

Research report

Galanin and perseveration

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Abstract

Galanin is a 29/30 amino acid neuropeptide that has been shown to impair learning and memory task performance and also have roles in somatosensation, stress responses, sexual behavior, and feeding regulation. However, little is known about galanin involvement in higher cognitive processes, especially executive processes. Perseveration is a classic sign of frontal cortex damage and failure of executive control. Galanin has been shown to disrupt the performance of maze delayed alternation tasks and the operant, spatial delayed nonmatch-to-position (DNMTP) working memory task, tests especially sensitive to perseverative responding. To better understand this potential involvement of galanin in executive control, the present study tested the hypothesis that galanin induces perseveration. The first experiment examined the effects of galanin (10, 20 µg i.c.v.) on the performance of a simple operant response alternation task in which stimuli were assigned to one of two spatially distinct locations to produce extended sequences of presentations to one location, separated by a 10-s intertrial interval. The second experiment looked at the effects of galanin (5, 20 µg i.c.v.) on the performance of non-delayed match-to-position and nonmatch-to-position conditional discrimination operant tasks in which a minimal 1.0 s time interval separated responses. Finally, the effects of galanin (10, 20 µg i.c.v.) on delayed match-to-position (DMTP) performance were examined to determine whether response alternation (i.e., nonmatching) was critical to observing a galanin-induced impairment in this task. Galanin reduced the rate of trial completion in all the tasks, but did not alter simple or conditional discrimination accuracy. Galanin (10 µg) impaired DMTP performance in a delay-independent manner. Together, these data suggest that galanin does not produce perseveration, but are consistent with a galanin-induced decrease in reinforcer strength.

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1. Introduction

Galanin is a 29/30 amino acid neuropeptide that generally inhibits the actions of ascending serotonin, acetylcholine, norepinephrine, and dopamine neurotransmitter systems [2,3,28], and has also been implicated in mediating several psychological and behavioral processes

in rodents, including somatic sensation, stress responses, sexual behavior, and feeding [6,15,16,20]. Centrally administered galanin has also been shown to impair the performance of a variety of learning and memory tasks in rats, including T-maze delayed alternation [10,13], plus-maze spontaneous alternation [27] and the operant spatial delayed nonmatch-to-position tasks (DNMTP; [14,21–23]). Tasks such as these that require an alternation response are, by definition, impaired if the subject engages in pattern perseveration, a classic indicator of dysfunction of the prefrontal cortex in humans and rats [1,7,8,12,18,25]. Indeed,

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the midline frontal cortical region is rich in galanin receptors and receives monoaminergic projections that are inhibited by galanin in the rat [9,11,18], which raises the hypothesis that galanin induces perseveration. However, to definitively implicate perseverative responding as the source of the task performance impairments, one needs to compare the effects of galanin under conditions in which both non-alternation and alternation responses are assessed while in the presence and absence of intervening time (i.e., retention) intervals. The present studies were designed to directly test the hypothesis that galanin causes perseverative responding by examining the effects of centrally administered galanin on three operant variants of the DNMT task that disentangle the alternation from retention components in this manner.

The DNMT task requires that the subject first respond to a visual stimulus presented in one of two locations, then to remember that stimulus for a variable retention interval ranging from 1 s to longer intervals during which the subject responds on a lever mounted at the rear of the chamber. After the completion of the retention interval, an alternation (“nonmatch”) response is required to produce a reinforcer and a subsequent intertrial interval. An inability to alternate responses would result in accuracy deficits regardless of the length of the retention interval, thus manifesting as the memory “delay-independent” pattern of impairment that was produced by galanin administered intracerebroventricularly [21]. The present experiments first addressed the hypothesis that galanin selectively disrupted the ability to alternate responses separated temporally and spatially by using a simple response alternation procedure (Experiment 1), in which cued responses were separated by a 10-s interval. Next, the hypothesis that galanin selectively impairs the ability to perform a conditional response sequence with no intervening time interval (Experiment 2) by using non-delayed nonmatch and match-to-position conditional discrimination procedures. Finally, the hypothesis that galanin would not impair the entire four component DNMT complex when the alternation response was switched to a non-alternation response was tested using a delayed *match-to-position* (DMTP) procedure (Experiment 3). A simple working hypothesis would predict impairments to be observed when alternation responses were required and no impairment and perhaps even enhanced performance of the task when a “perseverative” match response is required for successful performance.

