Cognition-Impairing Effects of Benzodiazepine-Type Drugs: Role of GABA_A Receptor Subtypes in an Executive Function Task in Rhesus Monkeys

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Abstract

The present studies evaluated the role of $\alpha 1$ and $\alpha 5$ subunit-containing GABA_A receptors ($\alpha 1$GABA_A and $\alpha 5$GABA_A receptors, respectively) in the ability of benzodiazepine (BZ)-type drugs to alter performance in the cognitive domain of executive function. Five adult female rhesus monkeys (ages 9–17 years old) were trained on the object retrieval with detours (ORD) task of executive function. For the ORD task, monkeys were required to retrieve food items from a clear box with one open end that was rotated to different positions along with varying placements of food. When the non-selective BZ triazolam and the $\alpha 1$GABA_A-preferring agonists zolpidem and zaleplon were evaluated in the ORD task, deficits in performance occurred at doses that did not increase the latency of monkeys to initiate responding and/or increase the percentage of reaches that were incorrect (i.e., reaches in which food was not obtained). Cognition-impairing effects of triazolam and zolpidem in ORD were blocked by the $\alpha 1$GABA_A-preferring antagonist, βCCT, whereas the $\alpha 5$GABA_A-preferring antagonist XLi-093 blocked the effects of triazolam but not zolpidem. While these findings suggest a role for both $\alpha 1$GABA_A and $\alpha 5$GABA_A receptor mechanisms, $\alpha 1$GABA_A receptor mechanisms appear to be sufficient for impairments in executive function induced by BZ-type drugs.

1. Introduction

Drugs that act at the γ-aminobutyric acid type-A (GABA_A) receptor, such as the benzodiazepines (BZs) have been shown to alter learning and memory processes (Arolfo and Brioni, 1991, Buffett-Jerrott and Stewart, 2002). Cognitive deficits are a major impediment to the clinical use of BZ-type drugs as anxiolytics and hypnotics, especially in populations already suffering from neurocognitive disorders. At present, relatively little is known about the mechanisms underlying cognitive deficits induced by BZ-type drugs, or if there are any differences among the clinically available compounds.

GABA_A receptors are heteropentameric chloride ion channels that are assembled in a typical stoichiometry of 2 α, 2 β, and 1 γ subunits, and conventional BZs bind specifically to

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GABA$_A$ receptors containing α1, α2, α3 or α5 subunits (α1GABA$_A$, α2GABA$_A$, α3GABA$_A$ or α5GABA$_A$ receptors, respectively; for review, see Rudolph and Knoflach, 2011). The α1GABA$_A$ receptor has been shown to account for approximately 60% of all GABA$_A$ receptors and is particularly dense on hippocampal and cortical interneurons (Fritschy *et al.*, 1992, Gao and Fritschy, 1994, McKernan and Whiting, 1996, Rudolph and Knoflach, 2011). The α2GABA$_A$ and α3GABA$_A$ receptors represent a smaller percentage of the GABA$_A$ receptor population, with the α2GABA$_A$ receptor particularly dense in hippocampus, cortex, and basal ganglia, whereas the α3GABA$_A$ receptor is expressed in cortex and thalamus. In contrast, the α5GABA$_A$ receptor is found almost exclusively in hippocampus and deep layers of cortex (for review, see Rudolph and Knoflach, 2011).

BZs and related drugs have long been documented to disrupt memory in human and non-human subjects (e.g., Duka *et al.*, 1996, Ghoneim and Mewaldt, 1975, McNaughton and Morris, 1987; for review, see Stewart, 2005). Broadly stated, in human subjects, administration of BZ-type drugs can result in a loss of the ability to form new memories (i.e., anterograde amnesia). Early reports in human subjects concluded that BZs blocked the acquisition of new information (e.g., Ghoneim *et al.*, 1984a, Ghoneim *et al.*, 1984b), and a number of clinically available BZ-type drugs have been shown to impair memories for facts that are explicitly stored and retrieved (explicit, or declarative, memory; cf. Mintzer *et al.*, 1997, Mintzer and Griffiths, 1999). Although the exact cognitive processes underlying these types of memory deficits are unknown, it has been suggested that BZ-type drugs specifically impair memory for contextual information, i.e., information peripheral to an event, such as time and place in which an event is experienced (Brown and Brown, 1990, Duka *et al.*, 1996, Mintzer and Griffiths, 1999).

Relatively little information is available on the role that GABA$_A$ receptor subtypes play in the ability of BZ-type drugs to impair memory processes. One finding from human laboratory studies is that the hypnotic BZ agonist zolpidem did not disrupt memory for spatial contextual information, in contrast to the non-selective BZ agonist triazolam (Mintzer and Griffiths, 1999). Zolpidem displays highest affinity for the α1GABA$_A$ receptor subtype and does not bind appreciably to the α5GABA$_A$ receptor (Hadingham *et al.*, 1993); the latter of which are densely expressed in hippocampus (for reviews, see Rudolph and Knoflach, 2011, Sieghart and Sperk, 2002). Based on these observations, Mintzer and Griffiths (1999) suggested that zolpidem's apparent lack of impairment of a visual-cues based task (see also Balkin *et al.*, 1992) is consistent with hippocampal regulation of spatial memory. Interestingly, studies in non-human animals generally suggest α1GABA$_A$-selective agonists are less effective than non-selective BZs in engendering memory deficits (Noguchi *et al.*, 2002, Sanger *et al.*, 1986). Moreover, some memory-impairing effects of BZs are not blocked by the α1GABA$_A$-selective BZ antagonist, β-carboline-3-carboxylate-3-butyl-ester (βCCT; Belzung *et al.*, 2000, Savic *et al.*, 2008).

