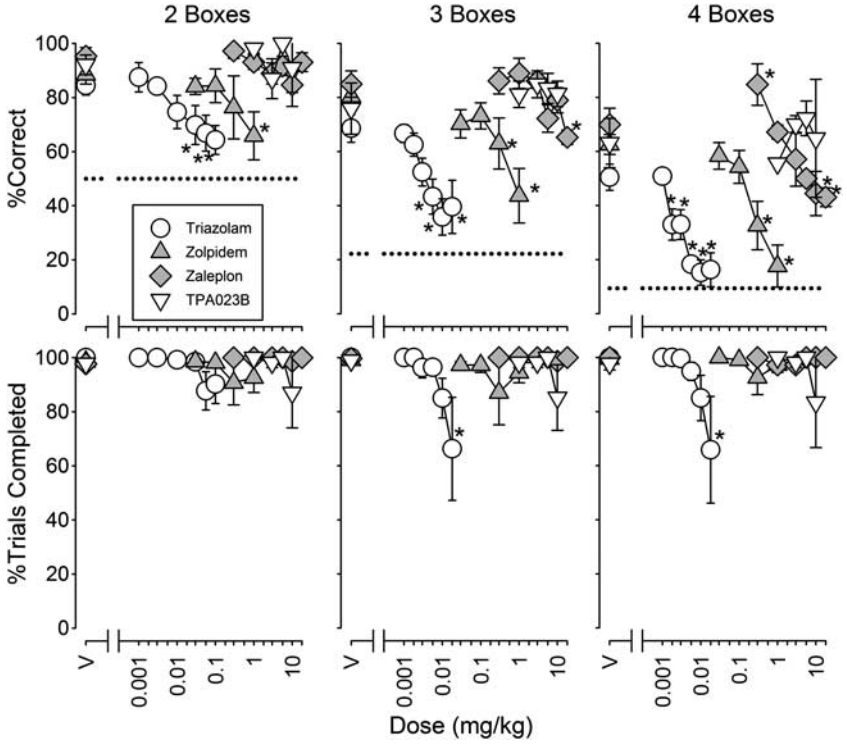

Figure 2 Effects of triazolam ($n = 6$), zolpidem ($n = 6$), zaleplon ($n = 4$), and TPA023B ($n = 4$) on self-ordered spatial search (SOSS) accuracy (top) and trial completion (bottom) for trials with each number of boxes in the SOSS procedure. The horizontal dotted lines in the top row of graphs represent chance accuracy on two-box (50%), three-box (22.2%), and four-box (9.38%) trials.

Table 2 MED Values (mg/kg) for Reducing Accuracy (Defined as 85% of Vehicle Control Accuracy Values) and for Decreasing Trial Completion by the Same Amount for Each Drug that had Statistically Significant Effects on the Relevant-Dependent Measure

Drugs	DMTS delays		
	2-s	30-s	300-s
		Accuracy	
Triazolam i.m.	0.012 (0.003–0.613)	0.004 (0–0.023)	0.004 (0–0.016)
Zolpidem i.m.	NS	0.180 (0–0.489)	0.172 (0.005–0.402)
Zaleplon p.o.	21.7 (3.50–[a])	4.05 (0.857–518.8)	0.891 (0.004–6.724)
		Trial completion	
Triazolam i.m.	0.007 (0.003–0.013)	0.006 (0.002–0.011)	0.007 (0.003–0.013)
RY-23 p.o.	1.75 (0.294–3.064)	1.62 (0.182–2.919)	1.22 (0.014- 2.493)
RY-24 i.m.	0.022 (0.011–0.035)	0.041 (0.024–0.056)	0.038 (0.018–0.054)
	SOSS number of boxes		
	Two boxes	**Three boxes**	**Four boxes**
		Accuracy	
Triazolam i.m.	0.022 (0.009–0.079)	0.004 (0.001–0.008)	0.002 (0.001–0.006)
Zolpidem i.m.	NS	0.162 (0.004–0.358)	0.098 (0.012–0.188)
Zaleplon p.o	NS	14.2 (5.70–194)	2.65 (1.53–4.37)
		Trial completion	
Triazolam i.m.	NS	0.050 ([a]–[a])	0.049 ([a]–[a])
RY-24 i.m.	0.048 (0.024–0.069)	0.049 (0.025–0.070)	0.048 (0.024–0.070)
RY-23 p.o.	6.038 (0.993–7.829)	5.837 (0–7.811)	5.954 (0.46–7.777)
RY-24 p.o.	0.414 ([a]–[a])	0.426 ([a]–[a])	0.415 ([a]–[a])

