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Investigations on the CNS Sites of Action of the Discriminative Stimulus Effects of Arecoline and Nicotine[1]

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MELTZER, L. T. AND J. A. ROSECRANS. *Investigations on the CNS sites of action of the discriminative stimulus effects of arecoline and nicotine.* PHARMAC. BIOCHEM. BEHAV. 15(1) 21–26, 1981.—The role of the dorsal hippocampus (DH) and mesencephalic reticular formation (MRF) in mediating the discriminative stimulus (DS) effects of nicotine and arecoline was assessed. In rats trained to discriminate nicotine (1.14 mg/kg) from saline, peripherally administered nicotine generalized to injection of nicotine, but not arecoline, directly into the DH and MRF. The stimulus effect of centrally administered nicotine was antagonized by peripherally administered mecamylamine, but not atropine. Response rate decreases were also observed after nicotine injection into either central site. In rats trained to discriminate arecoline (1.74 mg/kg) from saline, peripherally administered arecoline did not generalize to the direct injection of arecoline into the DH and MRF. However, a decrease in response rates was observed after arecoline injection into either site. Thus, the DH and MRF are important in mediating the DS effects of nicotine but not arecoline.

Arecoline Nicotine Drug discrimination Sites of action Hippocampus Reticular formation

THE DISCRIMINATIVE stimulus (DS) effects of arecoline and nicotine have been demonstrated to be dependent upon central muscarinic ([10]; Meltzer and Rosecrans, unpublished observation) and nicotinic receptors [13], respectively. The role of specific brain sites in mediating these effects have not been evaluated.

This study was designed to investigate the role of the mesencephalic reticular formation (MRF) and the dorsal hippocampus (DH) in mediating the DS effects of arecoline and nicotine. The selection of these two sites was based on data from other areas of research. Iontophoretic studies have demonstrated the presence of neurons responsive to both nicotinic and muscarinic agonists in both structures [1, 2, 15]. Nicotine and arecoline have been demonstrated to produce electroencephalographic (EEG) changes, through both direct and indirect actions, on the DH and cerebral cortex [6,9]. The indirect effects of arecoline and nicotine on cortical and DH EEG may be mediated through an action on the MRF [6,7].

The binding of nicotinic and muscarinic ligands have been demonstrated in the DH, suggesting the presence of nicotinic and muscarinic receptors [16,18]. However, no one has investigated the presence or absence of nicotinic or muscarinic receptors in the MRF. In a previous study, bilateral administration of nicotine (0.5 $\mu g/\mu l$/site) into the DH produced a partial generalization to the DS effect of peripherally administered nicotine [13]. The present investigation will extend this work by examining the dose-effect relationship for intracerebral nicotine at both the DH and MRF and by conducting parallel studies in rats trained to discriminate arecoline from saline.

METHOD

Subjects

Male Sprague-Dawley rats (175–200 g) with no previous drug or experimental experience were purchased from Flow Research Animals, Dublin, VA, and used in all experiments. These rats were individually housed in a temperature-controlled environment under a 12 hour light/dark cycle. Initially food (Purina Rodent Chow) and water were available ad lib. After allowing two to four weeks for acclimation, rats were reduced to 80% of their expected free-feeding weight by restricted feeding. For the remainder of the study, water was freely available in the home cages and adjusted amounts of rodent chow were offered after each experimental session to maintain the animals at 80% of their expected free-feeding weight.

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Apparatus

The experimental space was a standard operant test chamber (Lehigh Valley Electronics, Model 1417 or Coulbourn Model E10-10). One wall of the chamber contained two levers with a dipper centered between them for delivery of liquid reinforcement. Above the dipper was a white house light that was on for the entire session. The experimental chamber was located in a larger sound-insulated and light-proof isolation cubicle. Solid-state and electromechanical programming equipment were used to control sessions. Data were recorded automatically in the form of response and reinforcement totals. Equal parts of sugar and non-fat powdered milk (Land O Lakes, Inc.) mixed in tap water and delivered by the dipper (0.01 ml) was the reinforcer.