Although research in recent years has focused on the role of hippocampal α5GABA$_A$ receptors in the memory-impairing effects of BZ-type drugs, as noted, BZ-sensitive GABA$_A$ receptors exist throughout the CNS (for reviews, see Rudolph and Knoflach, 2011, Sieghart and Sperk, 2002). Consistent with the relatively ubiquitous distribution of GABA$_A$ receptor subtypes throughout the brain, BZ-type drugs impair performance on tasks that likely involve regions other than hippocampal/temporal areas (e.g., attentional set-shifting, paired-associates learning, (Coull *et al.*, 1999, Coull *et al.*, 1995).

A cognitive domain often associated with prefrontal cortical areas and not the hippocampus is executive function. This domain characteristically includes processes such as goal formation, planning, initiation, preservation and alteration of goal-directed behavior, problem solving, response inhibition, and cognitive flexibility (Kehagia *et al.*, 2010). To our

knowledge, no previous studies have evaluated systematically the effects of BZ-type drugs on executive function. However, Ballard et al. (2009) did demonstrate enhancement of performance in an executive function task in monkeys by administration of an $\alpha5GABA_A$-selective inverse agonist. To the extent that agonists act in an opposite fashion to inverse agonists, these data would suggest that "positive" intrinsic efficacy at the $\alpha5GABA_A$ receptor would result in impairments of executive function

The object retrieval with detours (ORD) task in monkeys is often described as providing a measure of executive functioning (Ballard *et al.*, 2009, Jentsch *et al.*, 1999, Taylor *et al.*, 1990). Lesion studies in monkeys have indicated the involvement of prefrontal cortex-striatal circuitry in mediating behavior in the ORD task, with no involvement of the $\alpha5GABA_A$ receptor-enriched hippocampus (e.g., Diamond *et al.*, 1989, Dias *et al.*, 1996). It is important to note, however, that $\alpha5GABA_A$ receptors are found in deep cortical layers, albeit at relatively low expression levels (Rudolph and Knoflach, 2011) in rodents, and importantly, the expression of $\alpha5GABA_A$ receptors in primate prefrontal cortex is unknown.

This experiment is part of a series of studies designed to evaluate the extent to which BZ-type drugs alter cognitive function via $\alpha1GABA_A$ and/or $\alpha5GABA_A$ receptor subtypes. Our overall hypotheses, based on the relative distributions of the two subtypes, are that (1) the $\alpha1GABA_A$ receptor is involved in cognitive impairments in tasks involving cortical regions; and (2) the $\alpha5GABA_A$ receptor plays a primary role in cognitive tasks that engage the hippocampus. For this report, we evaluated the role of $\alpha1GABA_A$ and $\alpha5GABA_A$ receptors in the ORD task of executive function described by Ballard et al. (2009), with the prediction that $\alpha1GABA_A$, but not $\alpha5GABA_A$ receptors, would mediate impairments in ORD performance. In contrast to this prediction, evidence was obtained for a role of both $\alpha1GABA_A$ and $\alpha5GABA_A$ receptors in the ORD task.

2. Methods

2.1. Animals

Subjects were five adult female rhesus monkeys (*Macaca mulatta*), age 9 to 17 years (9, 11, 13, 17, and 17 years old) with no history of exposure to drug (except for occasional analgesics, anesthetics and/or sedatives for clinical exams/surgeries) or experimental compounds. The monkeys weighed between 6 and 9 kg during the course of the study. Monkeys were individually housed and maintained on a 12 hour lights on/12 hour lights off cycle, with water available ad libitum. All animals were maintained on 20–30 biscuits per day of commercially-available macaque food (LabDiet 5038), which allowed maximum allotment of food availability without a decrease in daily performance during experimental sessions, with the monkeys maintaining steady weights and body conditions as assessed by the clinical veterinary staff. Three times per week, the monkeys were given a small allotment of fruits and/or vegetables as part of the New England Primate Research Center's environmental enrichment program. Animals in this study were maintained in accordance with the guidelines of the Committee on Animals, Office of Research Subject Protection, of the Harvard Medical School and the Guide for the Care and Use of Laboratory Animals (8th edition, 2011). Research protocols were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

2.2. Surgery

Monkeys were prepared with chronic indwelling venous catheters (polyvinyl chloride, i.d.: 0.64 mm; o.d.: 1.35 mm) following the general surgical procedures described by Platt *et al.* (2011). Monkeys initially were anesthetized with 10–20 mg/kg i.m. injection of ketamine for transport to the surgical suite and preparation for the procedure. Throughout surgery,

anesthesia was maintained by an isofluorane/oxygen mixture. Under aseptic conditions, a catheter was implanted in the femoral, brachial, or jugular vein and passed to the level of the right atrium. The distal end of the catheter was passed subcutaneously and exited in the mid-scapular region. The external end of the catheter was fed through a fitted jacket and tether system (Lomir Biomedical, Toronto, Canada) and attached to a fluid swivel mounted to the animal's cage. The exit site of the catheter was inspected routinely and the catheters were flushed two-three times per week with heparinized saline (150–200 U/ml). Physical exams were conducted that occasionally included contrast-dye infused into the catheter, followed by radiography to verify catheter patency and proper placement.

2.3. Overall design

The general design consisted of two phases: (1) determination of dose-response functions for the non-selective BZ triazolam and the $\alpha 1 GABA_A$ receptor-preferring drugs zolpidem and/or zaleplon, and if effects were evident, then (2) evaluation of blockade of a maximally-effective dose of triazolam and zolpidem (supplies of zaleplon were too limited for the antagonism studies) by the $\alpha 1 GABA_A$ receptor-preferring antagonist βCCT and the $\alpha 5 GABA_A$ receptor-preferring antagonist, XLi-093 (1,3-bis(8-ethynyl-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5a][1,4]benzodiazepine-3-carboxy)propyl diester; Li *et al.*, 2003). All drugs/compounds were administered by the i.v. route, in order to make direct comparisons with other procedures in our laboratory that use this route (e.g., i.v. self-administration). In general, animals were trained in the ORD task until performance reached a priori criteria (see below), and tests of individual drugs/compounds, their respective vehicles, or selected doses of agonists plus antagonists were conducted up to 3 times per week, with training days interspersed. A test session consisted of a 5-min pretreatment with one of a range of doses of agonist or vehicle (phase 1) or a dose of agonist (determined in phase 1) with one of a range of doses of antagonist or vehicle (phase 2). For the phase 2 studies, the two injections were given in separate syringes, with the antagonist administered first and the agonist second. Within a phase, the doses were tested in irregular order, except that an agonist or agonist + antagonist combination was completed before studying the next agonist or agonist + antagonist combination.