Abbreviations: DMTS, delayed matching-to-sample; i.m., intramuscular; p.o., per os; SOSS, self-ordered spatial search.
Numbers in parentheses are 95% confidence limits (CLs).
NS = nonsignificant linear regression.
[a]95% CL could not be calculated.

with a potency of $\sim$0.05 mg/kg. Zolpidem and zaleplon did not alter SOSS trial completion (Figure 2). TPA023B was without effect on SOSS accuracy and trial completion (Figure 2).

Negative Allosteric Modulators: PWZ-029, RY-23, and RY-24

The low-efficacy α5GABA$_A$R-selective NAM PWZ-029 (i.m.) produced a small, but statistically significant, improvement in performance (Figure 3; $F_{5,25} = 3.72$, $P = 0.012$), but post-hoc tests did not reveal a dose at which the performance differed significantly from vehicle performance and when administered orally, PWZ-029 was without statistically significant effect on accuracy or trial completion (Figure 3). The high-efficacy α5GABA$_A$R-selective NAMs RY-23 (i.m. or p.o.) and RY-24 (i.m.) were without statistically significant effects on DMTS accuracy (Figure 3, top row). Trial completions at all delays were dose dependently decreased by i.m. RY-24 (Figure 3; $F_{4,20} = 7.34$, $P < 0.001$) and p.o. RY-23 ($F_{5,10} = 10.22$, $P = 0.001$), but not i.m. RY-23. The MED values of i.m. RY-24 and p.o. RY-23 in reducing trial completion were 0.022–0.041 and 1.22–1.75 mg/kg, respectively (Table 2).

In the SOSS procedure, RY-23 (i.m. and p.o.), RY-24 (i.m. and p.o.), and PWZ-029 (i.m. and p.o.) were without significant effects on accuracy (Figure 4), despite an apparent decreasing trend in accuracy with RY-24 (i.m.) on two- and three-box trials. Trial completions at all box numbers were decreased by RY-23 (p.o.) and RY-24 (i.m. and p.o.). Decreases in SOSS trial completion were statistically significant for p.o. RY-24 and p.o. RY-23 (Figure 4; $F_{4,12} = 4.65$, $P = 0.017$; and $F_{4,16} = 8.05$, $P < 0.001$, respectively) and for i.m. RY-24 when the two subjects that were tested with the highest dose of 0.18 mg/kg were used in the ANOVA ($F_{6,6} = 17.1$, $P = 0.002$). (Only two monkeys were tested at 0.18 mg/kg because there was $\geqslant$50% suppression of trial completion at 0.1 mg/kg for the other monkeys, and we were concerned about adverse effects if we tested the higher dose in those monkeys.) MED values for reducing SOSS trial completion were $\sim$0.05, 0.4, and 0.6 mg/kg for RY-24 i.m., RY-24 p.o., and RY-23 p.o. (Table 2).

Table 3 Percentage of Trials Correct and Completed After Administration of Vehicle ('V') and Doses (mg/kg) of SH-053-2′F-S-CH_3 ($n=4$) and SH-053-2′F-R-CH_3 ($n=3$) During DMTS Trials of 2-, 30-, and 300-s Delays

Dose	%Trials correct (SEM)			%Trials completed (SEM)		
	2-s	30-s	300-s	2-s	30-s	300-s
SH-053-2′F-S-CH_3 (p.o.)						
V	93.1 (6.94)	83.3 (2.41)	52.8 (7.35)	91.7 (8.33)	91.7 (8.33)	91.7 (8.33)
3	91.7 (4.17)	85.0 (2.50)	41.7 (9.30)	100 (0.00)	87.5 (12.5)	91.7 (4.17)
5.6	93.8 (6.25)	77.4 (12.4)	66.7 (16.7)	95.8 (4.17)	91.7 (4.17)	91.7 (8.33)
10	95.8 (4.17)	91.7 (8.33)	36.3 (4.17)	100 (0.00)	95.8 (4.17)	91.7 (4.17)
SH-053-2′F-R-CH_3 (i.m.)						
V	89.2 (9.26)	84.3 (6.16)	54.2 (6.16)	98.4 (0.90)	99.2 (0.78)	99.2 (0.78)
0.03	84.4 (15.6)	86.2 (10.1)	56.3 (14.9)	100 (0.00)	96.9 (3.13)	100 (0.00)
0.3	84.4 (9.38)	78.1 (10.7)	53.6 (13.4)	100 (0.00)	100 (0.00)	96.9 (3.13)
1	78.1 (12.9)	83.5 (6.49)	64.3 (8.15)	100 (0.00)	96.9 (3.13)	96.9 (3.13)
1.8	87.5 (7.22)	83.3 (16.7)	60.7 (7.43)	100 (0.00)	100 (0.00)	95.8 (4.17)

