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Intraseptal Galanin Potentiates Scopolamine Impairment of Delayed Nonmatching to Sample

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Galanin coexists with ACh in the basal forebrain and medial septal region. The present study investigated the interactions of the muscarinic receptor antagonist scopolamine and the neuropeptide galanin on an operant spatial delayed nonmatching to sample task (DNMTS) in rats. Scopolamine administered both intraperitoneally and microinjected into the medial septum impaired performance on DNMTS. Galanin administered alone into the medial septum did not disrupt DNMTS, but potentiated the disruptive effects of intraperitoneal administered scopolamine. These findings raise the possibility that endogenous galanin may exacerbate cognitive impairments associated with forebrain cholinergic deficits.

[Key words: ACh, coexistence, learning, medial septum, memory, microinjection, neuropeptide, septohippocampal pathway, retention]

Galanin is a 29 amino acid neuropeptide that coexists with ACh in neurons of the medial septum in rats (Melander et al., 1985a), and with ACh in both the nucleus basalis of Meynert and medial septum/nucleus of the diagonal band cells in nonhuman primates (Melander and Staines, 1986; Walker et al., 1991). In addition, galanin is found in small interneurons in the region of the nucleus basalis of Meynert in humans (Chan-Palay, 1988b; Kordower and Mufson, 1990; Walker et al., 1991). Galanin inhibits cholinergic actions, including blocking EPSPs of hippocampal pyramidal cells following electrical stimulation of cholinergic Schäffer collaterals (Dutar et al., 1989), attenuation of scopolamine-induced release of ACh in ventral hippocampus *in vitro* and *in vivo* (Fisone et al., 1987), and inhibition of carbachol-stimulated phosphoinositide hydrolysis (Palazzi et al., 1991).

The inhibitory actions of galanin in basal forebrain cholinergic function have stimulated interest in this peptide as a possible contributing factor in the behavioral and cognitive deficits associated with Alzheimer's disease, in which patients have substantial degeneration of cholinergic neurons in the region of the nucleus basalis of Meynert and medial septum (Drachman, 1977; Coyle et al., 1982). Post mortem studies show that small galanin-immunoreactive neurons appear to hyperinnervate large ChAT-immunoreactive neurons in Alzheimer's patients (Chan-Palay, 1988a; Mufson et al., 1992). Additionally, an increase in galanin-like immunoreactivity was concomitantly observed with a decrease in ChAT levels in post mortem samples of the nucleus basalis of Meynert area of Alzheimer's patients (Beal et al., 1990), and galanin-containing neurons appeared in close proximity to plaques in Alzheimer's disease patients (Kowall and Beal, 1989), further suggesting an association of galanin to disease conditions.

Hökfelt et al. (1987) proposed that galanin serves as an inhibitory modulator of cholinergic function under normal conditions, possibly as a rate-dependent, negative feedback mechanism. In the disease state, the activity of surviving cholinergic neurons could increase their firing rate, possibly inducing greater release of galanin at the higher activities. As the synaptic concentration of galanin increased, galanin-induced inhibition of the surviving cholinergic neurons would further reduce cholinergic functions, contributing to greater cognitive disfunction.

Rodent learning and memory paradigms suggest that galanin is an inhibitory modulator of ACh (Crawley and Wenk, 1989; Robinson and Crawley, 1993a). Mastropaolo et al. (1988) found that galanin injected intraventricularly or into the ventral hippocampus blocked the restorative effects of ACh on impairments in T-maze delayed alternation performance in rats with basal forebrain lesions. Galanin in nanomolar concentrations impaired the acquisition of the Morris water maze task (Sundstrom et al., 1988) and the sunburst maze task (Malin et al., 1992), and galanin infused intraventricularly impaired operant, spatial delayed nonmatching to sample performance (DNMTS; Robinson and Crawley, 1993). The first study with a galanin receptor antagonist, M35, reported an improvement in the acquisition of a water maze task (Ögren et al., 1992). Finally, galanin infused directly into the medial septum of rats impaired performance on a T-maze delayed alternation task while simultaneously disrupting the hippocampal theta rhythm (Givens et al., 1992), suggesting that the medial septum may be one likely site for the disruptive effect of galanin on memory task performance.

The present study investigates the effects of galanin in the medial septum using an operant, spatial DNMTS paradigm. Performance of this task is inhibited by intraventricularly administered galanin and intraperitoneally administered scopolamine, a muscarinic receptor antagonist (Robinson and Crawley, 1993b). The effects on DNMTS performance of galanin infused into the septum were compared to the effects of both intraperitoneal and intraseptal scopolamine. The present ex-

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periments test the hypothesis (Hökfelt et al., 1987) that intraseptally administered galanin might produce a greater impairment of memory under conditions in which cholinergic function is already compromised, by quantitating the effects of galanin administered into the medial septum during scopolamine treatment on DNMTS.