2. Materials and methods

2.1. Subjects

The subjects were four separate groups of adult, male Sprague–Dawley rats ($N = 13$ for the simple response alternation experiment; $N = 6$ for the match-to-sample conditional discrimination experiment; $N = 5$ for the nonmatch-to-position conditional discrimination experi-

ment; $N = 8$ for the delayed match-to-position experiment). Each subject was housed individually in a plastic tub cage (measuring $L = 46.5$ cm $W = 24.5$ cm, $H = 20.5$ cm), on a multi-shelf aluminum rack, in a humidity and temperature regulated room with a 12-h (7 a.m. on and 7 p.m. off) light–dark cycle. Their access to water was limited to 30 min, and was provided 1 h following the experimental session, Monday through Friday. They had unlimited access to standard lab chow (Labdiet Prolab RMH 3000) in the home cage Monday through Friday. The experiments were carried out Monday through Friday and all subjects had unlimited access to both food and water Saturday and Sunday. All procedures were performed in compliance with the NIH Guide for the Care and Use of Laboratory Animals [29] and with the consent of the State University of New York at Stony Brook Institutional Animal Care and Use Committee.

2.2. Apparatus

The behavioral investigation was performed in six identical standard operant test chambers (MED Associates, East Fairfield, VT), all of which were enclosed in sound-attenuating enclosures. The stainless steel apparatus consisted of two front levers with one cue lamp mounted above each one, and a rear cue lamp with a lever beneath it. The water dispenser was placed in between the two frontal levers. The entire experiment was controlled and data recorded by a Dell PC computer running MED Notation programming language.

2.3. Surgical procedures

Each animal received a cannula made of stainless-steel hypodermic tubing (24 gauge and 1.7 cm long) into the right lateral ventricle under 50 mg/kg i.p. Ketamine and 10 mg/kg i.p. Xylazine anesthesia (From Bregma, 0.5 anterior/posterior, -3.5 dorsal/ventral, and $+1.0$ laterally from Bregma (from [19])). Four stainless steel screws and epoxy were used to secure the small plastic tubing protecting the cannula and to hold it in place. The animals were allowed to recover for 1 week after surgery prior to resuming behavioral testing.

At the completion of the experiments, the rats were sacrificed under deep sodium pentobarbital anesthesia and the correct placement of the cannula into the lateral ventricle was verified histologically.

2.4. Drug preparation and injection

Rat galanin (>99% purity) was purchased from Bachem Bioscience (King of Prussia, PA, USA) and was dissolved in saline. Galanin or saline control was administered using a Hamilton syringe connected by plastic tubing to a 31 gauge, 1.9 cm stainless steel injector. Each injection delivered a total 5 μ l solution into the ventricles over 25 s. The injectors were then left in place for an additional 60 s. The testing

sessions began immediately after injection due to the reported limited half-life of galanin in vivo [26].

In the simple response alternation experiment, the subjects received 10 and 20 μg of galanin and saline injections. In the nonmatch/match-to-position conditional discrimination experiment, the subjects received 5 and 20 μg of galanin and saline injections. In the DMTP experiment, the subjects received 10 and 20 μg of galanin and saline injections. The doses expressed as nmol are as follows (5 μg = 1.56 nmol; 10 μg = 3.1 nmol; 20 μg = 6.25 nmol).

2.5. Simple response alternation

The subjects were pretrained to press a front response lever to greater than 90% accuracy when one of the front cue lamps was illuminated above that lever. The cue lamp was illuminated unpredictably, but sequences of illuminations on the same side (e.g. left-left-left. . .) occurred with a probability of 0.67 (see Table 1 for the series of experimental events).

Table 1
Summary of the sequences of experimental events

1. Simple response alternation task
Trial 1: One of two cue lamps illuminated (e.g. left lamp); response on lever beneath it produces 0.1 ml water reinforcer.
↓
Trial 2: After 10 sec, the same cue lamp as trial 1 illuminated with $p = 0.67$.
↓
Trial 3: After 10 sec, the same cue lamp as trial 2 illuminated with $p = 0.67$.
↓
Trial 4: etc.
2a. Match-to-position conditional discrimination
Phase 1: One of two cue lamps is illuminated (e.g. left lamp); response on lever beneath it produces phase 2 after 1 sec interphase interval.
↓
Phase 2: Both cue lamps illuminated; response on same lever as Phase 1 produces 0.1 ml water reinforcer.
↓
Phase 3: 10 sec intertrial interval.
2b. Nonmatch-to-position conditional discrimination
Phase 1: One of two cue lamps illuminated (e.g. left lamp); response on lever beneath it produces Phase 2 after 1 sec interphase interval.
↓
Phase 2: Both cue lamps illuminated; response on alternate lever as Phase 1 produces 0.1 ml water reinforcer.
↓
Phase 3: 10 sec intertrial interval.
3. Delayed match-to-position
Phase 1: One of two cue lamps illuminated (e.g. left lamp); response on lever beneath it produces phase 2.
↓
Phase 2: 1 or 7 sec retention interval; at least one response on lever in rear of chamber after completion of retention interval produces Phase 3.
↓
Phase 3: Both cue lamps illuminated; response on same lever as Phase 1 produces 0.1 ml water reinforcer.
↓
Phase 4: 10 sec intertrial interval.

