Theses and Dissertations   Graduate School

1980

Psychopharmacological Analysis of Central Muscarinic and Nicotinic Receptors

Leonard T. Meltzer

Psychopharmacological Analysis of Central
Muscarinic and Nicotinic Receptors

by

Leonard T. Meltzer

B.A., University of Cincinnati, 1973

M.S., University of Maryland, 1977

Thesis

submitted in partial fulfillment of the

requirements for the Degree of Doctor of Philosophy

in the Department of Pharmacology

at the Medical College of Virginia

Virginia Commonwealth University

Richmond, Virginia

This thesis by Leonard Theodore Meltzer is accepted in its present form as satisfying the thesis requirement for the degree of Doctor of Philosophy.

Date: Approved:

6/27/80
....
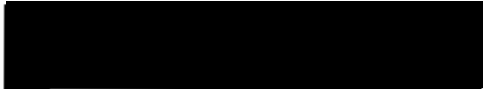
⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛
Advisor, Chairman of Graduate Committee

6/27/80
....

⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛

6/27/80
....

⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛
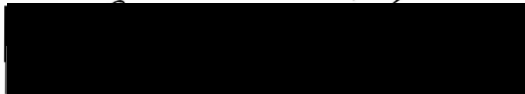
6/27/80
....

⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛

8/11/80
....

⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛

....

APPROVED: ⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛⬛ ....
          Chairman, MCV Graduate Council, Dean, School of Basic Sciences

CURRICULUM VITAE
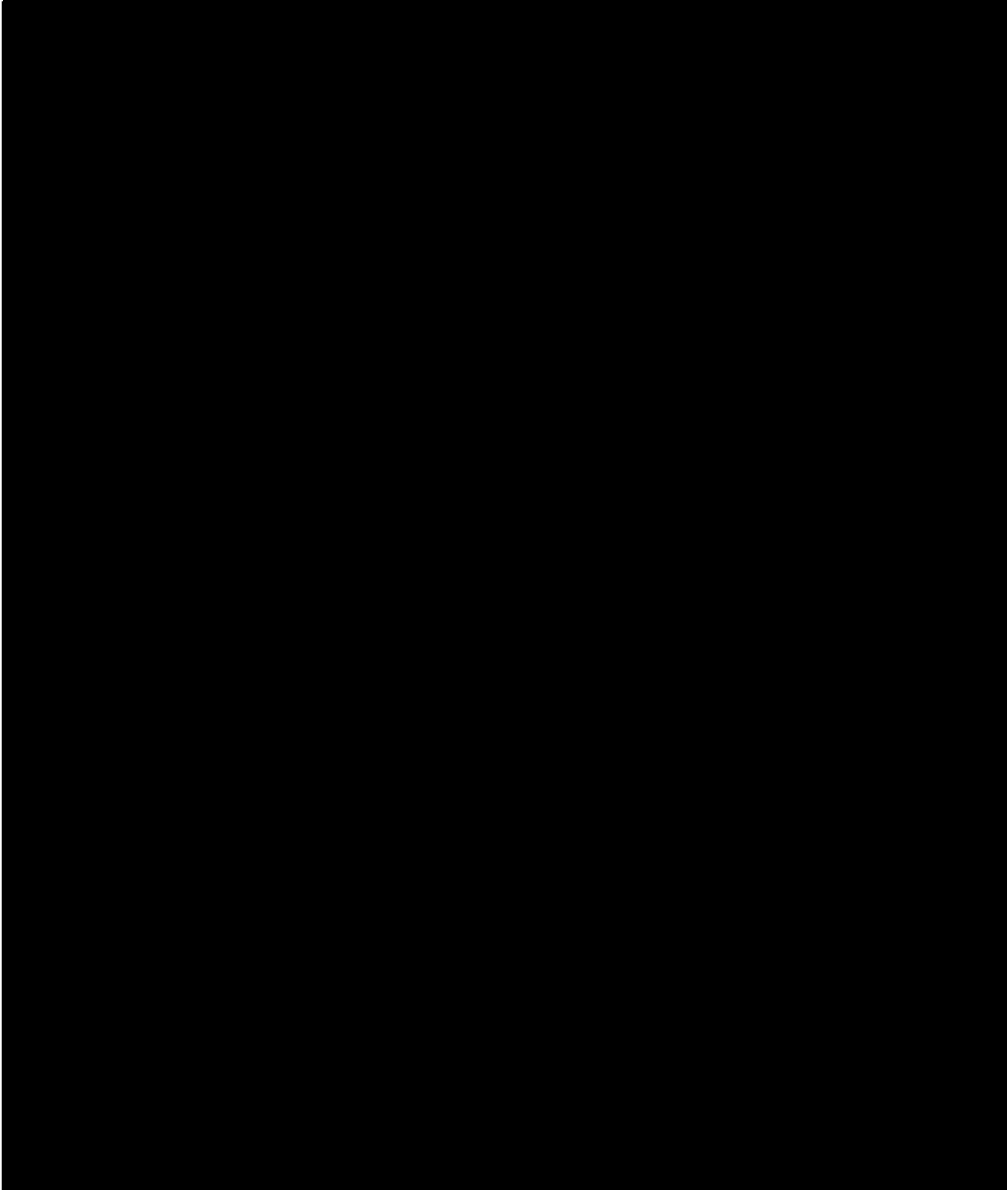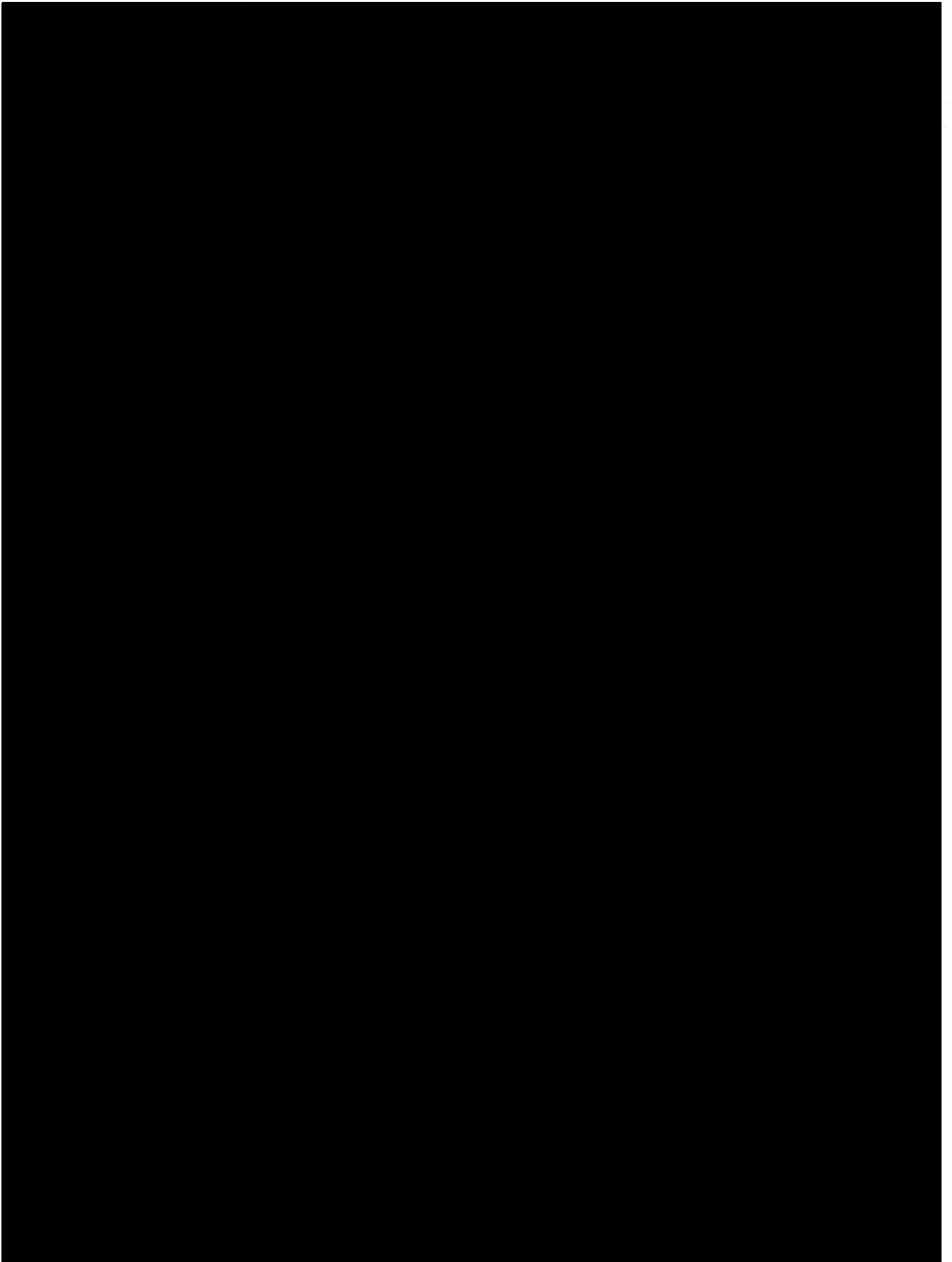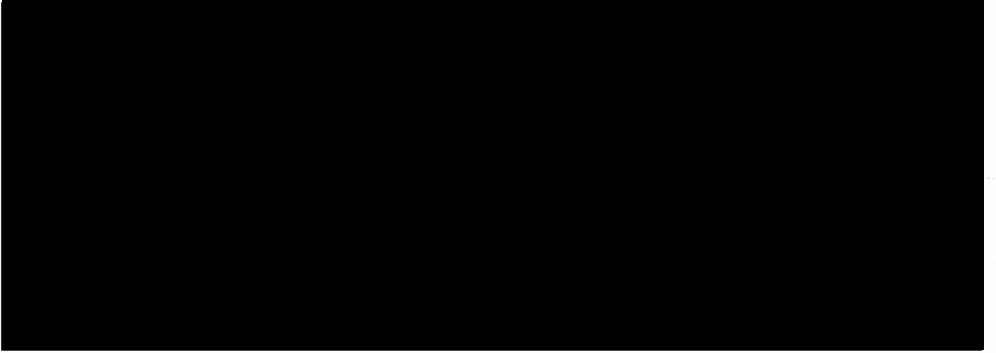
ACKNOWLEDGEMENTS

DEDICATION

To Leslee, for her love and patience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACh	acetylcholine
AChE	acetylcholinesterase
α-BT	alpha-bungarotoxin
Are	arecoline
Atr	atropine sulfate
ChE	cholinesterase
CNS	central nervous system
CRF	continuous reinforcement
CS	conditioned stimulus
DH	dorsal hippocampus
DRL	differential reinforcement of low rates
DS	discriminative stimulus
EEG	electroencephalographic
FI	fixed interval
FR	fixed ratio
gm	gram
Hex	hexamethonium
i.p.	intraperitoneal
i.v.	intravenous
kg	kilogram
MA	atropine methylnitrate
Mec	mecamylamine
mg	millgram
ml	milliliter
MRF	mesencephalic reticular formation
NE	norepinephrine
Nic	nicotine
% DBR	percent drug bar responding
Phy	physostigmine
PNS	peripheral nervous system
QNB	quinuclidinyl benzilate
RPM	responses per minute
Sal	saline
s.c.	subcutaneous
UR	unconditioned response
US	unconditioned stimulus
VI	variable interval

ABSTRACT

PSYCHOPHARMACOLOGICAL ANALYSIS OF CENTRAL MUSCARINIC AND NICOTINIC
RECEPTORS

Leonard T. Meltzer, Ph.D.

Medical College of Virginia - Virginia Commonwealth University, 1980

Major Professor: Dr. J. A. Rosecrans

Arecoline and nicotine are two psychoactive cholinergic alkaloids.
Arecoline is primarily a muscarinic agonist while nicotine, at low
doses, is a nicotinic agonist. The experiments in this dissertation
investigated two major areas: (1) the role of different factors in the
development of tolerance to the behavioral effects of arecoline and
nicotine, and (2) the possible mechanism and site of action of the
discriminative stimulus (DS) effects of arecoline and nicotine.

The role of dispositional and physiological factors compared to beha-
vioral factors in the development of tolerance to the effects of are-
coline and nicotine on operant behavior was assessed in Experiments I
and II, respectively. In part one of Experiment I, rats were trained to
respond on a variable-interval 15 second (VI-15) schedule for milk
reinforcement. Dose-effect relationships were assessed prior to and
during chronic arecoline (1.74 mg/kg/day) treatment. After 21 days of
arecoline administration prior to the session, the dose-effect rela-
tionship for total responses was not shifted. However, the dose-effect
relationship for total reinforcements was shifted to the right. In part
two of Experiment I, rats were trained to respond on a fixed-ratio 20
(FR-20) schedule for milk reinforcement. Dose-effect relationships were
assessed prior to and during chronic arecoline (0.87 mg/kg/day) adminis-

tration. One group of rats received daily injections of arecoline prior to the session and a second group received arecoline injections after the session. Daily administration of arecoline resulted in a greater shift to the right of the dose-effect relationship in the pre-session group compared to the post-session group. These data demonstrate the importance of behavioral factors in the development of tolerance to arecoline.

In Experiment II, rats were trained to respond on a VI-15 second schedule of milk reinforcement. Dose-effect relationships were determined prior to and during chronic nicotine (2.28 mg/kg/day) administration. One group of rats received daily injections of nicotine prior to the session, another group received nicotine injections after the session. After 36 days of chronic treatment, similar degrees of tolerance were observed in both groups, however the group receiving post-session nicotine developed tolerance at a faster rate. The data suggested that a complex interaction of nicotine and the experimental environment affected the rate of tolerance development.

Experiment III characterized the DS effect of arecoline. Using a two-lever operant paradigm, rats were trained to discriminate arecoline from saline on a VI-12 second schedule of milk reinforcement. Rats could learn to discriminate 1.74 mg/kg arecoline from saline, but not 0.58 mg/kg from saline. Agonist and antagonist studies demonstrated that the DS effect of arecoline is mediated through central muscarinic receptors.

In Experiment IV, the ability of physostigmine to interact with the DS effect of nicotine (1.14 mg/kg) and arecoline (1.74 mg/kg) was assessed. Physostigmine (0.125 mg/kg) pretreatment shifted the dose-

effect relationship for arecoline to the left but did not affect that of nicotine. Physostigmine (0.25 mg/kg) almost completely generalized to the DS effect of arecoline but not to the DS effect of nicotine. These data suggest an interaction of endogenous acetylcholine with muscarinic receptors but not with nicotinic receptors.

In Experiment V, the ability of arecoline and nicotine injected directly into the dorsal hippocampus (DH) and mesencephalic reticular formation (MRF) to generalize to the DS effect of peripherally adminis-tered arecoline (1.74 mg/kg) and nicotine (1.14 mg/kg) was assessed. Nicotine injected into these sites generalized in a dose-related manner to nicotine. The MRF was slightly more sensitive than the DH. Areco-line injected into either site did not generalize to the DS effect of peripherally administered arecoline. However, a decrease in response rates was observed.

I. INTRODUCTION

Nicotine and arecoline are two psychoactive cholinergic alkaloids. Nicotine is present in tobacco, which is used mainly for smoking purposes. The pharmacology of nicotine is very complex. Nicotine acts on the cardiovascular, nervous, gastrointestinal, and endocrine systems. However, it is thought that people are reinforced for smoking tobacco by the central nervous system (CNS) effects of nicotine. It has been demonstrated that through inhalation of tobacco smoke, nicotine reaches the brain as rapidly as after an intravenous injection (Russell and Feyerabend, 1978). The premise was put forth by Domino (1973) that the doses of nicotine inhaled produce definite mild and transient neuro-psychopharmacological effects which are positively reinforcing and thus promote repetition of smoking. These effects include: (a) modulation of conditioned behavior; (b) mixed depression and facilitation of neural substrates of reward; (c) transient (in minutes) electroencephalographic (EEG) and behavioral arousal; and (d) skeletal muscle relaxation.

Arecoline is the most biologically active substance in the areca catechu Linn, commonly known as the areca or betel nut. It is a widely cultivated plant in Eastern countries such as India, Ceylon, the Phillippines, and Japan. Chewing of this nut produces a mild euphoriant and stimulatory effect (Coutts and Scott, 1971).

These two drugs produce their major effects through the different components of the cholinergic system. Arecoline is predominantly a muscarinic agonist, and nicotine, in low doses, is a nicotinic agonist. The CNS actions of these two drugs have been evaluated and compared in a variety of experimental paradigms. These previous studies were con-

ducted with the objective of investigating functional aspects of central cholinergic systems, as well as delineating the psychopharmacological effects of these agents. Many of the previous studies have investigated only the effects of acute administration of arecoline and nicotine. The objective of the present investigations is to assess the effects of arecoline and nicotine in two paradigms that involve chronic drug administration.

The first set of experiments will investigate the effects of repeated administration of arecoline and nicotine on operant behavior. It has been demonstrated for some drugs, that with repeated administration there is a diminution in the originally observed effect (i.e., tolerance development). Tolerance to the effects of nicotine has been demonstrated while no studies have investigated the development of tolerance to arecoline. Various factors can play a role in tolerance development. These include dispositional, physiological, and behavioral factors. The present studies will investigate the role played by behavioral factors in the development of tolerance to nicotine and arecoline.

The second set of experiments will investigate the mechanism and site of action of the discriminative stimulus (DS) effects of arecoline and nicotine. A DS is any event in the environment that signals the availability of reinforcement. The observation that psychoactive drugs can serve as controlling or discriminative stimuli in a behavioral paradigm (Barry, 1974) indicates that these drugs produce effects which animals can distinguish from the non-drug condition, as well as from the effects produced by drugs that differ pharmacologically. Thus, the DS paradigm provides a specific and sensitive task by which to assess the mechanism and site of action of a drug. It is well documented that nicotine can

serve as a DS (Morrison and Stephenson, 1969; Schechter and Rosecrans, 1971a; Hirschhorn and Rosecrans, 1974). Arecoline has also been demonstrated to serve as a discriminative stimulus (Schechter and Rosecrans, 1972b). Through the use of the DS procedure, in conjunction with pharmacological pretreatments, and the injection of nicotine and arecoline into discrete areas of the brain, possible mechanisms and sites of action of the DS effects of arecoline and nicotine will be ascertained. It is hoped that from these studies, information will be gained on the actions of arecoline and nicotine, as well as on the functionality of central muscarinic and nicotinic systems.

In order to provide the proper background for the studies to be conducted, research on arecoline, nicotine, and the central cholinergic system from various disciplines is summarized below.

General Pharmacology

Dale (1914) was the first to propose the differentiation of the receptor-mediated effects of acetylcholine (ACh) in the peripheral nervous system (PNS) into nicotinic and muscarinic components. The nicotinic and muscarinic effects of ACh are mediated through two different receptors which have different structural requirements. These requirements are met by the alkaloids nicotine and muscarine and the other selective agonists. The divergence of actions of ACh is due to the flexibility of the ACh molecule, which can mimic the structure of the two more rigid molecules, nicotine and muscarine (Goldstein et al., 1974). The major actions of ACh at the neuromuscular endplate and autonomic ganglia (sympathetic, parasympathetic, and adrenal medullary) are mimicked by the alkaloid nicotine and referred to as nicotinic

effects. Low doses of nicotine stimulate the cholinergic receptors in these areas while high doses lead to paralysis or desensitization of these sites. Lobeline and tetramethylammonium are two other nicotinic agonists. Nicotinic effects are specifically blocked by d-tubocurarae, gallamine, and alpha-bungarotoxin (α-BT) at the neuromuscular junction and by hexamethonium and mecamylamine at autonomic ganglia.

Excitation of nicotinic receptors on autonomic ganglia has been shown to elicit the release of ACh at postganglionic parasympathetic synapses (muscarinic site) and norepinephrine (NE) at postganglionic sympathetic synapses (adrenergic site). Generalizing these facts to the CNS, one may hypothesize that nicotine may affect the brain through any of three mechanisms: (1) directly at a nicotinic receptor site, (2) indirectly by the release of ACh at a muscarinic receptor site, or (3) indirectly by releasing NE at an adrenergic receptor site.

The actions of ACh at autonomic effector cells are mimicked by the alkaloid muscarine and referred to as muscarinic effects. Stimulation of muscarinic receptors can produce either cell depolarization (smooth muscle contraction) or cell hyperpolarization (cardioinhibitory effects). The muscarinic effects of ACh are mimicked by arecoline and oxotremorine. These effects are selectively blocked by atropine and scopolamine.

Both the nicotinic and muscarinic actions of endogenous and exogenous ACh are increased by administration of inhibitors of acetylcholinesterase (AChE), the enzyme responsible for the degradation and inactivation of ACh. Cholinesterase (ChE) inhibitors include neostigmine (a quaternary amine), physostigmine (a tertiary amine), and the organophosphate and carbamate insecticides. Table 1 presents the names of some commonly used nicotinic and muscaranic agonists and antagonists.

TABLE 1

Cholinergic Agonists and Antagonists

	Muscarinic	Nicotinic
Agonists	Arecoline	Nicotine
	Oxotremorine	Lobeline
	Pilocarpine	Tetramethylammonium
Antagonists		
Peripheral	Atropine methylnitrate	Chlorisondamine
	Methylscopolamine	Hexamethonium
		Trimethidinium
Peripheral and Central	Atropine	Mecamylamine
	Scopolamine	β-erythroidine

The validity of the dichotomy of nicotinic and muscarinic effects of ACh, as proposed by Dale in 1914, is well established in the PNS. However, as our technical and pharmacological sophistication has grown, so has our understanding of the complexity of the PNS. Under proper experimental conditions one can demonstrate the presence of muscarinic receptors on the cell bodies of postganglionic neurons (Volle, 1966). There is also evidence demonstrating the presence of muscarinic and nicotinic receptors on presynaptic nerve terminals of cholinergic and adrenergic neurons, that may play a role in regulating transmitter release (Westfall, 1977; Langer, 1977; Starke et al., 1977). There is also evidence for the existence of these presynaptic receptors in the CNS. It is not the intention here to completely review the literature, but merely to point out the growing sophistication of our understanding of physiological and pharmacological systems. These facts must be taken into account when assessing drug actions and will be considered, where appropriate, in later sections.

The dichotomy of nicotinic and muscarinic effects has been extended to the central cholinergic nervous system. This has been accomplished using the electrophysiological, biochemical, and behavioral techniques. In the following sections the use of these techniques in establishing the existence of separate central nicotinic and muscarinic cholinergic systems will be reviewed.

Anatomy of Central Cholinergic Systems

The delineation of central cholinergic pathways has been hampered by the lack of a simple definitive histochemical technique for cholinergic systems, similar to that which exists for catecholamines. Therefore, a

variety of biochemical and histochemical techniques have been used to investigate central neuronal systems in which ACh is the neurotransmitter. These biochemical markers include: ACh (Cheney et al., 1975; Hoover et al., 1978), choline acetyltransferase (CAT) (Cheney et al., 1975; Hoover et al., 1978; Olivier et al., 1970; Palkovitz et al., 1974; Brownstein et al., 1975), and high affinity choline uptake (Yamamura et al., 1974). AChE has been extensively investigated by histochemistry (Jacobowitz and Palkovitz, 1974; Lewis and Shute, 1967; Palkovitz and Jacobowitz, 1974; Shute and Lewis, 1967). More recently CAT has been investigated through the use of immunohistochemical techniques (Rossier, 1976; McGeer et al., 1979).

The high levels and widespread distribution in brain of the above listed cholinergic indices indicates that the few pathways that have been defined or suggested must include only a small fraction of all the cholinergic cells in the brain (McGeer and McGeer, 1979). The presently identified cholinergic pathways of a brain are summarized below.

Limbic System. The septo-hippocampal pathway, which has its cell bodies in the medial septal nucleus and projects to the hippocampus, is the major cholinergic pathway in the limbic system. Lesions of the septal area cause large decreases in ACh, AChE, CAT, and high-affinity choline uptake in the hippocampus on the operated side (Fonnum, 1970; Kuhar et al., 1973; Lewis et al., 1967; McGeer et al., 1969; Pepeu et al., 1971). ACh is released in the hippocampus after electrical stimulation of the septum (Smith, 1972; 1974).

There is evidence that fibers of the septo-hippocampal tract branch off to innervate the cingulate cortex (Kuhar et al., 1973; Lewis and Shute, 1967; Pepeu, 1971; Shute and Lewis, 1967).

Within the hypothalamus, there is histochemical and biochemical evidence for a cholinergic projection from the arcuate nucleus to the median eminence (Walaas and Fonnum, 1978; Carson et al., 1977).

The interpeduncular nucleus has the highest concentration of CAT thus far found in the brain (Hoover et al., 1978). Afferents to the inter-peduncular nucleus from the medial habenular nucleus and the nucleus of the diagonal band of Broca have been demonstrated to be cholinergic (Gottesfeld and Jacobowitz, 1978; Kataoka et al., 1973).

The nucleus accumbens and olfactory tubercle contain intrinsic choli-nergic interneurons. This is evidenced by a decrease in indices of cho-linergic function after local injection of kainic acid (cell body neuro-toxin) but not hemitransection (Fonnum et al., 1977).

Extrapyramidal System. All or almost all of the cholinergic activity in the caudate nucleus and the putamen is in intrinsic neurons. Evidence comes from experiments showing that lesioning the known afferents and efferents did not cause any significant decrease in either CAT or AChE activity in the caudate-putamen (Butcher and Butcher, 1974; McGeer et al., 1971).

Cortical Systems. The presence of cholinoceptive cells in the cerebral cortex and the release of ACh in the cortex have long been established (Phillis, 1975), but there is some doubt as to the structure of the cholinergic systems. It seems probable that there are both cholinergic interneurons and, in some cortical areas, cholinergic projections to or from subcortical regions (McGeer and McGeer, 1979). Physiologic evi-dence has suggested a diffusely projecting ascending cholinergic system

(reticular activating system) that reaches most of the cortex (Phillis, 1975). Chemical data to its existence are less clear.

Receptor Binding Studies

Through the use of radiolabeled, high affinity antagonists, putative nicotinic and muscarinic receptors have been identified. Muscarinic receptors in the brain have been identified through the use of tritiated quinuclidinyl benzilate (QNB) (Yamamura et al., 1974; Snyder et al., 1975; Kobayashi et al., 1978). Total binding is highest in the caudate-putamen, with the cerebral cortex, hippocampus, amygdala, thalamus, and superior colliculus also showing high levels of binding, suggesting the greatest number of receptors in these areas.

The distribution of nicotinic receptors in the brain has been determined through the use of α-BT (Schechter et al., 1978; Segal et al., 1978). Highest binding levels are found in the hypothalamus and hippocampus. Other areas that have high binding are the cerebral cortex, ventral lateral geniculate, and the mesencephalic dorsal tegmental nucleus. It was suggested (Segal et al., 1978) that the limbic forebrain, midbrain structures, as well as sensory nuclei, are the main nicotinic cholinoceptive structures in the brain.

Muscarinic receptors appear to be more abundant than nicotinic receptors in the brain. There is a different regional distribution of muscarinic and nicotinic receptors. The regional distribution of QNB binding throughout rat and monkey brain parallels to a major extent other cholinergic markers, suggesting that the majority of cholinergic synapses in the brain are muscarinic (Snyder et al., 1975). However, there are brain regions which have high levels of cholinergic indices,

and low levels of QNB binding; these areas have been demonstrated to have α-BT binding. These two receptor sites are also pharmacologically separable. Only muscarinic agents interact with QNB binding, while only nicotinic drugs interact with α-BT binding. ACh interacts with the binding of both compounds.

Effects of Cholinergic Drugs on Electrical Activity of the Brain

The ability of cholinergic agents to produce changes in the animal electroencephalogram (EEG) has been extensively investigated. The action of nicotine on the EEG can be summarized as follows: small doses produce an activation of the EEG while high doses produce seizure-like spike discharges (Longo et al., 1967). In unrestrained animals, these EEG changes are accompanied by the corresponding behavioral states, arousal and convulsions, respectively. The electrographic changes produced by low doses of nicotine, which are characterized by desynchronization of neocortical activity (Longo et al., 1954) and the appearance of a theta rhythm in the hippocampus (Stümpf and Golgolák, 1967) are similar to those seen during an arousal reaction elicited by sensory or reticular stimulation (Green and Arduini, 1954). Arecoline, like nicotine, produces an activation of the cortex and hippocampus (Riehl et al., 1962; Herz, 1963; Yamamoto and Domino, 1967; Kawamura and Domino, 1969). Also similar to nicotine, the EEG activation response is indistinguishable from the activation response evoked by auditory stimulation or electrical stimulation of the medial reticular formation (Riehl et al., 1962).