Materials and Methods

Subjects. The subjects were 30 male Sprague–Dawley rats (Taconic Farms), aged 120 d (approximately 250 gm) at the beginning of the experiment. They were housed individually in stainless steel cages in a temperature and humidity controlled vivarium, with rat chow available ad libitum, and maintained on a 7 A.M. on/7 P.M. off, light/dark cycle.

Apparatus. Behavioral testing was conducted in three identical operant test chambers (MED Associates, East Fairfield, VT), enclosed in sound-attenuating chambers, described in Robinson and Crawley (1993b). An aperture centered on the front panel contained a stainless-steel cup into which droplets of water could be delivered. Two response levers were mounted on the front panel 4 cm above the cage floor on either side of the water aperture. A single response lever was similarly mounted on the rear panel. A white cue lamp was mounted above each lever, and a houselight was mounted above the rear cue lamp. Experimental events and data recording were controlled by an MED PC computer, using MEDstate Notation programming language.

Surgery. Stereotaxic surgery was conducted in accordance with NIH Guide for the Care and Use of Laboratory Animals (1985), using aseptic techniques, and under chloral hydrate anesthesia. Each subject was implanted unilaterally with two guide cannulas made of stainless steel hypodermic tubing, 1.7 cm, 24 gauge in the lateral ventricle (not used in the present experiment), and into the medial septum, 0.7 mm anterior and 1.5 mm lateral to bregma, and 5.0 mm ventral to the surface of the skull, after Paxinos and Watson (1986), and at a 15° angle toward the midline (Givens et al., 1992). A 31 gauge stylet closed the cannulas. Dental cement and four jewelers screws held the guide cannulas in place and anchored to the skull. Subjects were given 1 week for recovery before any behavioral training began.

Behavioral testing. Subjects were exposed initially to a series of pretraining conditions prior to DNMTS training as previously described (Robinson and Crawley, 1993b). Throughout all training, the subjects were allowed 30 min of access to water in the home cage at the completion of the session, and free access to water on weekends. A DNMTS trial consisted of three phases, the "sample phase," the delay period or "retention interval," and the "choice phase," as well as an intervening intertrial interval (ITI). In the sample phase, one of the cue lamps over the front levers was illuminated on a random basis, and the chamber light was turned on. Following a barpress on the lever mounted below the light, the cue lamp was extinguished and the retention interval was initiated. After the retention interval, the first barpress on the rear lever illuminated both front cue lamps, beginning the choice phase. A press on the lever opposite the one cued in the sample phase produced a water reinforcer. The front cue lamps and the overhead illumination were then extinguished, beginning the ITI. A "correction rule," which repeated the sample stimulus on the same side on the subsequent trial following an error choice on that previous trial, was employed to prevent the development of position biases.

The subjects were initially exposed to a DNMTS condition in which the retention interval was 1 sec and the ITI was 1 or 3 sec. Later the ITI was gradually lengthened to 10 sec (over approximately 15 sessions). When a subject exceeded a performance criterion of three consecutive sessions at 90% choice accuracy, the final condition of DNMTS was begun. In this condition, the retention interval on a given trial was either 1, 5, or 15 sec, in a randomized order that yielded a total of thirty 1 sec, twenty 5 sec, and ten 15 sec retention interval trials per session. Drug administration began after performances at the 1 sec retention interval exceeded 90% correct in a majority of a series of several consecutive trials. Sessions were terminated at the completion of 60 trials or if 60 min passed since session start.

Pharmacological treatments. Injections [i.p. and intraseptal (i.s.)] were routinely given twice weekly, on Tuesdays and Thursdays, with intervening baseline days (Mondays, Wednesdays, and Fridays) to assess stability of performance. Treatments were randomized, such that subjects received only one injection of each dose of compound, and no subject received more than five injections into the medial septum. Scopolamine (Sigma, St. Louis, MO; 0.12 mg/kg and 0.25 mg/kg, i.p.) was dissolved in 0.9% sodium chloride solution (saline vehicle). Intraperitoneal scopolamine was administered 10 min prior to the start of the session. Scopolamine administered intraseptally (1.0 μg, 5.0 μg, 20.0 μg) and galanin (Bachem Biosciences, Philadelphia, PA; 0.2 μg, 1.0μg, 3.2 μg) were dissolved in Ringer's solution. Scopolamine and galanin were infused into the medial septal region though a 1.9 cm, 31 gauge stainless steel microinjection tube, 0.5 μl over a period of 57 sec. The injector was left in place for an additional 1 min before removal and reinsertion of the stylet. The behavioral session began immediately after completion of the injection.

