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Amnestic Drugs in the Odor Span Task: Effects of Flunitrazepam, Zolpidem and Scopolamine

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Abstract

The odor span task is an incrementing non-matching-to-sample procedure designed to provide an analysis of working memory capacity in rodents. The procedure takes place in an arena apparatus and rats are exposed to a series of odor stimuli in the form of scented lids with the selection of new stimuli reinforced. This procedure makes it possible to study drug effects as a function of the number of stimuli to remember. In the present study, the non-selective positive allosteric GABA_A receptor modulator flunitrazepam impaired odor span performance at doses that did not affect a control odor discrimination. In contrast, the alpha-1 selective positive GABA_A receptor modulator zolpidem and the cholinergic receptor antagonist scopolamine only impaired odor span at doses that produced more global impairment, including decreased accuracy in the control discrimination and increased response omissions in the both the odor span and control discrimination procedures. Even though the effects of flunitrazepam were selective to odor span performance, they did not depend on the number of stimuli to remember—the same degree of impairment occurred regardless of the memory load. These findings suggest that flunitrazepam interfered selectively with conditional discrimination performance rather than working memory and tentatively suggest that flunitrazepam's selective effects in the odor span task relative to the control odor discrimination are mediated by one or more non-alpha1 GABA_A receptor subtypes.

Keywords

GABA-A modulator; flunitrazepam; scopolamine; zolpidem; odor span task; working memory

1.0 Introduction

Theories of human working memory posit several key features that are thought to separate it from other forms of memory including a relatively brief duration and a limited capacity (Baddeley and Hitch, 1974; Gathercole, 2009). A wide variety of procedures have been used

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as models of working memory in animals in order to permit pharmacological analysis of working memory including the Morris water maze, the radial arm maze, the delayed alternation task, the novel object task, and delayed matching- and non-matching-to sample tasks (Dudchenko, 2004; Dudchenko, Talpos, Young & Baxter, 2013). What these procedures have in common is that successful performance requires a “short term memory for an object, stimulus, or location that is used within a testing session, but not typically between sessions” (p. 700, Dudchenko, 2004). These techniques have generally been successful in showing forgetting functions: decreases in accuracy with increases in the retention interval (White, 2013). Such forgetting functions provide some validation for the limited duration of working memory, but different procedures are required to study memory capacity.

One procedure for studying memory capacity is the self-ordered spatial search (SOSS) task which has been used to study drug effects on working memory in non-human primates (e.g., Soto et al., 2013 Taffe, 2012; Taffe, Davis, Gutierrez & Gold, 2002; Taffe, Weed & Gold, 1999). For example, in the Taffe et al. (1999) study, rhesus monkeys were given touchscreen presentations of stimuli in 16 possible locations in a 4 X 4 array. On any given trial, 2, 3 or 4 stimuli were presented in random screen locations and each non-repeating touch on a stimulus was reinforced with food. Repeat touches terminated the trial, and if all stimuli were touched with no repetitions, the trial was scored as correct. Accuracy in the SOSS procedure was sensitive to the number of stimuli; accuracy decreased as the number of stimuli in the array increased. Taffe et al. (1999) further showed that the effects of muscarinic anticholinergic compound, scopolamine, depended on the number of stimuli in the array. Doses of scopolamine as low as .03 mg/kg disrupted SOSS accuracy on trials with four stimuli, but accuracy on three-stimulus trials was not disrupted until doses of 1.4 mg/kg were administered. Finally, accuracy on trials with two stimuli was not impaired at any of the scopolamine doses tested. Thus, Taffe et al. (1999) showed that the effects of scopolamine depended on the number of stimuli to remember.

Soto et al. (2013) studied the effects of the non-selective positive GABA_A modulator, triazolam, on SOSS performance and found that those effects also depended on the number of stimuli in the array. That is, the minimally effective dose of triazolam that impaired SOSS accuracy was lowest with the 4-stimulus array and highest with the 2-stimulus array. Similar memory-load dependent functions were produced following administration of the selective alpha-1 GABA_A receptor modulators zolpidem and zaleplon. However, compounds selective for other GABA_A receptor subtypes (including a positive alpha-2/3 modulator and alpha-5 positive and negative modulators) failed to produce any evidence of memory-load dependent effects or memory effects, *per se*. These results suggest a critical role for alpha-1 GABA_A receptors in working memory capacity in non-human primates. Importantly, in both the Taffe et al. (1999) and Soto et al (2013) studies, drug effects were also evaluated using a more traditional delayed-matching-to-sample procedure and none of the compounds produced delay-dependent effects on accuracy—that is, neither scopolamine nor positive GABA_A receptor modulators affected rate of forgetting. Taken together, these results suggest that procedures that permit the manipulation of the number of stimuli to remember may be more useful in detecting drug effects on working memory than DMTS.