Abbreviations: DMTS, delayed matching-to-sample; i.m., intramuscular; p.o., per os.

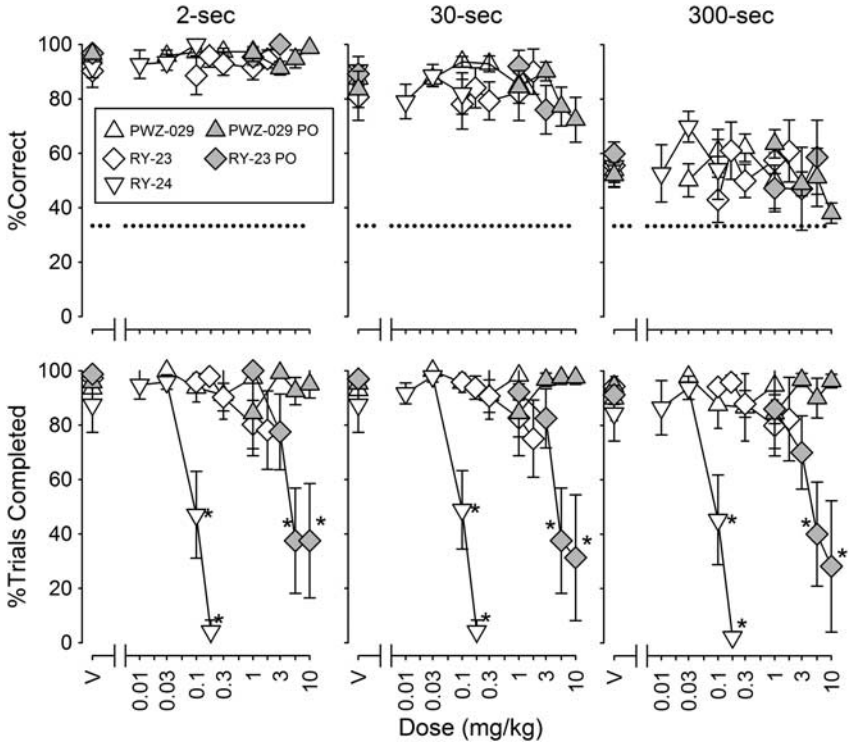

Figure 3 Effects of PWZ-029 i.m. (intramuscular) ($n=7$) and p.o. (per os) ($n=5$), RY-23 i.m. ($n=6$) and p.o. ($n=5$), and RY-24 i.m. ($n=6$) on delayed matching-to-sample (DMTS) accuracy (top) and trial completion (bottom). Other details are as in Figure 1.

Comparison of Effects of Triazolam, Zolpidem, and Zaleplon on DMTS and SOSS Performances

For triazolam, zolpidem, and zaleplon, the relative impairment of accuracy did not depend on DMTS delay. The slopes of the dose-response curves for reducing DMTS accuracy at each delay value were not significantly different (Figure 5, top panels). In contrast, in the SOSS procedure, relative impairment of accuracy depended on the number of locations to be remembered. The slopes of the triazolam, zolpidem, and zaleplon dose-response curves varied significantly as number of boxes increased (Figure 5, bottom panels; $F_{2,98}=4.83$, $P=0.01$; $F_{2,66}=3.43$, $P=0.0384$; and $F_{2,66}=12.3$, $P<0.001$, respectively).