Experimental Procedure

Ten rats were trained to discriminate 1.74 mg/kg arecoline from saline and ten rats were trained to discriminate 1.14 mg/kg nicotine from saline using a two-lever operant procedure. State (drug or saline) correct responses were reinforced on a variable-interval 12 second schedule with sweetened milk. Drug and saline injections were administered in a double-alternation sequence (D,D,S,S,D,D, etc.). Arecoline and saline were injected five minutes prior to the session in the arecoline-trained group. Nicotine and saline were injected ten minutes prior to the session in the nicotine-trained group. Experimental sessions had a total duration of 15 minutes. Baseline discrimination was assessed in a two-minute non-reinforced period on the first day of each alternation. Discrimination is expressed as percent drug bar responding (%DBR) which is the number of responses on the drug correct level divided by the total responses on both levers during the non-reinforced session.

When discrimination had stabilized (85% DBR under drug state, 10% DBR under saline state), standard stereotaxic techniques were used to implant the rats with unilateral, stainless steel intracerebral cannulas. Under ketamine anesthesia (Ketalar, Parke-Davis, Detroit, MI; 100–150 mg/kg, IP), five rats in each drug condition had cannulas implanted that were aimed at the DH, and five rats had cannulas implanted that were aimed at the MRF. One nicotine rat with a cannula in the MRF lost its cannula after only one nicotine test. This animal's data is not included in any analysis.

The coordinate system based on bregma was used for cannula implantation [20]. The stereotaxic coordinates for the DH were: rostral-caudal: −3.0 mm; lateral±2.2 mm; dorsal-ventral: −3.0 mm. The stereotaxic coordinates for the MRF were: rostral-caudal: −4.2 mm; lateral: ±2.2 mm; dorsal-ventral: −7.0 mm. For each group, approximately one-half of the implants were in the right side of the brain and the rest were in the left side. Rats received oral antibiotic treatment (ampicillin mixed in milk) for two days after surgery. They were allowed to recover for five to seven days before resuming on the double-alternation discrimination procedure.

After surgery, rats were run two double-alternations before intracerebral drug administration began. During this time, the rats were acclimated to the injection procedure (described below). All rats were first injected intracerebrally with artificial cerebrospinal fluid (CSF). Next, intracerebral arecoline, in arecoline-trained rats, or nicotine in nicotine-trained rats, was injected in an ascending dose order. The effects of peripherally administered antagonists, as well as replicates of previous injection doses, were assessed in a counter-balanced order. Approximately one-half of the injections followed drug training days, the rest followed saline training days. At least five days separated intracerebral drug administration days. Antagonists were administered 25 minutes prior to the test sessions.

Cannula Construction and Implantation

On a lathe, using number 74 drill bits, holes were drilled through 3/8″ brass flathead machine screws. Stainless steel hypodermic tubing (25-gauge, thin wall, Small Parts, Inc., Miami, FL) was cemented into the screw (using a commercially available super glue), so that the tubing was flush with the threaded end on the screw and extended beyond the head portion. The stainless steel tubing extended 16 and 12 mm beyond the screw head, for the MRF and DH cannulas, respectively. This tubing was the part lowered into the brain.

Styli as well as injector cannulas were constructed from 31-gauge stainless stell hypodermic tubing (Small Parts, Inc., Miami, FL) and when inserted in the guide cannula, extended 1.5 mm past the end of the guide cannula. The lengths were kept constant by cementing a collar of 25-gauge thin wall tubing at the proper distance. The stylus head extended for three to four mm above the screw.

The injector cannula extended approximately 10 mm past the screw. A cover cap was made by tapping threads into the end of a plastic centrifuge tube. The guide cannula with stylus inserted was implanted using standard stereotaxic techniques. Four stainless steel machine screws (#0-80; 1/8 in.; small Parts, Inc., Miami, FL) were screwed into the skull. The cannula assembly was held in place by covering its base and the small screws with dental acrylic.