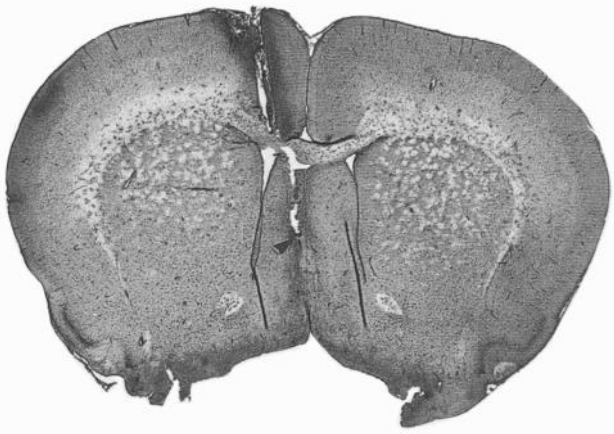

Figure 1. Photomicrograph of thionin-stained coronal section showing cannula track into medial septum. *Arrowhead* indicates termination of tract.

Histology. The subjects were killed by decapitation under deep chloral hydrate anesthesia, and the brains removed and stored in 10% formalin solution. The brains were sectioned and stained with thionin, and the anatomical locations of the cannula tracks were then determined by two independent observers. Data from subjects whose cannula placements were greater than 1.0 mm outside of the medial septum were removed from the analyses ($N = 2$). Figure 1 shows a photomicrograph of a representative medial septum cannula placement. Fast green dye (0.5 μl of 2% solution) injected to the present coordinates in anesthetized rats killed 5 min following injection spread to a radius of approximately 1.0 mm from the tip of the injector, showing that the present injections were contained within the septum.

Statistical analyses. ANOVAs and post hoc analyses were conducted on SUPERNOVA (version 1.0) statistical software. Repeated-measures ANOVAs were used to analyze the choice accuracy data, with delay as a within-groups factor, and dose as a between-groups factor. Secondary measures were analyzed by one-way ANOVAs. Where post hoc comparisons were made between experimental groups and a saline or Ringer's solution control group, Dunnett's post hoc test was used. Simultaneous multiple post hoc testing employed the Newman-Keuls test. In addition, in some cases, pairwise comparisons between two orthogonal means were made where there was a priori interest (Kirk, 1982; Rosenthal and Rosnow, 1985).

Results

Intraperitoneal scopolamine

The percentage of correct responses as a function of retention interval length for each intraperitoneal dose of scopolamine is presented in Figure 2. As in previous studies (e.g., Dunnett, 1985; Robinson and Crawley, 1993b), scopolamine reduced choice accuracy in a dose-dependent manner, with choice accuracy reduced relative to saline control at all delays ($F_{2,28} = 9.6$, $p < 0.0007$). Dunnett's post hoc test (one-tailed) revealed that only the 0.25 mg/kg dose of scopolamine was significantly different than saline ($p < 0.01$). No significant dose × delay interaction was determined ($F_{4,56} = 2.1$).

Performance measures collected concomitantly to choice re-

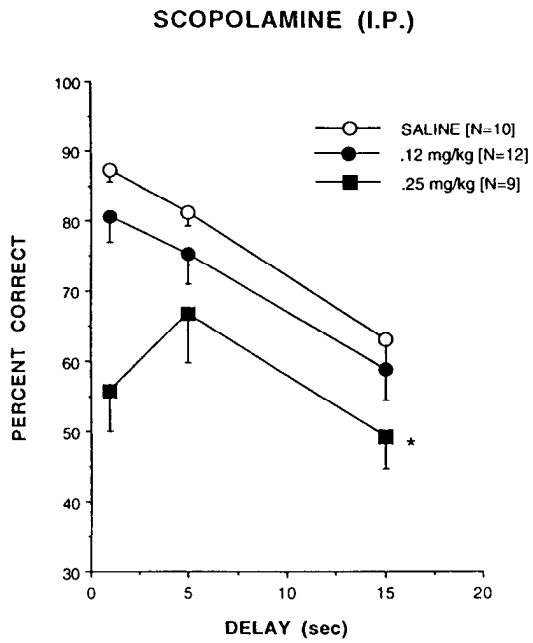

Figure 2. Scopolamine administered intraperitoneally reduced the percent correct of DNMTS choice responses at all delay values in a dose-dependent manner. *, main effect of dose significant for choice accuracy at all delays for the 0.25 mg/kg dose, $p < 0.05$ by Dunnett's post hoc test as compared to saline control. For Figures 2–5 data are plotted as mean ± SEM. The number of animals in each treatment group is given in the key.

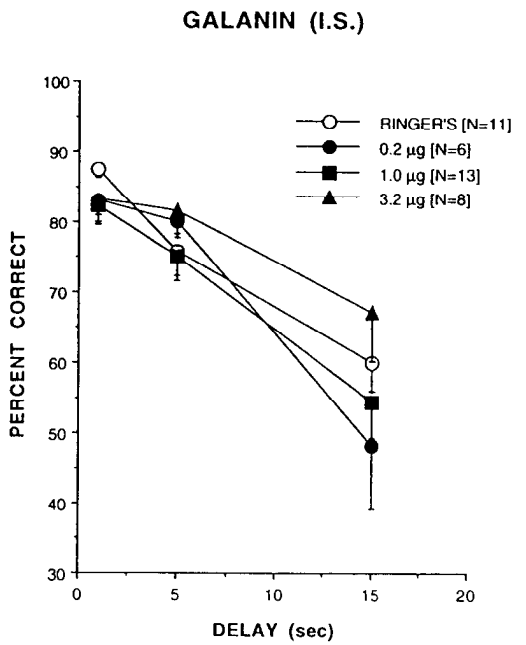

Figure 3. Galanin (0.2, 1.0, and 3.2 µg) microinjected into the medial septum (i.s.) did not significantly reduce the percentage of correct choice responses on DNMTS.