2.6. Nonmatch/Match-to-position conditional discrimination

Two separate groups of subjects were pretrained to press a front response lever to greater than 90% accuracy when one of the a cue lamps was illuminated randomly (the “sample” stimulus). Incorrect sample responses were recorded, but were followed by no scheduled consequence. A correct sample lever press was followed by a 1-s inter-response interval and the illumination of both front cue lamps. In the match-to-position group, a 0.1-ml water reinforcer was provided for pressing the same lever as the sample, and in the nonmatch-to-position experiment, the reinforcer followed a press on the opposite lever of the one pressed during the sample.

2.7. DMTP

A DMTP trial consisted of three consecutive phases: (1) a sample phase, in which one of the front cue lamps was illuminated and a response on the corresponding response lever was required, (2) a retention phase in which the subjects responded on the rear lever during which a retention interval of either 1.0 s or 7.0 s was in effect, and (3) a choice phase, in which both front cue lamps were illuminated and a response on the same response lever as during the sample phase produced a 0.1-ml water reward (a “match” response). This procedure employed an adjusting retention interval procedure [24] in which overall choice accuracy was held at approximately 75% or greater by presenting 1.0 s delay trial exclusively when accuracy fell below 75% and 7.0 s delay trials when accuracy was 75% or greater.

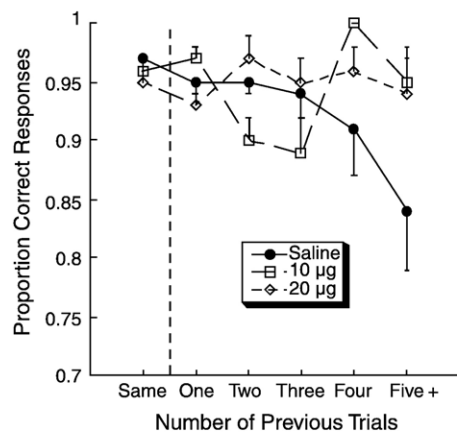
2.8. Experiment design and data analyses

The simple response alternation and DMTP experiments employed Latin square designs in which all subjects received each dose of the galanin and saline. Injection days were Tuesday and Thursday, and Monday–Wednesday–Friday were no injection baseline days. Because of the trend for an enhancement of performance by galanin observed in the first experiment, the nonmatch/match-to-position conditional discrimination experiments employed a single-subject design where Monday–Wednesday–Friday were saline injection days to enhance sensitivity to detecting a modest effect against a stable performance baseline. Both the nonmatch- and match-trained groups received either 5 or 20 μg galanin injections on Tuesdays and Thursdays counterbalanced within each group.

3. Results

Fig. 1 shows data from the simple response alternation. A repeated measures analysis of accuracy as a function of

A. Choice Accuracy - Light/Dark Discrimination



B. Trials/Session - Light/Dark Discrimination

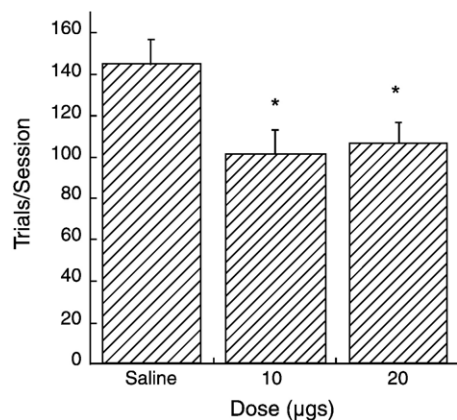


Fig. 1. Choice accuracy as a function of the number of trials during which the discriminative stimulus appeared in one location. The leftmost points represent accuracy on all trials where the stimulus appeared on the same side as the previous trial. The remaining points represent trials where the stimulus appeared on the side different from the previous 1–5 or more trials. Galanin (i.c.v.) produced a significant enhancement in accuracy when the stimulus appeared on a new side only after an extended sequence (shown in A). Galanin also significantly reduced the number of trials completed per session (B). For all figures, * = Fishers PLSD post hoc test ($P < 0.05$); and error bars show the SEM.