2.4. Object Retrieval with Detours (ORD)

Five female rhesus monkeys were trained in the ORD task. Monkeys were individually housed in stainless-steel primate cages (Lab Products Inc, Seaford, DE) which also served as the experimental chamber, allowing the object retrieval apparatus (Metal Smiths, Boston, MA) to be attached to the home cage. A transparent barrier was affixed to the front of an open cage during test sessions. Three arm holes in the transparent barrier allowed for the subject to reach the testing apparatus and to discourage hand preference when obtaining food rewards (either a single marshmallow or dried cranberry, depending on each monkey's preference). An additional screen was placed in front of the barrier when a trial was not in session and lifted off of the barrier at the beginning of each trial. A transparent test box (6″ × 6″) with only one open face was situated on a tray attached to the barrier. When the screen was lifted from the barrier, the monkeys were expected to reach through one of the three holes to obtain the reward. The reward was located in one of three positions relative to the open face of the box: (1) directly outside of the open face, considered "easy" trials; (2) directly on the inside near the open face, considered "easy" trials; (2) deep within the box, considered a "difficult" trial. Additionally, orientation of the open face varied from line of sight (LOS), left and right from the monkey's perspective. Complexity of the trial was also dependent on the location of the open face. When the open face was in the LOS, this was considered "easy" and when positioned to the left and right, the complexity of the trial was dependent on the depth of the reward inside the box. The position of the box and the

position of the reward in the box were randomly predetermined prior to a daily session so that the animal was given approximately 50% easy and difficult trials.

A session consisted of 15 trials and monkeys were given up to 2 minutes to complete each trial. Correct trials were indicated by the animal reaching for the correct hole and successfully obtaining the treat. An "incorrect reach" occurred if the animal reached for the correct hole, but did not successfully obtain the treat, usually due to the monkey dropping the treat or not completing the act of retrieving the treat. A "barrier reach" occurred when the animal touched a closed side of the test apparatus. An incorrect or barrier reach resulted in termination of the trial. Monkeys were trained until they reached 75% percent successful responses, for 3 consecutive sessions. Data were calculated as percentage of successful trials in a session. The latency for the animal to initiate the trial was also recorded. Barrier reaches were analyzed as the percentage of total reaches.

2.5. Data analysis

The measures analyzed were the percentage of correct trials, the latencies to initiate a response, and the percentage of touches that consisted of barrier reaches. These data were analyzed for each drug with one-way repeated measures ANOVA, with dose as the factor. Multiple comparisons were made using Bonferroni t-tests, comparing each dose to the vehicle condition. For all comparisons, the α level was set at $p < 0.05$.

2.6. Drug/compound preparation

Triazolam and zolpidem (zolpidem hemitartate) were purchased from Sigma-Aldrich. Zaleplon was a gift from Wyeth Pharmaceuticals. βCCT and XLi-093 were synthesized by the laboratory of Dr. James M. Cook at the University of Wisconsin-Milwaukee. Triazolam, zolpidem and zaleplon were dissolved in propylene glycol and diluted in 0.9 % sterile saline (final concentration of propylene glycol of 50%) and sterilized by filtration. βCCT was dissolved in 5% 1N HCl, 80% propylene glycol and 15% sterile distilled water and XLi-093 was dissolved in 80% propylene glycol and 20% sterile distilled water. Doses were expressed as the base forms of the drugs. All experimental compounds were administered in injections volumes ranging from 0.03 to 0.15 ml/kg, via the i.v. route, approximately 5 min prior to the initiation of a task. Injections were administered by hand over an approximately 10 second period.

3. Results

3.1. Triazolam, zolpidem, and zaleplon alone

In the ORD task, monkeys consistently achieved ~75% successful trials once trained to criterion levels (number of training sessions ranged from 6 to 30) with very short latencies to initiate the task (often less than 1 second). Most errors during the latter stages of training, as well as during vehicle tests, were barrier reaches (84 – 100% of total errors). Administration of i.v. doses of triazolam prior to an ORD session resulted in a significant effect of dose ($F[4,14]= 20.39$, $p<0.05$). Bonferroni t-tests showed that both the 0.03 and 0.1 mg/kg doses of triazolam significantly reduced the percentage of successful trials (Figure 1A). In contrast, no significant effect of triazolam was found with ANOVA performed on the latency to initiate a trial; however, Bonferroni t-tests demonstrated that latencies were significantly increased at the highest triazolam dose tested (Figure 1B, 0.1 mg/kg). For barrier reaches, no significant effect of triazolam was found with ANOVA, but with Bonferroni t-tests, the highest dose of triazolam reduced the percentage of errors that consisted of barrier reaches (i.e., reaches in which a closed side of the box was touched, expressed as means ± SEM: vehicle, 98% ± 2.0; triazolam 0.1 mg/kg, 68% ± 7.3), due to an

increase in incorrect reaches (i.e., reaches in which the treat was dropped or otherwise not placed in the mouth).