sponses revealed other effects of scopolamine administration, as previously described (Robinson and Crawley, 1993b. These measures are summarized in Table 1. The number of choice responses per session where the latency to respond exceeded 4 sec following the completion of the delay interval ("long-latency responses") was increased slightly but significantly by the 0.25 mg/kg dose of scopolamine ($F_{2,28} = 6.0$, $p < 0.007$; Dunnett's post hoc test, two-tailed $p < 0.05$). These long-latency responses occur rarely under control conditions, and are not used in calculating choice accuracy percentages.

The proportion of total error trials that occurred following a previous error trial increased at the 0.25 mg/kg dose of scopolamine ($F_{2,28} = 6.0$, $p < 0.007$; Dunnett post hoc test, two-tailed $p < 0.01$), suggesting that subjects were more likely to "perseverate" (see Milner, 1982) under this dose, but not under the 0.12 mg/kg dose of scopolamine.

The proportion of trials that contained a press on the unsignaled lever during the sample phase (a simple light/dark discrimination error) also increased in a dose-dependent manner under scopolamine ($F_{2,28} = 5.8$, $p < 0.008$), significant at the 0.25 mg/kg dose by Dunnett post hoc test (one-tailed $p < 0.01$). This result, which has not been reported in earlier studies of scopolamine and DNMTS performance (e.g., Robinson and Crawley, 1993), suggests that some loss of attentional or discriminative abilities may be caused by scopolamine.

Several measures were not significantly influenced by scopolamine, including session duration ($F_{2,28} = 1.3$), number of trials completed in a session ($F_{2,28} = 2.4$), the rear-lever variable-interval (VI) response rate ($F_{2,28} = 1.9$), and the proportion of errors in the first versus second half of the session (within-session error distribution; $F_{2,28} = 0.39$). The first two of these measures are related indicators of general response slowing, though not necessarily indicators of ataxia. Rear-lever response rate is a more valid selective indicator of motoric changes. The within-session error distribution proportion (errors in first half of session/total errors) is typically 0.5 under control conditions. An increase in the proportion suggests a diminishing of drug effects over time, and an decrease can be interpreted as an increase in proactive interference.

Intraseptal galanin

Percentage correct responses as a function of length of retention interval for each dose of intraseptally administered galanin is shown in Figure 3. No significant effects of intraseptally administered galanin on choice accuracy were detected (repeated-measures ANOVA, no main effect, $F_{3,34} = 1.2$, and no dose × delay interaction, $F_{6,68} = 1.4$).

Effects of galanin were detected in four of the secondary measures (shown in Table 1). Session duration increased in a dose-dependent manner as a function of galanin ($F_{3,34} = 6.4$, $p < 0.002$). Dunnett's post hoc test (one-tailed) revealed that both the 1.0 µg ($p < .05$) and 3.2 µg ($p < 0.01$) doses were significantly different from the Ringer's control group. The rear-lever VI response rate was unchanged for the 0.2 µg and 1.0 µg doses, but decreased significantly for the 3.2 µg dose ($F_{3,33} = 3.7$, $p < 0.03$; Dunnett's post hoc test, two-tailed $p < 0.05$). The frequency of trials with discrimination errors went up slightly but significantly for the 1.0 µg dose of galanin ($F_{3,33} = 3.7$, $p < 0.02$; Dunnett's post hoc test, two-tailed $p < 0.05$). The frequency of long-latency choice responses also went up slightly but significantly at the 1.0 µg dose ($F_{3,34} = 3.2$, $p < 0.04$; Dunnett's post hoc test, one-tailed $p < 0.05$). Galanin did not affect measures of trials completed ($F_{3,33} = 0.65$), error trials following error trials ($F_{3,34} = 1.0$), and the within-session distribution of errors ($F_{3,32} = 0.52$).