The effects of arecoline, nicotine, and physostigmine on cortical and subcortical EEG, and the antagonism of these effects, were compared in

freely-moving cats (Yamamoto and Domino, 1967). I.v. injections of
arecoline produced a short-lasting but marked behavioral arousal and EEG
activation in both the cortex and hippocampus. Atropine blocked the
effects of arecoline, while methyl atropine, mecamylamine, and trime-
thidinium were without effect. The i.v. injection of nicotine also
produced EEG activation and behavioral arousal. Mecamylamine completely
blocked the nicotine-induced EEG and behavioral effects. Atropine
blocked the nicotine-induced activation of hippocampal EEG while not
affecting cortical desynchronization or behavioral arousal. This
represents a dissociation of the nicotine-induced behavioral effects and
hippocampal EEG activation. Trimethidinium reduced the nicotine-induced
EEG activation and behavioral arousal. This demonstrates that there is
a minor peripherally-mediated component to these effects of nicotine.
Methyl atropine was without effect. Physostigmine, similar to arecoline
and nicotine, produced EEG and behavioral arousal. Methyl atropine
slightly decreased, while atropine markedly reduced the physostigmine-
induced EEG and behavioral arousal. In contrast to the effectiveness of
the muscarinic cholinergic antagonists, the nicotinic cholinergic anta-
gonists trimethidinium and mecamylamine did not alter the EEG or beha-
vioral manifestations of physostigmine. It is important to note in
these studies that the EEG activating and behavioral arousal actions of
nicotine depend on the basal activity level, since they occur only when
nicotine is administered to an animal which is in a mild state of CNS
depression or asleep (Domino, 1967).

Central nervous system sites important in mediating the effects of
nicotine and arecoline on cortical and subcortical EEG have been inves-
tigated by transection of the brain stem at various levels and by selec-

tive lesioning of discrete nuclei. The level of brain stem transection effective in preventing the EEG activation induced by nicotine is different in different species. Nicotine induces neocortical desynchronization in the encéphale isolé preparation (transection at the first cervical segment of the spinal cord) (Longo et al., 1954), in the cerveau isolé preparation (transection at the level of the motor nuclei of the third nerve of the mesencephalon) of the rabbit (Floris et al., 1964), and in the inferior collicular-midpontine transected rabbit, cat, and dog (Knapp and Domino, 1962). In all these preparations, the midbrain reticular formation was at least partially connected to the forebrain.

Transection of the brain stem at the prepontine level (rostral border of the pons, which transects the pontine reticular formation) prevents the nicotine-induced EEG activation in dogs (Knapp and Domino, 1962), whereas more rostral lesions of the midbrain reticular formation are necessary to block the effect of nicotine in the cat (Kawamura and Domino, 1969). Nicotine alters the electrical activity of the isolated reticular slab in dogs (Knapp and Domino, 1962), but not in a slab of isolated cortex (Floris et al., 1964). These studies point to an important role of the reticular formation in mediating the EEG arousal effects of nicotine. Lesions of the medial septal nucleus abolish the hippocampal activation produced by nicotine, but does not affect the appearance of convulsive spikes (Stümpf and Goglák, 1967). Based on this evidence, a reticular formation-septum-hippocampus connection may mediate the hippocampal activation produced by low doses of nicotine (Stümpf and Gogolák, 1967). At high doses, a direct effect of nicotine on the hippocampus mediates the appearance of convulsive spikes. No

lesion or transection has been found effective in preventing nicotine-induced hippocampal or cortical seizures (Stümpf and Gogolák, 1967).

Kawamura and Domino (1969) examined the effects of nicotine and arecoline on cortical and subcortical EEG in midbrain transected cats. In caudal midbrain transected cats, which had an intact midbrain reticular formation, low doses of i.v. nicotine induced EEG activation in both neocortex and hippocampus. Bilateral lesions of the midbrain reticular formation in the same preparation blocked the EEG effects of nicotine. Mecamylamine blocked the activating effects of nicotine, while trimethidinium, a quaternary nicotinic ganglionic blocker, had no effect. Atropine produced a dose-related decrease in the EEG effect of nicotine. In the rostral midbrain cat, which lacks the midbrain reticular formation, no EEG activation in either the hippocampus or neocortex was seen after nicotine administration. Sporadic sharp waves appeared in the hippocampus with larger doses, indicating a convulsant site of action directly on the hippocampus. In contrast, in both caudal and midbrain-transected cats, low doses of arecoline produced hippocampal theta waves with no activation of the neocortical EEG. These effects were blocked by atropine. With higher doses of arecoline, both neocortical and hippocampal EEG activation was seen. It was concluded that the major site of action of nicotine on activating the rostral forebrain was located primarily in the midbrain reticular formation with some effects directly on the hippocampus. Arecoline acted on the midbrain reticular formation as well as above the level of the mesencephalon, on more rostral structures.

Nelsen et al. (1973) examined the effects of acute and chronic nicotine administration on the electrical activity of the reticular forma-

tion, hippocampus, and cortex in rats. Amplitude integration and frequency distribution of the cortical and subcortical EEG were used to assess the drug effects. The variance (or variability) of the EEG amplitude has been demonstrated to be an important index of changes in brain activity or functional state. Changes in variance levels can be used to measure the degree of mutual involvement between brain structures (Byford, 1965). Chronic nicotine treatment (0.1 mg/kg, four times a day, s.c.) produced changes in similar directions (decreased variability and mean EEG amplitude) for the hippocampus and cortex. There were no changes in these measures at the reticular formation. It was concluded that chronic nicotine administration produced a shift in control of cortical arousal from reticular formational to predominantly hippocampal influences. This study, which stresses the role of the hippocampus in mediating the chronic effects of nicotine, contrasts with the studies of Domino and coworkers, which stressed the role of the reticular formation in mediating the acute effects of nicotine.

The effects of nicotine, arecoline, and physostigmine on the evoked response of the rabbit sensorimotor cortex to electrical stimulation at the same site were assessed by Vazquez and Toman (1967). Nicotine enhanced, while arecoline reduced the amplitude of the evoked slow negative wave. These effects were selectively blocked by β-erythroidine (nicotinic blocker) and atropine, respectively. Physostigmine, after atropine had been administered to block its muscarinic action, affected the EEG in a manner similar to that of nicotine. After β-erythroidine pretreatment, physostigmine affected the evoked response similar to arecoline. This study is important because it shows that through the combined use of selective antagonists and physostigmine, one can selectively mimic either nicotinic or muscarinic receptor stimulation.

Microiontophoretic Studies

Through the use of microiontophoretic drug administration coupled with single-unit recording, the response of a single neuron to pharmacological agents can be studied. The effects of ACh, as well as pure nicotinic and muscarinic agents on single neuron activity in the CNS have been extensively investigated. Karczmar (1969) stressed the point that cholinoceptivity is not equivalent with cholinergicity. That is, the cholinoceptive response of a neuron cannot be accepted as proof that it has a cholinergic innervation unless further pharmacological and physiological data are available. Taken from the opposite view, a neuron that is not cholinergically innervated, but is cholinoceptive, may be affected by exogenous administration of cholinergic drugs, and may be important in mediating that drug's behavioral effects.

Neurons at all levels of the neuraxis respond to microiontophoretic ACh (Curtis and Crawford, 1969; Krnjević, 1969). Most studies have provided evidence for the existence of both nicotinic and muscarinic receptors on single neurons. Except for the feline neocortex (Krnjević and Phillis, 1963) and caudate nucleus (McLennan and York, 1966), where the receptors are mainly muscarinic, neurons have been found to be responsive to both muscarinic and nicotinic agents in the spinal cord (Curtis and Ryall, 1966; McLennan and Hicks, 1978), ventrobasal complex of the thalamus (Andersen and Curtis, 1964; McLennan and Hicks, 1978), hippocampus (Bird and Aghajanian, 1976; Segal, 1978) and brain stem (Bradley et al., 1966; Bradley and Wolstencroft, 1967).

In general, muscarinic agents produce both excitatory and inhibitory responses, whereas nicotinic responses are mainly, although not entirely excitatory. This is similar to cholinergic effects in the PNS where

excitatory (cell depolarization, smooth muscle contraction) and inhibitory (cell hyperpolarization, cardiac slowing) responses are seen following muscarinic stimulation, and excitatory (cell depolarization, striated muscle contraction) responses are observed after nicotinic stimulation.

In the Renshaw cell system on the cat spinal cord, the results of Curtis and Ryall (1966) indicate that these neurons possess two types of receptors, each mediating a separate response. Nicotinic receptors mediate a fast onset, short duration excitation, while muscarinic receptors mediate a slow onset, long duration excitation. The nicotinic response more closely resembles that produced by ACh.

In the rat, McLennan and Hicks (1978) found that the excitations in response to ACh of cortical, ventrobasal thalamic, and of Renshaw cells could be mimicked by both nicotinic and muscarinic agonists. The response to ACh was antagonized by both muscarinic and nicotinic antagonists, while specific agonists were blocked only by their respective antagonists. They concluded that the ACh receptors that they studied could not be classified within the classical distinction of nicotinic and muscarinic.

Similar results were found by Bradley and Wolstencroft (1967) in studying the response of undefined reticular neurons in the brain stem of cats. ACh had both excitatory and inhibitory effects on cells. The excitatory effects were mimicked by muscarinic and nicotinic agonists, while the inhibitory effects were mimicked by only muscarinic agonists. Both nicotinic and muscarinic antagonists blocked the excitatory response to ACh, while only the latter blocked the inhibitory response.

Bird and Aghajanian (1976) studied the response of rat hippocampal pyramidal cells to cholinergic agents. They concluded that these cells

are excited by muscarinic and some nicotinic agents (excited by phenyl-
trimethylammonium iodide but not nicotine). These agents were not
acting at two independent receptors since there was antagonism of
muscarinic agonists by nicotinic antagonists. In contrast to Bird and
Aghajanian (1976), Segal (1978) found a separation of nicotinic and
muscarinic receptors in the rat hippocampus. Segal classified hippo-
campal neurons into bursting (pyramidal) and non-bursting (non-pyra-
midal) cells based upon their firing characteristics. He found that ACh
excited almost all pyramidal cells but had little effect on non-pyramidal
cells. These effects were blocked by atropine and partially antagonized
by gallamine. On the other hand, nicotine inhibited firing of half of
the pyramidal cells and almost all non-pyramidal cells. Based on the
differential action of ACh and nicotine on the spontaneous activity of
the two cell types, Segal concluded that there were excitatory musca-
rinic receptors on pyramidal cells and inhibitory nicotinic receptors on
both pyramidal and non-pyramidal cells. There was also some evidence
for a nicotinic interaction with the muscarinic excitatory receptors.

In summary, based on microiontophoretic studies, it is difficult to
classify central cholinergic receptors into purely nicotinic and musca-
rinic. However, the effects seen in these microiontopheretic studies on
pyramidal cell firing do correlate with data on single-unit firing
recorded after systemic administration of nicotine. During nicotine-
induced arousal with concomitant hippocampal theta rhythm, there is an
increase in pyramidal cell firing (Stümpf and Gogolák, 1967). This
effect of nicotine may be mediated through a reticular formation-septal
nucleus-hippocampal connection (Stümpf and Gogolák, 1967). Electrical
stimulation of the medial septal nucleus produces hippocampal theta,

which is blocked by atropine (Vanderwolf et al., 1978). This agrees with the muscarinic-based excitation seen after ACh iontophoresis. During hippocampal seizure discharge, which may be a direct effect of nicotine on the hippocampus, there is a cessation of firing of pyramidal cells. This effect correlates with the depression of pyramidal cell firing produced by iontophoretic nicotine.

Behavioral Studies With Nicotine and Arecoline

The central cholinergic system is involved in an endless array of behavioral phenomena. There is hardly any behavior which is not affected by cholinergic agonists and antagonists or which in turn does not affect ACh, cholinesterase, or their turnover (Karczmar, 1975). This is consistent with the fact that sites for cholinoceptivity and for cholinergicity are widely distributed in the brain (Krnjević, 1969; Curtis and Crawford, 1969; see above). This widespread distribution of cholinoceptive sites makes it difficult to selectively affect one behavior exclusively.

Included in the list of functions involving the cholinergic system (predominantly muscarinic) are the following: (1) memory and learning, (2) appetitive behavior, (3) temperature control, (4) neurological syndromes and motor behavior, (5) EEG and behavioral arousal, and (6) nociception (Karczmar, 1977). Most studies involving the above listed functions are designed to examine the role of the cholinergic system in eliciting or mediating specific behaviors. A different approach to studying the central cholinergic system is not directly concerned with the role of the cholinergic agents in mediating certain behaviors or affecting their underlying basis, but is concerned with using behavior

to evaluate the pharmacology of cholinergic drugs as well as assessing the drug-behavior interaction. Of course, studies in these two areas are not mutually exclusive. Two behavioral categories which have been used to assess the effects of cholinergic agents are spontaneous motor activity and operant behavior. The effects of nicotine and arecoline on spontaneous motor activity and operant behavior are summarized below.

Spontaneous Motor Activity. The effect of nicotine on running wheel behavior in the rat is influenced by the animal's diurnal cycle and concommitant basal activity levels (Bovet et al., 1967). Running wheel activity, which is normally low during light hours, was increased by nicotine (0.4 mg/kg), while high rates of wheel activity during the dark hours were decreased by the same dose. Morrison and Lee (1968b) examined the effects of nicotine (0.4 mg/kg) and physostigmine (0.1 mg/kg) on spontaneous motor activity in the rat. The drug effects that they observed were also related to the baseline activity level of the rats. Both drugs reduced the activity of the more active rats and increased that of the less active animals. These studies are of great importance since they pointed to the role of baseline behavior in determining the effects of nicotine on that behavior and are examples of drug-behavior interactions.

Rosecrans (1971a; 1971b) replicated the findings of Morrison and Lee (1968b), that baseline behavior is an important determinant of the affect of nicotine on spontaneous motor activity. Based on the work of Kostowski et al. (1969) indicating that behavioral arousal may be mediated via the midbrain raphe nucleus from which forebrain serotonergic (5-HT) neurons originate (Anden et al., 1966; Sheard and Agha-

janian, 1968), Rosecrans (1971a) attempted to relate the behavioral data to neurochemical changes in the 5-HT system. It was found that the baseline rates of activity were related to the 5-HT levels, but there was no evidence that nicotine was specifically affecting the indoleamine system to yield the observed changes in behavior. There is no difference in brain region nicotine levels between high and low activity rats (Rosecrans, 1972).

In contrast to the baseline-dependent effects observed when moderate doses of nicotine (0.1-0.4 mg/kg) are used, higher doses of nicotine (0.75-2.0 mg/kg) invariably produce a decrease in locomotor activity in rats (Stolerman et al., 1973; Stolerman et al., 1974). Nicotine has been reported to produce catalepsy in rats at very high doses (7 mg/kg) (Zetler, 1968; 1971).

Arecoline has been demonstrated to consistently produce decreases in motility and exploratory activity in mice (Mattila et al., 1968) and rats (Pradhan and Dutta, 1970b) in doses of 0.25-4.0 mg/kg. Higher doses of arecoline (20 mg/kg) produce catalepsy (Zetler, 1968; 1971) and tremors (Holmstedt and Lundgren, 1967).

Operant Behavior. The behavioral effects of nicotine and arecoline in laboratory animals have been assessed on a number of different schedules of reinforcement utilizing different reinforcers. Different schedules of reinforcement engender different patterns of responding and different local and overall rates of responding (Ferster and Skinner, 1957). In general, the schedule of reinforcement, rather than the particular reinforcer employed in a study, is the major determinant of the response

pattern and the drug-behavior interaction (Kelleher and Morse, 1968). It has been demonstrated that for some drugs, the baseline rate of responding is an important determinant of the observed drug effect (Dews, 1958; Kelleher and Morse, 1968).

In general, the effects of nicotine and arecoline on operant behavior are similar to their effects on spontaneous motor activity. That is, the effect of nicotine on operant behavior is determined by the baseline rate of behavior (increases low rates and decreases high rates) and the dose of nicotine administered (Morrison, 1967; Olds and Domino, 1969; Pradhan, 1970; Stitzer et al., 1970; and Davis and Keasler, 1973). Arecoline produces mainly decreases in operant behavior (Olds and Domino, 1969; Pradhan and Dutta, 1970b).

Nicotine (0.4 mg/kg), had a biphasic effect on responding on a VI-2 minute schedule for water reinforcement in rats (Morrison, 1967). There was an initial decrease in responding of approximately 20 minutes followed by an increase in responding of approximately one hour. Decreasing the dose to 0.2 mg/kg decreased the initial depression, but had little effect on the secondary stimulatory phase. Doses of 0.1 and 0.05 mg/kg produced only a mild stimulation of response rate.

On a fixed-interval (FI)-2 minute schedule, 0.4 mg/kg briefly decreased responding, followed by a secondary increase in total responding (Morrison, 1967). Doses of 0.05, 0.1, and 0.2 mg/kg produced only increases in total responding. A relative enhancement of responding during the first half of the FI-2 minute period was observed after 0.1 and 0.2 mg/kg (Pradhan, 1970). This reflects a greater increase in low versus high rates of responding.

Nicotine has two characteristic effects on responding on a FR sche-
dule (Morrison, 1967; Pradhan, 1970; Domino and Lutz, 1973; Todd and
Dougherty, 1979). First there is a decrease in responding, the magni-
tude and duration of which are dose related. Nicotine also decreases
the characteristic post-reinforcement pause that is seen on a FR sche-
dule. Thus the overall effect of nicotine will be a composite of the
two separate effects.

Nicotine increased responding on a differential reinforcement of low
rate (DRL) schedule (Morrison and Lee, 1968a; Pradhan and Dutta, 1970a).
The effects of nicotine on the different schedules described above are
similar to those produced by amphetamine on the same schedules (Mor-
rison, 1967; Pradhan, 1970).

Morrison et al. (1969) studied the effects of cholinergic antagonists
on the effect of nicotine on responding on a VI 2 minute schedule for
water reinforcement. As in a previous study (Morrison, 1967), nicotine
(0.4 mg/kg) first produced a decrease, then an increase in responding.
Mecamylamine (0.5 mg/kg) blocked all effects of nicotine. Atropine (0.5
mg/kg), atropine methylnitrate (0.5 mg/kg) and chlorisodamine (0.05
mg/kg) blocked only the initial depressant phase. Scopolamine (0.05
mg/kg) did not block any of the effects of nicotine. The doses of the
quaternary antagonists that blocked the depressant effect of nicotine,
were approximately ten times as great as the doses at which these drugs
block peripheral cholinergic effects. Based on the evidence that the
blood-brain barrier to quaternary compounds is not absolute (Paul-David
et al., 1960), Morrison et al. (1969) hypothesized that the doses of
chorisondamine and atropine methylnitrate used were so high they they
had entered the CNS in sufficient amounts to antagonize the behavioral

effects of nicotine. Based on the data that atropine blocked the initial depressant phase produced by nicotine, Morrison et al. (1969) concluded that this phase of action was due to centrally-released ACh acting on muscarinic post-synaptic receptors. However, the rate dependent effects of atropine (selectively increases low rates of responding) (Boren and Navarro, 1959), may account for the blockade of the nicotine-induced behavioral depression. The lack of effect of scopolamine does not agree with the interpretation that centrally released ACh mediated the nicotine-induced depression. The secondary, rate-stimulatory phase, produced by nicotine on a VI 2-minute schedule, was postulated to be mediated through direct stimulation of nicotine receptors or through the release of brain transmitters besides ACh (Morrison et al., 1969).

On a FI-88 second schedule of water reinforcement, nicotine (0.4 mg/kg) produced an initial decrease in response rates, after which local rates of responding were altered differentially (Stitzer et al., 1970). Baseline rates above 30 responses/minute were decreased, whereas rates below 20 responses/minute were either increased, decreased, or unchanged. These results differed from those of Morrison (1967) utilizing a FI-2 minute, in which secondary rate increases were observed. This may be due to the higher baseline rates in the study by Stitzer compared to that of Morrison. Mecamylamine (0.3 mg/kg and higher) and hexamethonium (10 and 20 mg/kg but not 5 mg/kg) but not scopolamine (0.02 mg/kg) antagonized the initial rate-depressant effects of nicotine. However, only mecamylamine antagonized the rate dependent effects of nicotine. Considering the high doses of hexamethonium used, it is possible that some drug entered the CNS to exert its effects (McIsaac, 1962).

Pradhan and Dutta (1970b) assessed the effects on arecoline on water-reinforced FR, FI, DRL, and continuous shock-avoidance (Sidman) schedules of reinforcement in rats. Arecoline generally decreased responding, especially at doses of 1-2 mg/kg. Slight increases in responding were observed at a lower dose (0.5 mg/kg) on FI and Sidman avoidance schedules. Scopolamine by itself decreased FR responding and did not antagonize the depressant effect of arecoline on that behavior.

Olds and Domino (1969) compared the effects of nicotine, arecoline, and physostigmine on responding for electrical brain stimulation in rats. Rats exhibited a high rate of baseline bar pressing. Arecoline (0.1-3.0 mg/kg, s.c.) and physostigmine (0.05-0.3 mg/kg, s.c.) produced dose-related decreases in responding. Their effects were blocked by scopolamine (0.5 mg/kg). Methylscopolamine, mecamylamine, and trimethidinium were without effect. Nicotine (0.025-0.6 mg/kg, s.c.) produced a biphasic effect; an initial depression was sometimes followed by facilitation, and actions were much less consistent. The effects of nicotine were blocked by mecamylamine (5.0 mg/kg) and scopolamine (0.5 mg/kg). Trimethidinium (5 mg/kg) and methylscopolamine (0.5 mg/kg) were much less effective. These results support the hypothesis of Morrison et al. (1969) that centrally released ACh acting on muscarinic sites may in part mediate the nicotine-induced behavioral depression. However, the rate-dependent effects of scopolamine (McKim, 1973) may account for the antagonism of the nicotine-induced behavioral suppression. Also important is the demonstration that the effects of physostigmine are blocked by muscarinic and not nicotinic antagonists. This result is similar to the effects on physostigmine-induced EEG and behavioral arousal (Yamamoto and Domino, 1967).

Tolerance Studies. Tolerance to a substance can be demonstrated by the observation that repeated administrations of a fixed dose leads to a diminution of effect. Alternately, one can demonstrate tolerance by showing that the original effect of a substance, diminished in magnitude after sequential exposures to a fixed dose, can be reinstated by an increase in that dose. The most precise manner for defining and quantifying tolerance is to determine dose-response relationships both before and after repeated exposure to a drug (Schuster, 1978).

The development of pharmacological tolerance to the effect of a drug is considered to be one of three distinct components of drug dependence (WHO Technical Report, 1964); physical dependence and compulsive abuse (psychic craving) being the other two. Tolerance development can be an important factor, either through the process of tolerance development to aversive properties of the drug, allowing increased drug intake, or through tolerance development to the reinforcing properties of the drug, necessitating increased drug intake to obtain the desired effects, or both.

Aversive effects are experienced by novice users of both arecoline and nicotine. Nonsmokers reported varying degrees of nausea and dizziness after intravenous (i.v.) nicotine injections, while smokers reported no ill effects (Beckett, 1971). Inexperienced betel nut chewers experience a disagreeable combination of symptoms including constriction of the esophagus, redness and congestion in the face, and dizziness (Arjungi, 1976). These effects are not observed in chronic users of tobacco or the betel nut. Thus, the development of tolerance to the aversive effects of nicotine and arecoline may be a factor in the widespread use of these two drugs.

However, tolerance does not develop to all the effects of nicotine. In heavy smokers of long duration, the heart rate still increases after each cigarette (Jarvik, 1979). Murphee (1979) examined the effects of i.v. nicotine in smokers and nonsmokers. No difference was found between the two groups in the increase in heart rate, increase in blood pressure, and EEG arousal produced by i.v. nicotine. They concluded that smokers were not tolerant to the effects of nicotine.

With repeated administration, tolerance develops to the behavioral depressant effects of nicotine on spontaneous motor activity (Morrison and Stephenson, 1972; Stolerman et al., 1973; Stolerman et al., 1974) and operant behavior (Domino and Lutz, 1973; Todd and Dougherty, 1979) in laboratory animals. Two phases in the development of tolerance to the behavioral effects of nicotine, an acute and a chronic phase, can be demonstrated. Following a single i.p. injection of nicotine, acute tolerance develops to the effects of a second injection. This persists for approximately 8 hours. Acute tolerance has been demonstrated for both spontaneous motor activity (Stolerman et al., 1973) and operant behavior (Todd and Dougherty, 1979). Compared to a saline pretreatment, a single nicotine pretreatment, two hours prior to testing, shifted the dose-effect relationship for nicotine-induced locomotor suppression to the right, indicating tolerance (Stolerman et al., 1973; Stolerman et al., 1974). Even though there was complete tolerance to the rate-suppressant effects of nicotine on a FR schedule eight hours after pretreatment, much less tolerance was evident 24 hours later (Todd and Dougherty, 1979). With continued twice daily injections of nicotine, tolerance to the first daily injection was complete after nine days.

Repeated i.p. doses of nicotine (1 mg/kg three times daily for eight days) elicited chronic tolerance to the effects of nicotine on locomotor activity, which persisted for at least 90 days after the end of drug treatment (Stolerman et al., 1974). Morrison and Stephenson (1972) examined the effect of daily administration of 0.8 mg/kg of nicotine on spontaneous motor activity in rats. For the first three days of treatment, nicotine reduced locomotor activity as compared to saline treated controls. From day five onwards, nicotine administration produced a stimulation of locomotor activity compared to controls. However, in the absence of a complete dose-effect curve it is difficult to determine if this represents tolerance development.

Three factors important in pharmacolgoical tolerance can be recognized by the processes they involve: dispositional, physiological, and behavioral (Dews, 1978). In dispositional tolerance, the physio-chemical processes handling the drug (absorption, distribution, metabolism, and excretion) are modified so that reduced concentrations of the drug reach the receptive cells. Physiological tolerance is a change in the sensitivity of the receptive cells, such that the effects of a dose of a drug are reduced, even though the cells are exposed to the same concentration of drug. Behavioral tolerance to a drug is a change in the effect of a drug due to behavioral mechanisms and stresses the interaction of the animal in the drug state with the experimental contingencies as an important factor.

One theory of behavioral tolerance is the reinforcement-loss hypothesis put forth by Schuster et al. (1966). In their experiments, Schuster et al. observed that with repeated administration, tolerance developed to the effects of amphetamine on a DRL-30 second and FI-30

second responding that decreased reinforcement density, but tolerance did not develop to the effects of amphetamine that did not decrease reinforcement density. In a second experiment, amphetamine increased the response rate and decreased the shocks received (increased reinforcement density) of rats that were poor responders on a free (Sidman) avoidance procedure. With repeated administration, no tolerance developed to this effect of amphetamine. They concluded that "Behavioral tolerance will develop in those aspects of the organism's behavioral repertoire where the action of the drug is such that it disrupts the organism's behavior in meeting the environmental requirement for reinforcements. Conversely, where the actions of the drug enhance or do not affect the organism's behavior in meeting reinforcement requirements, we do not expect the development of behavioral tolerance." This hypothesis stresses the interaction of the contingencies of reinforcement with the drug-induced behavioral change as an extremely important variable affecting the development of behavioral tolerance.

Since the initial formulation of this theory, there have been reports both confirming and failing to confirm this hypothesis (Cornfield-Summer and Stolerman, 1978). It is clear that other variables in the experimental situation can affect the development of tolerance, even when there is a decrease in reinforcement density. These variables include the dosage tested (Freedman et al., 1964), the type of schedule (interval versus ratio), and baseline reinforcement density (Harris et al., 1972).

A research strategy for separating dispositional and physiological factors from behavioral factors is the method of Chen (1968) which utilizes presession and postsession drug injections. In this design, one group of animals is injected with the drug prior to the experimental

session, while a second group of animals is injected with the drug some time after the experimental session. It can be reasoned that tolerance development due to dispositional and physiological variables would be a factor in both groups of rats, whereas the importance of behavioral variables would be evidence only in the presession injection group.

It is clear that experimentally, tolerance develops to the behaviorally disruptive effects of nicotine. However, the role of dispositional, physiological, and behavioral factors in tolerance development has not been fully evaluated.

In view of the use of nicotine by man, it is important to ascertain the contribution of the above discussed factors to nicotine tolerance. No studies have assessed the effects of chronic administration of arecoline on behavior. In view of the lack of data, it is important to assess the effect of chronic administration of arecoline on behavior and the role played by the different processes in tolerance development, if it occurs.