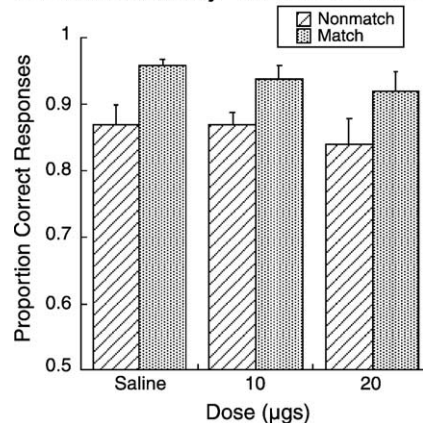
whether the previous trial was on the same side, or was one, two, three, four, five, or greater than five consecutive trials on the opposite side prior to changing over to the side of the present trial, revealed a modest but significant galanin dose \times number of consecutive trials interaction ($F(10,170) = 2.2$, $P < 0.02$; Fig. 1a) but no significant main effect ($F(1,34) = 1.1$, n.s.). Galanin also significantly reduced the number of trials completed per session ($F(1,36) = 4.5$, $P < 0.01$; Fig. 1b). Fishers PLSD post hoc test indicated that the 10 and 20 μg groups were significantly different than the saline-treated group (both $P < 0.05$).

Fig. 2 shows the effect of galanin on choice accuracy (Fig. 2a) and the rate of trial completion (Fig. 2b) for the nonmatch and match-to-position conditional discrimination groups. The two doses of galanin and saline were analyzed against the average trials per session and accuracy per

session for the Monday–Wednesday–Friday saline injection days. Analyses were conducted using MANOVA where dose of drug and match/nonmatch condition were each entered as factors. Galanin had no significant effect on accuracy (main effect of dose: $F(1,31) = 0.71$, n.s.; dose \times treatment interaction: $F(1,31) = 0.06$, n.s.; Fig. 2b). The MANOVA ($F(2,31) = 9.1$, $P < 0.006$) and a subsequent Fishers PLSD post hoc test ($P < 0.005$) indicated that the match and nonmatch conditions were significantly different. Galanin significantly reduced the number of trials completed per session for both groups ($F(1,27) = 4.4$, $P < 0.03$; Fig. 2a). Fishers PLSD post hoc test ($P < 0.05$) indicated that the 10 and 20 μg groups were significantly different than the saline treated group for the Match experiment and the 20- μg group from the Nonmatch experiment.

Fig. 3 shows the effect of galanin on choice accuracy (Fig. 3a) and the rate of trial completion (Fig. 3b) in the DMTP task. Galanin significantly reduced the number of trials completed per session ($F(1,18) = 4.4$, $P < 0.02$). Fishers PLSD post hoc test indicated that only the 20- μg

A. Choice Accuracy - Conditional Discrimination



B. Trials/Session - Conditional Discrimination

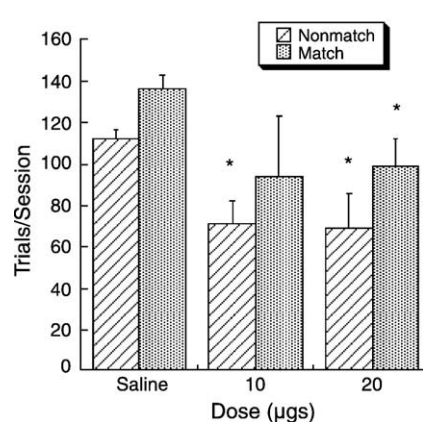


Fig. 2. The effect of Galanin (i.c.v.) on choice accuracy (A) and the number of trials completed per session (B) for the nonmatch and match-to-position conditional discrimination groups. Galanin had no significant effect on accuracy, though the match and nonmatch condition groups were significantly different. Galanin significantly reduced the number of trials completed per session for both groups (B).