Intraperitoneal scopolamine plus intraseptal galanin

Choice accuracy as a function of length of retention interval for a single dose of intraperitoneal scopolamine and intraperitoneal

Table 1. Secondary measures of DNMTS performance for each treatment

Scopolamine i.p. Measure	Saline	0.12 mg/kg	0.25 mg/kg
Session dur.	36.4 ± 3.3	34.3 ± 2.9	42.0 ± 4.3
Trials/session	59.3 ± 0.7	60.0 ± 0.0	54.1 ± 3.9
Long lat. resp.	1.0 ± 0.6	0.9 ± 0.3	4.4 ± 1.4*
VI resp. rate	2.7 ± 0.1	3.2 ± 0.1	2.8 ± 0.3
Errs. after errs.	0.21 ± 0.04	0.21 ± 0.06	0.45 ± 0.07*
Within sess. errs.	0.48 ± 0.04	0.47 ± 0.03	0.53 ± 0.07
Discrim. errs.	0.04 ± 0.01	0.09 ± 0.01	0.17 ± 0.05*

Galanin i.s. Measure	Ringer's	0.2 μg	1.0 μg	3.2 μg
Session dur.	25.5 ± 1.0	26.1 ± 0.70	37.4 ± 3.1*	41.5 ± 5.0*
Trials/session	60.0 ± 0.0	60.0 ± 0.0	56.7 ± 3.2	54.8 ± 5.3
Long lat. resp.	0.6 ± 0.3	0.5 ± 0.2	2.2 ± 0.5*	0.9 ± 0.6
VI resp. rate	3.7 ± 0.3	3.7 ± 0.3	3.6 ± 0.2	2.7 ± 0.2*
Errs. after errs.	0.15 ± 0.03	0.22 ± 0.05	0.26 ± 0.06	0.18 ± 0.06
Within sess. errs.	0.47 ± 0.05	0.57 ± 0.04	0.50 ± 0.05	0.53 ± 0.07
Discrim. errs.	0.10 ± 0.03	0.10 ± 0.03	0.19 ± 0.03*	0.08 ± 0.02

Scopolamine i.p. + Galanin i.s. Measure	Ringer's	Scop 0.12 mg	Scop + Gal 0.2 μg	Scop + Gal 1.0 μg
Session dur.	25.5 ± 1.0	34.6 ± 3.0*	29.0 ± 1.2	44.3 ± 4.1*†
Trials/session	60.0 ± 0.0	59.3 ± 0.8	60.0 ± 0.0	49.5 ± 5.4*†
Long lat. resp.	0.6 ± 0.3	3.3 ± 0.6*	0.8 ± 0.3	4.1 ± 1.2*
VI resp. rate	3.7 ± 0.3	4.0 ± 0.2	4.9 ± 0.7	3.2 ± 0.3
Errs. after errs.	0.15 ± 0.03	0.35 ± 0.06	0.33 ± 0.05	0.47 ± 0.09*
Within sess. errs.	0.47 ± 0.05	0.45 ± 0.04	0.51 ± 0.07	0.58 ± 0.04
Discrim. errs.	0.10 ± 0.03	0.20 ± 0.03	0.17 ± 0.02	0.17 ± 0.03

Scopolamine i.s. Measure	Ringer's	1.0 μg	5.0 μg	20.0 μg	5.0 μg + Gal 1.0 μg
Session dur.	28.1 ± 1.7	27.2 ± 2.0	40.0 ± 5.2	54.2 ± 2.6*	42.6 ± 8.8
Trials/session	60.0 ± 0.0	60.0 ± 0.0	53.0 ± 5.9	53.8 ± 4.6	47.8 ± 9.1
Long lat. resp.	0.1 ± 0.1	0.4 ± 0.2	2.1 ± 0.6	9.2 ± 2.7*	2.2 ± 1.3
VI resp. rate	3.2 ± 0.2	3.3 ± 0.3	3.1 ± 0.3	2.0 ± 0.2*	2.8 ± 0.4
Errs. after errs.	0.13 ± 0.05	0.18 ± 0.10	0.41 ± 0.10	0.51 ± 0.10*	0.27 ± 0.05
Within sess. errs.	0.50 ± 0.06	0.50 ± 0.02	0.47 ± 0.09	0.51 ± 0.05	0.48 ± 0.09
Discrim. errs.	0.10 ± 0.02	0.05 ± 0.02	0.10 ± 0.02	0.11 ± 0.04	0.07 ± 0.02

Mean ± SEM shown for each dose. Scop, scopolamine; Gal, galanin; Discrim., discrimination; Dur., duration; Lat., latency; Resp., responses; VI, variable interval; Errs., errors. See Results for explanation of measures. *, Significantly different from saline or Ringer's solution control group by Dunnett's post hoc test. †, scopolamine + galanin 1.0 μg dose significantly different than scopolamine 0.12 mg/kg i.p. dose.

scopolamine plus galanin is shown in Figure 4. The 0.12 mg/kg dose of scopolamine and the 1.0 μg dose of galanin were chosen as borderline doses (Figs. 2, 3), such that coadministration of galanin could produce either an increase or decrease in DNMTS performance as compared to scopolamine alone. Scopolamine 0.12 mg/kg produced some reduction in choice accuracy at the 1 sec and 5 sec retention intervals. While the overall main effect of drug was significant ($F_{3,39} = 5.1$, $p < 0.005$), the scopolamine 0.12 mg/kg dose was not significantly different from Ringer's controls according to a Dunnett's post hoc test (one-tailed). Newman-Keuls post hoc testing showed the scopolamine 0.12 mg/kg plus galanin 1.0 μg group to be significantly different from the scopolamine 0.12 mg/kg group ($p < 0.05$), the scopolamine 0.12 mg/kg plus galanin 0.2 μg group ($p < 0.05$), and the Ringer's control group ($p < 0.01$). In addition, an a priori contrast between the scopolamine 0.12 mg/kg group and the scopolamine 0.12 mg/kg plus galanin 1.0 μg group also yielded a significant difference ($p < 0.02$). Dunnett's post hoc test also did not show the scopolamine 0.12 mg/kg plus galanin 0.2 μg group to be statistically different from Ringer's controls, and no significant drug × delay interaction was determined ($F_{6,78} = 1.3$).