Drug Discrimination Studies. The ability of nicotine to serve as a DS in animals is well documented (Morrison and Stephenson, 1969; Schechter and Rosecrans, 1971a; Hirschhorn and Rosecrans, 1974). Nicotine has served effectively as a DS in a T-maze (Schechter and Rosecrans, 1971a) and on two-lever VI, FR, and DRL schedules of reinforcement (Chance et al., 1977). The discriminative cue produced by nicotine has been well characterized pharmacologically. The strength of the cue shows a dose-response relationship, that is, increasing the training dose of nicotine increases the stimulus control (Hirschhorn and Rosecrans, 1974; Chance et al., 1977). The time-response curve for nicotine discrimination has been

shown to correlate with its time course of the entry into and passage from the brain (Hirschhorn and Rosecrans, 1974). Mecamylamine can antagonize, while hexamethonium and chlorisondamine are ineffective in antagonizing, the DS effect of nicotine (Morrison and Stephenson, 1969; Schechter and Rosecrans, 1971b; Hirschhorn and Rosecrans, 1974). In addition, intraventricular administration of nicotine generalized to peripherally administered nicotine (Chance et al., 1978; Rosecrans and Chance, 1977). These latter two studies demonstrate a central origin for the DS effect of nicotine. The specificity of the cue was demonstrated by the lack of generalization to nicotine by physostigmine, gallamine, caffeine, chlordiazepoxide, pentobarbital, adrenaline, apomorphine, lobeline, and arecoline (Morrison and Stephenson, 1969; Schechter and Rosecrans, 1972c). Atropine, dibenamine (α-adrenergic antagonist), propranolol (β-adrenergic antagonist), and α-methyl-para-tyrosine (catecholamine synthesis inhibitor) did not block the nicotine cue (Hirschhorn and Rosecrans, 1974). Amphetamine has been shown to produce either a lack of generalization (similar to saline responding) or partial generalization (responding split between saline and nicotine choices) to nicotine (Morrison and Stephenson, 1969; Schechter and Rosecrans, 1972c; Chance et al., 1977). The degree of generalization is influenced by the training schedule and the training dose of nicotine (Chance et al., 1977).

Preliminary studies have supported a role for the hippocampus as an important area in mediating the DS effects of nicotine. In rats trained to discriminate 0.4 mg/kg (s.c.) of nicotine from saline, bilateral injections of nicotine (0.5 μg/μl/site) directly into the hippocampus

partially generalized to peripheral nicotine administration (Rosecrans and Chance, 1977). After unilateral application of 2-4 μg/0.5 μl (0.5 μl total) into the hippocampal-superior colliculus area, a generalization nearly equal to that produced by the training dose was observed in rats trained to discriminate 0.2 mg/kg of nicotine from saline (Newlon and Rosecrans, unpublished observations). Thus, discrimination data, as well as receptor binding studies, EEG studies, and microiontophoretic studies support an important role for the hippocampus in mediating the effects of nicotine.

Arecoline can serve effectively as a DS and its effects are blocked by atropine (Schechter and Rosecrans, 1972b). Rats can be trained to discriminate arecoline from nicotine in a T-maze (Schechter and Rosecrans, 1972a). Mecamylamine blocks the nicotine cue in this procedure, but has no effect on the arecoline cue. In contrast to nicotine, the pharmacological basis of the DS properties of arecoline have not been extensively evaluated. There is clearly much more basic research that needs to be carried out to characterize DS properties of arecoline.

II. GENERAL METHODS

SUBJECTS. Male Sprague-Dawley rats (175-200 g) with no previous drug or experimental experience were purchased from Flow Research Animals, Dublin, Virginia, 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 libitum. 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.

APPARATUS. The experimental space was a standard operant test chamber (Lehigh Valley Electronics, Model 1417 or Coulburn Model E10-10). One wall of the chamber contained two levers with a dipper centered between them for delivery of liquid reinforcement. Except where noted, both levers were always in the chambers. 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 and cumulative response recordings. 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.

DRUGS. The following drugs were used in these experiments:

Arecoline hydrobromide (Chemical Dynamics Co., Plainfield, N.J.); atropine methylnitrate, atropine sulfate, hexamethonium chloride, and oxotremorine sesquifumarate (Sigma Chemical Co., St. Louis, MO); mecamylamine hydrochloride (Merck, Sharp, and Dohme, West Point, PA); pilocarpine nitrate (Nutrional Biochemical Corp., Cleveland, OH); and optically pure (-)-nicotine 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 gm body weight. Neostigmine methylsulfate (Hoffmann La Roche, Nutley, N.J.) and physostigmine salicylate (O'Neal, Jones, and Feldman, St. Louis, MO) were obtained in aqueous solution from the Hospital pharmacy in injection vials. These drugs were diluted with 0.9% saline to a concentration that resulted in an injection volume of 0.1 ml/100 gm body weight. All injections were s.c. with a 26-gauge 3/8" needle attached to a 1-ml syringe. In all experiments, drug dosage is expressed as the salt.

Free base and μmole equivalents of salt of the drugs used in the present investigation are as follows:

Drug	Salt (mg/kg)	Free base (mg/kg)	μmole (μmole/kg)
Arecoline HBr	1.74	1.14	7.41
Nicotine bitartrate	1.14	0.40	2.47
Oxotremorine sesquifumarate	0.1	0.053	0.25
Pilocarpine nitrate	2.0	1.54	7.41
Neostigmine methylsulfate	0.10	0.07	3.0
Physostigmine salicylate	0.125	0.08	3.0
Atropine methylnitrate	2.0	1.58	5.46

Drug	Salt (mg/kg)	Free base (mg/kg)	μmole (μmole/kg)
Atropine sulfate	4.0	3.33	11.50
Hexamethonium Cl	1.0	0.87	3.66
Mecamylamine HCl	2.35	1.43	11.50

DATA ANALYSIS. All analyses of variance were performed by following the procedures outlined in Computational Handbook of Statistics by J.L. Bruning and B.L. Kintz (1968). The data points used in each analysis are discussed in the appropriate sections.

Linear regression analyses were performed on a Texas Instruments SR-52 calculator with a prewritten program. The linear portion of the dose-effect curve was used in each analysis. The slope represents the percent control by one log cycle.

III. EXPERIMENT 1. ACUTE AND CHRONIC EFFECTS OF ARECOLINE ON SCHEDULE-
CONTROLLED BEHAVIOR

INTRODUCTION

There have been no animal studies examining the effect of repeated
arecoline administration on behavior. The experiments in the present
study were designed to investigate the effects of daily administration
of arecoline on operant behavior in rats. The first study, which used a
VI schedule of reinforcement, assessed tolerance development to the
effects of arecoline administered prior to the session. The second
study, which utilized a FR schedule of reinforcement, evaluated the role
of behavioral factors in the development of tolerance to the effects of
arecoline, by using the strategy of administering one group daily pre-
session injections and a second group daily post-session injections
(Chen, 1968).

Arecoline produces peripheral, as well as central, muscarinic stimu-
lation, and has been reported to exert nitotinic-like activity at high
doses (Herz et al., 1967). To test whether the behavioral effects that
were being measured were of central muscarinic origin, the interaction
of selective cholinergic antagonists with the acute behavioral changes
induced by arecoline was also assessed.

METHODS

Experiment A. Effects of arecoline on variable-interval behavior.
Five male Sprague-Dawley rats, approximately 110 days of age, were
food deprived to 80% of normal body weight. They were trained, by the
method of sucessive approximation, to lever press for sweetened milk

reinforcement (0.01 ml) on a continuous reinforcement (CRF) schedule. After two to three days of responding on a CRF, a VI-3 second schedule of reinforcement was instated. This was increased by three seconds per day to a final schedule of VI-15 seconds. Sessions were 30 minutes in duration. During this training, saline was injected (s.c., 0.1 ml/100 gm body weight) immediately prior to each daily session.

When stable responding was achieved, the dose-effect relationship of arecoline (0.58, 1.16, and 1.74 mg/kg) on VI behavior was assessed. The blockade of the arecoline-induced behavioral effects by selective anta-gonists was also assessed. Antagonists (atropine sulfate, 2 and 4 mg/kg, atropine methylnitrate, 2 mg/kg, and mecamylamine HCl, 1 mg/kg) were injected (s.c.) 25 minutes prior to the session and arecoline was injected (s.c.) immediately prior to the session. Doses of antagonists were based on previous research in this laboratory. The different doses of arecoline and antagonists were administered in a randomized sequence. At least four control days separated test days. Seven days separated tests after antagonists were administered.

The dose-effect relationship for nicotine and the effect of anta-gonists was also assessed in these rats after the determination of the acute effects of arecoline and prior to beginning chronic arecoline treatment. These results are presented in Experiment Two.

Tolerance to the effects of arecoline on behavior were assessed after the initial agonist and antagonist studies. Rats were injected with 1.74 mg/kg of arecoline immediately prior to the session. Operant sessions were conducted four to five consecutive days, with one to two days in between. On days when no sessions were conducted, rats were injected with 1.74 mg/kg arecoline in their home cages. The dose-effect

relationship, as well as saline data points, were redetermined after 21 days of chronic treatment. Different doses of arecoline (0.58 and 1.16 mg/kg) or saline were substituted for the daily injection of 1.74 mg/kg immediately prior to the session. At least four days separated tests. Administration of 1.74 mg/kg arecoline continued throughout redetermination of the dose-effect relationship. On days when the saline response was redetermined, rats were injected with 1.74 mg/kg arecoline 15-30 minutes after the session. No supplemental injections were administered after other doses of arecoline.

Experiment B. Effects of arecoline of fixed-ratio behavior. Ten male Sprague-Dawley rats, treated similar to those in Experiment A, were trained to respond on a CRF schedule for sweetened milk reinforcement. A fixed-ratio (FR) schedule was instated. The response requirement was doubled, approximately every day, until a FR-20 was reached. Daily experimental sessions were 30 minutes in duration and were immediately preceeded by a saline injection (s.c., 0.1 ml/100 gm body weight).

When stable responding was achieved, the dose-effect relationship for arecoline was assessed. Arecoline (0.58, 0.87, 1.16, and 1.74 mg/kg in a counter-balanced order) was injected (s.c.) immediately prior to the session. At least four control days separated test days.

After the determination of the effects of single injections of arecoline, the animals were divided into two groups (five rats/group) matched for equivalent baseline response rates. For the remainder of the experiment, one group received 0.87 mg/kg arecoline immediately prior to the session (presession group), while the other group received the same dose of arecoline 30-45 minutes after the session (post-session group). One

rat in the post-session group that exhibited very unstable responding during the period of chronic treatment was removed from the study and data from this subject was not used in any analysis. The dose of arecoline chosen for chronic treatment (0.87 mg/kg) produced an effect similar to 1.74 mg/kg arecoline on the VI schedule in the initial study.

Operant sessions were conducted four to five consecutive days, with one to two days in between. On days when no sessions were conducted, rats were injected with 0.87 mg/kg arecoline in their home cages. In both groups, the dose-effect relationship, as well as saline data points, were redetermined after 25 days of arecoline administration. Two saline tests and doses of 0.58, 0.87, 1.16, and 1.74 mg/kg arecoline, in a counter-balanced sequence, were administered immediately prior to the session. In addition, the effect of 2.32 mg/kg arecoline was assessed at the end of the experiment. On saline test days, all rats received their daily arecoline injections 30-45 minutes after the session. The day after a dose-effect redetermination, rats received their normal injection in their home cages. At least four days separated test days. The pre- or post-session administration of 0.87 mg/kg arecoline continued throughout redetermination of the dose-effect relationship.

Data analysis. The parameters used to evaluate the effect of arecoline on operant behavior in the VI schedule were as follows: (1) total responses/session; (2) total reinforcements/session; (3) responses for consecutive five minute segments (1-5 minutes, 6-10 minutes, etc.) of the 30-minute session; and (4) reinforcements for consecutive five-minute segments. In contrast to the VI schedule, reinforcements obtained for

FR responding are directly related to response rate. Thus, only total reinforcements and reinforcements for consecutive five-minute segments were used to evaluate the effect of arecoline on FR responding. Due to the individual differences in total responses and reinforcements, the data is presented as percent change from baseline averaged across animals.

For analysis of acute drug effects and their antagonism, the two pre-ceeding saline days for each test were averaged and used as the control for that test. For analysis of the chronic drug regimens, the four saline days preceeding the chronic regimen were averaged and used as control baseline. Data for the entire period of chronic drug treatment is expressed as percent control of this four day baseline. The saline points that were redetermined with the dose-effect relationship during chronic drug treatment are expressed as percent control of this four day baseline and represent changes that may have occurred in baseline res-ponding due to drug effects and/or time. These saline points were used as the baseline in redetermining the dose-effect curve, and data are expressed as percent control of them.

The ED_{50} values and correlation coefficients for the dose-response determinations were derived from linear regression analysis. The ED_{50} values represent the dose that produced a 50% decrease in the response being measured. The data for peak effects was derived from the five-minute period for each rat in which responding was decreased the most by a given dose.

RESULTS

Experiment A.

Acute effects of arecoline on VI responding and their antagonism.

The pattern of responding engendered by a VI schedule of reinforcement

is characterized by a constant rate of responding throughout the session
(Figure 3A). This pattern of responding was observed in all rats.
Control rates were stable within subjects, but varied between subjects
Baselines varied from 0.35 ± 0.03 to 1.16 ± 0.03 response/ second, with
a mean response rate for the five rats of 0.80 ± 0.14 response/second.
The average reinforcements per session was 98.4 ± 4.4.

The effect of 1.74 mg/kg arecoline, alone or after pretreatment with
different selective cholinergic antagonists, is presented in Table 2 and
Figure 1. This dose of arecoline decreased total responses to approxi-
mately 30% of control. As evidenced by the cumulative records, (Figure
3G) the onset of the behavioral suppression occurred two minutes after
injection and the maximum disruption of behavior occurred five to ten
minutes after drug administration. The effect of 1.74 mg/kg arecoline
on VI-15 second responding was antagonized in a dose-dependent relation-
ship by atropine sulfate, but was not antagonized by the doses tested of
either atropine methylnitrate or mecamylamine (Table 2, Figure 1). Four
mg/kg atropine sulfate antagonized both the decrease in total responding
(df=4; t=12.6; p<0.05) induced by arecoline, while 2 mg/kg atropine
sulfate produced only a decrease in the period of greatest decrease in
responding (df=4; t=3.34; p<0.05).

Figure 1 presents the time course of the effect of 1.74 mg/kg areco-
line alone and after pretreatment with different antagonists. Four
mg/kg atropine sulfate antagonized the arecoline-induced decrease in
responding at all time points as reflected by a shift to the left
(decreased effect at the same time points) in the arecoline time-effect
curve. In contrast, 2 mg/kg atropine sulfate did not antagonize the
arecoline-induced decrease in responding over the initial five minutes,

Figure 1.   Time-course of effect of 1.74 mg/kg arecoline on VI respon-

ding and interaction with antagonists.   Each point is the mean of five

rats, except mecamylamine, which is four rats.   All antagonists were

injected 25 minutes before session.   Arecoline was injected immediately

before the session.
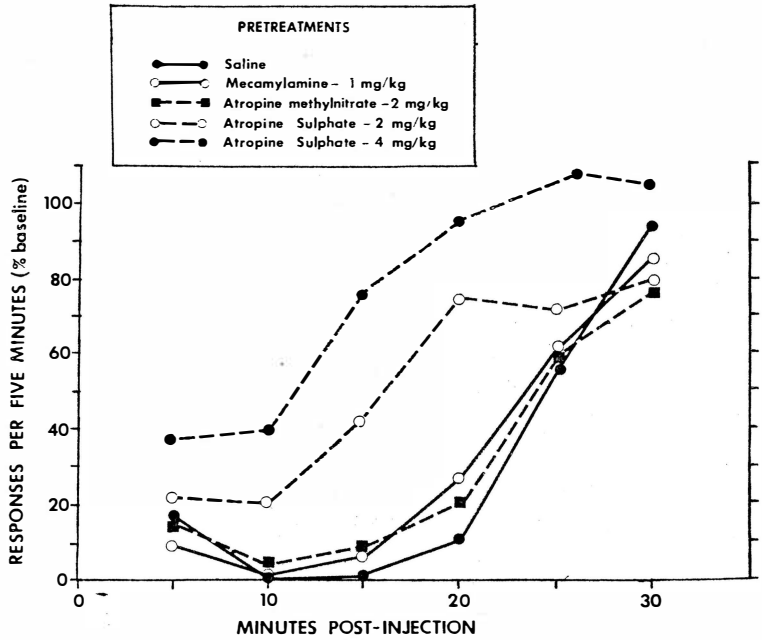
TABLE 2

Group Comparison of Arecoline (1.74 mg/kg)

and Effects of Antagonists on VI Responding

Drug	Dose (mg/kg)	N	% Control Total Respondings	% Control Greatest Decrease
Saline	0.1 ml/100 gm	5	96.6 ± 2.5	77.9 ± 3.8
Saline + Atropine Sulfate	4	5	103.4 ± 17.9	75.0 ± 19.6
Arecoline	1.74	5	28.6 ± 2.3	0.2 ± 0.2
+ Mecamylamine HCl	1.0	4	30.9 ± 3.2	0.5 ± 0.5
+ Atropine Methylnitrate	2	5	29.0 ± 5.0	3.2 ± 1.8
+ Atropine Sulfate	2	5	52.2 ± 11.6	14.2 ± 4.2*
+ Atropine Sulfate	4	5	74.0 ± 4.5*	34.8 ± 10.0*

Antagonists were administered (s.c.) 25 minutes prior to the session
and arecoline and/or saline were administered (s.c.) immediately prior
to the session. Significant effects of antagonists were assessed
by paired Student's t-tests. *Significantly different from 1.74
mg/kg arecoline, $p < 0.05$.

but did antagonize the effect of arecoline from 5 to 20 minutes. Neither mecamylamine HCl (1 mg/kg) nor atropine methylnitrate (2 mg/kg) affected the time course of the arecoline-induced decrease in responding. These data suggest that the behavioral suppressive effects of arecoline on operant behavior were due to stimulation of central muscarinic receptors.

Dose-effect relationship prior to and during the chronic drug regimen. Figures 2A and 2B show the initial and the redeterminated (after 21 days of arecoline treatment) dose-effect relationships for arecoline on total responses and total reinforcements. Saline administration had no effect on operant behavior (Figure 2). Acute administration of arecoline produced a dose-related decrease in total responses and total reinforcements (Figure 2A and 2B, respectively). The ED_{50} for the initial decrease in total responses was 1.12 mg/kg. The correlation coefficient of -0.87 was significant (df=13; t=6.20; p<0.01). The slope of the line was -115.5. The ED_{50} for the initial decrease in total reinforcements was 1.45 mg/kg. The correlation coefficient of -0.91 was significant (df=13; t=6.60; p<0.01). The slope of the line was -124.3.

After 21 days of chronic arecoline administration, the total responses and total reinforcements after saline injection did not change compared to the initial determination. Thus, the overall baseline response rate and reinforcement total did not change as a consequence of chronic drug administration. Compared to the initial determination, there was no change in the dose-effect relationship for total responses. The ED_{50} for the redetermined decrease in total responses was 1.12 mg/kg, which was equal to that for the initial determination. The cor-

Figure 2. Dose-effect relationship for arecoline on total responses (A)
and total reinforcements (B) prior to and during chronic drug adminis-
tration. Points above SAL indicate the results of vehicle (saline)
injection. Each point is the mean ± S.E.M. of one administration in
each of five rats.

A. Total Responses

B. Total Reinforcements

- ●——● Initial Determination
- ○——○ Redetermination

PER CENT BASELINE

DOSE OF ARECOLINE (mg/kg)
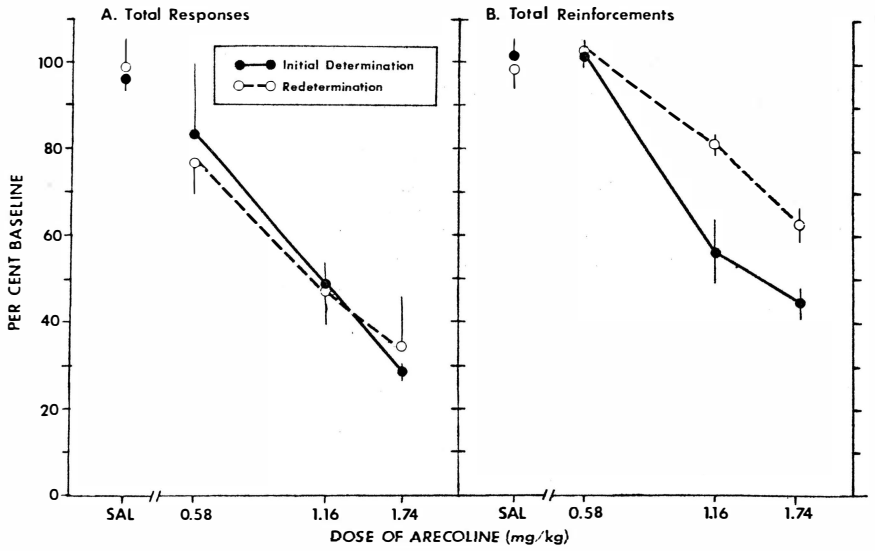
relation coefficient for the redetermination of the dose-effect relationship of -0.71 was significant (df=13; t=3.6; p<0.01). The slope of the line was -88.3. A two-factorial, repeated measures, analysis of variance was carried out on the initial and redeterminated dose-effect relationship for saline and arecoline on total responses. The two factors that were analyzed were dose (saline and the three doses of arecoline) and determinations. This analysis yielded a significant effect for the dose factor (df=3,12; F=51.1; p<0.001), while the determination factor (df=1,4; F=1.04; p>0.2) and the dose x determination interaction (df=3,12; F=1.0; p>0.2) were nonsignificant. This analysis showed that there was a significant dose-effect relationship, whereas there was no difference between the two determinations, and that the dose-effect relationship was similar in both determinations.

In contrast to the data for total responses, there was a shift to the right of the dose-effect relationship for arecoline on total reinforcements. The ED_{50} for the redetermination was 2.52 mg/kg, compared to 1.45 mg/kg for the initial determination. The ratio of the two ED_{50}'s is 1.74. This reflects the development of tolerance to the decrease in total reinforcements induced by arecoline administration. The correlation coefficient for the redetermination of 0.92 was significant (df=13; t=8.58; p<0.01). The slope of the line was -83.6.

A two-factorial, repeated measures analysis of variance was performed on the initial and redeterminated dose-effect relationship for saline and arecoline on total reinforcements. This analysis yielded a significant effect of the dose factor (df=3,12; F=34.06; p<0.001) and the determination x dose interaction (df=3,12; F=57.12, p<0.001) while the determination factor was not significant (df=1,4; F=4.76; p>0.2). This

analysis showed that there was a significant dose-effect relationship and that there was no overall difference in the determinations, but the dose-effect relationship was different between the two determinations. This last point is seen by inspection of the graph (Figure 2B), in which there was no shift in the effect of saline and 0.58 mg/kg arecoline, but there was a change in the effect of 1.16 and 1.74 mg/kg arecoline when comparing the initial and the redetermination of the dose-effect curve.

Cumulative records obtained during the initial and redeterminated dose-effect relationship are shown for a representative rat (Figure 3). Examination of the cumulative records, from the initial determination, revealed an onset of action of approximately two minutes. The peak effect occurred approximately five to ten minutes after injection (Figure 3). The lowest dose (0.58 mg/kg) produced an initial decrease in the rate of responding, but did not completely abolish responding. The two higher doses (1.16 and 1.74 mg/kg) produced a complete cessation of responding, the duration of which was dose related. The duration of action was also dose related, as reflected by the onset and time course of recovery of responding. The most evident differences in comparing the cumulative records between determinations are: (1) a decrease in responding during the initial few minutes after 0.58 mg/kg in the redetermination, and (2) a decrease in the time of complete cessation of responding after 1.16 and 1.74 mg/kg in the redetermination.

The individual responses observed in the cumulative records are also seen in the group data for the time course of the effect of arecoline on responses and reinforcements (Figures 4 and 5, respectively). Control responses (Figure 4A) and reinforcements (Figure 5A) fluctuated by plus or minus 10% over the time course of the session. After initial adminis-

Figure 3. Cumulative records from one rat showing the effects of saline and three doses of arecoline prior to and during the period of chronic administration. Upward movement of the pen reflects responses and short diagonal deflections indicate the delivery of milk reinforcement.

INITIAL DETERMINATION REDETERMINATION

A. B. SALINE

C. D. 0.58 mg/kg

E. F. 1.16 mg/kg

5 MINUTES

G. H. 1.74 mg/kg

500 RESPONSES
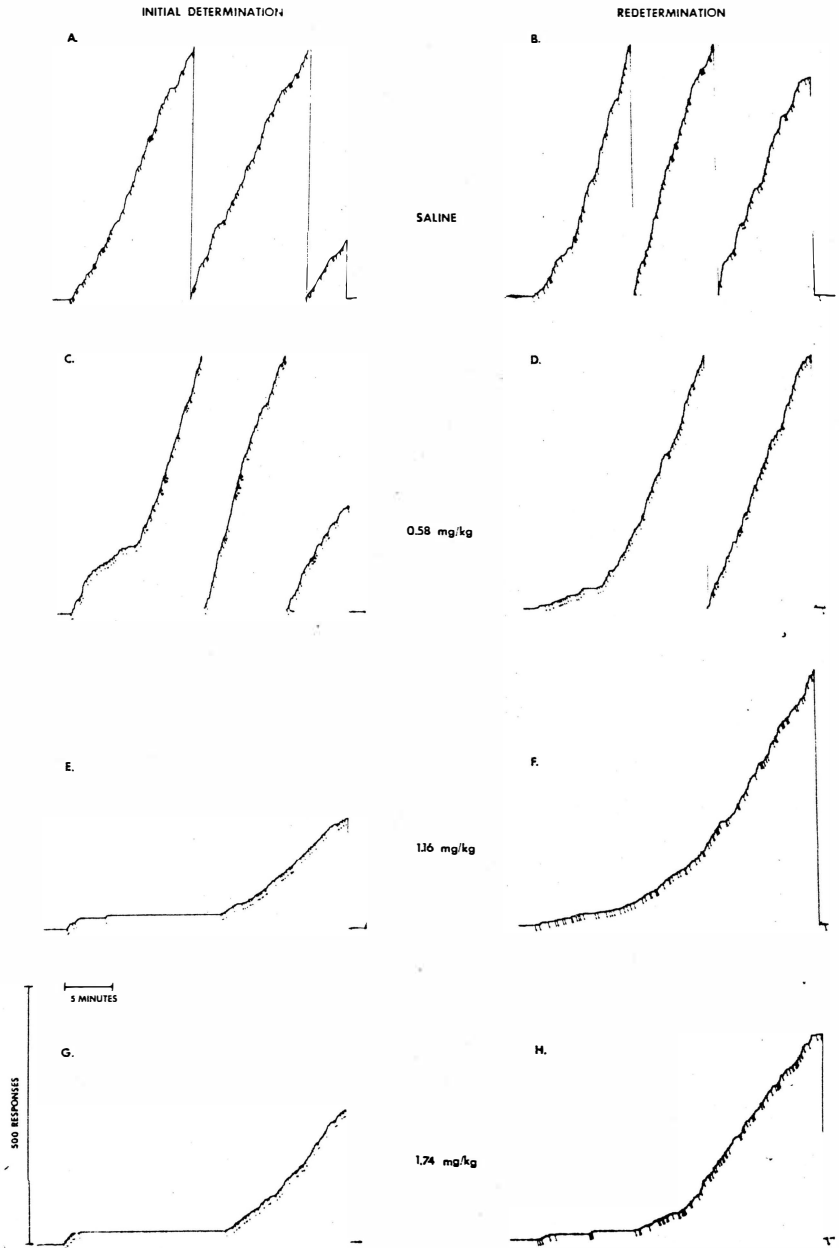
Figure 4. Initial and redetermined time-effect relationships for saline and three doses of arecoline on VI responding. Each point is the mean ± S.E.M. of one administration in each of five rats.