As with triazolam, zolpidem reduced the percentage of successful trials (F[4,15]= 5.05, p<0.05) in a dose-dependent manner, with the averages for the two highest doses (1.0 and 3.0 mg/kg) significantly lower than those obtained after vehicle treatment (Figure 2A). For latency to initiate a trial, the ANOVA approached, but did not achieve significance (F[4,15]= 2.82, p= 0.078) and Bonferroni t-tests revealed that only the highest dose of zolpidem (3.0 mg/kg) significantly reduced average latencies compared with the vehicle control condition (Figure 2B). For barrier reaches, no significant effect of zolpidem was found with ANOVA, but with Bonferroni t-tests, the highest dose of zolpidem reduced the percentage of errors that consisted of barrier reaches (means ± SEM: vehicle, 94% ± 2.9; zolpidem 3.0 mg/kg, 51% ± 9.3).

Another $\alpha 1GABA_A$-preferring drug, zaleplon, was evaluated in the ORD task (Figure 3). This drug, similar to triazolam and zolpidem, significantly altered the percentage of successful trials (F[4,15]= 15.25, p<0.05). Bonferroni t-tests showed that the 1.0 and 3.0 mg/ kg doses of zaleplon resulted in average percentage of successful trials that were lower than these values following vehicle administration (Figure 3A). Interestingly, the ANOVA and Bonferroni tests revealed no significant differences for average latencies to initiate the trial (Figure 3B). For barrier reaches, no significant effect of zaleplon was found with ANOVA, but with Bonferroni t-tests, the highest dose of zaleplon reduced the percentage of errors that were barrier reaches (means ± SEM: vehicle, 98% ± 2.0; zaleplon 3.0 mg/kg, 61% ± 7.5).

3.2. Antagonism studies

In initial studies, the highest doses of the two antagonists βCCT and XLi-093 (1.0 and 3.0 mg/kg for both) had no effects on performance in the ORD task (ANOVA, F's<1.0; data not shown). For the $\alpha 1GABA_A$-preferring antagonist βCCT, a dose range of 0.1 to 3.0 mg/kg, i.v., was evaluated, except when it became clear that this antagonist was more potent at blocking the effects of zolpidem than triazolam and a lower dose (0.03 mg/kg) was added. For the $\alpha 5GABA_A$-preferring antagonist XLi-093, a dose range of 0.1 to 3.0 mg/kg, i.v., was evaluated. In both sets of studies, the doses of triazolam and zolpidem chosen were those that reduced the percentage of successful trials without altering the latency to initiate a trial or the percentage of errors that were barrier reaches (0.03 mg/kg and 1.0 mg/kg, respectively). Note that zaleplon was not evaluated in the antagonism studies due to limited supplies of this drug.

When administered prior to ORD test sessions with 0.03 mg/kg of triazolam, βCCT treatments resulted in a significant alteration in the percentage of successful trials (F[4,16]= 6.57, p<0.05). Bonferroni t-tests comparing βCCT doses to vehicle revealed a significant increase in the percentage of successful trials at doses of 1.0 and 3.0 mg/kg, such that performance essentially returned to untreated levels (represented by the horizontal dashed lines, upper and lower SEMS; Figure 4A). Similarly, XLi-093 altered the percentage of successful trials (F[4,16]= 8.11, p<0.05), with the 3.0 mg/kg dose significantly enhanced compared to vehicle levels (Bonferroni t-tests, Figure 4B). As above, the increase in percentage of successful trials essentially returned to untreated levels.

Increasing doses of βCCT (0.1–3.0 mg/kg) resulted in an increase in the percentage of successful trials that occurred after 1.0 mg/kg of zolpidem (F[5,20]= 12.36, p<0.05 and Bonferroni t-tests; Figure 5A). In contrast to triazolam, βCCT was approximately 10-fold more potent at blocking zolpidem in the ORD task, based on the lowest significant doses (0.1 mg/kg for zolpidem, 1.0 mg/kg for triazolam). The percentage of successful trials returned to untreated levels, represented by the horizontal dashed lines (upper and lower

SEM values) in the Figure. Interestingly, over the dose range tested, XLi-093 had no effects on the percentage of successful trials after treatment with 1.0 mg/kg of zolpidem (Figure 5B).

4. Discussion

Drugs that act at the GABA$_A$ receptor have been shown to alter learning and memory processes (Arolfo and Brioni, 1991, Buffett-Jerrott and Stewart, 2002, Coull *et al.*, 1999, Mintzer and Griffiths, 2005). Cognitive deficits induced by BZ-type drugs in particular are a major impediment to their clinical use as anxiolytics and hypnotics; however, little is known about the mechanisms underlying cognitive deficits induced by BZ-type drugs. Previous studies have illustrated that the diverse behavioral effects of BZ-like drugs may reflect action at different subtypes of the GABA$_A$ receptor (Rudolph and Knoflach, 2011). Based on findings in human and non-human animals, we hypothesized that tasks involving primarily frontal circuitry would involve mostly α1GABA$_A$ receptors, whereas the α5GABA$_A$ receptor is involved in hippocampal-dependent tasks.

The frontostriatal-based ORD task is often described as providing a measure of executive functioning (Ballard *et al.*, 2009, Jentsch *et al.*, 1999, Taylor *et al.*, 1990). In the present study, triazolam, zolpidem, and zaleplon all reduced accuracy in terms of the percentage of successful trials. These deficits occurred for at least one dose in which there were no changes in either latency to initiate a trial or percentage of errors as barrier reaches. Based on these findings, we predict that BZ-type drugs may impair at least some aspect of executive functioning, irrespective of selectivity for α1GABA$_A$ receptor subtypes.