Secondary measures of DNMTS performance revealed other effects of scopolamine and galanin coadministration (shown in Table 1). The 0.12 mg/kg scopolamine group and the 0.12 mg/kg scopolamine plus 1.0 μg galanin group showed significantly longer session durations than Ringer's controls (main effect of dose $F_{3,39} = 8.3$, $p < 0.0002$; Dunnett's post hoc test, $p < 0.05$ for scopolamine i.p. alone; $p < 0.01$ for scopolamine + galanin). Newman-Keuls post hoc testing ($p < 0.05$) showed the intra-

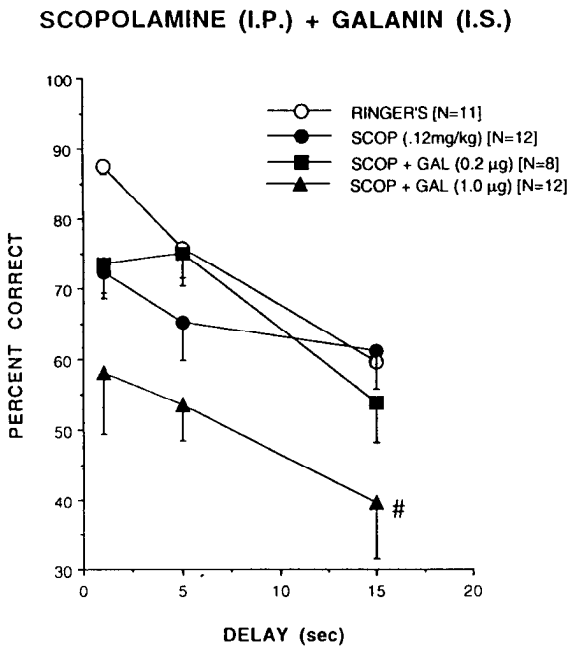

Figure 4. Galanin (1.0 μg, into the septum) coadministered with scopolamine (0.12 mg/kg, i.p.) reduced the percentage of correct choice responses at all delay values significantly more than scopolamine (0.12 mg/kg, i.p.) alone. #, significantly different than scopolamine 0.12 mg/kg, i.p., control group for choice accuracy at all delays for this dose at $p < 0.05$ by Newman-Keuls post hoc test.

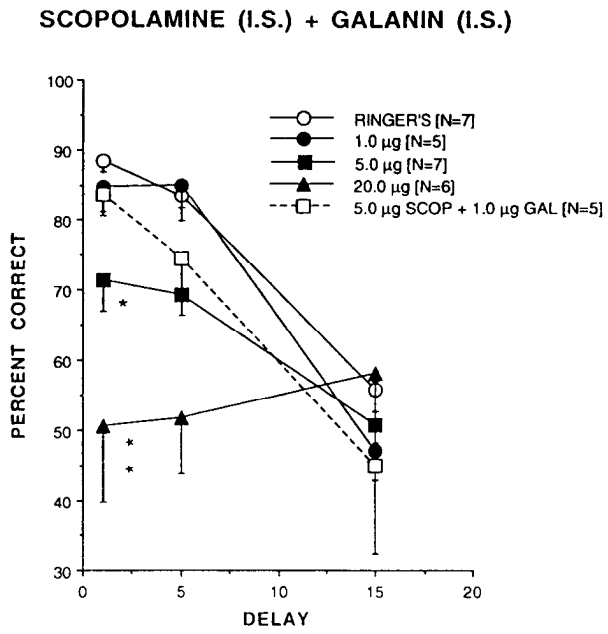

Figure 5. Intraseptal scopolamine at 5.0 μg and 20.0 μg reduced the percentage of correct choice responses at the 1 sec and 5 sec delay values in a dose-dependent manner. Galanin (1.0 μg into the medial septum, i.s.) coadministered with scopolamine (5.0 μg, i.s.) produced performance deficits not significantly different from scopolamine (5.0 μg, i.s.) alone, as compared by Newman-Keuls post hoc test. *, main effect of dose significant for choice accuracy at all delays for this dose at $p < 0.05$ by Dunnett's post hoc test as compared to Ringer's solution control. **, main effect of dose significant for choice accuracy at all delays for this dose at $p < 0.01$ by Dunnett's post hoc test as compared to Ringer's solution control.

peritoneal scopolamine and scopolamine plus galanin 1.0 μg groups to be significantly different, and this difference was corroborated by an a priori contrast between the two groups ($p < 0.05$).