Injection Procedure

The injector cannula was attached by polyethylene tubing (PE-20, #7406, Clay Adams; Becton, Dickinson, and Company, Parsippany, NJ) to a 10 μl Hamilton syringe. The tubing and injector cannula were flushed with 70% ethanol and sterile water before being filled with the drug solution. The microliter syringe was filled with sterile water, and was attached to the tubing by a 26-gauge needle. An air bubble was introduced into the tubing between the drug solution and the sterile water. Movement of this bubble against a mm ruler was used to monitor the injection volume. Advancement of the bubble by 6 mm was equal to an injection volume of 0.5 μl. This injection volume was used for all injections.

Rats were gently restrained by wrapping in a cloth towel so that only their heads were exposed. The plastic cover cap and stylus were removed from the cannula and the injection cannula was inserted. The plunger on the microliter syringe was manually advanced over a period of five to ten seconds to provide the injection volume. The cannula was left in place for 20 seconds after the injection was complete. It was then removed and the stylus and cover cap replaced. The rats were then immediately placed into the operant chambers. The time between the cessation of the injection and placing the rats in the chamber was 60 to 75 seconds.

Drugs

The following drugs were used in these experiments: Arecoline hydrobromide (Chemical Dynamics Co., Plainfield, NJ); atropine sulfate (Sigma Chemical Co., St. Louis, MO); mecamylamine hydrochloride (Merck, Sharp, and Dohme, West Point, PA); and optically pure (–)-nicotine

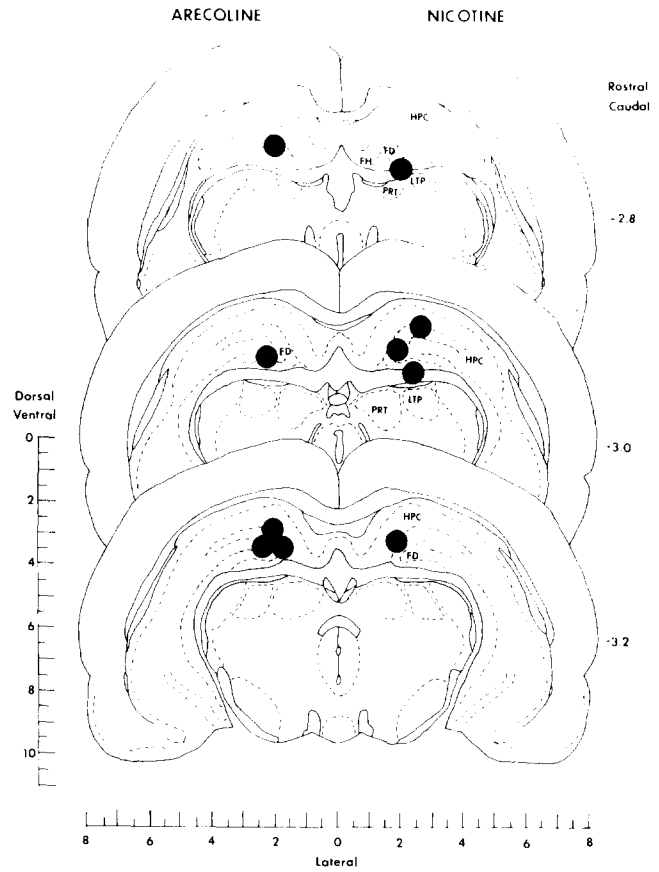

FIG. 1. Cannula placements in the dorsal hippocampus. Left side of figure is for arecoline trained rats; right side of figure is for nicotine trained rats. Key: FD=Dentate Gyrus; FH=Hippocampal fissure; HPC=Hippocampus; LTP=Lateral nucleus of the thalamus, posterior part; PRT=Pretectal area. Sections traced from Pellegrino and Cushman.

di-l-tartrate (synthesized and kindly supplied by Dr. Everette L. May), were obtained as the salt. These drugs were dissolved in 0.9% saline in a concentration that resulted in an injection volume of 0.1 ml/100 g body weight. All injections were SC with a 26-gauge 3/8 in. needle attached to a syringe. Drug dosage is expressed as the salt for peripheral administration.