Figure: RESPONSES (% baseline) vs MINUTES POST-INJECTION. Legend: ●—● Initial Determination; ○- -○ Redetermination. Panels: Saline, 0.58 mg/kg, 1.16 mg/kg, 1.74 mg/kg.
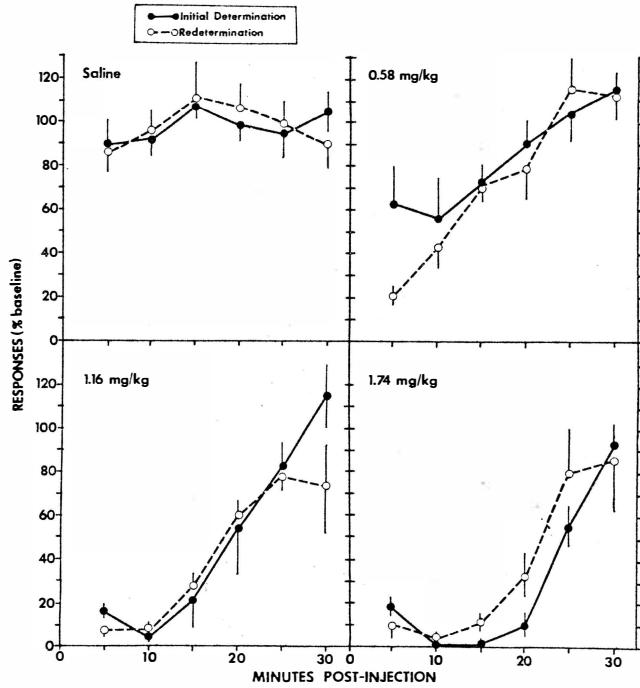
Figure 5.   Initial and redetermined time-effect relationships for saline and three doses of arecoline on reinforcements on a VI-15 schedule. Each point is the mean ± S.E.M. of one administration in each of five rats.
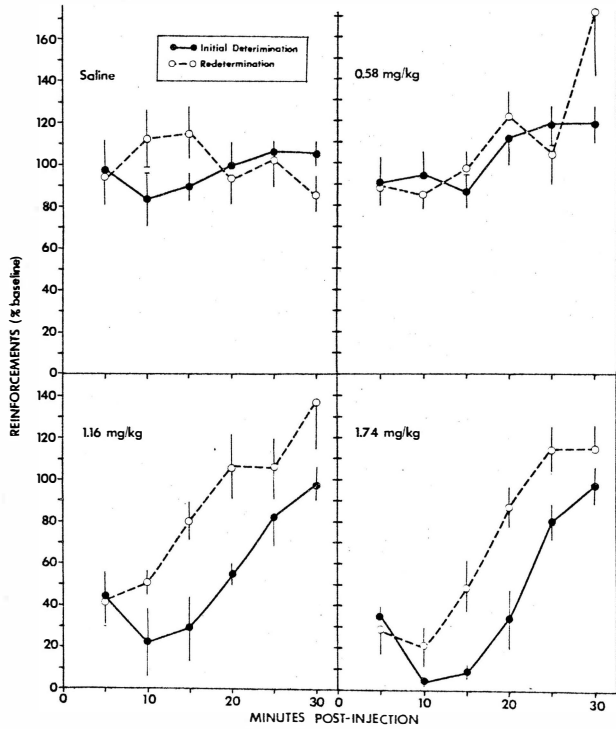
tration of 0.58 mg/kg, (Figure 4B), there was a moderate decrease in the responses for the first 10 minutes, after which response totals returned to control values. The time course of the redetermination of the effect of 0.58 mg/kg on responses was similar to the initial effect, except that there was a greater decrease over the first five minutes. The time course of the initial and redeterminated effect of 0.58 mg/kg on rein- forcements was similar to that observed after saline (Figure 5B). Thus, despite a decrease in responses emitted, over a five-minute period to 60 or 20% of control, there was no change in the number of reinforcements received. After initial administration of 1.16 and 1.74 mg/kg, there was a dose- and time-related decrease in both responses (Figures 4C and 4D) and reinforcements (Figures 5C and 5D). Upon redetermination, the time course of the effect of 1.16 mg/kg on responses was similar, although slightly shifted to the left, compared to the initial determi- nation. The time course of the redetermined effect of 1.74 mg/kg was slightly shifted to the left, especially from 15 to 25 minutes, indi- cating small increases in responding at these time points. In contrast, the redetermined time course for both 1.16 and 1.74 mg/kg on reinforce- ment showed shifts to the left, indicating tolerance had developed.

Effects during the daily injection regimen. The effect of 1.74 mg/kg arecoline on total responses on day 1 of chronic treatment was similar to that produced during the initial determination (43.8% vs. 42%, respec- tively), while the decrease in total responses on day 1 was slightly greater (28.6% vs 20.2%) (Table 3). Over days two through five (Block 1), the animals exhibited an apparent increased sensitivity to the effects of arecoline on total responses and reinforcements. With

TABLE 3

Effects of Arecoline (1.74 mg/kg/day) During Chronic Treatment

	Percent Control Total Responses	Percent Control Total Reinforcements
Day 1	20.2 ± 6.1	42.0 ± 5.4
Block 1	12.6 ± 4.2	34.0 ± 7.7
Block 2	22.6 ± 7.3	47.4 ± 4.5
Block 3	24.6 ± 9.9	50.6 ± 6.0
Block 4	27.2 ± 7.2	56.0 ± 4.5
Block 5	33.6 ± 11.4	61.0 ± 3.0
Block 6	29.0 ± 6.9	54.4 ± 2.2
Block 7	27.0 ± 6.6	58.6 ± 3.9

All values are the group mean ± S.E.M. Except for Day 1, all data are derived from four to five day means for each animal, which are averaged across animals.

repeated administration of 1.74 mg/kg arecoline, there was a decrease in the behavior disruptive effects of arecoline. Both measures reached a plateau at 17 and 21 days (Block 4) of chronic treatment. Total responses emitted increased to approximately 30% of control (which was similar to the effect of 1.74 mg/kg on the initial determination), and total reinforcements received increased to approximately 60% of control.

Experiment B.

Baseline Behavior. A FR-20 schedule of reinforcement engenders a pattern of responding that is characterized by a high rate of responding during completion of the ratio, followed by a brief pause after the reinforcement (post-reinforcement pause). This pattern of responding was observed in all rats. Control rates were stable within subjects. Between subjects, baselines varied from 0.49 ± 0.02 to 1.17 ± 0.03 response/second. The average baseline response rate 0.78 ± 0.12 response/second for the presession group and 0.93 ± 0.17 response/second for the post-session group. The mean ± SEM reinforcements per session was 74.2 ± 13.2 and 84.8 ± 14.2 for the pre- and post-session groups, respectively.

Dose-effect relationship before and during the daily injection regimen. Figures 6A and 6B present the effects of arecoline on total reinforcements in the initial and redeterminated dose-effect relationship in both groups of rats. Saline had no effect on total reinforcements in the initial determination and the dose-effect relationship for arecoline was similar in both groups of rats. The lowest dose (0.58 mg/kg) decreased total reinforcements less in the presession vs. post-session group, while the two intermediate doses (0.87 and 1.16 mg/kg) decreased the

Figure 6. Initial and redetermined dose-effect relationships in pre-
(A) (n=5) and post-session (B) (n=4) groups. Each point is the mean ±
S.E.M. of one administration in each rat.

A. Presession Group (n=5)

B. Post-session Group (n=4)

● Initial Determination
○ Redetermination

REINFORCEMENTS (% baseline)

DOSE OF ARECOLINE
(mg/kg)
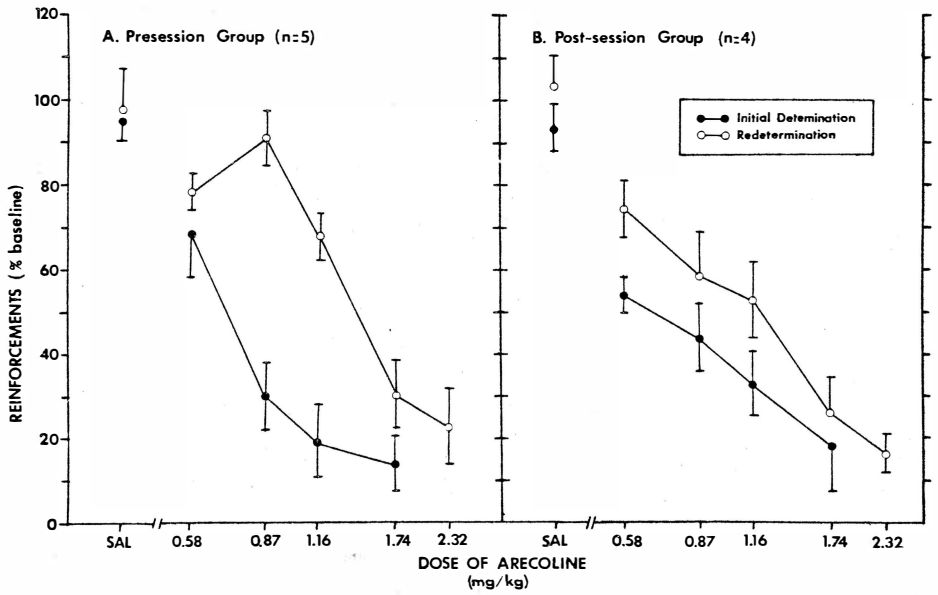
total reinforcements less in the post-session group. The effect of 1.74 mg/kg was similar in both groups.

The ED_{50}'s for the initial dose-response determination were 0.71 and 0.66 mg/kg for the pre- and post-session groups, respectively. For the presession group, the correlation coefficient of -0.73 was significant (df=18; t=4.57; p<0.01). The slope of the line was -111.2. For the post-session group, the correlation coefficient of -0.69 was significant (df= 14; t=3.59, p<0.01). The slope of the line was -75.1. Based on the initial dose-effect relationship, 0.87 mg/kg produced an effect on FR responding similar to that produced by 1.74 mg/kg on the VI schedule. Thus this dose was chosen for chronic treatment.

After approximately 25 days of the daily injection regimen, the dose-effect relationship as well as saline reinforcement totals were redetermined. For both groups, there was no change in total reinforcements after saline, relative to the initial determination. Thus, the baseline rate of responding did not shift during the period of chronic drug administration. Relative to the initial determination, the redetermined arecoline dose-effect curve was shifted to the right in both groups. Greater changes were observed in the dose-effect curve for the presession versus the post-session group, mainly at the intermediate doses of 0.87 and 1.16 mg/kg. This is not entirely due to the greater initial effect of these doses in the pre- versus post-session group. The redetermined effects of 0.87 and 1.16 mg/kg were less in the pre- versus post-session group (Figures 6A and 6B). The effects of 0.58, 1.74, and 2.32 mg/kg arecoline were similar in both groups of rats.

The ED_{50} for the dose-effect redetermination in the presession group was 1.48 mg/kg. The correlation coefficient of -0.86 was significant

(df=18; t=7.15; p<0.01). The slope of the line was -165.6. The ratio of the ED_{50}'s for the initial and redetermined dose-effect relationship was 1.38/.71 = 1.94. The ED_{50} for the dose-effect redetermination in the post-session group was 1.07 mg/kg. The correlation coefficient of -0.84 was significant (df=18; t=6.45; p<0.01). The slope of the line was -98.6. The ratio of the ED_{50}'s for the initial and redetermined dose-effect relationship was 1.07/0.66 = 1.62. Thus, the development of tolerance in the presession group was approximately twice that in the post-session group.

The initial and redetermined dose-effect relationship for both groups were compared by a three-factorial, repeated measures analysis of variance. The factors were groups (pre- versus post-session), determinations (initial versus redetermination), and doses (four doses of arecoline, 0.58 through 1.74 mg/kg, and saline). The results of the analysis are interpreted as follows. There was no overall difference between the two groups (df=1,7; F<1; p>0.2). There was a significant effect of dose levels (df=4,28; F=107.2; p<0.001) indicating a dose-effect relationship, and a significant effect of determinations (df=1,7; F=829.3; p<0.001) indicating the development of tolerance. The group x determination interaction (df=1,7; F=72.6; p<0.001) and determination x dose interaction (df=4,28; F=3.79; p<0.01) were significant, demonstrating that the two groups responded differently in the determinations and that the dose-effect relationship varied between the determinations. The group x dose interaction (df=1,28; F<1; p>0.2) and group x determination x dose interaction (df=4,28; F=2.27; p>0.1) were not significant.

The initial and redetermined time-effect curves for saline and dif-
ferent doses of arecoline in both groups of rats are seen in Figures 7A
and 7B. For the post-session group (Figure 7B), even though the total
reinforcements received were not affected by saline administration, the
initial time course of the responding after saline showed great varia-
bility. The time-course of responding was much more stable during the
redetermination, probably due to the subjects' experience with the
schedule. In the presession group, the reinforcements received after
saline were decreased in the first five minutes of the redetermination,
relative to the initial time course. This was due to the presence of
long intervals in the cumulative records of some rats (not shown) when
initially placed in the operant chamber, before responding began.

In comparing the initial and redetermined time-effect curves, a
greater shift to the left in the curves for 0.58, 0.87, and 1.16 mg/kg
is observed in the pre- versus the post-session groups. The extremely
high values, expressed as percent control in the last five-minute seg-
ment, are due to the fact that some animals would decrease their res-
ponding during the last five minutes under the saline condition. When
responding was suppressed in the initial segments of the session by
arecoline injections, the animals would usually respond at normal rates
over the last five minutes, which when compared to low control values
for the last segment, yields a high percent control value. The large
standard error of the mean reflects the variability in this last segment
of the session.

Effects during the daily injection regimen. Chronic post-session
arecoline administration had no effect on total reinforcements received

Figure 7. Initial and redetermined time-effect relationships for saline

and arecoline on reinforcements on a FR-20 schedule in pre- (A) (n=5)

and post -session (B) groups (n=4). Each point is the mean ± S.E.M. of

one administration in each rat.
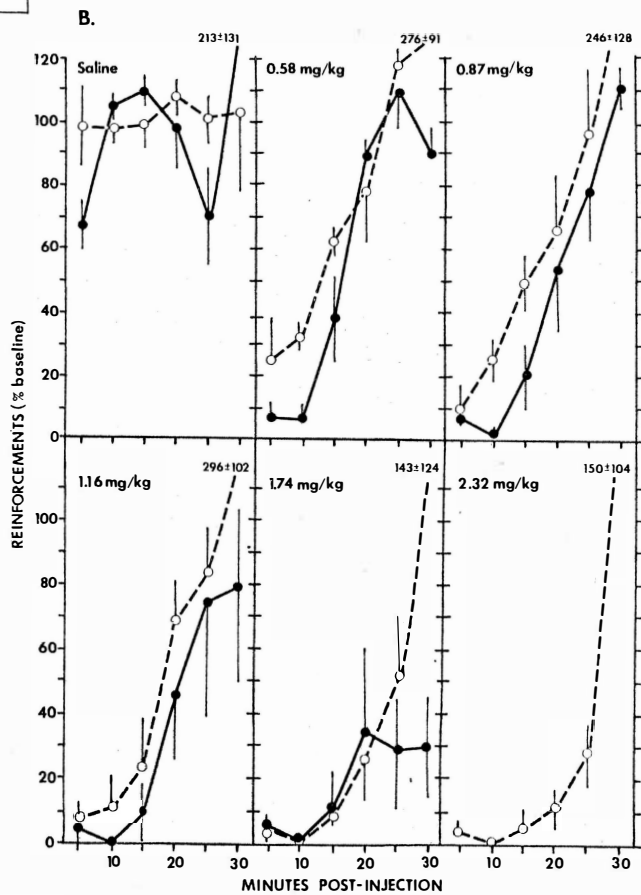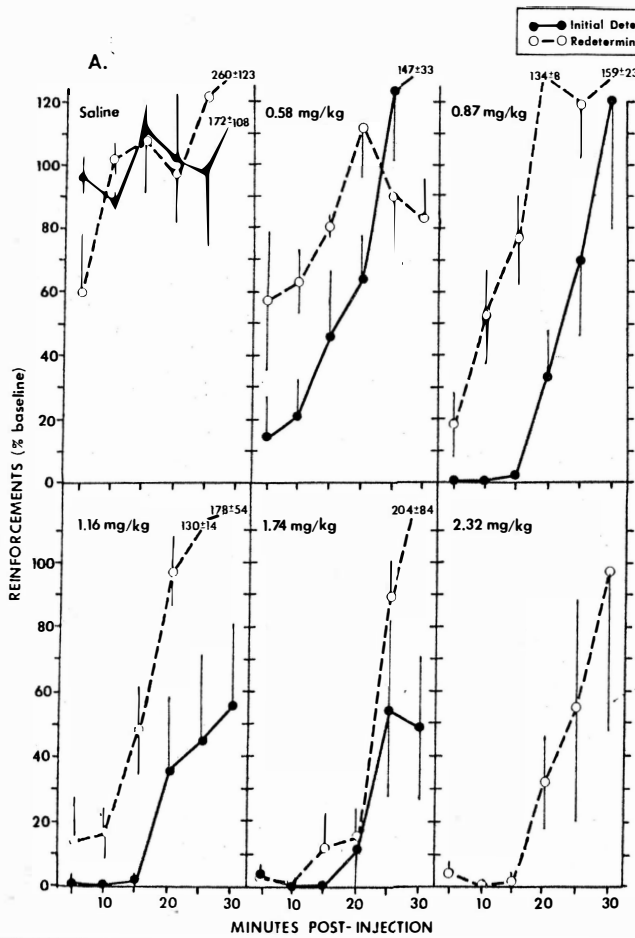
(Table 4). The effect of 0.87 mg/kg arecoline on day 1 of chronic treatment in the presession group was significantly less than the response to the same dose during the initial determination (paired Student's t-test; df=4; t=3.97; p<0.02). By days 7 through 11 (Block 2) of chronic treatment, the response of the presession group after 0.87 mg/kg had plateaued at approximately 85% of control.

On day 12 of chronic treatment, the post-session group was injected with 0.87 mg/kg prior to the session, to test for tolerance development, and the presession group was injected with saline to assess for baseline changes. The data is compared with the response of the pre-and post-session group on day 11 to their normal injections of arecoline and saline, respectively (Table 5). The saline response in the presession group was slightly increased over its baseline level, but was not significantly different from the response of the post-session group on day 11 (Student's t-test; df=7; t=1.45; p>0.2). The effect of 0.87 mg/kg of arecoline in the post-session group on day 12 was not significantly different than the effect of the presession group. Thus, after two weeks of chronic treatment, the degree of tolerance was similar in both groups.

For the post-session group, the effect of 0.87 mg/kg on day 12 was similar to that during the dose-effect determination (67.8 ± 9.3 and 58.7 ± 9.8, respectively). In the presession group, the effect of 0.87 mg/kg on day 11 was less than that during the dose-effect determination (72.0 ± 7.7 and 91.1 ± 6.6, respectively), although the difference was not significant (paired Student's t-test, df=4; t=1.84; p>0.2).

TABLE 4

Effect of Arecoline (0.87 mg/kg/day) on Total Reinforcements
on an FR-20 Schedule During Chronic Treatment

	Presession Group (n=5)	Post-Session Group (n=4)
Day 1	58.8 ± 8.4	112.3 ± 5.1
Block 1	55.2 ± 5.3	101.0 ± 4.9
Block 2	83.2 ± 4.9	110.5 ± 7.0
Block 3	85.4 ± 7.4	97.8 ± 13.8
Block 4	88.8 ± 5.3	108.5 ± 7.9
Block 5	82.8 ± 6.9	99.3 ± 10.3
Block 6	74.8 ± 7.1	76.8 ± 13.9
Block 7	86.6 ± 8.1	106.8 ± 7.8
Block 8	77.4 ± 6.9	97.5 ± 13.8
Block 9	85.4 ± 4.1	104.3 ± 11.3

Each value is the group mean ± S.E.M. Except for Day 1, all data are
derived from three to five day means for each animal, which are aver-
aged across animals.

TABLE 5

Effect of Saline and Arecoline (0.87 mg/kg) in Pre- and Post-Session

Groups on Days 11 and 12 of Chronic Treatment

	SALINE	ARECOLINE
Presession (n=5)	121.2 ± 14.6	72.0 ± 7.7
Post-session (n=4)	96.8 ± 4.2	67.8 ± 9.3

On day 12, arecoline (0.87 mg/kg) and saline were injected immediately before the session, in the post-session and presession groups, respectively. This data is compared to the saline and arecoline response in the post-session and presession groups, respectively, from day 11. Each value is the mean ± SEM.

DISCUSSION

In the present study arecoline induced a dose-dependent decrease in VI and FR responding. Arecoline was approximately twice as potent in decreasing FR as VI responding. The dose-effect and time course of the arecoline effect was similar to that observed by Olds and Domino (1969) who studied the effects of arecoline on responding for electrical stimulation of the lateral hypothalamus. Pradhan and Dutta (1970b) also observed a dose-related decrease in spontaneous motor activity as well as FR, FI, DRL, and Sidman avoidance responding after arecoline administration.

The response of rate decreases produced by arecoline in the present study were antagonized in a dose-related manner by atropine sulfate and were not affected by the doses tested of either atropine methylnitrate or mecamylamine. This suggests that the behavior-suppressant effects of arecoline are mediated through stimulation of central muscarinic receptors. Even though other doses of atropine methylnitrate and mecamylamine were not tested, it is expected that higher doses of these two drugs would not specifically antagonize the behavioral effects of arecoline. Olds and Domino (1969) observed similar effects of selective antagonists on the behavioral effects of arecoline, when testing scopolamine, methylscopolamine, and mecamylamine. Pradhan and Dutta (1970b) antagonized the effect of arecoline on spontaneous motor activity with scopolamine but not with methylscopolamine or mecamylamine. In contrast, they could not antagonize the depressant effect of arecoline on FR responding with a dose of scopolamine which by itself decreased responding.

An alternative explanation for the antagonism of the arecoline-induced behavioral disruption by atropine is that atropine, which has been demonstrated to possess rate-dependent effects (Boren and Navarro, 1959) acted to increase low rates of behavior, by nonpharmacological antagonism. According to the rate-dependent hypothesis, atropine would act to increase low rates of behavior, whether they were schedule or drug-induced. However, in the same rats used in this study, atropine did not antagonize the response-depressant effect of nicotine. This supports the hypothesis that the antagonism of the arecoline effect is due to pharmacological antagonism and not rate-dependent effects.

When arecoline was administered immediately prior to the session to rats responding on a VI-15 second schedule of reinforcement, tolerance developed to some of the behavioral effects of the drug. No tolerance was observed when comparing the initial and redetermined dose-effect curves for total responses for the session. In contrast, tolerance developed to the decrease in total reinforcements received after 1.16 and 1.74 mg/kg arecoline. The degree of tolerance that developed was not very great, being approximately 2.0 fold. To try to account for the lack of tolerance to the effect on total responses, while tolerance developed to the effect on total reinforcements, the time-course of the initial and redetermined dose-effect curves was compared. Examination of cumulative records revealed that the period of response suppression appeared attenuated. This was observed as a group trend upon examination of the time-effect curves. Examination of the response-reinforcement relationship on a VI schedule of reinforcement can explain how a small increase in percent control responses can produce a large increase in percent control reinforcements. On a VI schedule, the first response

after a variable interval of elapsed time produces a reinforcement. Thus it is possible to alter moderate or high rates of responding (either increase or decrease) without changing the number of reinforcements received. However, small increases, in very low rates of responding can produce large increases in the relative number of reinforcements received. It appears that in the present experiment, with chronic arecoline treatment, the rats altered their pattern of responding, and increased it at points where it was low, to enable them to earn more reinforcements.

The second experiment examined the development of tolerance to the effects of arecoline on a FR-20 schedule of reinforcement. In contrast to the VI schedule, it was anticipated that on a schedule where responses were directly related to reinforcements, that tolerance to the response suppressant-effects of arecoline would develop. This was in fact observed.

Greater tolerance, as observed by a shift in the ED_{50}'s for the dose-effect relationship, was observed in the presession group compared to the post-session group. This interpretation is partially confounded by the change in the effect of 0.87 mg/kg in the presession group when comparing the initial determination and the first day of chronic treatment. The reason for this change is not evident. The initial decrease in reinforcement in the redetermined saline point in the presession group may be attributed to a conditioning of the drug effect in the animals, in which they anticipate the onset of the drug effect, and hence do not begin to respond immediately.

Three factors in tolerance development, that can be recognized by the processes they involve, are dispositional, physiological and behavioral

factors. One theory of behavioral tolerance is the reinforcement-loss hypothesis put forth by Schuster et al. (1966). This hypothesis stresses the interaction of the contingencies of reinforcement with the drug-induced behavioral change as an extremely important variable affecting the development of behavioral tolerance.

The data from the present experiments support the role of reinforcement loss as a factor in the development of tolerance to the behavioral effects of arecoline. In the first experiment, tolerance developed to the reinforcement-suppressant effect of arecoline. Tolerance to the effect of arecoline on total responses was not observed, but a change in the pattern of responding, which enabled the animals to earn more reinforcements, was observed.

The second study demonstrated that when drug administration produced reinforcement loss (presession group), more tolerance developed than when drug administration did not produce reinforcement loss (post-session group). This has been termed behaviorally augmented tolerance by LeBlanc et al. (1976). Similar results have been reported for ethanol (LeBlanc et al., 1978), phenobarbital (Tang and Falk, 1978) and phencyclidine (Woolverston and Balster, 1979). The development of tolerance in the post-session group demonstrates that other factors, such as dispositional or physiological mechanisms, play a role in the development of tolerance to the effects of arecoline on behavior, as well.

IV. EXPERIMENT 2. ACUTE AND CHRONIC EFFECTS OF NICOTINE ON SCHEDULE-CONTROLLED BEHAVIOR

INTRODUCTION.

Nicotine, via tobacco, is one of the most widely and most often used drugs in our society. In man, tolerance develops to the initially aversive effects of nicotine (nausea, dizziness) (Beckett, 1971). In animals, tolerance to the effects of nicotine on locomotor activity can develop rapidly and persists for a long time after drug administration (Stolerman et al., 1974). Tolerance also develops to the effect of nicotine on operant behavior. However, few studies have sought to study the relative role of behavioral, physiological, and dispositional factors in the development of a tolerance to nicotine. The experimental design of using two groups, one receiving pre- and the other post-session injections (Chen, 1968), was used to assess the role of behavioral factors in the development of tolerance to nicotine.

To test if the effects of nicotine on operant behavior that we were assessing were of central origin, the interactions of selective cholinergic antagonists with the behavioral effects of nicotine were also assessed.

METHODS

Experiment A. Acute effects of nicotine on VI responding alone and in the presence of cholinergic antagonists. Five male Sprague-Dawley rats trained to respond on a VI-15 second schedule of reinforcement were used in this experiment. These animals also served as experimental subjects in Experiment I. The effects of three doses of nicotine (0.29, 0.57,

and 1.14 mg/kg) on operant behavior were assessed in all rats. The
interaction of selective cholinergic antagonists (mecamyline HCl, hexa-
methonium Cl, and atropine sulfate) with 1.14 mg/kg nicotine was assessed
in three rats per antagonist. Doses of antagonists were based on pre-
vious research in this laboratory. Antagonists were injected (s.c.) 25
minutes prior to, and nicotine (s.c.) and saline (s.c.), were injected
immediately prior to the session. Sessions had a 30 minute duration.
At least four control days separated nicotine tests. Seven days sepa-
rated tests after antagonists were administered. The different doses of
nicotine and antagonists were administered in a counter-balanced se-
quence. This data was collected after the acute arecoline dose-effect
and antagonism study, and prior to the beginning of the arecoline toler-
ance study.

Experiment B. Effects of chronic nicotine administration. Ten male
Sprague-Dawley rats were trained to respond on a VI-15 second schedule
of reinforcement for sweetened milk. Sessions had a duration of 30
minutes. Saline was injected (s.c., 0.1 mg/100 gm body weight) imme-
diately prior to each daily session. When responding was stable, the
dose-effect relationship of the behavioral effects of nicotine on VI
responding was assessed. Four doses of nicotine (0.29, 0.52, 1.14, and
2.28 mg/kg nicotine) were tested in a counter-balanced order. At least
four control days separated test days.

After determination of the effects of single injections of nicotine
on behavior, the animals were divided into two groups (five rats/group),
matched for response rates and the initial dose-effect relationship.
For the remainder of the experiment, one group received 2.28 mg/kg

immediately prior to the session (presession group), while the other group received the same dose of nicotine after the session (post-session group). For the first 12 days of chronic treatment, the post-session group received its nicotine injection five to ten minutes after the session. The post-session injection interval was increased to 30 to 45 minutes for the remainder of the experiment. One rat in the presession group died of respiratory illness after 15 days of chronic treatment. The data from this subject is not included in the analyses.

On the twelfth and twenty-fifth days of treatment, the effect of saline and 2.28 mg/kg nicotine were assessed in the pre- and post-session groups, respectively. This was done to determine if the post-session group was developing tolerance to nicotine and if the presession group's baseline (saline) responding had altered.

Between sessions 36 and 66, dose-effect functions of nicotine were redetermined. During this period, rats continued to receive nicotine pre- and post-session, except that every fifth or sixth day, a different dose of nicotine or saline (in the presession group) was substituted for the normal injection. Doses of 0.29, 0.57, 1.14, and 2.28 mg/kg of nicotine were tested in a counter-balanced order. In addition, at the end of the dose-effect redetermination, the effect of 3.42 mg/kg nicotine was assessed in all animals.

Data analysis. The parameters used to evaluate the effect of nicotine on operant behavior in the VI schedule were as follows: (1) total responses/session; (2) total reinforcements/session; (3) responses for consecutive five-minute segments (1-5 minutes, 6-10 minutes, etc.) of the 30-minute session; and (4) reinforcements for consecutive five-

minute segments. Due to individual differences in total responses and reinforcements, the data is presented as percent change from baseline, averaged across animals.

For analysis of acute drug effects and their antagonism, the two preceeding saline days for each test were averaged and used as the control for that test. For analysis of the chronic drug regimen, the four saline days preceeding the chronic regimen were averaged and used as control baseline. Data for the entire period of chronic drug treatment is expressed as percent control of this four day baseline. The saline points that were redetermined with the dose-effect relationship during chronic drug treatment are expressed as percent control of this four day baseline and represent changes that may have occurred in baseline responding due to drug effects and/or time. These saline points were used as the baseline in redetermining the dose-effect curve, and data are expressed as percent control of them.