Both the intraperitoneal scopolamine and the scopolamine plus galanin 1.0 μg groups made significantly more long-latency responses per session than Ringer's controls (mean ± SEM for saline = 0.64 ± 0.28, for scopolamine i.p. = 3.3 ± 0.25, and for scopolamine + galanin = 1.0 μg = 4.1 ± 1.2; Dunnett's post hoc testing, $p < 0.05$; main effect of dose $F_{3,39} = 4.8$, $p < 0.006$). Both an a priori contrast and Newman-Keuls post hoc testing did not show the intraperitoneal scopolamine and scopolamine plus galanin 1.0 μg groups to be significantly different.

The proportion of error trials that followed error trials was shown to increase for the intraperitoneal scopolamine, scopolamine plus galanin 0.2 μg, and scopolamine plus galanin 1.0 μg groups as compared to Ringer's control ($F_{3,39} = 4.2$, $p < 0.02$), but significantly only for the scopolamine plus galanin 1.0 μg group according to Dunnett's post hoc test ($p < 0.05$). Neither an a priori contrast or Newman-Keuls post hoc testing showed the intraperitoneal scopolamine and scopolamine plus galanin 1.0 μg groups to be significantly different from each other on this measure. These perseverative responses could represent a delay-dependent perseveration, since choice accuracy for the scopolamine plus galanin 1.0 μg at the 15 sec delay was less than chance. To test this possibility, a delay × proportion errors following errors interaction was examined. This interaction was not determined to be significant ($F_{2,76} = 1.7$).

The number of trials completed per session was shown to decrease for the scopolamine plus galanin 1.0 μg group according to Dunnett's post hoc test ($p < 0.05$; effect of dose, $F_{3,39} = 3.0$, $p < 0.05$). An a priori contrast and Newman-Keuls post hoc test also showed that the intraperitoneal scopolamine and scopolamine plus galanin 1.0 μg groups were significantly different. ANOVA detected a main effect of dose for rate rear-lever VI responding, but no significant increase in barpress rate was shown by Dunnett's post hoc test for any dose as compared to the Ringer's control group. No differences were detected between the intraperitoneal scopolamine and the scopolamine plus galanin 1.0 μg groups by either a priori contrast or Newman-Keuls post hoc testing. No significant differences were detected among the groups on measures of discrimination error trials ($F_{4,26} = 0.73$), or on the within-session distribution of errors ($F_{4,23} = 0.09$).

Intraseptal scopolamine

Percentage correct responses for scopolamine (1.0 μg, 5.0 μg, and 20.0 μg) administered into the medial septum is shown in Figure 5. Both the 5 μg and 20 μg doses significantly decreased choice accuracy (main effect of dose, $F_{4,26} = 4.3$, $p < 0.009$). Dunnett's post hoc test (one-tailed) showed both the 5 μg and 20 μg doses to be significant ($p < 0.01$), at the 1 sec and 5 sec retention intervals. The lack of effect at the 15 sec interval is a result of all the groups being at chance level. A dose × delay interaction was also significant ($F_{8,52} = 2.9$, $p < 0.009$), reflecting the decreases in choice accuracy that occurred only at the 1 sec and 5 sec delays.

Several secondary measures of DNMTS performance also revealed significant dose-dependent effects of intraseptal scopolamine. These measures are summarized in Table 1. The number of long-latency responses per session was increased by scopolamine, significant at the 20 μg dose ($F_{4,26} = 7.5$, $p <$

0.0004; Dunnett's post hoc test, one-tailed $p < 0.01$). Session duration increased, significant at the 20 μg dose ($F_{4,26} = 5.6$, $p < 0.002$; Dunnett's post hoc, one-tailed $p < 0.01$). The proportion of error trials that followed error trials increased at the 20 μg dose ($F_{4,26} = 3.6$, $p < 0.02$; Dunnett's post hoc test, $p < 0.05$). Finally, the rate of rear-lever VI responses decreased significantly at the 20 μg dose ($F_{4,26} = 3.1$, $p < 0.03$; Dunnett's post hoc test, $p < 0.05$). Other measures were unaffected by intraseptal scopolamine, including within-session error distribution ($F_{4,23} = 0.09$), discrimination error trials ($F_{4,26} = 0.74$), and the number of trials completed per session ($F_{4,26} = 0.91$).

Intraseptal scopolamine plus intraseptal galanin

Galanin 1.0 μg coadministered with scopolamine 5.0 μg into the medial septum produced no significant additional impairment in choice accuracy as compared with scopolamine 5.0 μg alone (evaluated by a priori contrast and Newman-Keuls post hoc test). Table 1 shows measures for intraseptal scopolamine plus intraseptal galanin as compared to intraseptal scopolamine.