Although the SOSS has only been studied with primates, a procedure that permits analysis of memory load in rodents is the odor span task (OST--Dudchenko, Wood & Eichenbaum, 2000). The OST is an incremental non-matching-to-sample task generally conducted in an arena in which rats or mice are exposed to scented stimuli. In a variation of the OST used to study drug effects (Galizio, Deal, Hawkey & April, 2013; MacQueen, Bullard & Galizio, 2011), rats are initially exposed to an arena with a single cup filled with sand and covered with an opaque scented lid (Odor A). Removal of the lid is reinforced with a food pellet and the rat is removed from the apparatus. On the next trial, two cups are placed in the arena in new locations. One is covered with a new lid scented with Odor A and the other with a new odor (B). Responding to the new odor is always reinforced in the OST whereas responding to previously presented odors is never reinforced. Thus, on Trial 3, the A and B odors serve as negative comparison stimuli and responding to a new odor (C) is reinforced. This incrementing procedure continued for 24 trials with responding to each stimulus producing food reward the first time the odor stimulus was presented, but not on subsequent presentations. In order to avoid the potential confounding of the number of stimuli in the arena with the number of odors to remember, the number of comparison stimuli in the arena was permitted to increment up to five, but held constant at five as the number of stimuli to remember continued to increment through the session. Under such conditions rats generally develop accurate responding and average 6–10 trials before an error (span length) with overall accuracy decreasing as the number of odors to remember increases during the course of the session, which is often taken as providing some validation of the OST as measure of working memory capacity (Dudchenko et al., 2013).

In order to adapt the OST for behavioral pharmacology research, additional controls are generally added to separate the effects of drugs on working memory from potential actions related to sensory-motor impairment, motivational change or reference memory impairment. Galizio et al. (2013) and MacQueen et al. (2011) added a simple discrimination control (SDC) task to the basic OST. Five odors not used in the OST were presented in the arena on control trials with one odor designated correct throughout the experiment and the other four never associated with food reinforcement. Thus, the SDC task allows one to measure drug effects that do not depend on within-session/working memory for direct comparison to effects on OST performances. When impairment of OST accuracy is observed at doses that do not affect simple discrimination, it suggests that these actions are selective to within-session or working memory.

Research on the behavioral pharmacology of the OST is in its early stages, but some clear findings have emerged (see Galizio, 2016 for a review). For example, NMDA antagonists consistently produced impairments in OST accuracy at doses that spared performance under SDC and other control conditions (Davies, Greba & Howland, 2013; Galizio et al., 2013; MacQueen et al., 2011; MacQueen, Dalrymple, Drobles, & Diamond, 2016). Further, the effects of the NMDA antagonist, MK-801, were shown to depend on the number of stimuli to remember with virtually no effect when the memory load was small and increasing impairment relative to control as the load increased (Galizio et al., 2013; MacQueen et al., 2011).

A number of other putatively amnesic drugs have been studied using the OST. Of central importance to the present experiment, two studies investigated the effects of positive GABA_A modulators and found that both chlordiazepoxide (Galizio et al., 2013) and flunitrazepam (Galizio et al., 2016) produced impairments in the OST at doses that did not affect SDC performances. These findings appear consistent with the Soto et al. (2013) results showing that, in monkeys, the accuracy-decreasing effects of the positive allosteric GABA_A receptor modulators, triazolam and zolpidem, became stronger as the number of stimuli to be remembered in the SOSS increased.

The cholinergic receptor antagonist scopolamine also impaired OST performance in rats (Rushforth, Allison, Wonnacott & Shoaib, 2010). However, the Rushforth et al. study did not include an SDC condition to assess non-amnesic effects of scopolamine. In a follow-up study, Galizio et al. (2013) assessed the effects of scopolamine in a version of the OST that also included SDC trials. Scopolamine impaired OST performance, but only at doses that also produced equal impairments on the SDC trials. These findings were surprising given the results of Taffe et al. (1999) using the SOSS and cast doubt on a working memory account of scopolamine effects in the OST. However, some features of the Galizio et al. (2013) study make firm conclusions about scopolamine effects on memory capacity premature. In a review of scopolamine effects on memory, Klinkenberg and Blokland (2010) noted that disruption of simple discrimination and attentional processes can be observed at relatively low doses. The use of five comparison stimuli in both OST and SDC trials in Galizio et al. (2013) may have made the task particularly sensitive to the attentional effects of scopolamine. Perhaps a task with fewer distractors would be more sensitive to amnesic effects of drugs.