RESULTS

Acute effects of nicotine on behavior and interaction with antagonists. The response pattern and baseline rates for these rats is reported in Experiment 1. Nicotine administration produced a dose-related decrease in total responses for the session and for the five-minute period of greatest decrease in responding (Table 6). The onset of the nicotine induced response-suppressant effect was approximately two minutes after injection. The peak effect occurred approximately five to ten minutes after administration. The effects of 1.14 mg/kg nicotine on total responses and on response rate at the time of peak effect were antagonized by mecamylamine (0.5 mg/kg) but not by either hexamethonium (1 mg/kg) or atropine sulfate (4 mg/kg) (Table 6).

TABLE 6

Group Comparison of Dose-Effect Relationship of Nicotine

and Effects of Antagonists

Drug	Dose (mg/kg)	N	% Control Total Respones	% Control Greatest Decrease
Saline	0.1 ml/100 gm	5	96.6 ± 2.5	77.9 ± 3.8
Nicotine	0.29	5	104.8 ± 5.7	51.4 ± 7.6
	0.57	5	65.2 ± 14.4	30.8 ± 13.4
	1.14	5	48.4 ± 14.7	7.6 ± 2.0
Nicotine	1.14			
+ Mecamylamine	0.5	3	100 ± 15.4	80.0 ± 12.5
+ Hexamethonium	1.0	3	45 ± 12.4	5.3 ± 2.0
+ Atropine sulfate	4.0	3	44 ± 15.0	15.3 ± 8.9

Antagonists were administered (s.c.) 25 minutes prior to the session. Nicotine and saline were administered (s.c.) immediately prior to the session. Each value is the mean ± S.E.M.

The time-courses of the initial effect of different doses of nicotine on VI-15 second responding are presented in Figure 8. The data for both the pre- and post-session group were combined. The magnitude of the initial decrease in responding was dose-related. The peak effect occurred approximately five minutes after injection, responding then returned towards control levels. The highest dose (2.28 mg/kg) suppressed responding for the entire session.

Dose-effect functions before and during the daily injection regimen. Under control conditions, typical VI responding was observed in all animals. Response rates were stable within rats, whereas there were individual response rate differences between subjects. The range of response rates between rats was from 0.47 ± 0.02 to 1.54 ± 0.22 responses/ second. The average response rates for the pre- and post-session groups were 1.01 ± 0.22 and 0.87 ± 0.13 responses/second, respectively. Due to the individual differences in response rates, the data was calculated as percent change from baseline rates, averaged across animals. The average total reinforcements received for the 30-minute session for the pre- and post-session groups were 95.3 ± 4.4 and 87.6 ± 5.1, respectively.

Tables 7A and 7B show the effects of nicotine on overall responding in the initial determination and redetermination of the dose-effect function in both groups of rats. Under baseline conditions, total responding varied by approximately 10 to 15% of the previous day's total. The initial dose-effect relationships for nicotine on total responding were similar in both groups of rats. At the three lower doses tested (0.29, 0.57, and 1.14 mg/kg), the effect of nicotine was

Figure 8. Time-effect relationship for saline and four doses of nico-
tine on VI-15 second responding. Each point is the mean of one adminis-
tration of each of nine rats.
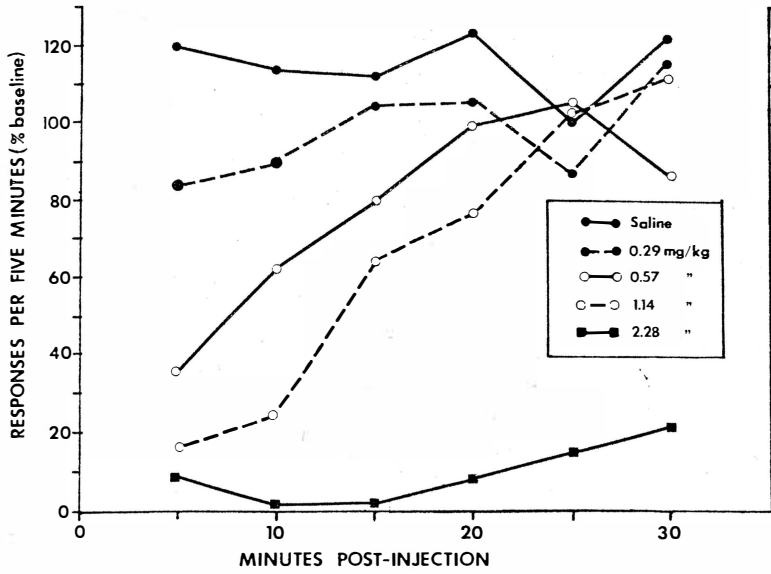
TABLE 7

Initial and Redetermined Dose-Effect Relationships
for Nicotine in Presession and Post-Session Groups

A.   Total Responses (% Baseline)

Dose	Presession Group (n=4)		Post-session Group (n=5)	
(mg/kg)	Initial	Redetermination	Initial	Redetermination
Saline	114.6 ± 5.6	117.8 ± 21.4	106.8 ± 5.1	123.4 ± 9.0
0.29	79.5 ± 11.6	102.0 ± 7.3	107.2 ± 25.8	86.4 ± 9.9
0.57	88.9 ± 8.9	84.3 ± 18.1	72.8 ± 30.2	96.8 ± 15.5
1.14	59.5 ± 19.8	65.8 ± 16.4	63.8 ± 15.2	80.6 ± 14.9
2.28	6.3 ± 4.6	63.0 ± 7.2	12.6 ± 4.9	56.2 ± 17.6
3.42	-	35.0 ± 13.4	-	19.8 ± 2.9

B.   Total Reinforcements (% Baseline)

Dose (mg/kg)	Initial	Redetermination	Initial	Redetermination
Saline	104.6 ± 4.3	101.0 ± 8.0	106.2 ± 3.8	100.6 ± 5.1
0.29	95.3 ± 2.8	96.0 ± 4.5	96.0 ± 7.3	100.2 ± 6.3
0.57	98.5 ± 4.7	95.5 ± 7.6	70.4 ± 17.9	90.4 ± 3.4
1.14	66.5 ± 15.1	87.0 ± 8.2	87.0 ± 6.3	93.8 ± 3.3
2.28	19.3 ± 15.0	94.0 ± 8.2	30.8 ± 10.3	86.6 ± 5.5
3.42	-	56.0 ± 21.9	-	59.2 ± 3.4

Values for total responses (A) and total reinforcements (B) are mean ± S.E.M.

variable. Most rats exhibited either no change or a 25 to 50% decrease from baseline responding. The effects of nicotine were usually characterized by an initial decrease in responding, the duration and magnitude of which were dose related. Responding then resumed at rates which eventually approached or exceeded control values. However, there were individual exceptions at different doses. The responding of one rat in the presession group was almost completely suppressed by 1.14 mg/kg. In the post-session group, one rat increased its responding to 200 and 175% of control after 0.29 and 0.57 mg/kg, respectively. The responding of a different rat in this group was almost completely suppressed by 0.57 mg/kg. The variability was not related to baseline response rates. At 2.28 mg/kg, the responding of most rats was almost completely and uniformly disrupted. This dose was selected for chronic administration since it consistently produced a decrease in total responses and reinforcements, whereas lower doses did not.

After 36 days of chronic treatment, control (saline) response totals as well as the nicotine dose-effect curve were redetermined (Table 7A). Compared to the initial baseline, total responses after saline administration were increased in the post-session group, while no change was observed in the presession group. The data for the redetermination of the nicotine dose-effect curve is expressed as percent control of the redetermined saline baseline. The redetermined dose-effect curves for nicotine were similar in both groups of rats. Compared to the initial determination, there was very little or no change in total responses after 0.29, 0.57, and 1.14 mg/kg nicotine. In both groups, the response suppression induced by 2.28 mg/kg was decreased from approximately 10 to 60% of baseline values in the initial and redetermined dose-effect

relationships, respectively. Increasing the dose of nicotine to 3.42 mg/kg produced a slightly greater decrease in responding in the post-session versus the presession group (20 and 35% of baseline, respectively).

A three-factor, mixed, repeated measures, analysis of variance was performed on the initial and redetermined dose-effect relationships for total responses in both groups of rats. The factors were determinations (initial versus redetermination), groups (pre- versus post-session), and doses (saline plus 0.29, 0.57, 1.14, and 2.28 mg/kg nicotine). The analysis yielded the following results. There was a significant effect cf the dose factor (df=4,28; F=162.1; p<0.001), indicating a dose-effect relationship. The determinations factor was almost significant (df=1,7; F=3.9; 0.05>p<0.1), indicating that the difference between determinations was almost significant. There was no significant effect of the following factors: (1) groups (df=1,7; F<1.0; p>0.2), indicating that the overall response of the two groups was similar; (2) groups x determinations interaction (df=1,7; F<1.0, p>0.2), indicating that the two groups responded similarly within each determination; (3) groups x doses interaction (df=4,28; F=1.1; p>0.2), indicating that the groups responded similarly at the different dose levels; and (4) group x determination x dose interaction (df=4,28; F<1.0; p>0.2), indicating that there was no significant overall interaction.

The dose-effect relationships for nicotine on total reinforcements, before and during chronic drug treatment in the pre- and post-session groups, are shown in Table 7B. For both groups, the total reinforcements received after saline were approximately 100% of control in the initial as well as the redetermined dose-effect relationships. In

general, the initial dose-effect relationship for nicotine was similar in both groups of rats. The lowest dose (0.29 mg/kg) had no effect on the total reinforcements received in either group. The intermediate doses (0.57 and 1.14 mg/kg) had little or no effect on total reinforcements received. The decrease observed after 1.14 mg/kg in the presession group and 0.57 mg/kg in the post-session group can be accounted for by an exaggerated response in one animal in each group, as compared to the other rats. The highest dose (2.28 mg/kg) decreased reinforcements received to 20 and 30% of control in the pre- and post-session groups, respectively.

The redetermined dose-effect curves were similar in both groups of rats. Doses of 0.29, 0.57, and 1.14 again produced very little or no change in the total reinforcements received. The rats that had exhibited greater reinforcement decreases after 0.57 and 1.14 mg/kg of nicotine in the initial dose-effect determination were indistinguishable from the other rats in the dose-effect redetermination. Thus, these animals demonstrated tolerance to the decrease in reinforcements induced by intermediate doses of nicotine. The total reinforcements after 2.28 mg/kg was approximately 90% of baseline totals for both groups, demonstrating equal degrees of tolerance had developed. Increasing the dose of 3.42 mg/kg decreased the reinforcements received to 60% of control in both groups.

A three factor, mixed, repeated measures, analysis of variance was performed on the initial and redetermination of the dose-effect relationships in both groups of rats. The factors were determinations (initial versus redetermination), groups (pre- versus post-session), and doses (saline plus 0.29, 0.57, 1.14, and 2.28 mg/kg nicotine). The

analysis yielded the following results. There was a significant dose effect (df=4,28; F=17.3; p<0.001), indicating there was a dose-effect relationship. The determination x dose interaction was significant (df=4,28; F=9.66; p<0.001), indicating that the dose-effect relationship changed between determinations. This may be accounted for by the lack of effect of 2.28 mg/kg in decreasing reinforcements in the redetermination. There was no significant effect of the following factors: (1) groups (df=1,7; F<1.0; p>0.2), indicating the overall response of the two groups was similar; (2) determinations (df=1,7; F<1.0; p>0.2), indicating no overall difference between the initial and redetermination of the dose-effect relationship (i.e., no significant overall tolerance development); (3) group x determination interaction (df=1,7; F<1.0; p>0.2), indicating that the two groups responded similarly within each determination; (4) group x dose interaction (df=1,28; F=1.66; p>0.2), indicating that the groups responded similarly at the different dose levels; and (5) group x determination x dose interaction (df=4,28; F=1.35; p>0.2), indicating that there was no significant overall interaction.

Effects during the daily injection regimen. The effect of 2.28 mg/kg of nicotine on the first day of chronic treatment in the presession group was similar to that observed during the initial dose-effect determination for both measures (percent control responses, Figure 9A and percent control reinforcements, Figure 9B). During the period of daily administration of 2.28 mg/kg nicotine, the percent control responding in the presession group increased from approximately 15% of control on days 2-5 (Block 1) to 75% of control by days 45-49 (Block 8) (Figure 9A). It remained at approximately 75% of control for the remainder of the study.

Figure 9. Effect of chronic nicotine treatment on total responses (A) and total reinforcements (B) in pre- (n=4) and post-session (n=5) groups. All points are group mean ± S.E.M. Except for Day 1, all data are derived from four to five day means for each animal, which are averaged across animals.

A. TOTAL RESPONSES

B. TOTAL REINFORCEMENTS

PER CENT BASELINE

PRESESSION GROUP
POST-SESSION GROUP

DAY 1

BLOCKS OF DAYS
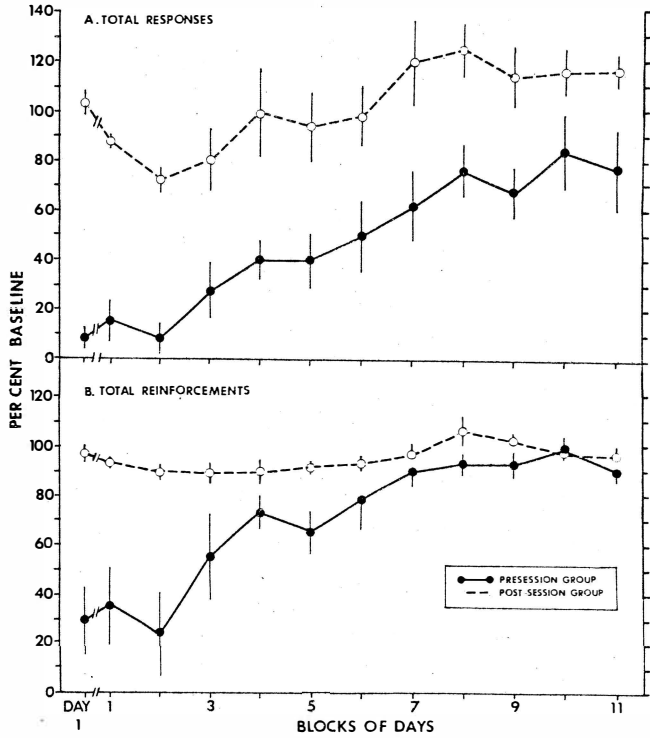
Over the first two five-day blocks of chronic treatment, there was a decrease in the percent control responding in the post-session group (Figure 9A). During this phase of the experiment, the rats received their nicotine injections five to ten minutes after the session. Beginning with the third five-day block, the time of injection was delayed to 30 to 45 minutes post-session. The response totals increased back towards and eventually increased by 15 to 20% over control levels after the post-injection was delayed.

Percent control reinforcements in the presession group increased from approximately 30% of control on day 1 to 90 to 95% of control by days 39-42 (Block 7) (Fig. 9B). They remained at this level for the remainder of the experiment. The reinforcements received by the post-session group remained at 90 to 100% of control throughout the period of chronic drug administration despite the changes that occurred in percent control responding (Figure 9B).

Figure 10 presents the effects of 2.28 mg/kg nicotine and saline on percent total responses and reinforcements in both groups at various time points in the experiment. Injection of 2.28 mg/kg initially produced a similar decrease in both groups on the measures of total responses and total reinforcements (Figures 10A and 10B, respectively). Administration of 2.28 mg/kg nicotine to the post-session group reduced total responding to approximately 40 and 80% of control on days 12 and 25, respectively (Figure 10A). In contrast, the same dose decreased total responding in the presession group to 10 and 40% of control on days 11 and 24, respectively. Thus, the presession group demonstrated less tolerance development than the post-session group on these days. Equal degrees of tolerance were evident when the dose-effect relationship was redetermined (days 36+).

Figure 10. Effect of 2.28 mg/kg nicotine (A and B) and saline (C and D)
at different times, prior to and during the period of chronic drug
administration. Effect on total responses (A and C) and total rein-
forcements (B and D) are presented. The "Initial" data represents the
initial determination. On Days 11-12 and 24-25, the normal presession
injections were switched, the presession group (n=4) received saline and
the post-session group (n=5) received 2.28 mg/kg nicotine. The data for
36+ represents the dose-effect redetermination. Bars represent the
group mean ± S.E.M. of one administration in each rat.
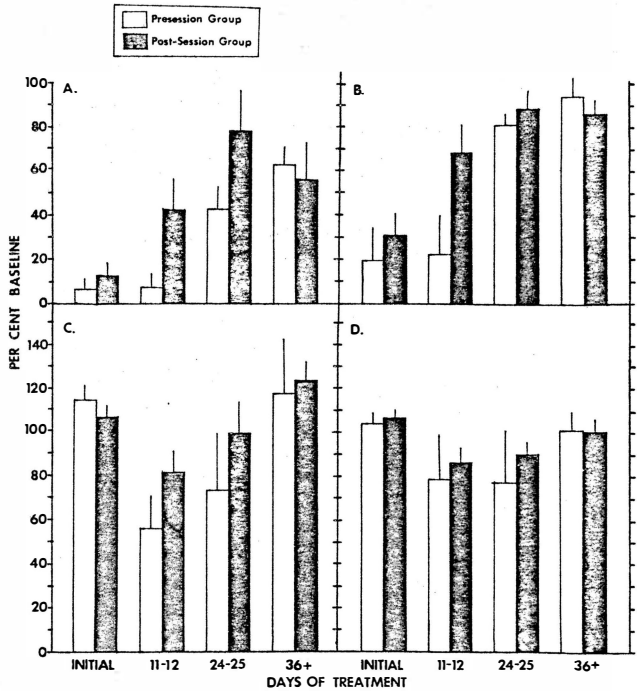
On days 11-12, 2.28 mg/kg decreased the reinforcements received to a greater extent in the presession versus the post-session group (Figure 10B). However, by days 24-25, despite the fact that there was a difference in total responses, the percent control reinforcements after 2.28 mg/kg were similar for both groups.

Initially, the percent of baseline total responses (Figure 10C) and total reinforcements (Figure 10D) after saline administration were similar for both groups. When the saline effect was re-evaluated on days 11-12, total responding in the pre- and post-session groups were 55 and 80% of control (Figure 10C), respectively. The total reinforcements were decreased to approximately 80 to 85% of control (Figure 10D). On days 24-25, saline behavior was at previous baseline levels for the post-session group, while total responses and total reinforcements for the presession group were approximately 75% of baseline. By the redetermination of the dose-effect relationship, the responses and reinforcements after saline were at prechronic treatment baseline levels. The reduction in the presession group's total responses after saline on days 12 and 25, as compared to the post-session group, may account for part of, but not all of, the difference in the effect of 2.28 mg/kg on total responding between the two groups on these days. In contrast, the total reinforcements after saline were similar on day 12 for both groups. Thus, the greater decrease in reinforcements after 2.28 mg/kg in the presession group compared to the post-session group on this day was not affected by a change in the baseline reinforcement total.

DISCUSSION

The acute effects of nicotine on VI responding observed in this study are different than those reported by Morrison (1967). Morrison observed an initial decrease with 0.4 and 0.2 mg/kg (free base; equivalent to 1.14 and 0.57 mg/kg nicotine bitartrate). This was followed by an increase in response rates. Lower doses produced only a stimulation of response rates. In the present study, only decreases in response rate were observed in a similar time frame as that in the study by Morrison (1967). The different results may be explained by the different VI schedules used in the two studies. Morrison (1967) used a VI-2 minute schedule compared to a VI-15 second used in the present study. Typically, long interval schedules produce lower rates of responding than do short-interval schedules. If the effects of nicotine are rate-dependent, as has been demonstrated in some situations, then only rate decreases will be observed on short-interval schedules with a high rate of responding and both rate decreases and increases will be observed on long-interval schedules with low rates of responding.

In the present study, the response rate decreasing effects of nicotine were antagonized by mecamylamine and not by atropine sulfate or hexamethonium. Vaillant (1967) and Stitzer et al. (1970) also observed that atropine and scopolamine, respectively, did not antagonize the behavioral effects of nicotine. These studies contrast with those of Morrison et al. (1969) and Olds and Domino (1969), in which atropine and scopolamine, respectively, antagonized the rate-depressant effects of nicotine. The contradictory results of the previous studies may be due to differences in doses tested, baseline response rates, and species used. In the present study, it is expected that higher doses of atro-

pine would have antagonized the response-rate decreasing effects of
nicotine through rate-dependent actions, not through pharmacological
antagonism. A systematic comparison of the interaction of atropine and
scopolamine with behavioral paradigms and other drugs would provide
useful information. Doses of hexamethonium (Stitzer et al., 1970) and
chlorisondamine (Morrison et al., 1969) that were in excess of the doses
needed for peripheral cholinergic antagonists have been demonstrated to
antagonize the response-rate suppressant effects of nicotine. These
effects may be due to the entry of these quaternary compounds into the
CNS (McIsaac, 1962; Paul-David et al., 1960). These studies, using high
doses of peripheral antagonists, that may enter the CNS, only confuse
the issue of whether or not the response-rate suppressant effects of
nicotine are peripherally or centrally mediated.

The major objective of this experiment was to investigate the role of
behavioral factors in influencing the development of tolerance to the
disruption of operant behavior produced by nicotine administration. It
was hypothesized that rats receiving daily injections of nicotine imme-
diately prior to the behavioral session would either develop tolerance
at a faster rate or to a greater degree than rats that received nicotine
injections after the behavioral session. The findings were the opposite
of the prediction. The rats that received the post-session nicotine
injections developed tolerance to nicotine at a faster rate than did the
presession group. After 12 days of chronic treatment, the post-session
group exhibited approximately four-fold tolerance to the response sup-
pressant effects of nicotine. In contrast, there was no evidence of
tolerance in the presession group at this time. However, similar
degrees of tolerance were observed in both groups after nicotine injec-

tions later in the study. These results differ from studies with
ethanol (LaBlanc et al., 1978), phenobarbital (Falk and Tang, 1978), and
phencylidine (Woolverton and Balster, 1979) in which presession drug
administration led to either a greater or faster tolerance development
than did the post-session group. The reasons for the different results
between the present studies and the other studies are not evident.
There were no grossly-observable changes in either group (body weight
change, respiratory illness, etc.). The explanation for this data must
take into account an interaction between the drug, the environment, and
the experimental contingencies. One possible explanation is that 2.28
mg/kg nicotine has aversive properties that produce response rate sup-
pression, and that with repeated pairings of the aversive effect of
nicotine with the operant chamber in the presession group, the aversive
effect of nicotine became associated with (conditioned to) the experi-
mental situation. Thus, after repeated pairings, the chamber elicited
the response-suppressant effect produced by nicotine administration.
Explained in classical conditioning terms, the aversive effects of
nicotine (unconditioned stimulus, US) elicited a suppression of level
pressing (unconditioned response, UR). The UR was paired with the
experimental chamber and injection procedure (conditioned stimulus, CS),
such that the CS could now elicit the suppression of lever pressing.
This is partially supported by the decreased response and reinforcement
totals when saline was adminstered to the presession group on day 12.
Further controlled experiments are necessary to test this hypothesis of
conditioned aversion to the effects of nicotine.

An alternate explanation is that nicotine administration had a debi-
litating (toxic) effect on the animals, that was not observable by gross

observations, but did affect operant behavior. This debilitating effect carried over to the next day and acted to potentiate the behavioral effect of the next injection of nicotine. This carry over effect may explain the decreased saline response totals in the presession group on day 12 of chronic treatment, and why the saline responding of the post-session group was suppressed over the first 14 days of chronic drug administration.

Upon redetermination of the dose-effect relationship, tolerance was observed at two doses. Tolerance was demonstrated by a decreased effect of 2.28 mg/kg on both total response and reinforcements. Tolerance to the effects of 3.42 mg/kg, which was tested only in the redetermination, is inferred, since on retest it produced effects that were less than the original effects of 2.28 mg/kg. The lack of tolerance development at lower doses may be related to the fact that initially their effects were negligible and variable.

Morrison and Stephenson (1972) reported the development of tolerance to the decreases produced by 2.28 mg/kg on locomotor activity. After three to five days of chronic treatment, 2.28 mg/kg increased motor activity. This change in the response was not observed in this study. The present data supports and extends the findings of Domino and Lutz (1973) and Todd and Dougherty (1979) that tolerance develops to the effects of nicotine on operant behavior in rats. In the two previous studies, the effects of 0.25 mg/kg nicotine (i.p.) administered twice a day, on FR responding was assessed. Both studies reported rapid toler-ance and complete development (within 7 to 15 days). The present study demonstrated complete tolerance to the effect on 2.28 mg/kg nicotine on reinforcements received after 25 days of chronic treatment. Less toler-ance was observed to the effect of nicotine on total responses.

V. EXPERIMENT 3. CHARACTERIZATION OF THE DISCRIMINATIVE STIMULUS EFFECTS
OF ARECOLINE

INTRODUCTION

The discriminative stimulus (DS) paradigm provides a specific and
sensitive task in which to study the pharmacological properties of drugs
which produce CNS effects. Arecoline, a central muscarinic agonist,
studied in several neuropharmacological systems, as yet has not under-
gone a systematic evaluation of its DS properties. The present experi-
ments were designed to study dose parameters under which arecoline can
exert DS control and to characterize the pharmacological specificity of
the DS produced by this drug.

METHODS

Initial Training: One lever in chamber. Twelve 60-90 day old male
Sprague-Dawley rats, reduced to approximately 80% of their normal body
weight by restricted feeding, were trained to press one lever in a
two-lever operant chamber for milk reinforcement. This lever was
designated as the saline lever. After three to four days of responding
on a CRF, rats were trained to respond on the second (drug) lever. Rats
were injected with either 0.58 (n=6) or 1.74 (n=6) mg/kg of arecoline
and five minutes later were placed in the operant chamber, with only the
drug lever present. Rats usually spontaneously initiated responding on
the lever; some were trained if necessary. Session durations were 15
minutes. After two to three days of CRF responding on the drug lever,
training under saline and drug conditions were alternated. Saline was
administered for two consecutive days, arecoline for two to four conse-

cutive days, with only the state appropriate lever in the chamber. At this time, a VI schedule of reinforcement was instated. The schedule was slowly increased from a VI-3 second to a VI-12 second. The schedule was increased in 3 second increments on each bar, individually. The criteria for advancement was 40 responses in the first five minutes for two cumulative days in each state. When a rat attained a VI-12 second on both levers, discrimination training began.

For one-half of the rats in each group, the left lever was the saline correct lever, and the right lever was the arecoline correct lever. The conditions were reversed for the remaining rats. The 0.58 mg/kg training dose was chosen because it is equimolar with 1.14 mg/kg nicotine bitar-trate, the dose that is optimal for nictoine discrimination studies. The use of the 1.74 mg/kg training dose was based on pilot studies, which suggested that this dose was effective as a DS.

Discrimination training: Both levers in chamber. Rats were injected with arecoline or saline five minutes before being placed in the operant chamber. Both levers were in the chamber. Responses on the state correct lever were reinforced on a VI-12 second. Responses on the incorrect lever had no consequence. Saline and arecoline were adminis-tered in a double-alternation procedure (A,A,S,S, etc.). Responses on each *lever* as well as total reinforcements received were automatically recorded. Discrimination learning was assessed during a two minute non-reinforced period that began the first day of each alternation. The data collected during this non-reinforced period is presented as Percent Drug Bar Responding (% DBR) which is the responses on the drug correct lever/total responses. The overall rate of responding on both levers

during this two-minute period and during training sessions is a measure of drug-induced behavioral disruption. If a rat did not emit at least five responses during the two minute non-reinforced period, this session was extended until the rat emitted five responses. The total time required was recorded and used to calculate the response rate.

Agonist and antagonist testing. The rats that learned to discriminate 1.74 mg/kg arecoline from saline were used in these experiments. Animals continued to receive 1.74 mg/kg of arecoline and saline according to the double alternation sequence. The effects of the following experimental manipulations were assessed in these rats: (1) generalization of different doses of arecoline and antagonism of their effects by atropine, (2) the time course of the discriminative effects of the training dose (1.74 mg/kg) and 1.16 mg/kg arecoline, (3) the effects of selective cholinergic antagonists on the training dose, and (4) the generalization of cholinergic agonists to the DS effect of arecoline. Tests were conducted in a two minute non-reinforced session following the second day of an alternation (A,A,Test,S,S,Test...). This procedure did not disrupt the baseline discrimination. Animals were removed from the chambers after two minutes or after five responses were emitted if animals took longer than two minutes to respond. Sessions were conducted for six consecutive days. At least four training days (one double-alternation) separated antagonist tests. Testing of drugs and doses was randomized so that approximately one-half of the test trials followed an arecoline training day; the rest followed a saline training day. Doses of agonists and antagonists were based on values obtained from a survey of the literature.

The discrimination data are expressed as percent drug bar responding (% DBR) and responses per minute (RPM). ED_{50}'s were derived from linear regression analysis and represent the derived dose that produced 50% DBR.

RESULTS

Acquisition of arecoline discrimination. Due to the disruption of responding produced by 1.74 mg/kg arecoline, the rats in this group were advanced at a slower rate on the VI schedule, as compared to the 0.58 mg/kg group. The total number of days (mean ± S.E.M.) under saline and drug conditions in the initial one lever training segment of the experiment was 24.0 ± 1.5 for the 0.58 mg/kg group and 44.3 ± 3.5 for the 1.74 mg/kg group.