Drug solutions for intracerebral injection were made in artifical cerebrospinal fluid (minus dextrose). The composition of this fluid (gram/liter) is: NaCl: 7.46; KCl: 0.19-$CaCl_2$ (anhydrous): 0.14; $MgCl_2$ $6H_2O$: 0.19; $NaHCO_3$: 1.76; and Na_2HOP_4: 0.18. These salts were dissolved in sterile water. Drug was added to the solution to yield the proper concentration for injection. The pH of the solutions was adjusted to between 7.0 and 7.8 by adding sodium hydroxide. The drug solution was filtered through a Millipore filter system (Type GS; pore size 0.22 μm) that had previously been autoclaved. The solution was then ready for injections. Doses of arecoline and nicotine for intracerebral injection are expressed as the free base.

Data Analysis

Test sessions were conducted in a two minute non-reinforced session. Animals were removed from the chambers after two minutes. If animals did not emit at least five responses in two minutes the session was extended until five responses were emitted. Sessions had a maximum duration of 15 minutes after which the animals were removed and considered disrupted. The data from these animals were not used in any analysis, but are reflected in the data showing the number of rats completing the response requirement/number rats tested. When doses were replicated, the mean of the replications for each animal was derived, and the group mean±SEM was derived from that. Data are expressed as %DBR and responses per minute (RPM). The ED_{50} values derived from linear regression analysis are the doses that produced 50% DBR. A two-factor, mixed, analysis of variance was performed on the nicotine generalization data according to the procedure in Bruning and Kintz [3].

Cannula Placement Verification

Rats were anesthetized with Na Pentobarbital and decapitated with a guillotine. The brain was removed and put in a 10% formaldehyde-sucrose solution. After approximately five days, brains were blocked and put on the platform of a freezing microtome (American Optical). The brain was frozen with CO_2 and was cut in 50 micron sections. Sections were washed in distilled water and then soaked in an agar-sucrose solution before being mounted on slides. Approximately one-half of the slides from each rat were stained with cresyl violet (cell body stain). The stained and unstained slides were examined on a projection microscope and cannula tract and tip location verified by comparison with sections in the stereotaxic atlas [12].

RESULTS

Cannula Placements

The DH cannula placements are presented in Fig. 1 and the MRF cannula placements are presented in Fig. 2. For both figures, the left half represents the cannula placements for the arecoline-trained rats and the right half the cannula placements for the nicotine-trained rats, regardless of the actual side of implantation in the rat. In the rostral-caudal plane, all hippocampal placements were −2.8 and −3.2 mm from bregma. Laterally they were between 2.0 and 2.5 mm from bregma and between −2.5 to −4.0 mm in the dorsal-ventral plane. Two implants in the nicotine rats demonstrated possible involvement of the lateral nucleus of the thalamus.

The reticular formation placements were between −3.8 and −4.2 mm from bregma in the rostral-caudal plane, between 2.0 and 3.0 mm from bregma in the lateral plane, and between −7.0 and −9.0 mm in the dorsal-ventral plane. Most of the placements were more ventral than aimed for and demonstrated involvement of the lateral tegmental area and substantia nigra. These placements must be considered when analyzing the data and making any general conclusions.

Generalization in Arecoline-Trained Rats

Peripherally administered arecoline did not generalize to arecoline administered directly into the DH and MRF (Table 1). Rats with cannulas in the DH had a higher percent DBR after CSF injection than did rats with cannulas in the MRF. Arecoline administration did not increase the percent DBR. Doses were tested that produced almost complete disruption of behavior. The MRF was more sensitive than the DH to the