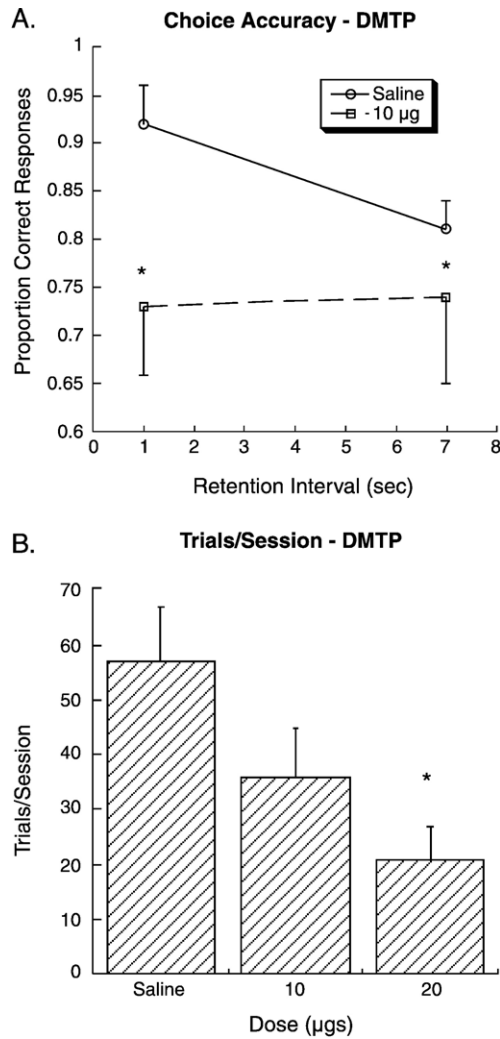


Fig. 3. Galanin (i.c.v.) impaired choice accuracy (A) delay-independently and reduced the rate of trial completion (B) in the DMTP task.

group was significantly different than the saline-treated group ($P < 0.05$). Because of the low number of trials completed by the 20-µg group, these scores were not included in the repeated measures analysis of accuracy as a function of retention interval. This analysis revealed a significant main effect ($F(2,14) = 5.5, P < 0.02$;) but not a significant interaction ($F(2,14) = 53, n.s.$) of galanin on accuracy.

4. Discussion

The present data provide no support for the theory that galanin induces perseveration. No impairments in the simple response alternation or conditional discrimination tasks were observed. Furthermore, galanin impaired the performance of a delayed match-to-position task, indicating that galanin induces performance deficits regardless as to whether or not response alternation is required for accurate task performance.

An additional finding of the present work is that intracerebroventricularly administered galanin produced consistent decreases in the rate of trial completion. This has been observed in other operant studies of galanin (20, 27–29). These observations suggest that galanin decreases the incentive value of the water reinforcers. Recent work from our lab has addressed this issue directly using rats that are water restricted to the same degree as those in the present experiment. In this study, galanin (20 µg i.c.v.) reduced both free water intake tested in a plastic tub cage and on a response-independent, fixed time operant schedule [4]. Furthermore, galanin (5, 10 and 20 µg i.c.v.) reduced persistence on a progressive ratio schedule of reinforcement in which the number of responses to produce each reinforcer increased after each reinforcer delivery. However, while these data argue for a role for galanin in mediating water intake regulation and reinforcement processes, it was also demonstrated that reducing the degree of deprivation by allowing pre-session water access (modeling galanin-induced reduction in water reward strength) only reduced the rate of delayed nonmatching-to-position (DNMTP) trial completion without affecting choice accuracy. Therefore, a change in reinforcer strength is an unlikely explanation for the DMTP deficits observed presently.

The present data suggest that response perseveration does not account for the galanin-induced performance deficits and suggest that a galanin-induced short-term memory deficit remains a viable explanation for the impairments observed, since in all maze studies, arm entrances were separated by a time interval [10,13,27]. The delay-independent DMTP deficits observed presently cannot argue against this working memory theory because they cannot differentiate when a process *in addition to* working memory is concomitantly altered from when a process *instead of* working memory is altered [5].

Another issue raised by the present results is why reduced performance was evident only in the most difficult of tasks (the DMTP). One explanation is that galanin produces reference memory impairment similar to “disorientation” or “confusion”, leading to inappropriate responding due to a loss of temporal or sequential organization within the four phases of the DNMTP/DMTP tasks (sample, retention interval, choice, and intertrial interval). This would result in the delay-independent deficits in choice accuracy we observed. Alternatively, it may be that even the 1.0-s retention interval, which also involves a rear panel lever press, is sufficient to distract the animal and disrupt retention. Future work could follow up on these issues by examining the effects of galanin on chained reinforcement schedules and tests of sustained attention.

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