After ten double-alternations, rats did not learn to discriminate 0.58 mg/kg arecoline from saline (Figure 11). In contrast, rats learned to discriminate 1.74 mg/kg from saline (Figure 11). In this group, rats could discriminate arecoline from saline (84% DBR and 26% DBR, respectively) by the second double-alternation. With continued drug and saline administration, the drug and saline appropriate responding increased. The mean percent DBR responding plateaued at approximately 95 to 100% after 1.74 mg/kg arecoline and 5 to 0% after saline.

Table 8 shows the group data for response rates, during both the non-reinforced test period and the reinforced training period, and total reinforcements received, under saline and drug conditions for both groups of rats. The response rates under the saline state, for both the test period and the reinforced period were similar when comparing between groups. The response rate was higher during the reinforced

Figure 11. Acquisition curves for discrimination of arecoline, 0.58 mg/kg (left panel) or 1.74 mg/kg (right panel) versus saline. Each point is the group mean ± S.E.M. of one test in each rat under each condition. N=6, except where noted.

0.58 mg/kg ARECOLINE VS. SALINE   1.74 mg/kg ARECOLINE VS. SALINE
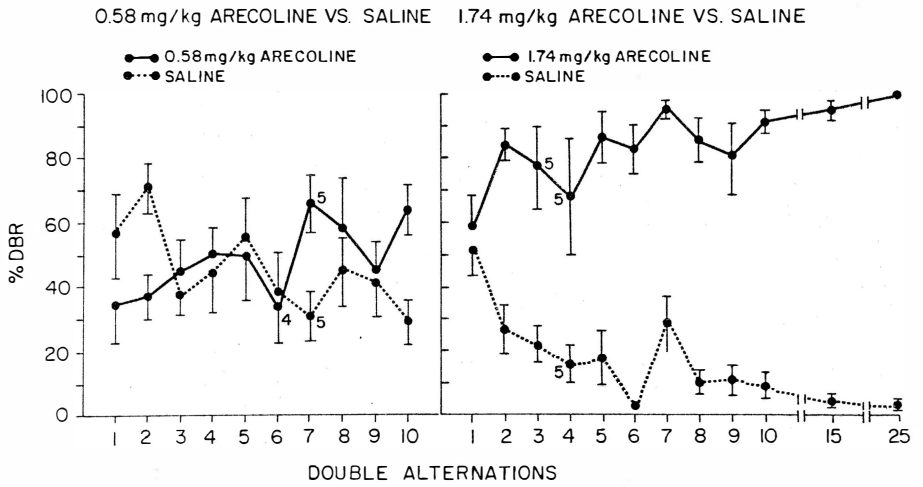
TABLE 8

Response Rate and Reinforcement Comparisons

for Acquisition Period of Arecoline Discrimination

Training Dose	Double Alternation Number	Test Response Rate (RPM)		Training Response Rate (RPM)		Total Training Reinforcements	
		Saline	Arecoline	Saline	Arecoline	Saline	Arecoline
0.58 mg/kg (n = 6)	1-2	14.0±3.3	10.1±1.5	33.2±4.4	24.7±4.6	49.9±1.2	54.3±3.6
	5-6	24.2±7.6	11.2±3.7	42.1±6.4	31.7±7.9	40.3±7.3	56.0±5.5
	9-10	33.8±7.8	11.2±2.1	43.1±7.5	34.8±8.1	53.7±2.0	56.8±2.8
1.74 mg/kg (n = 6)	1-2	17.8±3.7	4.0±0.8	21.0±4.1	15.2±2.4	48.0±4.9	46.6±3.0
	5-6	15.3±6.0	4.3±1.7	32.0±8.7	15.5±2.9	49.5±4.7	44.3±6.9
	9-10	21.9±4.1	4.5±1.3	35.5±6.9	14.1±2.6	47.9±2.4	43.3±2.8
	15	18.9±4.9	3.9±0.8	43.9±7.0	16.1±2.2	53.8±3.5	48.8±3.6
	25	18.4±5.3	5.8±2.1	33.3±6.1	16.0±2.6	50.0±4.3	44.5±4.6

Test response rate determined from two minute nonreinforced period on the first day of each alternation. Training response rate and reinforcement totals determined from 15 minute training session on second day of alternation. Data for each rat fo two double-alternations was averaged and the presented was derived from that group mean ± S.E.M.

training period, compared to the non-reinforced test period. The saline response rate increased with time for both groups under the training condition, while the test period response rate increased only in the 0.58 mg/kg group.

Compared to the saline data, arecoline administration produced a dose-related decrease in the response rates under both testing and training conditions. The test period response rate under the drug state was approximately 50% and 25% lower than saline values, for the 0.58 mg/kg and 1.74 mg/kg dose groups, respectively. Over time, the response rate during the test period remained the same for both groups, even after 25 double alternations in the 1.74 mg/kg group.

The response rates during the training period under the drug state were approximately 25% and 50% lower than the saline response rate for the 0.58 mg/kg and 1.74 mg/kg group, respectively. There was a slight increase in the training response rate for the 0.58 mg/kg group over time, while there was no increase in the response rate for the 1.74 mg/kg group.

In contrast to the arecoline-induced decrease in response rates, the total reinforcements received were similar when comparing within (saline versus arecoline) and between (0.58 mg/kg versus 1.74 mg/kg) groups.

Throughout the study, rats injected with 1.74 mg/kg arecoline exhibited signs of peripheral muscarinic stimulation (salivation, urination, and defecation). These effects were not observed in rats injected with 0.58 mg/kg arecoline.

Pharmacological specificity of the discriminative stimulus effect of arecoline. The six rats that learned to discriminate 1.74 mg/kg areco-

line from saline were used in these studies. The DS effect of arecoline demonstrated a dose-effect relationship (Figure 12A). In contrast, the arecoline-induced decrease in response rates did not appear to be dose related (Figure 12B). The training dose of arecoline, which produced approximately 92% DBR, decreased the responses/minute to 30% of saline response rates. Decreasing the dose to 0.87 and 0.58 mg/kg decreased the discrimination level to approximately 40 and 10% DBR, respectively, the latter value being similar to saline. After 0.87 and 0.58 mg/kg arecoline, the response rates were still decreased to 35 and 50% of the saline response rate, respectively. This data demonstrates a separation of the response-suppressant and discriminative-stimulus effects of arecoline.

The time-effect relationship on discrimination for two doses of arecoline is presented in Figure 13. When tested five minutes after injection, both the training dose (1.74 mg/kg) and a dose two-thirds the training dose (1.16 mg/kg) produced approximately 95% DBR. However, the two doses demonstrated different time-effect curves. At 15 minutes post-injection, 55% drug bar responding was observed after 1.74 mg/kg, while saline-like responding (5% DBR) was recorded after 1.16 mg/kg. At 25 minutes after injection of the training dose (1.74 mg/kg), saline-like responding (2% DBR) was observed. For both doses, when the percent DBR had returned to saline levels, the response rates had increased above saline levels (27.9 ± 10.8 RPM for 1.16 mg/kg at 15 minutes, 24.8 ± 6.5 RPM for 1.74 mg/kg at 25 minutes, compared to 14.8 ± 2.8 RPM for saline at 25 minutes.

Table 9 presents the effects of different antagonists on the percent DBR produced by the training dose of 1.74 mg/kg. Atropine methylnitrate

Figure 12. Dose-effect relationship for DS effects and response rate

suppression (B) for arecoline alone and with atropine pretreatment.

Each point is the group mean ± S.E.M. for one administration in each

rat.

A. DISCRIMINATION · B. RESPONSE RATE

●—● SALINE - ARECOLINE (n=6)
○···○ ATROPINE (4mg/kg) - ARECOLINE (n=6)

% DBR

% SALINE RESPONSE RATE

DOSE OF ARECOLINE (mg/kg)
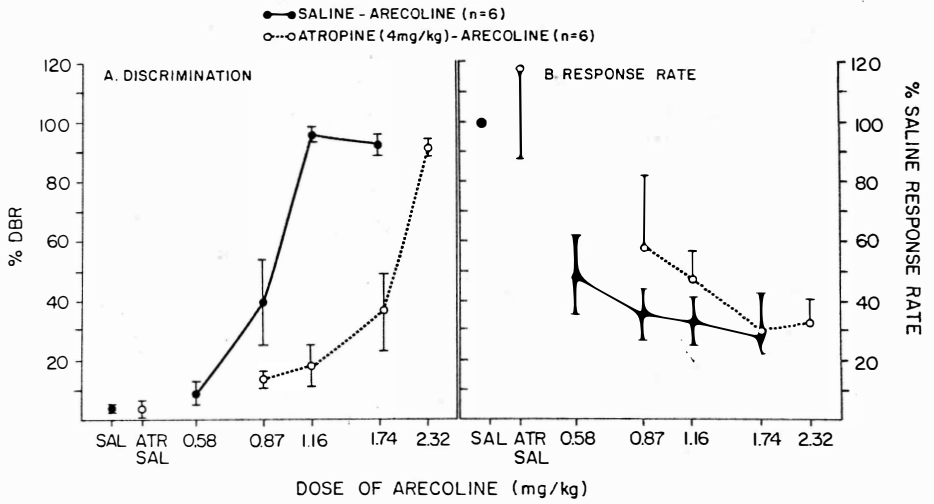
Figure 13. Time course of DS effect of arecoline. Each point is the group mean ± S.E.M. of one administration in each rat.
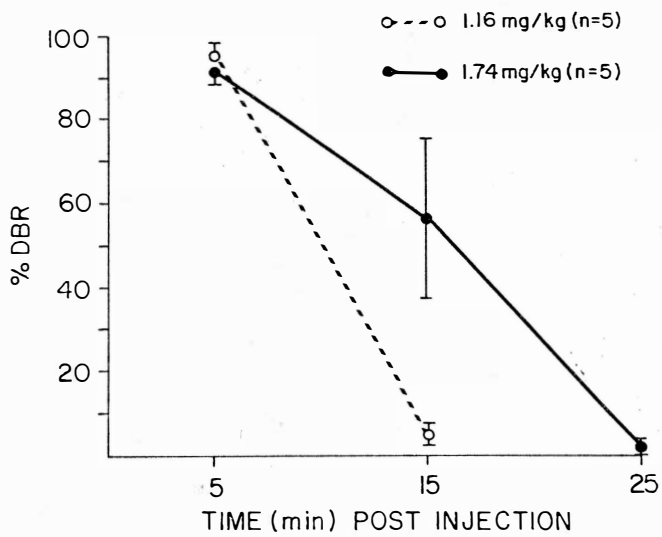
TABLE 9

Effect of Antagonists on Arecoline Cue

Pretreatment	Test	N	RPM	% DBR
Saline	Saline	6/6	17.5 ± 3.1	3.9 ± 1.4
Saline	1.74	6/6	4.3 ± 1.2	92.2 ± 3.8
Atropine Methylnitrate (2 mg/kg)	1.74	6/6	7.8 ± 3.1	96.0 ± 2.7
Atropine Methylnitrate (4 mg/kg)	1.74	6/6	5.8 ± 1.6	97.9 ± 2.1
Mecamylamine (1 mg/kg)	1.74	5/5	4.6 ± 1.8	99.1 ± 0.9
Mecamylamine (2.35 mg/kg)	1.74	6/6	4.2 ± 0.9	86.2 ±10.0
Atropine Sulfate (2 mg/kg)	1.74	6/6	5.5 ± 0.9	93.4 ± 3.8
Atropine Sulfate (4 mg/kg)	1.74	6/6	3.7 ± 0.8	36.0 ±13.1

Antagonists were administered (s.c.) 25 minutes prior to the test session. Arecoline (1.74 mg/kg) and saline were administered (s.c.) 5 minutes prior to the test session. N = number of subjects completing response require-ment/subjects tested. Each value is the mean ± S.E.M.

(2 and 4 mg/kg) and mecamylamine (1 and 2.35 mg/kg) did not antagonize the DS effect of arecoline. Two mg/kg atropine sulfate did not antagonize the DS effect of 1.74 mg/kg arecoline, but 4 mg/kg atropine sulfate decreased the percent drug bar responding from a control value of 92.2 ± 3.8 to 36.0 ± 13.1. Pretreatment with 4 mg/kg atropine sulfate produced a one-log shift in the arecoline dose-effect relationship (Figure 12). The ED_{50} for arecoline alone was 0.83 mg/kg, compared to 1.70 mg/kg after atropine pretreatment. However, there was no antagonism of the arecoline-induced suppression of response rates by atropine. Based on gross observations of the subjects, the doses of atropine sulfate and atropine methylnitrate used prevented the peripheral parasympathetic stimulation produced by arecoline. This data again demonstrates a separation of the DS and rate-suppressant effects of arecoline.

The generalization of different cholinergic agonists to the DS effect of arecoline is presented in Table 10. Different doses of oxotremorine at various time points were tested for generalization. Percent DBR equal to that produced by the training dose of arecoline (indicating complete generalization) was observed with 0.1 mg/kg oxotremorine sesquifumarate administered 20 minutes prior to testing. As was evident with arecoline, the suppression of the response rates induced by oxotremorine administration was unrelated to the percent DBR.

Administration of pilocarpine nitrate at different doses and various time points produced only partial generalization to the DS effects of arecoline. Between 30 to 55% drug bar responding was produced by the various time and dose combinations. Due to the strong peripheral parasympathetic stimulation produced by pilocarpine, it was necessary to pretreat rats with 2 mg/kg atropine methylnitrate. This dose of the

TABLE 10
Generalization of Cholinergic Agonists
to DS Effect of Arecoline

Drug	Dose (mg/kg)	Time (min)	N	RPM	% DBR
Oxotremorine	0.2	5	5/5	3.0 ± 2.0	60.2 ± 16.7
Sesquifumarate	0.1	5	5/5	3.8 ± 1.3	53.1 ± 13.5
	0.1	10	4/5	2.7 ± 0.3	52.2 ± 16.2
	0.1	20	5/5	6.8 ± 3.1	87.1 ± 6.2
	0.05	20	4/4	5.1 ± 1.0	23.3 ± 11.3
Pilocarpine	4.0	10	4/5	3.2 ± 1.0	44.5 ± 16.2
Nitrate	8.0	10	0/2	-	-
	2 MA + 8.0	10	5/5	9.6 ± 4.0	31.5 ± 16.7
	2 MA + 12.0	10	4/5	3.2 ± 1.5	55.2 ± 18.8
	2 MA + 16.0	10	4/5	2.1 ± 0.4	32.1 ± 16.0
	2 MA + 8.0	25	5/5	1.4 ± 0.5	38.0 ± 8.0
	2 MA + 12.0	25	4/4	1.8 ± 0.4	29.3 ± 12.4
	2 MA + 16.0	25	0/5	-	-
(-) Nicotine	0.57	15	6/6	18.7 ± 3.7	1.0 ± 1.0
Bitartrate	1.14	15	5/6	11.4 ± 2.4	1.2 ± 1.2
	1.14	5	5/6	4.4 ± 2.7	17.2 ± 8.4
Arecoline HBr	1.74	5	6/6	4.3 ± 1.2	92.2 ± 3.8
Saline	0.1 ml/100 gm	5	6/6	17.5 ± 3.1	3.9 ± 1.4

Agonists were administered (s.c.) at times indicated prior to test session.
N = number of subjects completing response requirement/number tested. 2 MA =
2 mg/kg atropine methylnitrate administered (s.c.) 10-15 minutes prior to
pilocarpine nitrate. Each value is the mean ± S.E.M. of subjects that res-
ponded.

peripheral antagonist completely blocked all observable signs of peripheral muscarinic stimulation (salivation, urination, and defecation). Increasing doses of pilocarpine completely disrupted behavior before complete generalization to arecoline could be attained.

Optically pure (-)-nicotine-di-l-tartrate did not generalize to arecoline. Administration of 1.14 mg/kg nicotine five minutes prior to testing produced 15% drug bar responding. Higher doses were not tested.

DISCUSSION

In this study, it was demonstrated that arecoline effectively exerted DS control of behavior at a dose of 1.74 mg/kg, while 0.58 mg/kg arecoline did not. An attempt was made to optimize the conditions for discrimination learning by training the rats to respond on the state appropriate levers from the beginning of the experiment (Overton, 1979). During the initial training period, the response requirement for advancement was used to try to control for drug-induced disruption of responding, which would interfer with a rat's interaction with the appropriate lever. Due to the behavioral disruption produced by 1.74 mg/kg arecoline, it required approximately twice as much time for this group to reach the final VI-12 second schedule and begin discrimination training as compared to the 0.58 mg/kg group. This difference in the duration of initial training should not account for the difference in discriminability of the two training doses. After ten double-alternations, the 0.58 mg/kg group had been exposed to drug and saline conditions for approximately 64 days, and the responding during the test session was not under stimulus control. In contrast, the 1.74 mg/kg group demonstrated stimulus control by the second double-alternation. The rats had been

exposed to drug and saline conditions for a total of 52 days. Thus, it was not the total duration of exposure, but the strength of the stimulus which was the critical factor in the discrimination learning. The acquisition of discrimination has been demonstrated to be dose-dependent for other classes of drugs. These include nicotine (Chance et al., 1977; Overton, 1974), quipazine (serotonergic agonist) (Appel et al., 1978), alcohol, scopolamine, pentobarbital, and ketamine (Overton, 1974).

Despite the fact that arecoline decreased the response rate in the training condition for both groups, the total reinforcements received were similar both within and between groups. It may be argued that if more reinforcements were received under one condition compared to another, that the rat's discrimination may be biased towards that lever. Thus the number of reinforcements received did not affect or bias the discrimination learning.

The failure of rats to learn to discriminate 0.58 mg/kg arecoline from saline differs from the study of Schechter and Rosecrans (1972), in which rats learned to discriminate 0.5 mg/kg arecoline from saline. The difference may be due to the procedures used in the two studies. Schechter and Rosecrans (1972b) utilized a two-lever operant paradigm in which correct responses were reinforced with sweetened milk on a CRF and incorrect responses were punished with shock. Discrimination was measured by the first response the rat made in the session. In the present experiment, rats were reinforced for correct lever choices on a VI-12 second schedule of reinforcement and incorrect responses had no consequence. Discrimination was measured in a two minute non-reinforced period.

Compared to nicotine, arecoline is a less potent, although an equi-effective DS. The 0.58 mg/kg training dose of arecoline is equimolar with 1.14 mg/kg of nicotine. Using a slightly different training procedure, i.e. the discrimination and schedule training were introduced together, 1.14 mg/kg nicotine has been demonstrated to serve effectively as a discriminate stimulus in a two-lever operant paradigm utilizing a VI-15 second schedule of reinforcement (Chance et al., 1977). Stimulus control by nicotine was evident by the third double-alternation and asymptotic learning was reached by the tenth double-alternation. This pattern of discrimination learning is similar to that observed with 1.74 mg/kg arecoline, a dose that is three times the molar concentration of 1.14 mg/kg nicotine.

The DS effect of arecoline appears to be mediated through central muscarinic receptors. This was demonstrated by the antagonism by atropine sulfate and not by atropine methylnitrate or mecamylamine, of the DS effects of arecoline. Complete generalization by oxotremorine and the lack of generalization by nicotine also demonstrates the involvement of muscarinic receptors. It should be added that the lack of antagonism by atropine methylnitrate and mecamylamine does not appear to be a function of the dose. Atropine methylnitrate has been demonstrated to be at least ten times as potent as atropine sulfate in blocking the effects of peripheral muscarinic stimulation by ACh (Morrison et al., 1969). The highest dose of mecamylamine tested was equimolar with 4 mg/kg atropine sulfate. It is also 2.5 times the dose required to completely block the discriminative stimulus effects of 1.14 mg/kg nicotine.

Optically pure (-) nicotine did not generalize to the DS effect of arecoline. This is similar to the lack of generalization of arecoline to nicotine, in rats trained to discriminate nicotine from saline (Schechter and Rosecrans, 1972c). Rats can also discriminate arecoline from nicotine (Schechter and Rosecrans, 1972a). These studies support the existence of separate muscarinic and nicotininc cholinergic systems.

Pilocarpine, in doses that were eight times the molar concentration of the training dose of arecoline, failed to produce complete generalization to the DS effect of arecoline. One explanation for this is that pilocarpine did not enter the CNS in a high enough concentration to mimic the arecoline effect. Herz et al. (1967) demonstrated that pilocarpine had an approximately eight-fold less heptane/water partition coefficient than arecoline. In that study, when administered to rabbits, pilocarpine was four to eight times less potent than arecoline in inducing cortical EEG activation (central muscarinic stimulation), while the two drugs were equipotent in producing salivation (peripheral muscarinic stimulation). However, Yamamoto and Domino (1967) demonstrated that the effects of pilocarpine on EEG and behavioral arousal in the cat were almost completely antagonized by atropine methylnitrate, suggesting a peripheral site of action. A difference in the gross behavioral and biochemical effects of arecoline and oxotremorine as compared to pilocarpine can be demonstrated. Arecoline and oxotremorine produce tremors and a rise in total brain ACh, that occurs within five minutes of drug administration, lasts for only 15 to 20 minutes, and is blocked by prior treatment with atropine sulfate (Holmstedt and Lundgren, 1966). Pilocarpine does not produce tremors, but produces clonic movements, that last several hours, and a rise in the ACh levels

that occurs within 30 minutes and lasts several hours (Haubrich and Reid, 1972). The overt behavioral effects of pilocarpine, but not the biochemical changes, are blocked by atropine sulfate (Haubrich and Reid, 1972). In the present study, rats were pretreated with atropine methylnitrate prior to pilocarpine administration. This antagonized all observable signs of parasympathetic stimulation. The fact that responding was completely disrupted by the highest doses of pilocarpine tested, even after blockade of peripheral muscarinic receptors, implies that pilocarpine did indeed reach behaviorally effective levels in the CNS. This data provides evidence for the existence of two central muscarinic systems; only one that mediates the DS effect of arecoline. Thus, the lack of complete generalization by pilocarpine may be due to either a lower receptor affinity or intrinsic activity for the receptors that mediate the DS effect of arecoline.

There was a dissociation of the dose-effect relationship of the DS effects and response rate suppressant effects of arecoline. The percent DBR produced by the lowest dose of arecoline was similar to saline values, but the response rate was 50% of the saline value. This may point to two separate muscarinic cholinergic systems in the brain, one that mediates the discriminative stimulus effects of arecoline and a second that mediates rates of responding. In addition, the sensitivity of the latter system to muscarinic stimulation appears to be greater than the former. An alternate, but not exclusive, explanation is that the DS effects of arecoline or muscarinic stimulation are very weak, and hence require a maximum stimulation of the muscarinic system by arecoline. In contrast, operant behavior response rates are very sensitive to disruption by muscarinic stimulation. Thus, discrimination can only

be attained at doses that disrupt behavior (decreased response rates). It may be concluded that the discrimination procedure is specific for muscarinic stimulation but is not very sensitive.

This differential sensitivity hypothesis may be confounded by the lack of tolerance to the response-rate suppressant effects of arecoline on a VI schedule (see Experiment 1). In the present discrimination study, the response rates under the arecoline condition were approximately 30% of the saline response rates. This differs from discrimination studies with other drugs (nicotine, see Experiment 4; LSD and amphetamine, Minnema, personal communication; and PCP, Brady, personal communication) in which the response rates are similar under both drug and saline conditions. All of these drugs suppress response rates upon initial administration. However, with repeated administration, tolerance develops to the response-rate suppressant effects of these drugs but not to this effect of arecoline. This lack of tolerance to the response-rate suppressant effects of arecoline may make the discrimination procedure appear insensitive to muscarinic stimulation. The relationship between response-rate and discrimination should be further evaluated. The DS effect of arecoline on a VI schedule should be further investigated to find the lowest dose that will maintain discrimination. In addition, the DS effect of arecoline on an FR schedule should be investigated, since on this schedule tolerance develops to the response rate suppressant effects of arecoline.

VI. EXPERIMENT 4. INTERACTION OF PHYSOSTIGMINE WITH THE DS EFFECTS OF
ARECOLINE AND NICOTINE

INTRODUCTION

This study was designed to investigate two issues. The first was to
determine whether there is a presynaptic cholinergic innervation which
impinges upon the central muscarinic and nicotinic receptors that medi-
ate the DS effects of arecoline and nicotine, respectively. The second
issue addressed is the question as to whether the DS effect of nicotine
is contingent upon the release of endogenous ACh. Both of these issues
were examined by studying the interaction of the cholinesterase inhibi-
tor, physostigmine, with the DS effects of both arecoline and nicotine.

It was hypothesized that if the receptors which mediate the DS
effects of arecoline and nicotine are innervated by neurons that release
ACh, then the DS effects of arecoline and nicotine would be mimicked or
potentiated by increasing ACh levels via cholinesterase inhibition.
One possible mechanism of action of nicotine is that it acts indirectly,
by releasing ACh, which interacts with muscarinic receptors (Morrison,
1967). Evidence for this comes from operant behavior and EEG studies
which demonstrated that atropine can partially or completely block
nicotine-induced rate depressant and EEG but not behavioral arousal
effects (Morrison et al., 1969; Yamamoto and Domino, 1967). In
addition, research from biochemical studies indicates that nicotine
administration induces an increased efflux of cortical ACh and a de-
crease in ACh levels (Madill and Parker, 1970). Behavioral studies have
also demonstrated that physostigmine can potentiate the rate depressant
effects of nicotine. If the DS effect of nicotine is mediated through
release of ACh, increasing ACh levels via cholinesterase inhibition
should potentiate or mimic the DS control of behavior by nicotine.

METHODS

Using a procedure similar to that described in Experiment 3, seven rats were trained to discriminate 1.74 mg/kg of arecoline from saline, and seven rats were trained to discriminate 1.14 mg/kg of nicotine from saline. These training procedures differed from that of Experiment 3 in that the schedule was returned to a CRF during the initial period of discrimination training, when both levers were in the chamber and was then increased up to a VI-12". These rats showed learning curves and asymptotic discrimination similar to that previously described. Once discrimination had stabilized, experiments investigating the interaction of physostigmine with the DS properties of arecoline and nicotine were conducted.

Experiment A. Interaction of physostigmine with the dose-effect relationship for arecoline and nicotine. The dose-response relationship, with and without physostigmine pretreatment, for arecoline and nicotine, were carried out in animals trained to discriminate arecoline and nicotine, respectively. Physostigmine or saline was administered (s.c.) 25 minutes prior to testing. Arecoline and nicotine were administered five and ten minutes prior to testing, respectively. The different test conditions were presented in a counter-balanced sequence. Previous studies had demonstrated that ACh levels in rat brain were maximally elevated 25 minutes after physostigmine administration (Rosecrans, Dren, and Domino, 1965). The dose of physostigmine used (0.125 mg/kg) was selected from pilot studies as one that did not completely disrupt responding. The interaction of neostigmine with the DS effect of arecoline was assessed after Experiment B was completed. The dose of neo-

stigmine used (0.10 mg/kg) was equimolar with the dose of physostigmine
(0.125 mg/kg) previously used. Neostigmine was administered (s.c.) 25
minutes prior to testing. Arecoline (0.58 mg/kg) was administered five
minutes prior to testing. Discrimination was assessed in a two-minute
nonreinforced session. If rats took longer than two minutes to respond,
they were removed after five responses were emitted. Test sessions were
extended to a maximum of 15 minutes, after which the rat was removed and
considered disrupted. The data from these rats were not included in any
analysis.

Experiment B. Generalization of physostigmine to the DS effects of
arecoline and nicotine. The generalization of physostigmine, adminis-
tered alone and with different antagonist combinations in rats trained
to discriminate arecoline or nicotine was assessed. Pilot experiments
had demonstrated that when administered alone, physostigmine (0.25
mg/kg), completely disrupted the responding of most animals. Rats
trained to discriminate arecoline or nicotine were similarly affected.
Thus in these experiments, nicotine-trained rats were pretreated with
hexamethonium (1.0 mg/kg) and either atropine sulfate (4.0 mg/kg) or
atropine methylnitrate (2.0 mg/kg), in an attempt to antagonize the
peripheral nicotinic and central and/or peripheral muscarinic effects of
physostigmine. Arecoline-trained rats were pretreated with atropine
methylnitrate (2.0 mg/kg) and mecamylamine (1.0 mg/kg) in an attempt to
antagonize the peripheral muscarinic and peripheral and central nico-
tinic effects of physostigmine. The most noticeable peripheral effects
produced by physostigmine are salivation and diarrhea (muscarinic stimu-
lation) and muscle fasiculation (nicotinic stimulation). Noticeable

signs of peripheral muscarinic stimulation were antagonized by the doses of atropine methylnitrate and atropine sulfate used in these studies. However, the production of muscle fasiculation by physostigmine, an effect on the neuromuscular junction, was not blocked by the nicotinic ganglionic blockers used. Physostigmine was injected 25 minutes prior to the test session, except once when it was injected 45 minutes prior to the session. Antagonists were administered 10 minutes prior to physostigmine. To control for possible disruptive effects of multiple injections or antagonist combinations, the discrimination of the training doses of arecoline and nicotine, as well as saline, were assessed after antagonist pretreatments. Antagonists were administered 35 minutes, and arecoline and nicotine five and ten minutes, prior to the test session, respectively. The same criteria for test sessions as in Experiment A were used.