DISCUSSION

The current results with triazolam are consistent with a number of studies demonstrating working memory impairment with other non-selective BZs in nonhuman primates (Baron and Wenger, 2001; Bradley and Nicholson, 1984;

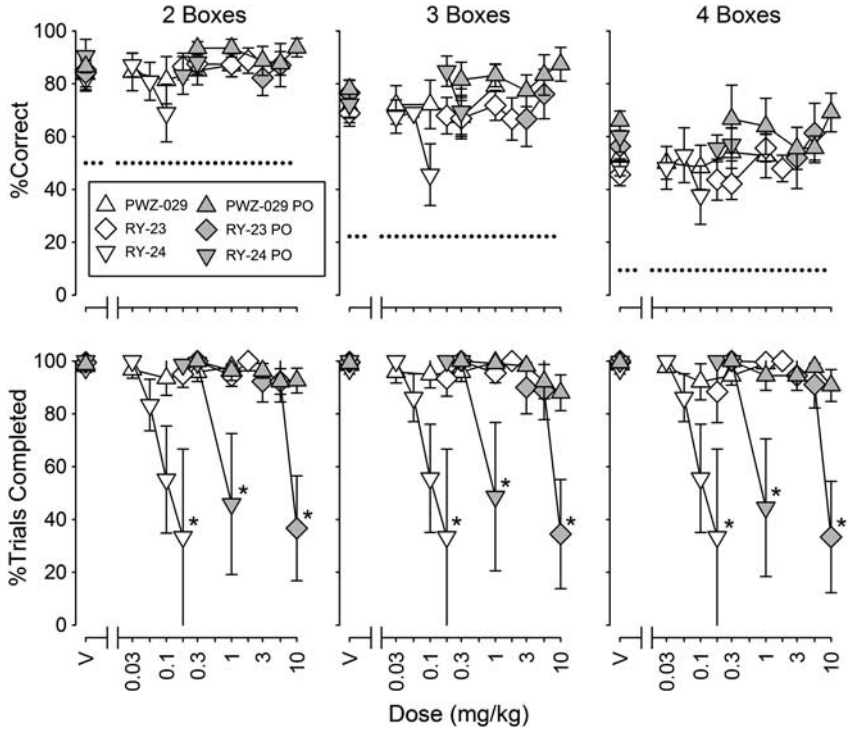

Figure 4 Effects of PWZ-029 i.m. ($n = 6$) and p.o. ($n = 6$), RY-23 i.m. ($n = 6$) and p.o. ($n = 5$), and RY-24 i.m. ($n = 6$ except that only two monkeys were tested at the highest dose as explained in Results) and p.o. ($n = 4$) on self-ordered spatial search (SOSS) accuracy (top) and trial completion (bottom). Other details are as in Figure 2.