The primary type of error that contributed to the percentage of successful trials measure was "barrier" reaches, i.e., reaches that resulted in the monkey touching a closed side of the box. In untreated monkeys, the other type of error—incorrect reaches, in which the reward was dropped—occurred relatively infrequently. The latter type of error increased significantly only at the highest doses tested for all three drugs, which also corresponded to doses that increased latency to respond. Based on research in our laboratories with these drugs and other procedures in rhesus monkeys (e.g., Rowlett *et al.*, 2005), the most parsimonious explanation for increased incorrect reaches and latencies likely is the emergence of motor coordination deficits and/or sedation. The parsing out of barrier and incorrect reaches was of interest due to the fact that changes in barrier reaches often have been attributed to alterations in "impulsivity" (Jentsch *et al.*, 1999), and some drugs do alter barrier reaches selectively (e.g., phencyclidine). Because the proportion of barrier reaches to incorrect reaches did not change at doses of BZ-type drugs that reduced the percentage of successful trials, we do not have evidence for BZ-induced changes in impulsive behavior, at least within the context of the ORD task.

To our knowledge, no previous studies have evaluated systematically the effects of BZ-type drugs in the ORD task. However, Ballard et al. (2009) did demonstrate enhancement of performance in ORD by administration of an α5GABA$_A$-selective inverse agonist. To the extent that agonists act in an opposite fashion to inverse agonists, these data would suggest that "positive" intrinsic efficacy at the α5GABA$_A$ receptor would result in a decline in performance. Lesion studies in monkeys, however, have indicated the involvement of prefrontal cortex-striatal circuitry in mediating the ORD task, with no involvement of the α5GABA$_A$ receptor-enriched hippocampus (e.g., Dias *et al.*, 1996). It is important to note, however, that α5GABA$_A$ receptors are found in deep cortical layers, albeit at relatively low expression levels (Rudolph and Knoflach, 2011) in rodents, and importantly, the expression of α5GABA$_A$ receptors in primate prefrontal cortex is unknown.

In order to explore further the role of $\alpha1GABA_A$ and $\alpha5GABA_A$ receptors in mediating the cognitive-impairing effects of BZ-type drugs in the ORD task, we evaluated performance of these drugs when co-administered with βCCT and XLi-093, antagonists with selectivity for $\alpha1GABA_A$ and $\alpha5GABA_A$ receptors, respectively. We found that both βCCT and XLi-093 dose-dependently reversed the impairments induced by the non-selective BZ triazolam. In contrast, while βCCT blocked the effects of the $\alpha1GABA_A$ receptor-preferring agonist zolpidem, XLi-093 did not block the deficits induced by zolpidem over the same dose range that was effective at blocking the effects of triazolam. Moreover, our results indicated that βCCT was approximately 10-fold more potent in blocking the effects of zolpidem than it was in blocking the effects of triazolam. Collectively, these findings suggest that BZ-type drugs may impair performance on the ORD task by different mechanisms, with both $\alpha1GABA_A$ and $\alpha5GABA_A$ receptors playing a role in the effects of a non-selective BZ, whereas only $\alpha1GABA_A$ receptors were important for the impairments induced by zolpidem. The latter results further suggest that stimulation of $\alpha1GABA_A$ receptors may be sufficient to induce deficits in this executive function task.

As discussed above, Ballard et al. (2009) demonstrated that an inverse agonist at $\alpha5GABA_A$ receptors enhanced performance on an ORD task similar to the one used in the present study. Our data with the $\alpha5GABA_A$ receptor-preferring antagonist, XLi-093, provide additional support to an unexpected role for this subtype in a prefrontal cortex-striatal task. Interestingly, a recent report by (Koh *et al.*, 2013)) demonstrated that compounds that act as functionally-selective agonists at $\alpha5GABA_A$ receptors can improve various indices of memory in cognitively-impaired aged rats, whereas a functionally-selective $\alpha5GABA_A$ inverse agonist had no effect, yet improved memory performance in young rats. In the present study, the monkeys generally fell into the category of "middle age" for rhesus macaques, according to analyses of survival rates, age of sexual maturity, and other environmental factors (Peters *et al.*, 1996). Moreover, the aged rats (~2 years old) in the Koh et al. (2013) study were pre-selected quantitatively for relatively impaired performance on spatial learning tasks. It is difficult to make direct comparisons regarding the relative ages of monkeys and rats across the studies; nevertheless, the extent to which $\alpha5GABA_A$ agonists vs. inverse agonists improve cognition in senescent (i.e., 20+ year old) rhesus monkeys is of considerable interest.

5. Conclusions

The BZ-like drugs characteristically are some of the most frequently used medications for the treatment of anxiety and insomnia. The use of these drugs in treating anxiety and sleep disorders is limited by unwanted side effects, and a major side effect of BZ-type drugs is the detrimental effects on memory processing (Coenen, 1989, Curran and Birch, 1991, Lister, 1985, Mintzer and Griffiths, 2005). At present, the mechanisms of action responsible for cognitive deficits associated with the use of BZ-type drugs are understood poorly. In the present study, we evaluated the ability of a BZ, triazolam, which binds non-selectively to all BZ-sensitive $GABA_A$ receptor subtypes, as well as the $\alpha1GABA_A$ receptor-preferring drugs zolpidem and zaleplon, on a task associated with executive function (ORD task). Contrary to expectations, all drugs impaired cognitive performance on the ORD task at doses that did not alter responding directly (as assessed by latency to initiate a response and percentage of barrier reaches). Studies with selective antagonists suggested that the $\alpha1GABA_A$ receptor subtype is sufficient to induce impairment of ORD performance by BZ-type drugs; however, the $\alpha5GABA_A$ receptor subtype can contribute to the deficits in an as-yet specified manner. These findings raise the possibility that BZ-type drugs can induce a deficit in at least certain aspect(s) of executive functioning via an $\alpha1GABA_A$ receptor mechanism.

Acknowledgments

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Highlights

- Triazolam (non-selective benzodiazepine), zolpidem, and zaleplon (both preferential agonists at $\alpha 1$ subunit-containing $GABA_A$ receptors) impaired performance on the object retrieval with detours (ORD) task of executive function.

- The effects of triazolam and zolpidem in the ORD task were reversed by an antagonist that binds preferentially to $\alpha 1$ subunit-containing $GABA_A$ receptors.