Only one study has directly examined the effects of number of distractor stimuli in the OST. April, Bruce & Galizio (2013) studied OST performance with either ten, five or two comparison choices in the arena and showed that accuracy was highest and that the effects of memory load were diminished with two choices compared to conditions with more distractors. Indeed, it could be argued that minimizing the number of comparison stimuli in the arena creates the purest test of working memory in the OST because it minimizes the influence of distractor stimuli which otherwise can be confounded with the number of stimuli to remember.

Thus, one major purpose of present experiment was to examine the effects of the muscarinic antagonist, scopolamine, and the positive GABA_A modulator, flunitrazepam, in a two-choice version of the OST to compare with previous studies with these drugs that used five or more choices (Galizio et al., 2013; 2016; Rushforth et al., 2010). It was hypothesized that a reduced number of distractors in this procedure might permit detection of the amnesic effects of scopolamine and enhance assessment of such effects with flunitrazepam. A second purpose was to systematically replicate findings of Soto et al. (2013) by comparing the effects of the of alpha-1 selective positive GABA_A receptor modulator zolpidem with those of the relatively non-selective GABA_A receptor modulator flunitrazepam. Based on the Soto et al. findings, it was hypothesized that zolpidem, like flunitrazepam, would produce selective and memory-load dependent impairment of OST accuracy.

2.0 Method

2.1 Subjects

Subjects were 14 male Sprague-Dawley rats 90–120 days old at the onset of testing. Rats were housed individually in self-ventilating, Plexiglas cages (34.5 cm L x 18 cm W x 24.5 cm H) and were maintained on a 12:12 reverse dark/light schedule with *ad lib* water. Food was restricted to keep animals at 85% of free feeding weight.

2.2 Apparatus

Testing was conducted in a circular arena 94 cm in diameter surrounded by metal baffling 32 cm high. The floor of the arena contained 18 holes arranged in two concentric circles into which 60 ml plastic cups were placed (see Galizio et al., 2013 for more details). Testing was conducted in a small room with white background noise (70 dB) presented during the sessions. Sessions were recorded with a video camera positioned in the ceiling, and to avoid cuing, the experimenter stood outside the rat's field of view and observed the animal's activity on a monitor.

2.3 Stimuli

Odor stimuli were presented via opaque scented lids which were placed over the cups in the arena. The lids were odorized by storage in containers in which aromatic oils and spices were placed. Odorants were purchased through commercial websites (Great American Spice Co., Nature's Garden) and included: allspice, anise, bay, bubblegum, caraway, celery, cherry, chocolate, cinnamon, clove, coconut, coriander, cumin, dill, fennel, fenugreek, garlic, ginger, honey, lilac, marjoram, marshmallow, mustard, nutmeg, onion, oregano, pecan, pine forest, rosemary, sage, savory, spinach, sumac, and thyme.

2.4 Procedure

2.4.1 Preliminary training—Rats were initially acclimated to the arena and permitted to consume sugar pellets (45 mg., Bio-Serv, Inc., Frenchtown, NJ) in the plastic cups. Once rats reliably consumed pellets from all cups in the arena, unscented lids were placed over part of the cup which forced the rat to remove the lid in order to obtain the pellets. Gradually, the lids were moved to cover more of the cup until rats reliably removed lids that completely covered the cup, at which time the OST training began. This preliminary training was completed in an average of 6.6 sessions.

2.4.2 OST training—Odorized lids were first introduced at this point of the study. OST trials involved first placing the animal in the arena with a single baited cup placed randomly in one of the 18 arena locations and covered with a lid scented with one of the odor stimuli (call it Odor A). The trial continued until the rat removed the lid and obtained the pellet after which the rat was removed to a holding cage for an inter-trial interval of approximated 30 s. On Trial 2, two stimulus cups placed in random locations were presented. One was covered with a lid scented with Odor A (note that fresh lids were used on each trial) and the other with a new odorant (Odor B). Responding to Odor B, but not Odor A, produced a sugar pellet (i.e., a non-matching-to-sample contingency). However, the OST span task is essentially an *incrementing* non-matching-to-sample contingency, so on Trial 3, three cups

were placed in random arena locations with Odors A, B and a new stimulus: Odor C. The Odor C cup was baited, whereas A and B cups were not. The number of comparison odors in the arena continued to increment with the addition of an odor that was new to the current session added on each trial and only responses to the new odor were reinforced. A correction procedure was used such that after an error the trial continued until the rat made a correct response. If the rat failed to make a correct response within 2 min, the trial was terminated and the animal was placed on the correct lid to ensure that the odor was contacted. The number of comparison cups in the arena continued to increment until the animal made an error. On the first trial following an error only a single stimulus cup was presented and the incrementing procedure began anew. Sessions terminated after 25 trials. These training conditions continued until the rat completed a longest run of 10 or more consecutive correct responses, or until two consecutive sessions with 5 or more consecutive correct. When either of these criteria was met, the Two-Comparison OST procedure began.