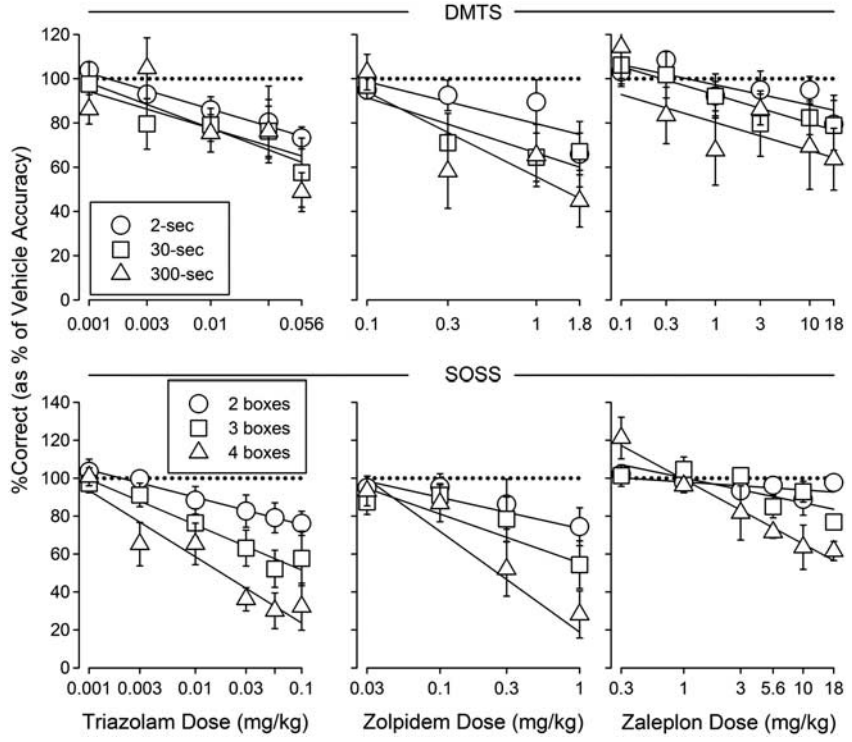

Figure 5 Normalized (% of vehicle accuracy values) dose-response curves along with best-fitting straight lines for triazolam's (left), zolpidem's (middle), and zaleplon's (right) effects on delayed matching-to-sample (DMTS) (top) and self-ordered spatial search (SOSS) (bottom) accuracy. Each data point represents the mean across monkeys. Error bars represent ± 1 SEM. The horizontal dotted lines are for reference and indicate 100% of vehicle accuracy. Solid lines represent best-fitting lines. Other details are as in Figures 1 and 2 for DMTS and SOSS, respectively.

Myers and Hamilton, 2011; Schulze *et al*, 1989). Our results with subtype-selective GABA$_A$R PAMs suggest that modulation of α1GABA$_A$Rs, but not α2/3 or α5GABA$_A$Rs impairs visual recognition and visuospatial working memory in nonhuman primates; and, by extension, our results suggest that cognitive impairment produced by non-selective

GABA$_A$R PAMs is primarily due to the modulation of α1GABA$_A$Rs. First and foremost, zolpidem, which has virtually no affinity for α5GABA$_A$Rs, and zaleplon, which has very low affinity for this subtype, produced dose-dependent reductions in DMTS and SOSS accuracy, suggesting a lack of involvement of α5GABA$_A$Rs in the effects of non-selective GABA$_A$R PAMs. Second, neither the α5GABA$_A$R-selective PAMs (SH-053-2′F-R-CH$_3$ and SH-053-2′F-S-CH$_3$) nor the α5GABA$_A$R-selective NAMs (PWZ-029, RY-23, and RY-24) affected DMTS or SOSS accuracy, even at doses of some of the NAMs that profoundly suppressed responding. Finally, the lack of effects of TPA023B, at mg/kg doses shown to produce close to 100% occupancy of α2/3GABA$_A$Rs in humans, baboons, rats, and mice (Atack *et al*, 2011), suggests that positive modulation of α2/3GABA$_A$Rs, at least at low levels, is not sufficient to produce impairments in visual recognition and visuospatial working memory. Interestingly, the lowest dose of zaleplon was associated with an improvement in SOSS accuracy on four-box trials, but this outcome was not shown with zolpidem, suggesting that such an improvement is not a general feature of low doses of non-selective or α1GABA$_A$R-preferential PAMs. Together, these results suggest that positive allosteric modulation of α1GABA$_A$Rs, compared with α2/3 and α5GABA$_A$Rs, is the major contributor to visual recognition and visuospatial working memory impairment produced by non-selective GABA$_A$R PAMs in nonhuman primates.

The lack of consistent, dose-dependent effects on DMTS and SOSS accuracy of the α5GABA$_A$R NAMs is partially consistent with results in elderly human participants that demonstrated no effect in a paired-associates learning task at a low dose and impairment at a high dose of the α5GABA$_A$R NAM α5IA (Atack, 2010). The lack of robust effects of the α5GABA$_A$R NAMs on DMTS and SOSS accuracy in the present study with rhesus monkeys do not appear consistent with results in rodents that demonstrated improved performance in Morris water maze and passive avoidance procedures with a variety of α5GABA$_A$R NAMs (Atack *et al*, 2006; Chambers *et al*, 2003, 2004; Collinson *et al*, 2006; Savic *et al*, 2008). Nor are the present results consistent with those in cynomolgus monkeys that demonstrated improved ORD performance with an α5GABA$_A$R NAM (Ballard *et al*, 2009). The variables responsible for these discrepancies are not clear, but may include both task and species. Perhaps the simplest explanation is that α5GABA$_A$R NAMs do not have pro-cognitive effects on short-term working memory performances maintained by positive reinforcement. This conclusion is consistent with the current results as well as the lack of improvement with Ro4938591 on delayed matching-to-position performance in rats (Ballard *et al*, 2009) and the reported pro-cognitive effects on performance in the Morris water maze and untrained behavior such as passive avoidance behavior and ORD reaching. Importantly, PWZ-029 improved novel object recognition in rats without improving Morris water maze performance (Milic *et al*, 2013), demonstrating that α5GABA$_A$R NAMs do not universally improve performance in aversively motivated working memory procedures such as the Morris water maze.