Data analysis. The discrimination data is presented as percent drug bar responding (% DBR) and response rate data as responses/minute (RPM). Data was analyzed using either paired Student's t-test or treatment-by-treatment-by-subjects analysis of variance. When doses were replicated, the mean of the replications for each animal was derived and the group mean ± S.E.M. was derived from that.

RESULTS

Experiment A. Interaction of physostigmine with the dose-effect relationship for arecoline and nicotine. Figure 14 presents the percent drug bar responding for different doses of nicotine and arecoline administered with a saline or physostigmine (0.125 mg/kg) pretreatment.

Figure 14.   Interaction of physostigmine with the DS effects of nicotine
(left panel) and arecoline (right panel).   Numbers inside bars indicate
number completing response requirement/number tested.   Each value is the
group mean ± S.E.M. of one administration in each rat.

100 ─

80 ─

60 ─

% DBR 40 ─

20 ─

0 ─

A.

6/7

7/7

5/7

7/7

6/7

B.

7/7

7/7

7/7

7/7

7/7

PRETREATMENT   PHY   SAL   PHY   SAL   PHY   PHY   SAL   PHY   SAL   PHY

TEST   SAL   0.29   0.58   SAL   0.58   0.87

NICOTINE
(mg/kg)
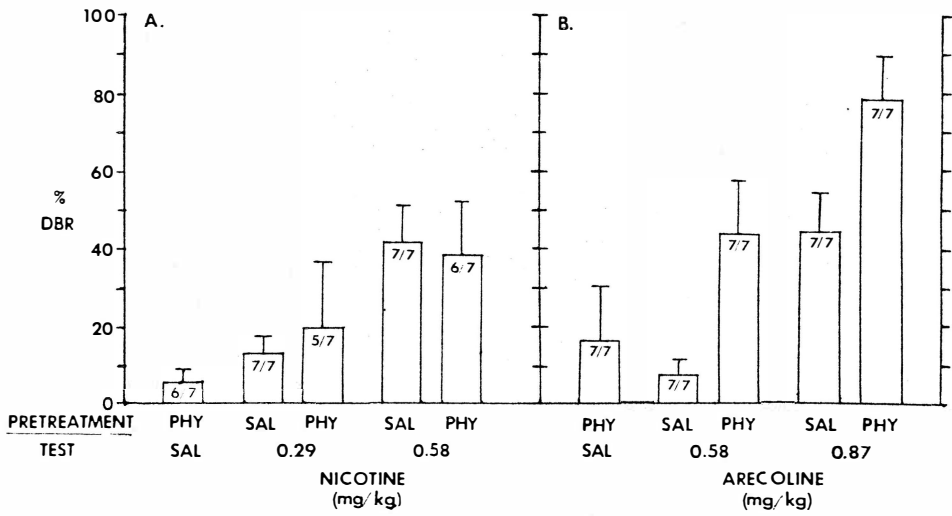
ARECOLINE
(mg/kg)

Physostigmine, administered alone produced 6 and 17% DBR in nicotine-
trained and arecoline-trained rats, respectively. The greater effect of
physostigmine in arecoline-trained rats is due to one rat that responded
100% on the drug bar, while the others responded zero to five percent on
the drug bar. There was a dose-effect relationship for both nicotine
and arecoline when preceeded by a saline injection. Physostigmine
pretreatment did not shift the nicotine dose-effect relationship. In
contrast, the arecoline dose-effect relationship was shifted to the left
(greater effect of the same dose) by physostigmine pretreatment. The
interaction of physostigmine with the arecoline and nicotine dose-effect
relationship was analyzed by a treatment-by-treatment-by-subjects analy-
sis for each training drug. For both analyses, the factors analyzed
were dose (of nicotine or arecoline) and pretreatment condition (saline
or physostigmine). For the nicotine-physostigmine interaction, there
was a significant dose effect ($df=1,6$; $F=4.45$; $p<0.05$), indicating a
dose-effect relationship. Both the pretreatment condition ($df=1,6$;
$F<1.0$; $p>0.2$) and the treatment x dose interaction ($df=1,6$; $F=1.22$;
$p>0.2$) were nonsignificant. These results indicated that there was no
difference between the nicotine dose-effect curve after saline and
physostigmine pretreatment and that the dose-effect relationship was not
different between the two pretreatments.

For the arecoline-physostigmine interaction, there was a significant
effect of the dose factor ($df=1,6$; $F=15.1$; $p<0.001$), indicating a
dose-effect relationship and of the pretreatment factor ($df=1,6$; $F=32.4$;
$p<0.001$), indicating that there was a significant facilitation of the
dose-effect relationship by physostigmine. The pretreatment x dose
interaction was nonsignificant ($df=1,6$; $F<1$; $p>0.2$), indicating that the

dose-effect relationship was not different between the two pretreatments.

After the experiment was completed, the interaction of neostigmine with the DS effect of arecoline was assessed in six rats. This was carried out to assess if physostigmine was producing its effects through the inhibition of the metabolism of arecoline, which has an esteratic linkage. Administration of 0.1 mg/kg neostigmine methylsulfate, a peripheral cholinesterase inhibitor (the dose is equimolar to the dose of physostigmine used) 25 minutes prior to administration of 0.58 mg/kg arecoline, produced a 25.6 ± 15.9 % DBR. This contrasts to the response after 0.58 mg/kg arecoline alone (9.0 ± 4.5) and with physostigmine pretreatment (51.3 ± 14.8). Thus, inhibition of the metabolism of arecoline may play a role in the potentiation by physostigmine of low doses of arecoline.

Experiment B. Generalization of physostigmine to the DS effects of arecoline and nicotine. The generalization of physostigmine with the discriminative stimulus effects of nicotine is presented in Table 11. When administered alone, physostigmine (0.125 mg/kg), produced approximately 5% DBR and only slightly decreased response rates compared to saline. Administration of 0.25 mg/kg physostigmine by itself (not presented), completely disrupted the responding of three out of four rats tested, and so was not tested any further. Thus, rats trained to discriminate nicotine were pretreated with hexamethonium (1 mg/kg) and either atropine methylnitrate (2 mg/kg) or atropine sulfate (4 mg/kg) in an attempt to partially block some of the peripheral nicotinic and peripheral and central muscarinic effects of physostigmine. Pretreat-

TABLE 11

Generalization of Physostigmine to the DS Effects of Nicotine

Pretreat-ments[a]	Dose Physostigmine mg/kg[b]	N	Repli-cations	RPM	% DBR
Sal.	0.125	6/7	1	10.4 ± 3.6	5.3 ± 4.0
MA Hex	0.25	3/7	1	1.1 ± 0.4	33.3 ± 19.3
Atr Hex	0.25 (45 min)	7/7	1	3.3 ± 0.6	23.9 ± 4.6
Atr(8) Hex	0.25	6/7	1	4.5 ± 1.9	5.6 ± 5.6
Atr Hex	0.25	7/7	2-3	4.1 ± 0.7	29.1 ± 12.4
Atr Mec	0.25	7/7	2	2.0 ± 0.3	18.2 ± 9.6
Atr Hex	0.5	7/7	2	7.1 ± 3.4	30.7 ± 8.0
Atr Hex	Sal	7/7	1	16.7 ± 5.0	0.0
Atr Hex	Nic	7/7	1	22.2 ± 6.8	92.8 ± 3.2
Atr Mec	Nic	7/7	1	3.8 ± 1.6	13.3 ± 8.6
	Sal	7/7	2	15.1 ± 2.9	1.0 ± 0.6
	Nic	7/7	2	22.6 ± 7.3	90.7 ± 5.2

N = number completing response required/number tested. Each value under RPM and % DBR is the mean ± S.E.M. Replications are the number of times a particular interaction was tested in each animal.

MA = 2 mg/kg atropine methylnitrate.
Hex = 1 mg/kg hexamethonium Cl.
Atr - 4 mg/kg atropine sulfate (except where noted)
Mec = 1 mg/kg mecamylamine HCl.
Nic = 1.14 mg/kg nicotine bitartrate.

[a] Given 10 minutes before physostigmine.
[b] Given 25 minutes before session, except where noted.

ment with atropine methylnitrate and hexamethonium prior to physostig-
mine administration did not block the disruptive effects of 0.25 mg/kg
physostigmine (three out of seven rats responded), indicating a central
action for the rate suppressant effects of physostigmine. When pre-
treated with either 4 or 8 mg/kg atropine sulfate and 1 mg/kg hexame-
thonium prior to physostigmine (0.25 mg/kg), all rats responded, how-
ever, response rates were still depressed. Due to the observed group
variability on percent DBR with physostigmine, some antagonist-physos-
tigmine interactions were replicated two to three times in each animal.
A mean value for each animal was calculated and these were averaged to
derive the group mean and standard error of the mean. Approximately 30%
DBR was observed with the atropine, hexamethonium, and 0.25 mg/kg phy-
sostigmine treatment. No change in percent drug bar responding was
observed when physostigmine (0.25 mg/kg) was administered 45 minutes
prior to testing. Increasing the dose of physostigmine to 0.5 mg/kg did
not increase the percent DBR. Pretreatment with atropine sulfate and
the central nicotinic antagonist, mecamylamine, did not affect the DBR
produced by physostigmine administration. Neither the discrimination
level nor response rate after saline and nicotine (0.4 mg/kg) were
affected by pretreatment with atropine and hexamethonium. Pretreatment
with atropine and mecamylamine antagonized the percent drug bar res-
ponding produced by nicotine administration, demonstrating that this
antagonist combination can block a centrally mediated nicotinic effect.
The response rate with this combination of drugs was low, however. This
combination was tested at the end of the experiment, and the decrease in
response rate may be due to the animals associating the multiple injec-
tion procedure with the nonreinforced test session.

The generalization of physostigmine to the discriminative stimulus effects of arecoline are presented in Table 12. Administration of physostigmine (0.125 mg/kg) after pretreatment with atropine methylnitrate and mecamylamine, produced 29% DBR. The effects of 0.25 mg/kg physostigmine were assessed after pretreatment with atropine methylnitrate (2 mg/kg) and mecamylamine (1 mg/kg). When tested 45 and 25 minutes after physostigmine administration, the percent DBR was approximately 40 and 67% respectively. Increasing the dose of physostigmine to 0.5 mg/kg completely disrupted the responding of all rats. Pretreatment of rats with atropine sulfate (4 mg/kg) and mecamylamine (1 mg/kg) significantly decreased the percent DBR produced by physostigmine (0.25 mg/kg). Pretreatment with atropine methylnitrate and mecamylamine did not affect the percent DBR after saline, but did decrease the percent DBR after the training dose of arecoline. The reason for this decrease is not evident, since when administered alone, neither antagonist affects the arecoline discrimination. When this antagonist combination preceeded the training dose, the discrimination of the rats was more variable than when arecoline was administered alone.

After atropine methylnitrate and mecamylamine pretreatment, the percent DBR for physostigmine (0.25 mg/kg) and arecoline (1.74 mg/kg) were similar (approximately 70% DBR), although both responses were below the baseline discrimination for arecoline. In addition, injection of atropine sulfate (4 mg/kg) and mecamylamine (1 mg/kg) antagonized the discrimination produced by physostigmine and arecoline to a similar extent (approximately 25% DBR).

TABLE 12

Generalization of Physostigmine to the DS Effect of Arecoline

Pretreat-ment[a]	Dose Physostigmine mg/kg [b]	N	Repli-cations	RPM	% DBR
MA Mec	0.125	6/6	1	11.9 ± 5.5	29.1 ± 12.5
MA Mec	0.25 (45 min)	4/6	1	3.8 ± 2.1	39.2 ± 19.7
MA Mec	0.25	6/6	2-4	2.5 ± 0.5	66.8 ± 12.9
MA Mec	0.5	0/6	1	-	-
Atr Mec	0.25	6/6	2	4.0 ± 1.6	22.2 ± 7.6*
MA Mec	Sal	6/6	1	27.9 ±10.8	0.0
MA Mec	Are	6/6	2	4.8 ± 2.3	76.6 ± 7.6
Atr Mec	Are	5/6	1	1.9 ± 0.3	25.8 ± 10.2
	Sal	6/6	2	15.3 ± 3.8	2.9 ± 1.7
	Are	6/6	2	3.9 ± 1.4	92.0 ± 4.3

N = number completing responses required/number tested. Replications are the number of times a particular interaction was tested in each animal. Each value under RPM and % DBR is the mean ± S.E.M.

MA = 2 mg/kg atropine methylnitrate
Mec = 1 mg/kg mecamylamine HCl
Atr = 4 mg/kg atropine sulfate
Are = 1.74 mg/kg arecoline HBr

[a] Given 10 minutes before physostigmine.
[b] Given 25 minutes before session, except where noted.

*Significantly different from MA-Mec 0.25 $p < 0.01$.

DISCUSSION

The results of the present study demonstrated that physostigmine can potentiate and partially generalize to the DS effect of arecoline. In contrast, physostigmine did not potentiate or generalize to the DS effect of nicotine. The ability of physostigmine to potentiate the DS effect of low doses of arecoline is thought to be due mainly to the inhibition of degradation of ACh by acetylcholinesterase. The ACh which is protected from hydrolysis can then interact with the central muscarinic receptors at which arecoline is acting, to produce a response summation. The potentiation by neostigmine of the DS effect of arecoline, although not as great as physostigmine, indicates that peripheral cholinesterase inhibition may also be important. Arecoline has a carboxylic ester group that may be susceptable to hydrolysis by esterases. Inhibition of the metabolism of arecoline may therefore be a factor in the potentiation of the DS effect of arecoline by cholinesterase inhibitors. No studies have investigated if arecoline is hydrolyzed by esterases and if cholinesterase inhibitors can affect the hydrolysis of arecoline. Liver homogenates can completely hydrolyze the carboxymethyl group on arecoline to the carboxylic acid (Nieschulz and Schmersahl, 1968). However, they used incubation times of 30 minutes. It is not known how much of a given dose of arecoline would be hydrolyzed within the five minutes from time of drug administration to testing.

The ability of physostigmine, administered after peripheral muscarinic and central and peripheral nicotinic antagonists, to partially generalize (approximately 70% DBR) to arecoline, indicates that there is a cholinergic innervation of the muscarinic receptors that may mediate the DS effect of arecoline. The specificity of this effect was demon-

strated by the antagonism of the physostigmine generalization by atropine sulfate. Similar to the antagonism of the DS effect of arecoline by atropine, atropine blocked the generalization of physostigmine to arecoline, but not the rate depressant effect.

The failure of physostigmine to potentiate or generalize to the DS effect of nicotine indicates that this action of nicotine is not mediated through the release of ACh. The data also indicate that there may be a lack of a cholinergic innervation to the receptors that mediate the DS effect of nicotine. An alternate explanation is that the nicotinic cholinergic system has a low level of spontaneous activity. The ability of physostigmine to enhance the action of ACh is dependent on ACh release and hence neuronal activity. Thus, if the nicotinic cholinergic system had a low level of spontaneous activity, physostigmine would not be able to greatly potentiate or mimic stimulation of the system by exogenous nicotinic agents. Thus, the neurons that mediate the DS effect of arecoline are cholinergic, while the neurons that mediate the DS effect of nicotine are cholinoceptive but may not be cholinergic.

Administration of physostigmine (0.25 mg/kg) by itself produced almost complete disruption of responding. After various antagonist pretreatments, the response rate decreasing effect of physostigmine was partially antagonized. The fact that the response rates in both groups of rats were slightly higher after atropine pretreatment compared to atropine methylnitrate pretreatment indicates that the response rate decreasing effect of physostigmine is due mainly to central muscarinic stimulation. Even when pretreated with 8.0 mg/kg atropine sulfate, the response rate was decreased by physostigmine. The inability of atropine to completely block the response rate decreasing effects of physostig-

mine contrasts with the studies of Vailant (1964, 1967), Olds and Domino (1969), Chait and Balster (1979) and Pfeiffer and Jenny (1957) in which central muscarinic antagonists blocked the behavioral effects of physostigmine. The difference in the present results and the previous studies may be in the different conditions under which response rates were assessed. The previous studies examined the drug interactions in acute administration situations, with the animal being reinforced for responding. In the present investigation, the drug effects were assessed in a nonreinforced test period in animals that were receiving chronic drug administration (double-alternation schedule of nicotine or arecoline administration).

Previous studies examining the interaction of selective cholinergic antagonists with physostigmine, demonstrated that the central effects of cholinesterase inhibition were mediated through muscarinic and not nicotinic receptors (antagonized by atropine or scopolamine, not by mecamylamine) (Olds and Domino, 1969; Yamamoto and Domino, 1967; and Vaillant, 1967). Consistent with this, the present study demonstrated that the effect of a muscarinic agonist could be mimicked by physostigmine, while the effect of a nicotinic agonist could not be mimicked. This contrasts with the study of Vazquez and Toman (1967) in which both the muscarinic and nicotinic effects on the electrical-evoked response in the rabbit sensurimotor cortex could be mimicked by physostigmine.

VII. EXPERIMENT 5. CNS SITES OF ACTION OF THE DS EFFECT OF ARECOLINE
AND NICOTINE

INTRODUCTION

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. Neurons respon-
sive to both nicotinic and muscarinic agonists are present in both
structures (Bradley et al., 1966; Bird and Aghajanian, 1976; Segal
1978). Nicotine and arecoline have been demonstrated to produce EEG
changes, through both direct and indirect actions, on the DH and cere-
bral cortex (Longo et al., 1967; Kawamura and Domino, 1969). The in-
direct effects of arecoline and nicotine on cortical and DH EEG may be
mediated through an action on the MRF (Kawamura and Domino, 1969).
Binding of nicotinic and muscarinic ligands have been demonstrated for
the DH, suggesting the presence of nicotinic and muscarinic receptors
(Segal et al., 1978; Yamamura et al., 1974). However, no one has inves-
tigated the presence or absence of nicotinic or muscarinic receptors in
the MRF.

Bilateral administration of nicotine (0.5 µg/µl/site) into the DH
produces a partial generalization to the DS effect of peripherally
administered nicotine (Rosecrans and Chance, 1978). The present inves-
tigation 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.

METHODS

Subjects. Using the training procedure described in Experiment IV, 10 rats were trained to discriminate 1.74 mg/kg arecoline from saline and 10 rats were trained to discriminate 1.14 mg/kg nicotine from saline using a two-lever VI-12 second schedule of milk reinforcement. When discrimination had stabilized, standard stereotaxic techniques were used to implant the rats with unilateral, stainless steel intracerebral cannulas. Under ketamine anesthesia (Ketalar, Parke-Davis, Detroit; 100-150 mg/kg, i.p.), 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 (Pellegrino and Cushman, 1967). 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. Rats were run for 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 intra-cerebral drug administration days.

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 steel 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"; 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 polyethy-
lene tubing (PE-20, #7406, Clay Adams; Becton, Dickinson, and Co.,
Parsippany, N.J.) 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. Drug solutions were made in artificial cerebrospinal fluid
(minus dextrose). The composition of this fluid (gram/liter) is: NaCl:
7.46; KCl: 0.19; $CaCl_2$ (anhydrous): 0.14; $MgCl_2 \cdot 6H_2O$: 0.19; $NaHCO_3$:
1.76; and Na_2HPO_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 injection. Doses of arecoline and nicotine for intracerebral injection are expressed as the free base.

Data analysis. The drug discrimination data was assessed by the procedure described in Experiment 3. The ED_{50} derived from linear regression analysis is the dose that produced 50% DBR. When doses were replicated, the mean of the replication for each animal was derived, and the group mean ± S.E.M. was derived from that

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 (Pellegrino and Cushman, 1967).

RESULTS

Cannula placements. The DH cannula placements are presented in Figure 15 and the MRF cannula placements are presented in Figure 16. For both figures, the left half represents the 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 hippcampal placements were between -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. Intracerebral administration of arecoline at the DH and MRF did not produce any generalization to the DS effect of peripherally administered arecoline (Table 13). Rats with cannulas in the DH had a higher percent DBR after CSF than did rats with cannulas 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 disruption of response rates produced by muscarinic stimulation.

Figure 15. Cannula placements in the dorsal hippocampus. Left side of
figure is for arecoline trained rats, right side of figure is for nico-
tine 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 Cush-
man (1967).
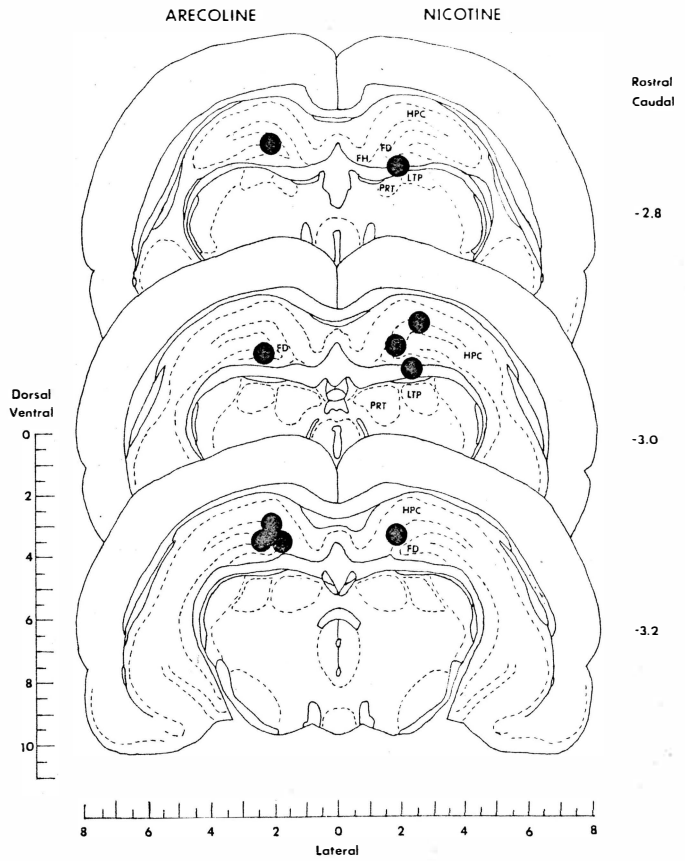
Figure 16.   Cannula placements in the mesencephalic reticular formation area.   Left side of figure is for arecoline trained rats, right side of figure is for nicotine trained rats.   Key: LM = Medial lemniscus; PL = Cerebral peduncle;   RF = Reticular formation of mesencephalon;   SN = Substantia nigra; TL = Lateral tegmental nucleus.   Sections traced from Pellegrino and Cushman (1967).
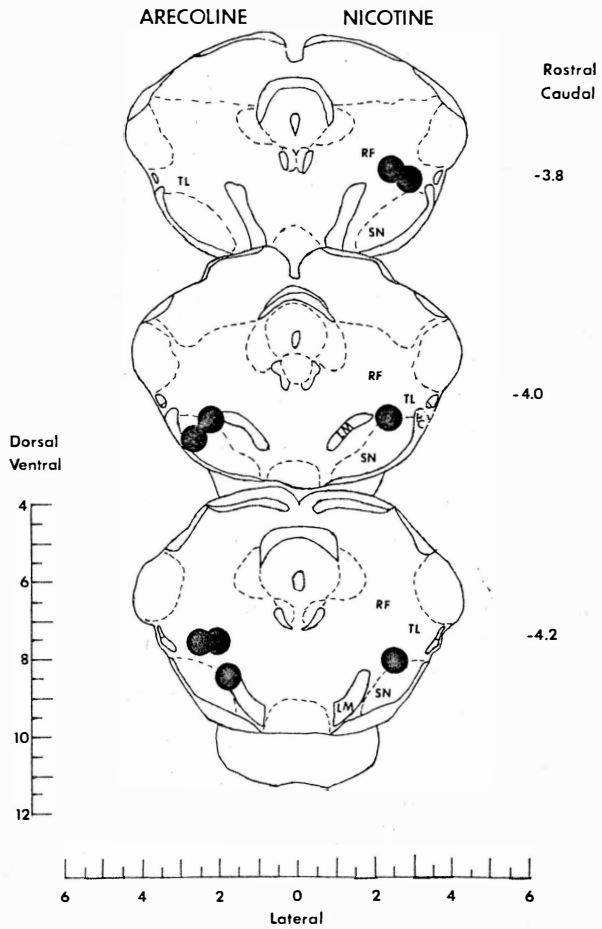
TABLE 13

Effects of Intracerebral Drug Injections in Rats
Trained to Discriminate Arecoline from Saline

Drug Injected	N	Reticular Formation Repli-cations	RPM	% DBR	N	Dorsal Hippocampus Repli-cations	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								
1.74mg/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. Antago-
nist 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 ± S.E.M.

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 hippo-campus appeared to be potentiated by peripheral administration of atropine. Administration of nicotine (8 μg) at either site did not generalize to peripheral arecoline administration, and produced beha-vioral disruption, as measured by the response rate and the number responding of the number tested.

Generalization in nicotine-trained rats. In both the MRF and DH, intracerebral nicotine produced a dose-related generalization to the DS effect of peripheral nicotine administration (Table 14 and Figure 17). The effects of some doses of nicotine were replicated two to four times in each animal. A mean value for each animal was calculated and these were averaged to derive the group mean and standard error of the mean. The MRF appeared to be more sensitive than the DH. The ED_{50}'s 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 generali-zation data. The factors were dose (2, 4, and 8 μg) and brain site. The dose factor was significant (df=2,15; F=15.6; p<0.001), indicating a significant dose-effect relationship. The brain site factor (df=1,6; F=2.07; p>0.2) was nonsignificant, indicating that the sensitivities of the two sites were not significantly different. The dose x site inter-action (df=2,15; F=1.1, p>0.2) was also nonsignificant, indicating that the dose-effect relationship was similar for both groups.

The generalization produced at both sites by administration of 8 μg nicotine was antagonized to a similar degree (decrease of 50% DBR) by

TABLE 14

Effects of Intracerebral Drug Injections in Rats
Trained to Discriminate Nicotine from Saline

Drug Injected	N	Reticular Formation Repli- cations	RPM	% DBR	N	Dorsal Hippocampus Repli- cations	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								
1.14mg/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. Antago-
nist 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 ± S.E.M.

Figure 17. Generalization of intracerebrally injected nicotine to the DS effect of peripherally administered nicotine. Each point is the group mean ± S.E.M. N=5 for DH, N=4 for MRF.
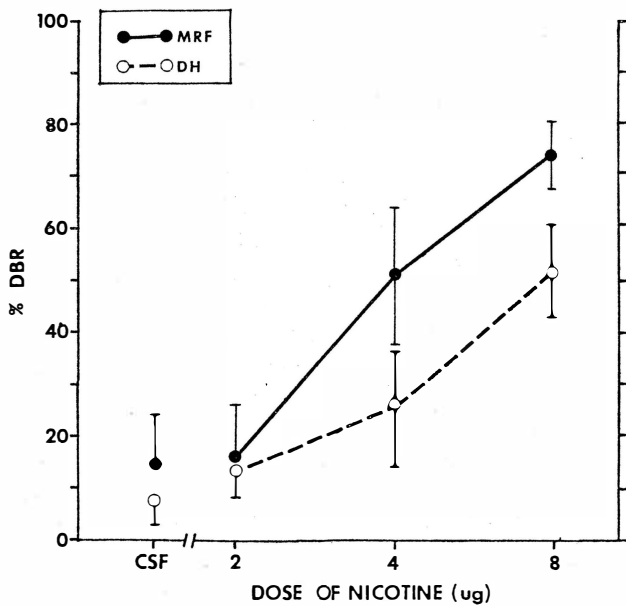
peripheral administration of 1 mg/kg mecamylamine, but was not anta-
gonized by 4 mg/kg atropine sulfate. Administration of 8 µg arecoline
into either site did not generalize to peripherally administered nico-
tine.

The dose-effect relationship was also examined taking the highest
percent DBR for each animal, at doses that were replicated, and obtain-
ing a group mean and standard error of the mean from this data (Table
15). Again, the MRF was more sensitive to nicotine injection than was
the DH. Complete generalization (95% DBR) was obtained with 8µg nico-
tine, directly into the MRF, compared with 76% DBR for the DH. In the
MRF rats, there were no differences in percent DBR related to exact
injection sites.

Response rates were decreased to a similar degree by nicotine injec-
tions 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.

Gross behavioral observations. After the discrimination studies were
conducted, the effects of intracerebral injection of 8 µg arecoline in
arecoline-trained rats, and 8 µg nicotine, in nicotine-trained rats, on
grossly observable behavior was observed. No saline controls were
performed. The rats were injected with the drugs and immediately placed
in plastic observation cages (26 x 48 x 21 cm). For both drugs, injec-
tion into the DH produced no grossly observable behavioral effects. The
rats exhibited exploratory behavior (sniffing, rearing, and locomotion).
These behaviors would be exhibited by any rat in a novel situation. In

TABLE 15

Peak Generalization of Centrally Administered Nicotine

to the DS Effect of Peripherally Administered Nicotine

| Brain Site | Dose of Nicotine | |
	4 µg	8 µg
MRF (n=4)	68.3 ± 15.4	95 ± 5.0
DH (n=5)	43.2 ± 17.9	76 ± 11.6

Data is expressed as percent drug bar responding.