2.4.3 Two-Comparison OST—During this phase of training, a new odor was introduced on each trial, but after Trial 1 the number of comparison stimuli was held constant at two. This was accomplished by selecting on each trial a single previously presented odor to serve as the negative comparison for that trial. Negative comparison stimuli were selected randomly with the constraints that the same S- was not presented more than three times consecutively, and at least three stimuli presented in the first half of the session were also presented in the second half. In this way, the confound between the number of stimuli to remember and the number of comparison stimuli among which to choose was eliminated. Another difference from initial training was that these two-comparison trials continued throughout the 25-trial session regardless of errors. When a criterion of two consecutive sessions with percent correct of 18/25 (72%) was met, a simple discrimination procedure was added to the OST.

2.4.4 Two-Comparison OST with Simple Discrimination Control—During this phase of training, the OST continued as described above, however, after 25 OST trials were completed, the rat was presented with 6 simple discrimination trials on which one odor was arbitrarily designated positive (S+) and always baited and another designated as negative (S-) and never baited. The S+ and S- stimuli differed across rats and were never used on OST trials. The purpose of this simple discrimination control (SDC) was to provide a way to evaluate drug effects that might interfere with various task demands of the OST unrelated to within-session memory such as disruption of olfactory perception, motor or motivational impairment, or effects on more general features of remembering (e.g., reference memory). When rats were performing at high levels on both OST and SDC trials, the 6 SDC trials were distributed throughout the session and this served as the final baseline for the drug study. Thus, throughout the remainder of the study each session was composed of 31 trials: 25 OST and 6 SDC. Animals were tested under these baseline conditions for a minimum of 10 sessions and until a stability criterion (difference between the mean percent correct of most recent five sessions and the previous five sessions differed no more than 10% from the 10-session mean) was met at which point the drug regimen began. Total number of training sessions required to meet criterion ranged from 20 to 71 and is presented for each subject in Table 1.

2.4.5 Drug protocol—Rats were tested five days per week with baseline sessions conducted on Mondays and Wednesdays and intraperitoneal injections were given 15 min prior to sessions on the other days. Vehicle was generally administered on Thursdays, and on Tuesdays and Fridays rats generally received a drug dose (all injections administered in a volume of 1 ml/kg). Flunitrazepam (National Institute of Drug Abuse Research Supply Program, RTI) was dissolved in a vehicle of 40% saline, 40% propylene glycol, and 20% ethanol. Zolpidem hemitartrate (RBI-Sigma) and scopolamine hydrobromine (RBI-Sigma) were dissolved in 0.9% saline solution. The dose range for flunitrazepam and scopolamine were selected on the basis of our previous research (Galizio et al., 2013; 2016), but zolpidem doses were determined empirically by establishing doses too low to have effects up to doses that impaired overall responding. Vehicle injections were administered for each rat until responding after vehicle was close to baseline levels (Mean = 3.5 sessions). The first determination of drug doses was administered in ascending order; in subsequent determinations, doses were administered in semi-random order with the constraint that each dose was presented before a new cycle of determinations was begun. Generally, two to four determinations were made of each dose, depending on how much variability across determinations was observed. At higher doses of some drugs, animals frequently failed to respond, and the experimenter terminated the trial if 2 min elapsed without a response. Such trials were analyzed separately as response omissions. If responding was largely eliminated on the first determination of a high dose, it was not repeated and higher doses were not tested. Four of the rats were exposed to two of the study drugs (see Table 1). In these cases, two weeks of testing without injections intervened between testing of the two drugs.

2.4.6 Unbaited Control Sessions—In order to monitor the possibility that responding might be influenced by detection of the smell of the sugar pellet, one session each week (generally Mondays) was programmed with six randomly selected trials, which were conducted with neither cup baited with a sugar pellet. On these trials, the pellet was manually dropped into the cup using a long tube after a correct response was made. Table 1 shows mean percent correct on these unbaited trials for each rat along with percent correct on baited trials for the same sessions. None of the animals showed significantly lower accuracy on unbaited trials compared to baited trials.