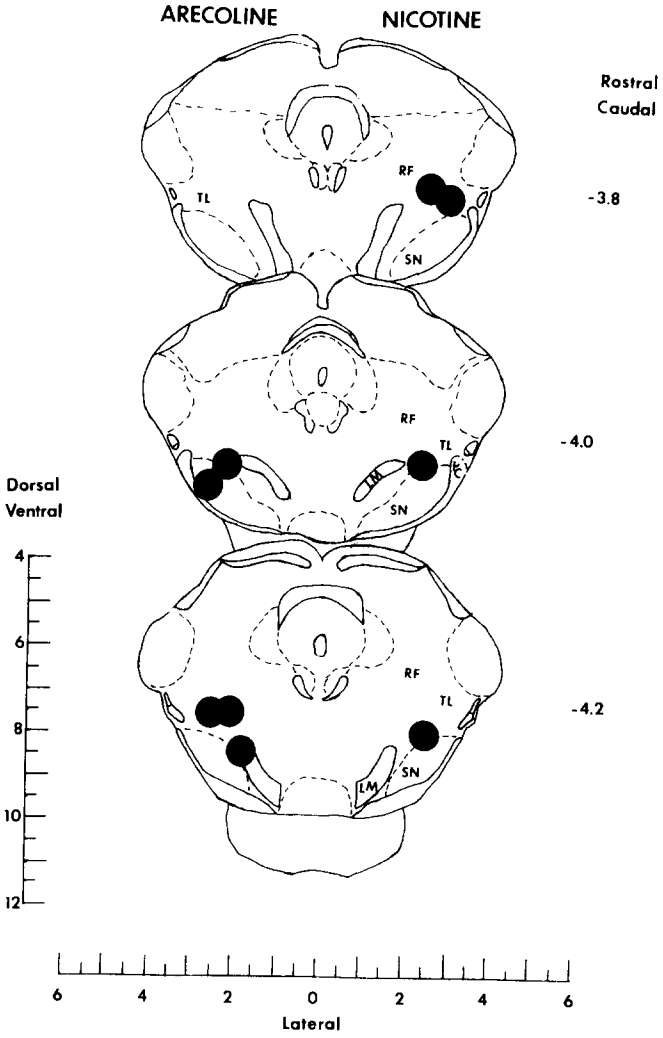

FIG. 2. Cannula placements in the mesencephalic reticular formation area. Left side of figure is for arecoline trained rats; right side of figure is for nicotine trained rats. Key: LM=Medial lemniscus; PL=Cerebral peduncle; RF=Reticular formation of mesencephalon; SN=Substantia nigra; TL=Lateral tegmental nucleus. Sections traced from Pellegrino and Cushman.

disruption of response rates produced by muscarinic stimulation.

Peripheral administration of 4 mg/kg atropine sulfate antagonized the decrease in response rate produced by 4 μg arecoline in the MRF. In contrast, the rate decreasing effect of 4 μg arecoline in the hippocampus appeared to be potentiated by peripheral administration of atropine. Peripherally administered arecoline did not generalize to nicotine (8 μg) injected into either site. Central nicotine administration did however produce behavioral disruption, as measured by the response rate and the number responding of the number tested.

Generalization in Nicotine-Trained Rats

Peripheral administration of nicotine generalized to nicotine injected directly into the DH and MFR (Table 2). The degree of generalization was dependent upon the dose of nicotine administered centrally. The MRF appeared to be more sensitive than the DH. The ED_{50}'s, derived from linear regression analysis, were 5.3 and 7.7 μg for the MRF and DH, respectively. A two-factor, mixed, repeated measures, analysis of variance was performed on the generalization data. The factors were dose (2, 4, and 8 μg) and brain site. The dose factor was significant, $F(2,15)=15.6$, $p<0.001$, indicating a significant dose-effect relationship. The brain site factor, $F(1,6)=2.07$, $p>0.2$, was nonsignificant, indicating that the sensitivities of the two sites were not significantly different. The dose×site interaction, $F(2,15)=1.1$, $p>0.2$, was also nonsignificant, indicating that the dose-effect relationship was similar for both groups.

The present DBR produced at both sites by administration of 8 μg of nicotine was antagonized to a similar degree (decrease of 50% DBR) by peripheral administration of 1 mg/kg mecamylamine, but was not antagonized by 4 mg/kg atropine sulfate. Peripherally administered nicotine did not generalize to administration of 8 μg of arecoline into either site.

Response rates were decreased to a similar degree by nicotine injections at the two sites. This effect was not antagonized by peripheral administration of either mecamylamine or atropine. At both sites, intracerebral arecoline (8 μg) produced a greater disruption of response rates than did similar doses of nicotine.