In addition, the failure of the α5GABA$_A$R PAMs SH-053-2′F-R-CH$_3$ and SH-053-2′F-S-CH$_3$ to affect either DMTS or SOSS performance is consistent with the results in rodents in which SH-053-2′F-R-CH$_3$ was without effect on Morris water maze performance (Savic *et al*, 2010) and consistent with the failure of the α5GABA$_A$R NAMs PWZ-029, RY-23, and RY-24 to alter DMTS or SOSS accuracy. Interestingly, a recent study demonstrated that an α5GABA$_A$R PAM improved radial arm maze performance in aged impaired rats at doses that produced no change in the performance in young rats (Koh *et al*, 2012), which suggests that the effects of positive allosteric modulation of α5GABA$_A$Rs may depend on the age of the subjects and/or the performance baseline.

The effects of zolpidem on DMTS accuracy compared with DMTS trial completion were more selective than those of triazolam. The ratios of the triazolam MED values for reducing accuracy and decreasing trial completion ranged from ∼0.6 to 1.8. In contrast, zolpidem was without significant effects on trial completion at doses 10-fold greater than its MED value for reducing accuracy, indicating that the selectivity of zolpidem to reduce accuracy compared with trial completion was greater than that of triazolam. Whether the effects of zolpidem on accuracy and trial completion in the SOSS procedure were more selective than those of triazolam is unclear. The selectivity of triazolam for impairing SOSS accuracy compared with suppressing SOSS trial completion, based on ratios of MED values for the two measures (∼13–25), was much higher than its selectivity in the DMTS procedure; a conservative estimate of the selectivity of zolpidem based on the highest dose tested (1 mg/kg) compared to its MED values for reducing SOSS accuracy (0.098–0.162) suggests a selectivity within that range.

The reliability of the effects of GABA$_A$R PAMs and NAMs in the current study is demonstrated by the replication of effects across the two procedures and in the two groups of monkeys. Also, the current results demonstrate that degree of impairment produced by GABA$_A$R PAMs varied in a load-, but not delay-dependent fashion; relative impairment increased with the number of locations to be remembered in the SOSS procedure, but did not vary with the delay for remembering a stimulus in the DMTS procedure. The delay-independent effects of triazolam are consistent with studies reporting delay-independent impairment of DMTS performance by the non-selective GABA$_A$R PAM diazepam in humans and monkeys (Robbins *et al*, 1997; Schulze *et al*, 1989). The current study extends those findings to α1-selective GABA$_A$R PAMs. The differential sensitivity of SOSS, but not DMTS, performance to triazolam, zolpidem, and zaleplon is consistent with the notion that the two procedures measure different aspects of working memory and suggests that positive allosteric modulation of GABA$_A$Rs has a greater relative impact on spatial working memory as the number of locations to be remembered increases, but not on visual recognition working memory as the delay for remembering increases.

In conclusion, the present results suggest a prominent role of α1GABA$_A$Rs in the effects of non-selective GABA$_A$R PAMs on visual recognition and visuospatial working memory in nonhuman primates. This finding implicates α1GABA$_A$Rs in nonhuman primate working memory under unperturbed conditions; however, this conclusion is tentative given the risk associated with extrapolating from

receptor function in perturbed (ie, drug-induced) conditions to receptor function in unperturbed conditions. The finding of a prominent role of α1GABA$_A$Rs in the effects of non-selective GABA$_A$R PAMs on visual recognition and visuospatial working memory further suggests that the efforts to separate sedative and working memory-impairing effects of GABA$_A$R modulation may not be feasible because it appears that α1GABA$_A$Rs are involved in both the sedative and memory-altering effects of drugs such as BZs. However, these studies suggest that positive modulators of α2/3GABA$_A$Rs, which have been pursued as non-sedating anxiolytics (eg, Atack, 2011a), would have minimal impact on cognitive function. The current results do not identify underlying brain regions or circuits involved in the effects of GABA$_A$R modulation on working memory, but future research might investigate local infusion of GABA$_A$R modulators to determine brain region involvement. Future research also might investigate the effects of allosteric modulation of GABA$_A$R subtypes on visual recognition and visuospatial working memory in aged monkeys or in animals with neurobiological or pharmacological impairment to determine whether the effects vary as a function of age or state of impairment.

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