Discussion

The present experiments demonstrate that galanin microinjected into the medial septum, at doses that did not affect DNMTS choice accuracy alone, potentiated the disruptive effects of intraperitoneally administered scopolamine on DNMTS choice accuracy. Scopolamine given alone into the medial septum produced deficits of choice accuracy qualitatively similar to deficits produced by scopolamine intraperitoneally.

The finding that scopolamine impairs choice accuracy when administered into the septum extends the observations of Dunnett and collaborators, who reported disruptive effects of scopolamine microinjected into the dorsal hippocampus and prefrontal cortex on DNMTS (Dunnett et al., 1990). In the hippocampus, performance deficits induced by scopolamine were significant only at the long retention intervals, a "delay-dependent" (see White, 1985; Ringo, 1988; Wixted, 1989, 1990) performance deficit (Dunnett et al., 1990). However, in the prefrontal cortex, performance deficits induced at all retention intervals, a "delay-independent" deficit, were reported for scopolamine into the prefrontal cortex (Dunnett et al., 1990). Delay-independent effects have also been reported for scopolamine administered intraperitoneally (e.g., Dunnett, 1985; Robinson and Crawley, 1993b). The deficits in DNMTS produced by scopolamine into the medial septum in the present study are delay independent. Because performance is impaired even at the shortest retention intervals, when the "memory" requirement is minimized, delay-independent effects are usually interpreted as representing impairments not specific to memory systems. These data suggest that the medial septum may be an important site at which systemically administered scopolamine produces the non-mnemonic-specific performance changes on DNMTS.

Changes in secondary measures for intraperitoneal and intraseptal scopolamine were not identical, suggesting that scopolamine microinjected into a single anatomical site may not produce all aspects of the behavioral changes induced by systemic scopolamine administration. Because multiple central cholinergic systems are probably affected when scopolamine is given intraperitoneally, different effects at several anatomical sites may combine to produce the full profile of changes in DNMTS performance. Both intraperitoneal and intraseptal routes of administration produced significant increases in long-latency choice responses and in errors following errors, but only intraperitoneal scopolamine produced an increase in trials with discrimination errors, and only intraseptal scopolamine produced an increase in session duration and a decrease in rear-lever VI response rate. Furthermore, the observation that galanin did not potentiate the disruptive effects of scopolamine when both were coadministered into the septum suggests that it is not only the septal site where the interaction between intraperitoneal scopolamine and intraseptal galanin occurred. The blockade of cholinergic receptors in the hippocampus, and/or other sites, by intraperitonal scopolamine, may be required before disruption of septohippocampal cholinergic functioning by galanin are detectable by DNMTS. However, since this experiment was the last to be conducted, the lack of interaction between galanin and scopolamine coadministered into the medial septum may also reflect increased resistance to change of the behavioral baseline or a loss of sensitivity at the septal injection site due to repeated injections.

The present observations are not consistent with the observations of Givens et al. (1992), which showed disruption of T-maze delayed alternation performance following administration of 0.2 μg and 1.0 μg of galanin into the medial septum at the stereotaxic coordinates used in the present studies. The explanation of this discrepancy may lie in the differences between the tasks, since the T-maze may draw to a greater degree on the spatial dimensions of the choice stimuli, and demands greater motor activity on the part of the subject for each choice response.

Interactions between galanin and other neurotransmitters, at anatomical sites relevant to learning, memory, and cognition, may be complex. Galanin is known to coexist with ACh in the medial septum, with norepinephrine in the locus coeruleus, and with 5-HT in the raphe nucleus (Melander et al., 1985a,b). Galanin receptor sites are found at these sites and in several other distinct anatomical locations thought to be relevant to learning and memory, including the amygdala, ventral hippocampus, and prefrontal and entorhinal cortices (Melander et al., 1985; Skofitsch and Jacobowitz, 1985; Skofitsch et al., 1986). Galanin administered intraventricularly has been shown to disrupt DNMTS in a delay-independent manner (Robinson and Crawley, 1993b), and this impairment may reflect the contributions of galanin working at a site other than the medial septum, or simultaneously at several different sites. Comparison of the effects of galanin on DNMTS when microinjected into other sites will be necessary before this question can be fully addressed. The present study of galanin infused into the medial septum suggests that at this site in this task, galanin is not inhibitory alone, but potentiates the inhibitory actions of scopolamine.

The present findings of galanin/scopolamine interaction are consistent with the hypothesis proposed by Hökfelt et al. (1987) that in Alzheimer's disease, galanin could be particularly deleterious in inhibiting the cholinergic system already compromised by disease. The ability of galanin to potentiate the effects of scopolamine in the medial septum may provide a heuristic model of the cognitive disruption observed in Alzheimer's disease. This model may be useful in testing drug combinations, including galanin antagonists with cholinergic agonists, for therapeutic potential as novel treatments for dementia. It is conceivable that interactions between neurotransmitter and neuromodulator systems produce the behavioral deficits found in some dementias (Decker and McGaugh, 1991), and that pharmacotherapies involving drug combinations may be a good approach to reversing those deficits (Sunderland et al., 1986).

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