- The effects of triazolam, but not zolpidem, were reversed by an antagonist that binds preferentially to $\alpha 5$ subunit-containing $GABA_A$ receptors.

- Positive modulation of the $\alpha 1$ subunit-containing $GABA_A$ receptor was sufficient to induce impairments in a task of executive function, although evidence for a role of $\alpha 5$ subunit-containing $GABA_A$ receptors was obtained.

A. Successful Trials
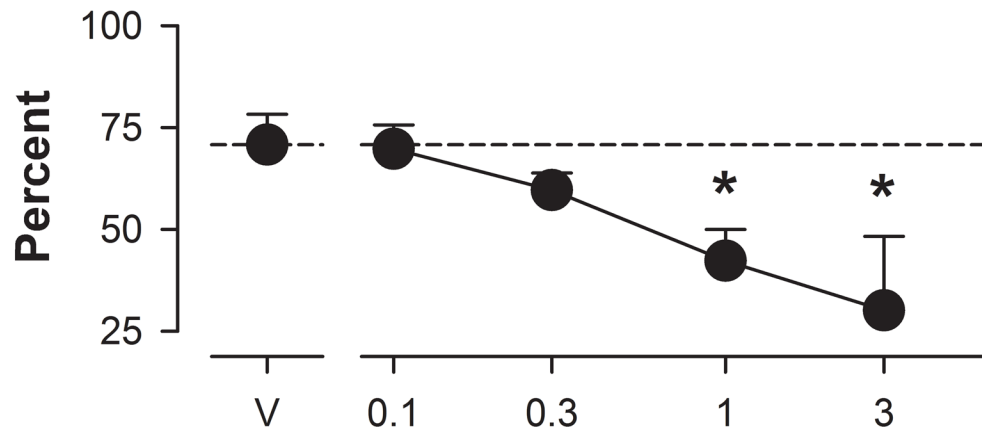

B. Latency

Triazolam (mg/kg, i.v.)
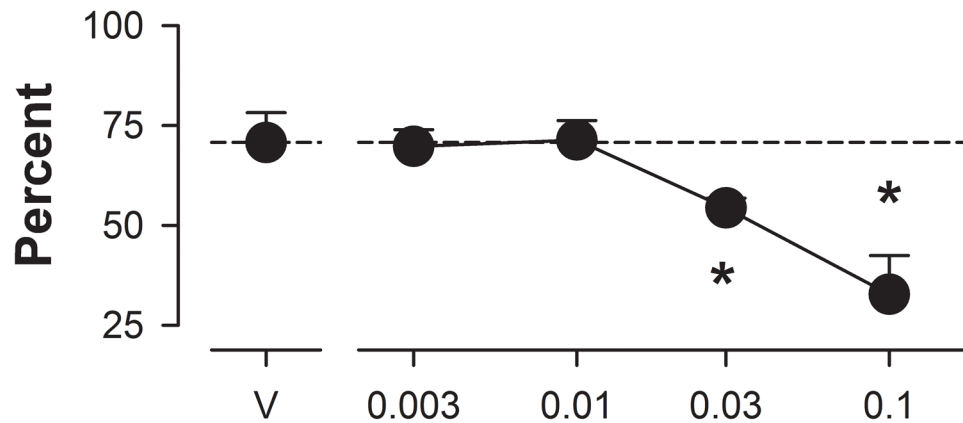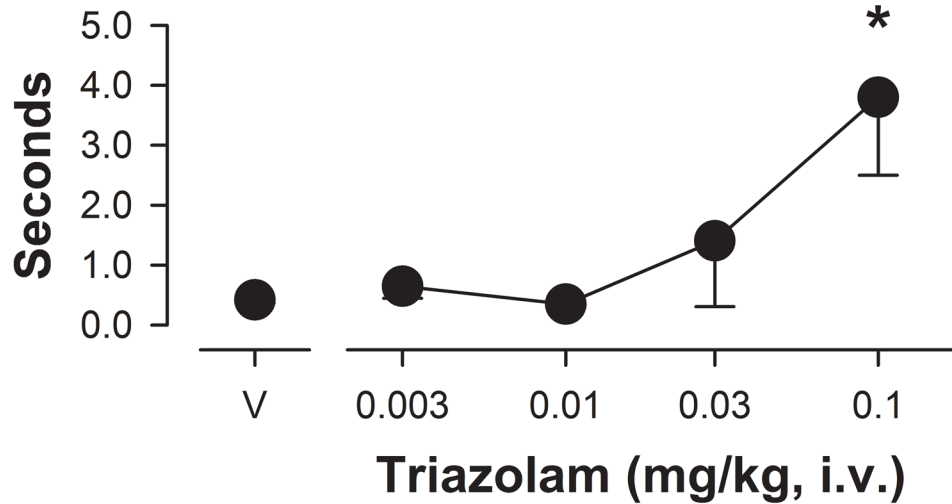
Figure 1.
Performance on the object retrieval with detours (ORD) task following triazolam administration in rhesus monkeys (N=5). *Panel A*: Mean (± SEM) percentage of successful trials, defined as a single reach into the apparatus, resulting in food retrieval into the mouth with no errors (i.e., dropping food, placing hand on closed end of box), following vehicle (V) or increasing mg/kg (i.v.) doses of drug. *Panel B*: Mean (± SEM) latency (in sec) to initiate a trial, defined as hand entering the platform area on which the box was mounted (see Methods for details of the apparatus). Note that *$p<0.05$ compared with vehicle, Bonferroni t-tests.