2.4.7 Dependent measures and statistical analysis—Span length was calculated as the number of consecutive correct responses that occurred before the first error minus one (the first trial with only one stimulus was excluded). Longest run (largest number of consecutive correct responses) was also calculated for each session. One-way, repeated measures ANOVAs were used to analyze span and longest run data. Percent correct on OST and SDC trials was calculated by dividing the number of correct responses by the number of trials in which a response was made and then multiplying by 100. Percent of programmed trials omitted (omissions were defined as trials that lasted 2 min without a response) was also calculated. Latency to the first response was also recorded and on omission trials, a latency of 120 s was recorded. Percent correct and latency were analyzed using a dose x task factorial ANOVA. Within-session analyses were also conducted to examine accuracy as a function of the number of stimuli to remember for doses of flunitrazepam that produced selective effects. These analyses are described below. Finally, to further assess selectivity of

effects on OST and SDC performance, individual average percent correct values at each drug dose were normalized as a percent of vehicle accuracy. A linear model was fitted using log dose and the pooled individual normalized percent correct values (Graphpad Prism version 6, Graphpad Software, San Diego, CA). To determine if differences in the linear fits to the OST and SDC results were statistically significant a model comparison approach was taken using Graphpad Prism: the slope values were compared via F -test with $\alpha=.05$ and if differences in the slope values were not statistically significant, then the intercept values were compared via F -test with $\alpha=.05$ (Motulsky and Christopoulos 2004)

3.0 Results

The top panel of Figure 1 shows the effects of flunitrazepam on accuracy on OST (black symbols) and SDC (white symbols) trials. Under vehicle conditions percent correct was high on both tasks, but flunitrazepam decreased OST accuracy in a dose-dependent fashion while largely sparing SDC performance. This selective impairment of OST was confirmed by a factorial ANOVA which yielded a significant Dose X Task interaction [$F(3, 15) = 6.22, p < .01$]. Post hoc LSD tests showed that OST accuracy was significantly lower than vehicle only at the 1.0 mg/kg dose ($p < .05$). Flunitrazepam administration also resulted in dose-dependent declines in span length and longest run (bottom panel of Figure 1) and one-way ANOVAs confirmed the significance of these decreases [span length, $F(3,15) = 7.11, p < .01$; longest run, $F(3,15) = 13.02, p < .01$]. Table 2 shows percent correct for individual subjects and supports the group analysis. Every rat showed a selective decrease in OST accuracy at one or more doses. Two animals (K11 and M26) were relatively sensitive to flunitrazepam, showing impairment of OST, but not SDC accuracy at the 0.1 mg/kg dose. Selective effects on OST performances were seen only at higher doses in the remaining four rats. In summary, flunitrazepam impaired OST accuracy in a dose-dependent and selective fashion. The decreases in OST accuracy were selective in that doses that did not affect SDC accuracy produced significant decreases in OST accuracy.

Figure 2 and Table 2 show a comparable analysis of the effects of zolpidem. Zolpidem effects were complicated by large individual differences in sensitivity. Interpretation of the mean data in Figure 2 is made difficult because data presented for the 5.6 and 10.0 mg/kg doses are based on fewer than 6 rats. Two animals (L24 and N32) showed substantial impairment at the 5.6 mg/kg dose and were not tested at the 10 mg/kg dose. Rat L24 responded on only two trials at the 5.6 mg/kg dose and Rat M10 failed to respond at the 10.0 mg/kg dose, so their data at those doses were not included in percent correct means of Figure 2 (see Table 2 for individual subject data for each rat). Note also that one rat (M22) was unaffected by zolpidem even at the 10.0 mg/kg dose and a higher dose of 18.0 mg/kg was administered for this rat. Despite this variability in sensitivity, it is evident from Table 2 that the overall dose-effect functions were similar across rats. Zolpidem reduced accuracies on both OST and SDC tasks with effects primarily occurring at the higher doses (5.6, 10.0 and 18.0 mg/kg) depending on the rat. But note that, unlike the effects of flunitrazepam, zolpidem effects were generally not selective to the OST. Note also in Figure 2 that the higher doses of zolpidem resulted in many response omissions (bars)—trials that terminated after 2 min without a response. As might be expected given the variability in sensitivity to zolpidem, ANOVA failed to show significant effects of dose or a dose X task interaction on

percent correct ($p > .05$). Span length, longest run and latency to respond were clearly impaired by zolpidem, but statistical analyses that included all 6 rats (excluding the 10.0 mg/kg dose) failed to reach significance ($p > .05$). However, ANOVAs that included the 10.0 mg/kg dose for the four rats tested were significant for span length [$F(4,12) = 21.56$, $p < .05$], but not longest run ($p > .05$).