DISCUSSION

One problem that arises in studies involving intracerebral drug administration concerns the extent of diffusion of a substance from the site of injection. Myers [11] demonstrated that the diffusion of different molecular weight dyes injected into the hypothalamus depended to a large extent on the injection volume. He suggested that 0.5 μl is the maximum volume that should be injected into a rat brain. In the present study, this injection volume was always used. Also, to insure that diffusion of drug away from the injection site was not a factor, the rats were placed in the operant chamber immediately after injection.

This study demonstrated that peripherally administered nicotine can generalize to the DS effect of unilateral injection of nicotine directly into the DH and MRF. Thus, both structures are probably involved in mediating the DS effect of nicotine. This is similar to the findings of Knapp and Domino [7] and Kawamura and Domino [6] that the acute EEG arousal effect of low doses of nicotine is dependent upon an intact MRF and that at higher doses nicotine produces a direct effect on the hippocampus. Thus, the site of action of the DS effect and the EEG arousal effect of nicotine appear to be similar. In considering the conclusions based on the MRF placements, it should be remembered that all cannulas were not directly in the MRF. These studies need to be replicated with more exact cannula placements.

In a previous study, nicotine injected bilaterally into the DH (0.5 μg/μl/site; total 1 μg) produced a discriminability (percent DBR after nicotine minus percent DBR after saline) of 33% [13]. Actual percent DBR was not presented. In the present study, after unilateral administration into the hippocampus, the discriminability, by that definition, was 18 and 44% for 4 and 8 μg nicotine, respectively. Thus, greater sensitivity of brain sites may be demonstrated through the use of bilateral cannuli.

The specificity of the central effect of nicotine was demonstrated by the antagonism with peripherally adminis-

TABLE 1

EFFECTS OF INTRACEREBRAL DRUG INJECTIONS IN RATS TRAINED TO DISCRIMINATE ARECOLINE FROM SALINE

Drug Injected	Reticular Formation N	Replications	RPM	% DBR	Dorsal Hippocampus N	Replications	RPM	% DBR
CSF	5/5	2	7.8 ± 3.0	0.7 ± 0.7	5/5	1	10.0 ± 6.8	23.8 ± 6.3
Arecoline								
4 μg	4/5	1	2.4 ± 0.2	11.7 ± 7.1	5/5	1	8.3 ± 2.6	19.7 ± 5.8
8 μg	5/5	1	1.5 ± 0.3	8.7 ± 6.5	3/3	1	8.2 ± 2.6	13.3 ± 2.1
12 μg	4/5	1	1.9 ± 0.6	10.0 ± 10.0	5/5	1	4.0 ± 1.0	20.5 ± 15.1
24 μg	—		—	—	5/5	1	1.3 ± 0.2	36.0 ± 4.8
4 μg + 4 mg/kg Atropine Sulfate	5/5	1	6.6 ± 3.9	4.9 ± 3.9	4/4	1	1.8 ± 0.3	8.3 ± 8.3
Nicotine								
8 μg	3/5	1	1.0 ± 0.1	38.9 ± 31.0	4/4	1	1.2 ± 0.2	32.5 ± 13.8
Peripheral Administration								
1.74 mg/kg Arecoline	5/5	1	2.3 ± 0.6	89.8 ± 4.3	5/5	1	4.4 ± 2.7	92.0 ± 4.9
Saline	5/5	1	16.9 ± 7.1	0.0 ± 0.0	5/5	1	19.2 ± 3.0	4.3 ± 2.7

Intracerebral drugs administered immediately prior to test session. Antagonist administered 25 minutes prior to test session. N=number completing response requirement/number tested. Replications=number of times dose-level was tested in each animal. RPM and % DBR values are mean ± SEM.