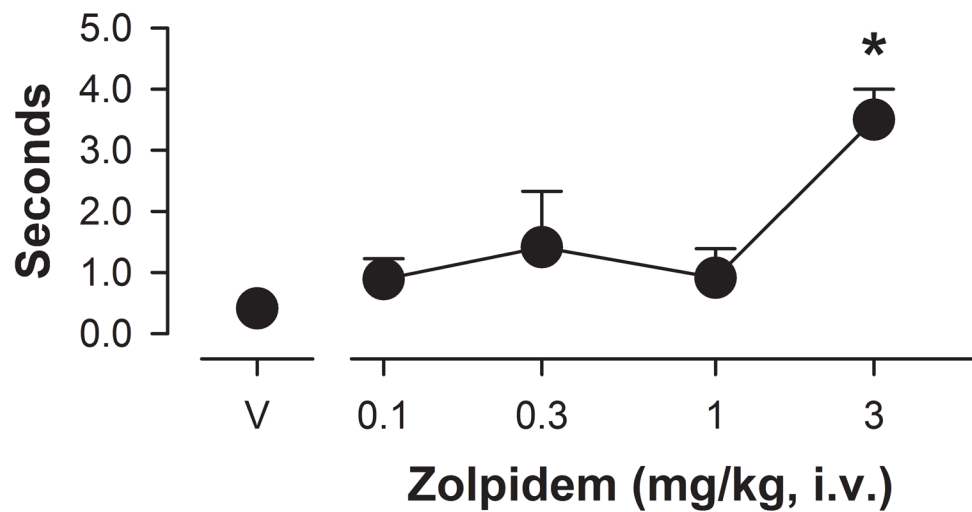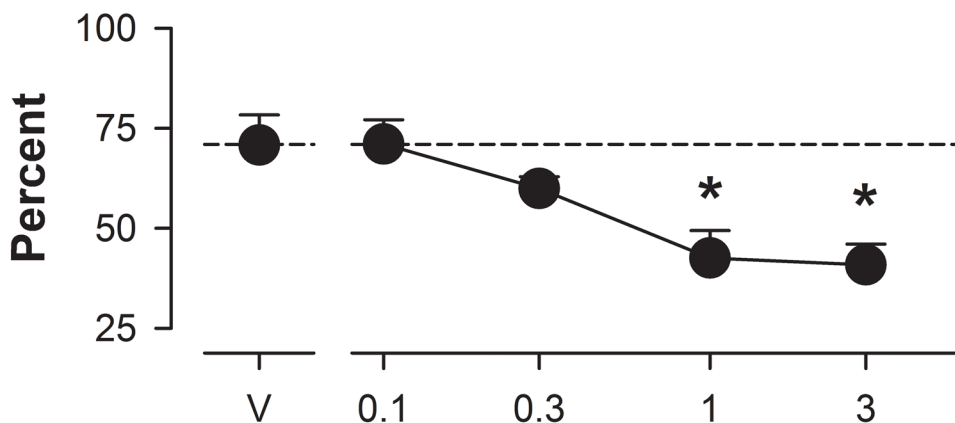## A. Successful Trials

B. Latency

Figure 2.
Performance on the object retrieval with detours (ORD) task following zolpidem administration in rhesus monkeys (N=5). Other details as in Figure 3.