Because of the individual subject differences in sensitivity to zolpidem, it is possible that selective effects might be evident at the individual subject level at different doses. However, Table 2 provided only a little support for this. Three rats (L24, M10 and N32) showed no effects of zolpidem on accuracy until doses caused frequent response omissions on both OST and SDC trials. Zolpidem did increase errors in Rats N36 and M16, but this occurred at doses that reduced accuracy on both OST and SDC tasks. However, one rat (M22) did show evidence of a selective effect. This rat was unaffected by 10.0 mg/kg zolpidem, but when exposed to 18.0 mg/kg OST accuracy dropped while the SDC performance remained high. In summary, zolpidem produced dose-dependent impairments in performance, but did so in a non-selective fashion for 5 of the 6 rats tested (i.e., errors and omissions were increased on SDC trials at the same doses that affected OST responding).

Scopolamine results are shown in Figure 3 and Table 2 and, like zolpidem, were characterized by considerable variability in sensitivity across subjects. Also like zolpidem, scopolamine generally produced dose-dependent, but non-selective, decreases in accuracy accompanied by frequent omissions at the higher doses. Responding was completely eliminated for one animal (K17) at the 0.3 mg/kg dose and this animal was not tested at the 1.0 mg/kg dose and not included in the statistical analysis. Factorial ANOVA conducted with the remaining rats confirmed a significant main effect for dose [$F(3,12) = 6.32$, $p < .01$], but no interaction between dose and task ($p > .05$). Post hoc tests showed that accuracy following both the 0.3 and 1.0 doses differed significantly from accuracy following saline ($p < .05$ and $p < .01$, respectively). Analysis of individual subject data also failed to reveal evidence of any selective effect of scopolamine on OST performance. OST errors increased in four of the rats (K10, L7, M16 and N33), but only at doses that also decreased SDC accuracy. The other two rats (K17 and M26) showed no effect until doses that reduced overall responding were reached. Span length and longest run both showed dose dependent decreases [Span length $F(3,12) = 21.93$, $p < .01$; longest run $F(3, 12) = 15.02$, $p < .01$]. The decreases in span length and longest run were largely due to the increased number of omission trials observed at the 0.3 and 1.0 mg/kg doses. In summary, scopolamine failed to show evidence of any effects that were selective to the OST.

To directly compare the dose-response curves of the three drugs studied, percent correct values were normalized (percent of vehicle control) and plotted along with best fitting straight line as a function of log dose. Figure 4 shows the results of these analyses for flunitrazepam (top), zolpidem (middle) and scopolamine (bottom). The selective nature of flunitrazepam effects shows clearly in Figure 4 with a much steeper slope for OST accuracy relative to the SDC slope ($F_{1,32} = 7.26$, $p = .01$). In contrast, note the virtual absence of selectivity for scopolamine: the two slopes are virtually identical as are the intercept values. Finally, the results for zolpidem were somewhat intermediate between flunitrazepam and scopolamine with some divergence between OST and SDC evident, particularly at the

highest dose. For zolpidem, the difference in slope values between OST and SDC was statistically significant ($F_{1,31} = 9.99$, $p = .004$), but this finding should be interpreted with caution because only one subject contributed to the data point at the highest dose and only two subjects contributed to the data point at the second highest dose.

Flunitrazepam was the only drug in the present study that produced clearly selective effects on OST performances. Such selective effects suggest the effects of flunitrazepam may have impaired memory capacity, however, examination of the effects as the memory load varied within the session is needed to confirm such a conclusion. Percent correct was calculated as a function of the number of stimuli to remember by dividing the sessions into bins of 4 trials (omitting Trial 1 on which only one stimulus was presented), and these results are shown in Figure 5 under vehicle and the two most effective flunitrazepam doses, 0.3 and 1.0 mg/kg. There was very little change in within-session accuracy on vehicle sessions as percent correct remained high throughout the session, dropping only slightly as the number of stimuli to remember reached 20 or more. The effects of flunitrazepam were evident early in the session when the memory load was small, but did not increase as the session continued and the number of stimuli to remember increased. A dose X bin ANOVA confirmed these conclusions with a main effect of dose [$F(2,10) = 19.62$, $p < .01$], but no statistically significant effect of bin and no dose X bin interaction. In sum, flunitrazepam impaired OST accuracy independently of the memory load.