TABLE 2

EFFECTS OF INTRACEREBRAL DRUG INJECTIONS IN RATS TRAINED TO DISCRIMINATE NICOTINE FROM SALINE

Drug Injected	Reticular Formation N	Replications	RPM	% DBR	Dorsal Hippocampus N	Replications	RPM	% DBR
CSF	4/4	2	11.2 ± 2.3	14.1 ± 10.1	5/5	2	7.0 ± 2.1	7.6 ± 5.0
Nicotine								
2 μg	4/4	1–2	7.7 ± 4.6	15.5 ± 11.2	5/5	1	3.5 ± 0.7	13.4 ± 5.8
4 μg	4/4	2–3	5.0 ± 2.0	50.7 ± 13.2	5/5	2	5.7 ± 2.3	25.6 ± 16.6
8 μg	4/4	2–4	3.1 ± 0.8	70.4 ± 7.2	5/5	3–4	3.2 ± 1.1	51.8 ± 8.6
8 μg + 1 mg/kg mecamylamine	4/4	1	18.8 ± 17.1	27.3 ± 17.9	5/5	1	2.3 ± 1.0	4.0 ± 4.0
8 μg + 4 mg/kg atropine sulfate	4/4	1	3.5 ± 2.2	57.5 ± 21.7	4/4	1	3.8 ± 1.0	37.5 ± 15.1
Arecoline								
8 μg	4/4	1	1.5 ± 0.4	8.3 ± 8.3	4/4	1	1.4 ± 0.3	15.0 ± 15.0
Peripheral Administration								
1.14 mg/kg Nicotine	4/4	1	8.4 ± 3.7	87.2 ± 7.8	4/4	1	7.3 ± 2.6	81.7 ± 9.5
Saline	4/4	1	7.3 ± 4.3	0.0 ± 0.0	4/4	1	4.7 ± 1.7	1.0 ± 1.0

Intracerebral drugs administered immediately prior to test session. Antagonist administered 25 minutes prior to test session. N=number completing response requirement/number tested. Replications=number of times dose-level was tested in each animal. RPM and % DBR values are mean ± SEM.

tered mecamylamine, the lack of antagonism by atropine, and the lack of generalization to intracerebrally injected arecoline. Even though only one dose of arecoline was tested, it disrupted responding to a level greater than that produced by 8 μg of nicotine, yet produced only saline like DBR. Thus, it may be concluded that peripheral nicotine does not generalize to centrally administered arecoline. This same argument follows for the lack of generalization of peripherally administered arecoline to centrally administered nicotine. Identical results were obtained when assessing the effects of mecamylamine and atropine on the DS effect of peripherally administered nicotine [5] and the generalization to peripherally administered arecoline [14]. Intracerebral arecoline administration however, did produce a decrease in response rate, demonstrating that there are separate muscarinic and nicotinic effects in both the MRF and DH. The separation of muscarinic and nicotinic effects in the MRF and DH is similar to the results of receptor binding studies that demonstrated a separation of muscarinic and nicotinic binding sites [16,17]. This contrasts with the results from microiontophoretic studies, which demonstrated an overlap between muscarinic and nicotinic responses.

In contrast to the generalization after intracerebral nicotine in nicotine-trained rats, peripherally administered arecoline did not generalize to arecoline administered directly into either brain site. Intracerebral, arecoline administration did, however, produce a disruption of behavior that was observed as a decrease in response rates. The MRF was more sensitive than the DH to this action of arecoline. This data demonstrates a separation of the discriminative stimulus and response rate effects of arecoline administration.

In a previous study ([10]; Meltzer and Rosecrans, unpublished observation), a separation of the DS and response rate suppressant effects of arecoline was also observed. In those studies, pretreatment with atropine antagonized the DS effect, but not the response rate suppressant effect of arecoline. In addition, administration of low doses of arecoline, which produced saline like percent DBR decreased response rates to 50% of the saline rate. These data were interpreted as suggesting the presence of two muscarinic systems or receptor populations in the brain with different affinities for arecoline. The DS effects of arecoline were mediated through one system (the least sensitive) while the other was involved in the modulation of motor output.