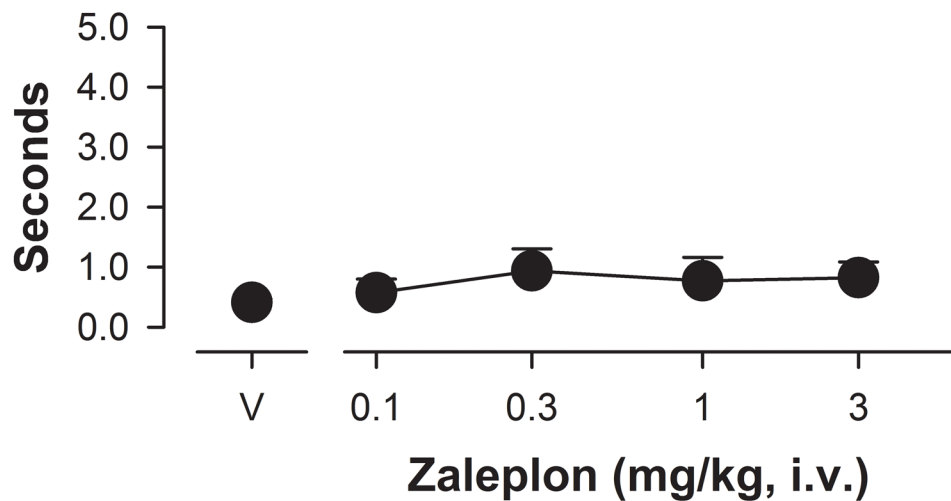## A. Successful Trials

B. Latency

Figure 3.
Performance on the object retrieval with detours (ORD) task following zaleplon administration in rhesus monkeys (N=5). Other details as in Figure 3.
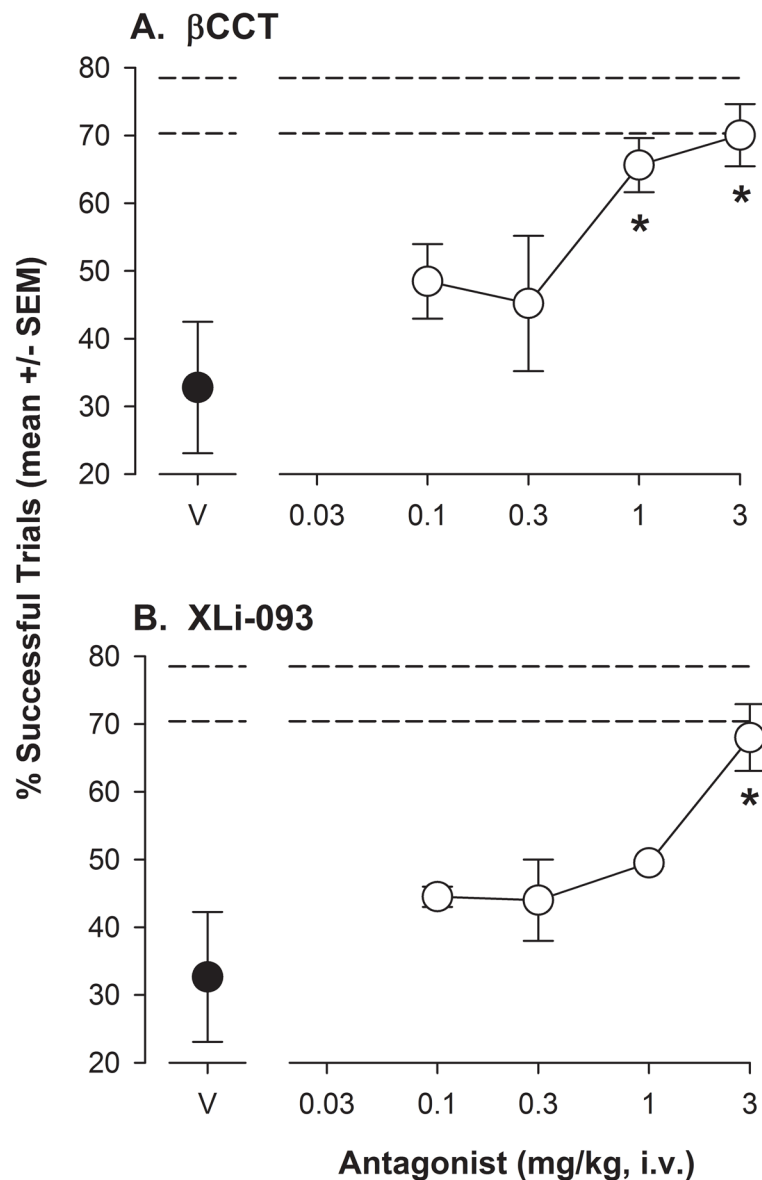
Figure 4.
Performance on the object retrieval with detours (ORD) task following 0.03 mg/kg triazolam and a range of doses of the antagonists βCCT or XLi-093 in rhesus monkeys (N=5). Both triazolam and the antagonists were administered i.v., 5 min before the session. Horizontal dashed lines represent upper and lower SEMs for performance on the ORD task without drug treatments. *Panel A*: Percentage of successful trials (see Figure 3 for details) following triazolam with βCCT vehicle (V) and increasing doses of βCCT. *Panel B*: Percentage of successful trials (see Figure 3 for details) following triazolam with XLi-093 vehicle (V) and increasing doses of XLi-093. Note that *p<0.05 compared with vehicle, Bonferroni t-tests.
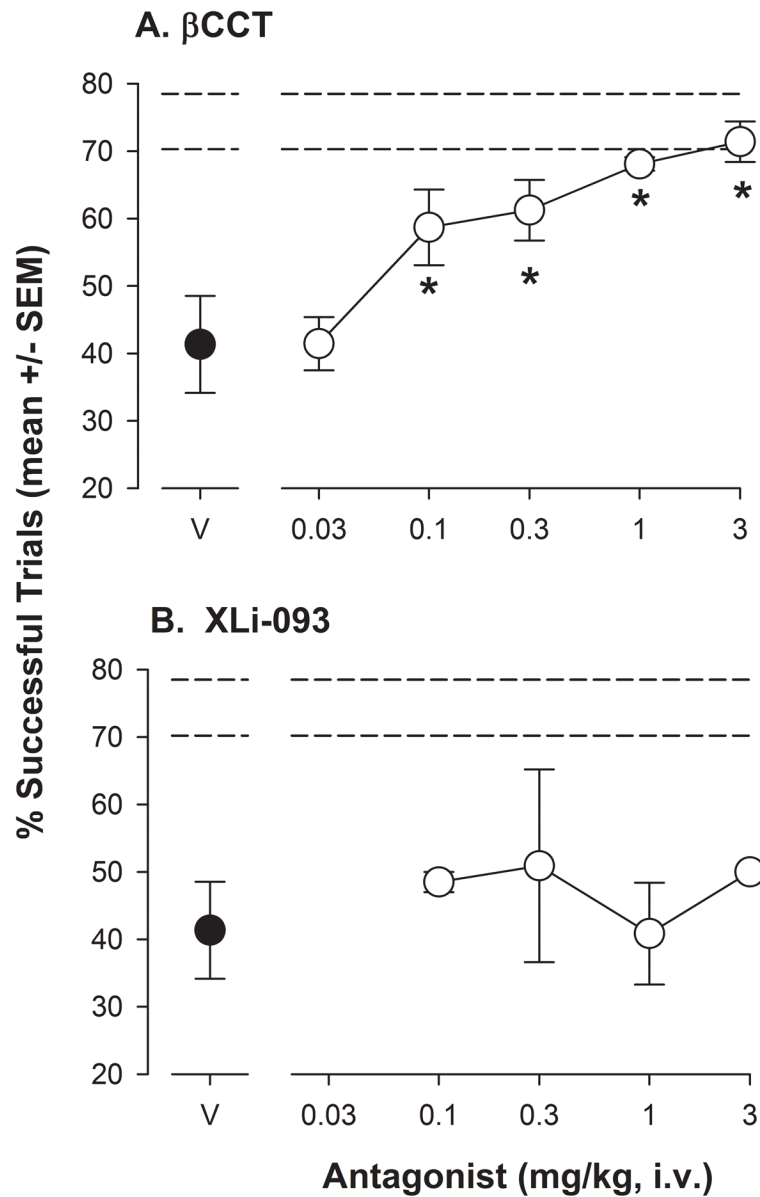
A. βCCT

B. XLi-093

Figure 5.
Performance on the object retrieval with detours (ORD) task following 1.0 mg/kg zolpidem
and a range of doses of the antagonists βCCT or XLi-093 in rhesus monkeys (N=5). Other
details as in Figure 6.