Neurobiology of Learning and Memory 145 (2017) 67–74

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Amnestic drugs in the odor span task: Effects of flunitrazepam, zolpidem and scopolamine☆

Mark Galizio[a,*], Michael Mathews[a], Madeleine Mason[a], Danielle Panoz-Brown[a], Ashley Prichard[a], Paul Soto[b]

[a] University of North Carolina Wilmington, United States
[b] Louisiana State University, United States

ARTICLE INFO

Keywords:
GABA-A modulator
Flunitrazepam
Scopolamine
Zolpidem
Odor span task
Working memory

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.

1. 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 & Hitch, 1974; Gathercole, 2009). A wide variety of procedures have been used 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 × 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 0.03 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

☆ This research was supported in part by grant DA029252. Michael Mathews is now at West Virginia University, Danielle Panoz-Brown is now at Indiana University and Ashley Prichard is now at Emory University. The authors thank Nancy Ator for assistance in developing the zolpidem study.

* Corresponding author at: Department of Psychology, University of North Carolina Wilmington, 601 S. College Rd., Wilmington, NC 28403, United States.
E-mail address: galizio@uncw.edu (M. Galizio).

http://dx.doi.org/10.1016/j.nlm.2017.09.006
Received 16 April 2017; Received in revised form 10 August 2017; Accepted 7 September 2017
Available online 08 September 2017
1074-7427/

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, Dalrymple, Drobes, & Diamond, 2016; MacQueen et al., 2011). 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 amnestic 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, Wonnacut, & Shoaib, 2010). However, the Rushforth et al. study did not include an SDC condition to assess non-amnestic 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 Blocklund (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 amnestic effects of drugs.

Only one study has directly examined the effects of number of distractor stimuli in the OST. April, Bruce, and 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 amnestic 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.