The present data suggest that two brain sites that mediate the effects of muscarinic stimulation on response rate, or more generally on motor behavior, are the MRF and DH. Alternately, the DS effect of arecoline may be an insensitive measure of muscarinic stimulation and it may be necessary to stimulate muscarinic receptors throughout the brain rather than in just individual nuclei to produce the DS effect. It is clear that it is necessary to investigate the role of additional brain areas in mediating the DS effect of arecoline. Areas for future research are the caudate-putamen, the nucleus accumbens, and cerebral cortex. These areas have high levels of muscarinic ligand binding and indices of cholinergic function [17].

REFERENCES

1. Bird, S. J. and G. K. Aghajanian. The cholinergic pharmacology of hippocampal pyramidal cells: a microiontophoretic study. *Neuropharmacology* **15:** 273–282, 1976.
2. Bradley, P. B., B. N. Dhawan and J. H. Wolstencroft. Pharmacological properties of cholinoceptive neurones in the medulla and pons of the cat. *J. Physiol., Lond.* **183:** 658–675, 1966.
3. Bruning, J. L. and B. L. Kintz. *Computational Handbook of Statistics*. Glenview, IL: Scott, Foresman and Company, 1968.
4. Domino, E. F. Some comparative pharmacological actions of (−)-nicotine, its optical isomer, and related compounds. In: *Tobacco Alkaloids and Related Compounds*, edited by U. S. von Euler. New York: Pergamon Press, 1965, pp. 303–313.
5. Hirschhorn, I. D. and J. A. Rosecrans. Studies on the time course and the effect of cholinergic and adrenergic receptor blockers on the stimulus effect of nicotine. *Psychopharmacologia* **40:** 109–120, 1974.
6. Kawamura, H. and E. F. Domino. Differential actions of *m* and *n* cholinergic agonists on the brain stem activating system. *Int. J. Neuropharm.* **8:** 105–115, 1969.
7. Knapp, D. E. and E. F. Domino. Action of nicotine on the ascending reticular activating system. *Int. J. Neuropharm.* **1:** 333–351, 1962.
8. Lichensteiger, W., D. Felix, F. Hefti and M. Schlumpf. Effects of nicotine on dopamine neurones of adult and prenatal mammals and of invertebrates. In: *Electrophysiological Effects of Nicotine*, edited by A. Remond and C. Izard. Amsterdam: Elsevier, 1979, pp. 15–30.
9. Longo, V. G., F. Guinta and A. Scotti de Carolis. Effects of nicotine on the electroencephalogram of the rabbit. *Ann. N. Y. Acad. Sci.* **142:** 159–169, 1967.
10. Meltzer, L. T. and J. A. Rosecrans. Discriminative stimulus effects of arecoline. *Fedn Proc.* **39:** 1095, 1980.
11. Myers, R. D. Injection of solutions into cerebral tissue: relation between volume and diffusion. *Physiol. Behav.* **1:** 171–174, 1966.
12. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Appleton-Century-Crofts, 1967.
13. Rosecrans, J. A. and W. T. Chance. Cholinergic and noncholinergic aspects of the discriminative stimulus properties of nicotine. In: *Discriminative Stimulus Properties of Drugs*, edited by H. Lal. New York: Plenum Press, 1977, pp. 155–186.
14. Schechter, M. D. and J. A. Rosecrans. Nicotine as a discriminative cue in rats: inability of related drugs to produce a nicotine-like curing effect. *Psychopharmacologia* **17:** 379–387, 1972.
15. Segal, M. The acetylcholine receptor in the rat hippocampus: Nicotinic, muscarinic or both? *Neuropharmacology* **17:** 619–623, 1978.
16. Segal, M., Y. Dudai and A. Amsterdam. Distribution of an α-bungaro-toxin-binding cholinergic nicotinic receptor in rat brain. *Brain Res.* **148:** 105–119, 1978.
17. Snyder, S. H., K. J. Chung, M. J. Kuhar and H. I. Yamamura. Biochemical identification of the mammalian muscarinic cholinergic receptor. *Fedn Proc.* **34:** 1915–1921, 1975.
18. Yamamura, H. I., M. J. Kuhar, D. Greenberg and S. H. Snyder. Muscarinic cholinergic receptor binding: regional distribution in monkey brain. *Brain Res.* **66:** 541–546, 1974.