4.0 Discussion

The three drugs tested in the present study all increased errors in a dose-dependent fashion. However, the drug effects differed in several respects. Flunitrazepam decreased OST accuracy without affecting SDC accuracy at the highest doses tested. Scopolamine reduced OST accuracy only at doses that had comparable effects on SDC accuracy and that also led to frequent response omissions. Effects of zolpidem were much like those of scopolamine, but there was at least some evidence of selectivity (Figure 4). The limited selectivity of the effects of zolpidem and scopolamine was somewhat surprising as both can produce working memory decrements when tested in some other procedures (Klinkenberg & Blokland, 2010; Soto et al., 2013; Taffe et al., 1999).

In the case of scopolamine, the present results were not completely unexpected as they replicate the previous findings of Galizio et al. (2013) which also showed no selective effects of scopolamine on OST accuracy. In that study, up to five comparison odors were present on both OST and SDC trials and it was thought that the complexity of this procedure might have made the task excessively sensitive to the globally disruptive effects that scopolamine often produces. The present study did not support such an interpretation as even with the simpler 2-choice test used on both OST and SDC trials, scopolamine disrupted accuracy and increased omissions on both tasks in a non-selective fashion.

On the other hand, the selective effects of flunitrazepam on OST performance previously found by Galizio et al. (2016) were replicated in the present study. However, it should be noted that within-session effects were not analyzed in that earlier study. The effects of positive alpha-1 GABA_A receptor modulator zolpidem were quite different from those of

flunitrazepam. Zolpidem, like scopolamine, generally had no effect until doses were reached that impaired accuracy on both OST and SDC trials and increased omissions. Interpretation of the zolpidem effects are complicated by differences in sensitivity to the drug with two animals virtually ceasing to respond at the 5.6 mg/kg dose which had no effect on the other four rats. Only one rat showed evidence of a selective effect of zolpidem and that occurred in the animal that appeared to be least sensitive to the drug and only at the highest dose given (Rat M22, 18.0 mg/kg see Table 2). Nonetheless, the differences between the effects of zolpidem and flunitrazepam are striking and suggest that the α -1 GABA_A receptor may not be involved in the selective actions of flunitrazepam on OST performances. This finding was unexpected based the findings of Soto et al. (2013) who found that α -1 GABA_A modulators zolpidem and zaleplon, but not α 2/3 or α -5 modulators, produced memory-load dependent impairments in the SOSS task in monkeys.

The apparent difference in outcomes between the Soto et al. (2013) experiment and the present findings might suggest a possible species difference between monkeys and rodents in GABA_A receptor subtype modulation. However, analysis of the within-session data (Figure 5) suggests that differences between the SOSS and OST tasks may be more important. Although the effects of flunitrazepam observed in the present study were selective to the OST, they did not seem to depend on the memory load. Rather, substantial impairment was observed even at the very beginning of the session when the number of odors to remember was only 1–4. This effect is in marked contrast to the memory-load dependent effects of positive GABA_A modulators observed by Soto et al. in the SOSS task. It is also very different from the selective actions of NMDA antagonists observed in previous OST studies (Galizio et al., 2013; MacQueen et al., 2011) which did depend on the number of stimuli to remember.

The fact that flunitrazepam effects were OST selective, yet did not depend on memory load raises questions about the mechanisms responsible for this selectivity. One possibility is that flunitrazepam may have disrupted the non-matching-to-sample conditional discrimination rather than exerting effects on working memory in the present study. Such an interpretation still leaves the puzzling differences between flunitrazepam and zolpidem to account for, but the interpretation would now be that zolpidem affected both the simple and conditional discrimination, whereas flunitrazepam affected only the more complex conditional discrimination task at the doses tested. It is worth noting that Soto et al. (2013) found that positive GABA_A modulators, including those selective to the α -1 receptor, produced delay-independent effects on accuracy in a visual delayed matching-to-sample task, which is consistent with the interpretation of disrupted conditional discrimination rather than memory. A similar account could be offered for the differences between Taffe et al. (1999) and the present study as they found that scopolamine produced memory load-dependent effects in the SOSS, but delay-independent effects on delayed matching-to-sample. Perhaps special features of the SOSS procedure make it less sensitive to the disruption of discrimination relative to the OST, and thus more sensitive to effects on memory capacity. It would certainly be worthwhile to explore SOSS-like procedures in rodents to test this hypothesis.

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Highlights

- The odor span task (OST) assesses rodent's ability to remember multiple odor stimuli
- Positive GABA_A modulator flunitrazepam selectively impaired OST accuracy
- However, flunitrazepam effects did not depend on the number of items to remember
- Alpha-1 selective positive GABA_A modulator zolpidem did not selectively impair OST accuracy
- Cholinergic antagonist scopolamine did not selectively impair OST accuracy

Figure 1.

Top panel shows mean percent correct as a function of flunitrazepam dose for OST (black circles) and SDC (white circles) trials. Lower panel shows mean span length (black circles) and longest run (white circles). Vertical lines indicate SEM.

Figure 2.

Top panel shows mean percent correct and omissions as a function of zolpidem dose for OST (black circles) and SDC (white circles) trials. Black bars show percent omissions for OST and white bars show SDC omissions. Lower panel shows mean span length (black circles) and longest run (white circles). Vertical lines indicate SEM.

Figure 3.

Top panel shows mean percent correct and omissions as a function of scopolamine dose for OST (black circles) and SDC (white circles) trials. Black bars show percent omissions for OST and white bars show SDC omissions. Lower panel shows mean span length (black circles) and longest run (white circles). Vertical lines indicate SEM.

Figure 4.

Normalized percent correct (calculated as percent of vehicle values) for OST (black circles) and SDC (black squares) plotted as a function of log drug dose for flunitrazepam (top), zolpidem (middle) and scopolamine (bottom). Vertical bars indicate SEM.

Figure 5. Percent correct on OST trials plotted as a function of the number of stimuli to remember for the 0.3 mg/kg and 1.0 mg/kg doses of flunitrazepam and vehicle. Vertical lines indicate SEM.

Table 1

Number of training sessions and baseline data for all study rats

Rat	Sessions	Baseline %	Unbaited %	Drug
K10	71	90	86	SCOP
K11	30	89	97	FLZ
K17	37	94	89	SCOP/FLZ
L7	30	86	92	SCOP
L24	41	90	88	ZOLP
M10	26	98	100	ZOLP
M16	20	93	97	SCOP/ZOLP
M22	31	94	96	ZOLP
M26	36	90	88	SCOP/FLZ
N11	28	88	89	FLZ
N32	51	85	83	ZOLP/FLZ
N33	24	86	85	SCOP
N36	48	92	93	ZOLP
O7	26	94	92	FLZ

Table 2

Individual subject percent correct for each drug

Flunitrazepam (mg/kg)											
OST						SDC					
Rat	VEH	.1	.3	1.0	VEH	.1	.3	1.0			
K11	90.1	72.8	62.0	59.0	98.3	96.7	100.0	87.5			
K17	95.2	96.0	91.0	78.0	100.0	100.0	95.8	100.0			
M26	86.3	77.3	56.0	60.0	90.5	89.0	83.3	77.8			
N11	94.0	94.0	84.0	60.0	100.0	100.0	88.9	100.0			
N32	78.7	68.0	60.0	54.0	100.0	100.0	100.0	100.0			
O7	93.6	92.0	92.0	70.0	100.0	100.0	100.0	91.67			

Zolpidem (mg/kg)												
OST						SDC						
Rat	SAL	1	3	5.6	10	18	SAL	1	3	5.6	10	18
L24	84.0	100.0	*	*	nt	nt	89.0	100.0	*	*	nt	nt
M10	97.7	100.0	97.3	97.9	*	nt	100.0	100.0	100.0	100.0	*	nt
M16	94.4	96.0	96.0	98.0	80.7	nt	100.0	100.0	100.0	100.0	94.4	nt
M22	96.3	100.0	92.0	91.7	89.4	69.0	96.9	100.0	100.0	91.7	100.0	91.7
N32	75.1	81.00	70.2	*	nt	nt	100.0	100.0	100.0	*	nt	nt
N36	89.0	92.0	87.8	79.3	*	nt	98.0	100.0	100.0	94.4	*	nt

Scopolamine (mg/kg)											
OST						SDC					
Rat	SAL	.1	.3	1	1.8	SAL	.1	.3	1	1.8	
K10	93.0	82.8	56.1	62.5	*	93.7	94.0	83.3	71.1	*	
K17	92.7	91.5	*	nt	nt	96.7	100.0	*	nt	nt	
L7	90.4	90.0	89.7	79.2	*	100.00	100.00	96.00	86.7	*	
M16	96.4	88.0	91.1	*	nt	98.15	100.0	83.0	*	nt	
M26	86.8	88.0	*	*	nt	96.67	94.4	*	*	nt	
N33	89.1	87.7	74.7	61.9	nt	95.2	91.7	83.3	58.3	nt	

* indicates more than 25% of the trials were omissions at that dose; nt indicates that the animal was not tested at that dose

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