Each value is the mean ± S.E.M. of the number of

rats indicated.

two arecoline rats with cannulas in the MRF, contralateral circling and body asymmetry were observed for approximately five to six minutes. The other two rats did not exhibit any unusual behaviors. In the nicotine rats with MRF cannulas, one rat exhibited contralateral turning and three rats exhibited ipsilateral turning. The duration of these effects was between two to five minutes. All rats that demonstrated turning behavior after drug injection (nicotine or arecoline) exhibited an arousal reaction immediately after the drug injection. This consisted of intense sniffing and struggling against restraint. These effects were also observed during generalization testing.

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 (1968) 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. When examined histologically 30 minutes after injection, this injection volume diffused over an area of approximately 1.0 mm in diameter. 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. At pH 7.4, nicotine is approximately 70% ionized (Domino, 1965b) and arecoline is approximately 44% ionized (Albert, 1952). Thus, the charges on the molecule may act to inhibit diffusion from the site of injection.

This study demonstrated that unilateral injection of nicotine directly into the DH and MRF and surrounding areas can generalize to the DS effect of peripherally administered nicotine. Although the difference was not statistically significant, injection of nicotine into the MRF produced greater generalization than injection into the DH. Thus, even though both structures are probably involved in mediating the DS effect of nicotine, the MRF may have a greater involvement than the DH. This is similar to the findings of Knapp and Domino (1962) and Kawamura and Domino (1969) 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. Indirectly, this data does not support the contention of Nelsen et al. (1973) that chronic nicotine administration increases the influence of the hippocampus on the cortex and decreases the influence of the MRF on the cortex, and hence, possibly behavior. The present study suggests that even after chronic treatment, nicotine exerts its major effect on behavior through the MRF. In considering the conclusions based on the MRF placements, it should be remembered that all cannulas were not directly in the MRF. These studies should 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% (Rosecrans and Chance, 1977). 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. Although technically more difficult, this is an area for future research.

The specificity of the central effect of nicotine was demonstrated by the antagonism with peripherally administered mecamylamine, the lack of antagonism by atropine, and the lack of generalization of intracerebrally injected arecoline. Identical results were obtained when assessing the effects of mecamylamine and atropine on the DS effect of peripherally administered nicotine (Hirschhorn and Rosecrans, 1974) and the generalization of peripherally administered arecoline (Schechter and Rosecrans, 1972c). 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 is similar to the results of receptor binding studies that demonstrated a separation of muscarinic and nicotinic binding sites (Segal et al., 1978; Snyder et al., 1975). This contrasts with the results from microiontophoretic studies, which demonstrated an overlap between muscarinic and nicotinic responses (Bradley et al., 1967; Bird and Aghajanian, 1976; Segal, 1978).

In contrast to the generalization after intracerebral nicotine in nicotine-trained rats, injection of arecoline into either brain site did not produce any generalization to the DS effect of peripherally administered arecoline. 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. As in prior experiments, this data demonstrates a separation of the discriminative stimulus and response rate effects of arecoline administration. 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, as

discussed previously, 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 and the nucleus accumbens. Both of these areas have high levels of muscarinic ligand binding and indices of cholinergic function (Snyder et al., 1975). Also, the role of the cerebral cortex in mediation of the DS effect of arecoline should be evaluated, even though there may be problems as to the exact choice of the areas to investigate. The cerebral cortex has been demonstrated to have a moderate level of cholinergic indices and muscarinic ligand binding (Snyder et al., 1975).

The bilateral injection of ACh or carbechol (both of which possess muscarinic and nicotinic activity) into the MRF produces behavioral and cortical EEG arousal, hyperreactivity to sensory input (Grossman, 1968), and decreased responding on a variable-ratio schedule for either food or water reinforcement (Grossman and Grossman, 1966; Grossman, 1968). The decrease in operant responding is similar to the effects observed in the present study, which used more specific muscarinic and nicotinic agonists. The rats in the present study demonstrated behavioral arousal after nicotine or arecoline injection into the MRF, but this lasted for only 30 to 60 seconds. The animals then appeared insensitive to simuli and some demonstrated body asymmetry.

The turning behavior observed after injection of either arecoline or nicotine into the MRF may be related to effects on the substantia nigra. The zona compacta of the substantia nigra projects a dopaminergic tract

to the caudate nucleus. This pathway has been implicated in extrapyramidal motor function. Unilateral drug administration, either at the substantia nigra or at the caudate nucleus can produce turning behavior. Whether the turning behavior is ipsilateral or contralateral to the injection site is dependent on the drug injected, the site, and if the system has been lesioned (Arnt and Scheel-Kruger, 1979; Glick et al., 1976). No studies have reported the effect of cholinergic agents injected directly into the substantia nigra on turning behavior. Based on the present observations, a more quantitative and controlled study should be performed and may yield new data on the cholinergic system in motor behavior.

VIII. FINAL DISCUSSION AND FUTURE RESEARCH

The present investigations were designed to compare the behavioral effects of arecoline and nicotine on central cholinergic systems. It was hoped that through these studies, new information on the effects of the drugs themselves as well as on central cholinergic systems would be obtained.

The acute and chronic effects of arecoline and nicotine on operant behavior were assessed in Experiments I and II, respectively. Comparing the acute effects of the two drugs, nicotine was slightly more potent than arecoline in disrupting VI behavior (similar effects were produced by 0.8 mg/kg, free base, nicotine and 1.14 mg/kg, free base, arecoline). The factors involved in tolerance development were different for the two drugs. When the dose-effect relationship of arecoline on VI behavior was redetermined after 21 days of chronic pre-session treatment, there was no tolerance to the effects of arecoline on total responses, but tolerance did develop to the effects of arecoline on total reinforcements, suggesting the importance of behavioral variables. A second experiment, investigating tolerance development to the effects of arecoline on FR behavior also provided evidence for the importance of behavioral factors in tolerance development. However, dispositional and physiological factors were also involved. This has been termed behaviorally augmented tolerance (LeBlanc et al., 1976).

In contrast, in rats responding on a VI schedule of reinforcement, after 25 days of pre-session nicotine administration there was tolerance to both the response and reinforcement decreases initially produced by nicotine. However, in rats receiving post-session nicotine injections,

tolerance developed at a faster rate than in the pre-session group. The degree of tolerance development was similar for both nicotine groups at the end of chronic treatment (66 days). Thus, the interaction of nicotine and the experimental situation may have impaired the development of tolerance, suggesting that behavioral factors may play an inhibitory role in tolerance development to nicotine. An alternate theory is that debilitating effects of nicotine may have contributed to the impairment of tolerance development. The importance of dispositional and/or physiological factors in the development of tolerance to nicotine are stressed by this study. In view of the uniqueness of the results obtained in the nicotine tolerance study, this study should be replicated. The effects of chronic treatment with the same dose, as in this study, as well as other doses, should be studied to examine if the observed effect is a factor of dose.

Experiment III was designed to pharmacologically characterize the DS effect of arecoline. Previously, very little research had been done on the DS effect of arecoline. The acquisition of the arecoline discrimination was demonstrated to be dose dependent. Rats could learn to discriminate 1.74 mg/kg from saline, but not 0.58 mg/kg from saline. The 1.74 mg/kg dose produced disruption of response rates, an effect to which tolerance did not develop. The ability of intermediate doses of arecoline to serve as a DS, possibly without as much behavioral disruption, is an area for future research. Through the use of agonist and antagonist studies, the DS effect of arecoline was demonstrated to be mediated through central muscarinic receptors. The muscarinic basis of the DS effect of arecoline contrasts with the nicotinic basis of the DS effect of nicotine. By comparing the effects of manipulations of the

central cholinergic system on the DS effect of these two drugs, it will be possible to gain information on central muscarinic and nicotinic systems.

Experiments IV and V examined the mechanism of action and the CNS site of action, respectively, of the DS effect of arecoline and nicotine. In Experiment IV, it was demonstrated that arecoline interacts with muscarinic receptors that are innervated by a cholinergic input. Thus these receptors may be classified as cholinergic. In contrast, it was demonstrated that the receptors that mediate the DS effect of nicotine may not receive a cholinergic input. Thus, these receptors may only be pharmacological and not physiological.

In Experiment V, it was demonstrated that both the MRF and DH are involved in the mediation of the DS effect of nicotine. In contrast, neither site could be demonstrated to play a role in mediating the DS effect of arecoline.

When Experiment IV and V are viewed together, one can make some conclusions about central cholinergic systems. Based on the lack of interaction of physostigmine with the DS effect of nicotine, and the involvement of the MRF and DH in mediating the DS effect of nicotine, one might conclude that the nicotinic receptors in these areas are non-cholinergic. Further tests on this hypothesis are warranted. One method of exploring this would be to assess the interaction of mecamylamine with electrical stimulation of the septal nucleus, the origin of the major cholinergic input to the hippocampus, on hippocampal EEG or single unit activity.

Even though it possesses a major cholinergic innervation, the DH does not mediate the DS effect of arecoline. Thus, other brain sites that

possess a high level of muscarinic cholinergic activity must be evaluated for their role in mediation of the DS effect of arecoline.

Through all the studies with arecoline, it was possible to separate DS effects from response rate effects. In the dose-effect relationship, 0.58 mg/kg produced almost saline-like DBR, but reduced response rates to 50% of saline levels. Atropine antagonized the DS effect of arecoline, but not the response rate depressant effect. The generalization of physostigmine to the DS effect of arecoline was antagonized by atropine, but the response rate depressant effect was not. The above data suggest the existence of two muscarinic systems, one that mediates the DS effect of muscarinic stimulation, the other, the response rate or motor output decreasing effects of muscarinic stimulation. Anatomical support for this hypothesis is given by the failure of the MRF and DH to mediate the DS effect of arecoline but to mediate response rate decreases. On the other hand, a sensitivity difference may account for the separation of the DS effects and response rate effects. Studies utilizing different doses of arecoline and different schedules of reinforcement may be able to examine this possibility more thoroughly. Further analysis of this separation of effects is indeed an area for research. Through investigations utilizing chemical modifications of muscarinic agonists, one may separate structurally, as well as anatomically, different receptors for these two muscarinic effects.

More research is required to fully understand the functional significance of central nicotinic and muscarinic systems and how they relate to behavior. It is hoped that the research presented in this dissertation has added something to that understanding and will provide the impetus for further research.

IX. REFERENCES

Albert, A.   Ionization, pH and biological activity.   Pharmacol. Rev.
    4: 136-167, 1952.

Anden, N.E., Dahlström, A., Fuxe, A., Larsson, K., Olson, L. and
    Ungerstedt, U.   Ascending monoamine neurons to the telencephalon
    and diencephalon.   Acta Physiol. Scand. 67: 313-326, 1966.

Andersen, P. and Curtis, D.R.   The pharmacology of the synaptic and
    acetylcholine-induced excitation of ventrobasal thalamic neurons.
    Acta Physiol. Scand. 61: 100-120, 1964.

Appel, J.B., White, F.J., and Kuhn, D.M.   The use of drugs as discrim-
    inative stimuli in behavioral pharmacodynamics.   In Stimulus Proper-
    ties of Drugs: Ten Years of Progress. ed. by F.C. Colpaert and J.A.
    Rosecrans, pp. 7-29, Elsevier, Amsterdam, 1978.

Arjungi, Von K.N.   Areca Nut: a review.   Arzneimittel-Forsch. 26:
    951-956, 1976.

Arnt, J. and Scheel-Krüger, J.   GABAergic and glycinergic mechanisms
    within the substania nigra: Pharmacological specificity of dopamine-
    independent contralateral turning behavior and interactions with
    other neurotransmitters. Psychopharpharmacology (Berl.) 62: 267-277,
    1979

Barry, H. III.   Classification of drugs according to their discriminable
    effects in rats.   Federation Proc. 33: 1814-1824, 1974.

Beckett, A.H., Gorrod, J.W., and Jenner, P.   The effect of smoking on
    nicotine metabolism in vivo in man.   J. Pharm. Pharmacol., 23:
    62S-67S, 1971.

Bird, S.J. and Aghajanian, G.K.   The cholinergic pharmacology of hippo-
    campal pyramidal cells: a microiontophoretic study.   Neuropharmaco-
    logy 15: 273-282, 1976.

Boren, J.J. and Navarro, A.P.   The action of atropine, benactyzine and
    scopolamine upon fixed-interval and fixed-ratio behavior.   J. Exp.
    Anal. Behav. 2: 107-115, 1959.

Bovet, D., Bovet-Nitti, F., and Oliverio, A.   Action of nicotine on
    spontaneous and acquired behavior in rats and mice.   Ann. N.Y. Acad.
    Sci. 142: 201-215, 1967.

Bradley, P.B., Dhawan, B.N., and Wolstencroft, J.H.   Pharmacological
    properties of cholinoceptive neurones in the medulla and pons of the
    cat.   J. Physiol., Lond. 183: 658-674, 1966.

Bradley, P.B. and Wolstencroft, J.H. Effects of acetylcholine, nico-
tine, and muscarine on brain stem neurons. Ann. N.Y. Acad. Sci. 142:
15-20, 1967.

Brownstein, M., Kobayashi, R., Palkovits, M., and Saavedra, J.M.
Choline acetyltransferase in diencephalic nuclei of the rat.
J. Neurochem. 24: 35-38, 1973.

Bruning, J.L. and Kintz, B.L. Computational Handbook of Statistics.
Scott, Foresman and Company, Glenview, 1968.

Butcher, S.G., and Butcher, L.L. Origin and modulation of acetylcholine
activity in the neostriatum. Brain Res. 71: 167-171, 1974.

Byford, G.H. Signal variance and its application to continuous measure-
ments of EEG activity. Proc. R. Soc. 161B: 421-437, 1965.

Carson, K.A., Nemeroff, C.B., Rone, M.S., Youngblood, W.W., Prange,
A.J., Jr., Hanker, J.S., and Kizer, J.S. Biochemical and histo-
chemical evidence for the existence of a tuberoinfundibular cholin-
ergic pathway in the rat. Brain Res. 129: 169-173, 1977.

Chait, L.D. and Balster, R.L. Effects of phencyclidine, atropine, and
physostigmine, alone and in combination, on variable-interval perfor-
mance in the squirrel monkey. Pharmac. Biochem. Behav. 11: 37-42,
1979.

Chance, W.T., Murfin, D., Krynock, G.M., and Rosecrans, J.A. A descrip-
tion of the nicotine stimulus and tests of its generalization to
amphetamine. Psychopharmacology (Berl.) 55: 14-26, 1977.

Chance, W.T., Kallman, M.D., Rosecrans, J.A., and Spencer, R.M. A
comparison of nicotine and structurally related compounds as dis-
criminative stimuli. Br. J. Pharmac. 63: 609-616, 1978.

Chen, C.S. A study of alcohol tolerance effect and an introduction of
a new behavioral technique. Psychopharmacologia (Berl.) 12: 433-440,
1968.

Cheney, D.L., LeFeure, H.F., and Racagni, G. Choline acetyltransferase
activity and mass fragmentographic measurement of acetylcholine in
specific nuclei and tracts of rat brain. Neuropharmacology 14:
801-809, 1975.

Cornfield-Sumner, P.K. and Stolerman, I.P. Behavioral tolerance. In
Contemporary Research in Behavioral Pharmacology, ed. by D.E. Black-
man and D.J. Sanger, pp. 391-448, Plenum Press, New York, 1978.

Coutts, R.T. and Scott, J.R. The chemistry and pharmacology of some
tetrahydropyridines including arecoline and its derivatives. Can.
J. Pharm. Sci. 6: 78-84, 1971.

Curtis, D.R. and Crawford, J.M. Central synaptic transmission - micro-
electroporetic studies. Ann. Rev. Pharmacol. 9: 209-241, 1964.

Curtis, D.R. and Ryall, R.W. The excitation of Renshaw cells by cholinomimetics. Expl. Brain Res. 2: 49-65, 1966.

Dale, H.H. The action of certain esters and ethers of choline, and their relation to muscarine. J. Pharmacol. Exp. Ther. 6: 147-193, 1914.

Davis, T.R.A. and Kensler, C.J. Comparison of behavioral effects of nicotine, d-amphetamine, caffeine and dimethylheptyl tetrahydrocannabinol in squirrel monkeys. Psychopharmacologia (Berl.) 32: 51-65, 1973.

Dews, P.B. Stimulant actions of methamphetamine. J. Pharmacol. Exp. Ther. 122: 137-147, 1958.

Dews, P.B. Behavioral tolerance. In Behavioral Tolerance: Research and Treatment Implications. ed. by N.A. Krasnegor. pp. 18-26. Natl. Inst. Drug Abuse Res. Monogr. Ser. 18, Washington, D.C., 1978.

Domino, E.F. Some behavioral actions of nicotine. In Tobacco Alkaloids and Related Compounds. ed. by U.S. von Euler, pp. 145-162. Pergamon Press, New York, 1965a.

Domino, E.F. Some comparative pharmacological actions of (-)-nicotine, its optical isomer, and related compounds. In Tobacco Alkaloids and Related Compounds. ed. by U.S. von Euler, pp. 303-313, Pergamon Press, New York, 1965b.

Domino, E.F. Electroencephalographic and behavioral arousal effects of small doses of nicotine: A neuropsychopharmacological study. Ann. N.Y. Acad. Sci. 146: 216-244, 1967.

Domino, E.F. Neuropsychopharmacology of nicotine and tobacco smoking. In Smoking Behavior: Motives and Incentives. ed. by W.L. Dunn, Jr. pp. 5-31, V.H. Winston and Sons, Washington, D.C., 1973.

Domino, E.F. and Lutz, M.P. Tolerance to the effects of daily nicotine on rat bar pressing behavior for water reinforcement. Pharmac. Biochem. Behav. 1: 445-448, 1973.

Ferster, C.B. and Skinner, B.F. Schedules of Reinforcement. Appleton-Century-Crofts, New York, 1957.

Floris, V., Morocutti, C., and Ayala, G.F. Effects of nicotine on the cortical, thalamic, and hippocampal electrical activity in rabbits. J. Neuropsychiat. 5: 247-251, 1964.

Fonnum, F. Topographical and subcellular fractionation in the rat hippocampal region. J. Neurochem. 17: 1029-1037, 1970.

Fonnum, F., Walaas, I., and Iverson, E. Localization of GABAergic, cholinergic and aminergic structures in the mesolimbic system. J. Neurochem. 29: 219-230, 1977.

Freedman, D.X., Appel, J.B., Hartman, F.R., and Molliuer, M.E. Tolerance to behavioral effects of LDS-25 in rat. J. Pharmacol. Exp. Ther. 143: 309-313, 1964.

Glick, S.D., Jerussi, T.P., and Fleisher, L.N. Turning in circles: The neuropharmacology of rotation. Life Sci. 18: 889-896, 1976.

Goldstein, A., Aronow, L., and Kalman, S.M. Principles of Drug Action: The Basis of Pharmacology. John Wiley and Sons, New York, 1974.

Gottesfeld, Z. and Jacobowitz, D.M. Cholinergic projection of the diagonal band to interpeduncular nucleus of the rat brain. Brain Res. 156: 329-342, 1978.

Green, J.D. and Arduini, A. Hippocampal electrical activity in arousal. J. Neurophysiol. 17: 533-557, 1954.

Grossman, S.P. Behavioral and electroencephalographic effects of micro-injections of neurohumors into the midbrain reticular formation. Physiol. Behav. 3: 777-786, 1968.

Grossman, S.P. and Grossman, L. The effects of chemical stimulation of the midbrain reticular formation on appetitive behavior. J. Comp. Physiol. Psych. 61: 333-338, 1966.

Harris, R.T., Waters, W., and McLendon, D. Behavioral effects in rhesus monkeys of repeated intravenous doses of Δ^9-tetrahydrocannabinol. Psychopharmacologia (Berl.) 21: 297-306, 1972.

Haubrich, D.R., Reid, W.D., and Gillette, J.R. Acetylcholine formation in mouse brain and effect of cholinergic drugs. Nature, New Biol. 238: 88-89, 1972.

Haubrich, D.R. and Watson, D.R. Effects of pilocarpine or arecoline administration on acetylcholine levels and serotonin turnover in rat brain. J. Pharmacol. Exp. Ther. 181: 19-27, 1972.

Herz, A. Excitation and inhibition of cholinoceptive brain structures and its relationship to pharmacological induced behavior changes. Int. J. Neuropharmacol. 2: 205-216, 1963.

Herz, A., Holzhäuser, H., and Teschemacher, H. Central nicotine- and muscarine-like properties of cholinomimetic drugs with regard to their lipid solubility. Ann. N.Y. Acad. Sci. 142: 21-26, 1967.

Hirschhorn, I.D. and Rosecrans, J.A. Studies on the time course and the effect of cholinergic and adrenergic receptor blockers on the stimulus effect of nicotine. Psychopharmacologia (Berl.) 40: 109-120, 1974.

Holmstedt, B. and Lundgren, G. Tremoregenic agents and brain acetylcholine. In Mechanisms of Release of Biogenic Amines, ed. by U.C. von Euler, S. Rosell, and B. Uvnäs, pp. 439-468, Pergamon Press, New York, 1966.

Holmstedt, B. and Lundgren, G. Arecoline, nicotine, and related compounds. Tremorgenic activity and effect upon brain acetylcholine. Ann. N.Y. Acad. Sci. 142: 126-142, 1967.

Hoover, D.B., Muth, E.A., and Jacobowitz, D.M. A mapping of the distribution of acetylcholine, choline acetytransferase and acetylcholinesterase in discrete areas of rat brain. Brain Res. 153: 295-306, 1978.

Jacobowitz, D.M. and Palkovits, M. Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. I. Forebrain (telencephalon, diencephalon). J. Comp. Neurol. 157: 13-28, 1974.

Jarvik, M.E. Tolerance to the effects of nicotine. In Cigarette Smoking as a Dependence Process. ed. by N.A. Krasnegor, pp. 150-157, Natl. Inst. Drug Abuse Res. Monogr. Ser. 23, Washington, D.C., 1979.

Karczmar, A.G. Is the central cholinergic nervous system overexploited? Fed. Proc. 28: 147-157, 1969.

Karczmar, A.G. Cholinergic influences on behavior. In Cholinergic Mechanisms, ed. P.G. Waser, pp. 501-529, Raven Press, New York, 1975.

Karczmar, A.G. Exploitable aspects of central cholinergic functions, particularly with respect to the EEG, motor, analgesic and mental functions. In Cholinergic Mechanisms and Psychopharmacology, ed. by D.J. Jenden, pp. 679-708, Plenum Press, New York, 1977.

Kataoka, K., Hakamura, Y., and Hassler, R. Habenulo-interpenduncular tract: A possible cholinergic neuron in rat brain. Brain Res. 62: 264-267, 1973.

Kawamura, H. and Domino, E.F. Differential actions of m and n choinergic agonists on the brain stem activating system. Int. J. Neuropharmacol. 8: 105-115, 1969.

Kelleher, R.T. and Morse, W.H. Determinants of the specificity of behavioral effects of drugs. Ergebnisse der Physiologie: 60: 1-56, 1968.

Knapp, D.E. and Domino, E.F. Action of nicotine on the ascending reticular activating system. Int. J. Neuropharmacol. 1: 333-351, 1962.

Kobayashi, R.M., Palkovitz, M., Hruska, R.E., Rothschild, R., and Yamamura, H.I. Regional distribution of muscarinic cholinergic receptors in rat brain. Brain Res. 154: 13-23, 1978.

Kostowski, W., Giacalone, E., Garattini, S., and Valzelli, S. Electrical stimulation of midbrain raphé: biochemical, behavioral and bioelectric effects. Eur. J. Pharmacol. 7: 170-179, 1969.

Krnjević, K. Central cholinergic pathways. Fed. Proc. 28: 113-120, 1969.

Krnjević, K. and Phillis, J.W. Pharmacological properties of acetyl-choline-sensitive cells in the cerebral cortex. J. Physiol., Lond. 166: 328-350, 1963.

Kuhar, M.J., Sethy, V., Roth, R.H., and Aghajanian, G.K. Choline: Selective accumulation by central cholinergic neurons. J. Neurochem. 20: 581-593, 1973.

Langer, S.Z. Presynaptic receptors and their role in the regulation of transmitter release. Br. J. Pharmacol. 60: 481-497, 1977.

LeBlanc, A.E., Kalant, H., and Gibbins, R.J. Acquisition and loss of behaviorally augmented tolerance to ethanol in the rat. Psycho-pharmacology (Berl.) 48: 153-158, 1976.

LeBlanc, A.E., Poulos, L.X., and Cappell, H.D. Tolerance as a beha-vioral phenomenon: Evidence from two experimental paradigms. In Behavioral Tolerance: Research and Treatment Implications, ed. by N.A. Krasnegor, pp. 72-89. Natl. Inst. Drug Abuse Res. Monogr. Ser. 18, Washington, D.C., 1978.

Lewis, P.R. and Shute, C.C.D. The cholinergic limbic system: Projec-tions to the hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system and the subfornical organ and supra-optic crest. Brain 90: 521-540, 1967.

Longo, V.G., Giunta, F., and Scotti de Carolis, A. Effects of nicotine on the electroencephalogram of the rabbit. Ann. N.Y. Acad. Sci. 142: 159-169, 1967.

Longo, V.G., von Berger, G.P., and Bovet, D. Action of nicotine and of the "ganglioplégiques centraux" on the electrical activity of the brain. J. Pharmacol. Exp. Therap. 111: 349-359, 1954.

Madill, H.D. and Parker, J.M. The potentiating effect of DFP upon the central tremorgenic potency of nicotine-N-oxide in mice. In Drugs and Cholinergic Mechanisms in the CNS, ed. by E. Heilbronn and A. Winter, pp. 529-542, Försvarets Forskningsantalt, Stockholm, 1970.

Mattila, N.J., Ahtee, L., and Saarnivaara, L. The analgesic and sedative effects of nicotine in white mice, rabbits, and golden hamsters. Ann. Med. Exp. Bio. Fenn. (Helsinki), 46: 78-84, 1968.

McGeer, E.G., McGeer, P.L., Hattori, T., and Singh, V.K. Immunohisto-chemistry of choline acetyltransferase. In The Cholinergic Synapse, ed. by S. Tucek, Elsevier, Amsterdam, 1979.

McGeer, E.G., Wada, J.A., Terao, A., and Jung, E. Amine synthesis in various brain regions with caudate or septal lesions. Exp. Neurol. 24: 277-284, 1969.

McGeer, P.L. and McGeer, E.G. Central cholinergic pathways. In Nutrition and the Brain. Vol. 5, ed. by A. Barbeau, J.H. Growdon, and R.J. Wurtman, pp. 117-199, Raven Press, New York, 1979.

McGeer, P.L., McGeer, E.G., Fibiger, H.C., and Wickson, V. Neostriatal choline acetylase and acetylcholinesterase following selective brain lesions. Brain Res. 35: 308-314, 1971.

McIsaac, R.J. The relationship between distribution and pharmacological activity of hexamethonium-N-methyl C^{14}. J. Pharmacol. Exp. Ther. 135: 335-343, 1962.

McKim, W.A. The effects of scopolamine on fixed-inteval behavior in the rat: a rate dependency effect. Psychopharmacologia (Berl.) 32: 255-264, 1973.

McLennan, H. and Hicks, T.P. Pharmacological characterization of the excitatory cholinergic receptors of rat central neurones. Neuropharmacology 17: 329-334, 1978.

McLennan, H. and York, D.H. Cholinergic mechanism in the caudate nucleus. J. Physiol., Lond., 187: 163-175, 1966.

Morrison, C.F. Effects of nicotine on operant behavior of rats. Int. J. Neuropharmacol. 6: 229-240, 1967.

Morrison, C.F., Goodyear, J.M., and Sellers, C.M. Antagonism by anti-muscarinic and ganglion-blocking drugs of some of the behavioral effects of nicotine. Psychopharmacologia (Berl.) 15: 341-350, 1969.

Morrison, C.F. and Lee, P.N. A comparison of the effects of nicotine and amphetamine on the DRL performance in the rat. Psychopharmacologia (Berl.) 12: 176-180, 1968a.

Morrison, C.F. and Lee, P.N. A comparison of the effects of nicotine and physostigmine on a measure of activity in the rat. Psychopharmacologia (Berl.) 13: 210-221, 1968b.

Morrison, C.F. and Stephenson, J.A. Nicotine injections as the conditioned stimulus in discrimination learning. Psychopharmacologia (Berl.) 15: 351-360, 1969.

Morrison, C.F. and Stephenson, J.A. The occurance of tolerance to a central depressant effect of nicotine. Br. J. Pharmac. 45: 151-156, 1972.

Murphee, H.B. EEG effects in humans of nicotine, tobacco smoking, withdrawal from smoking and possible surrogates. In Electrophysiological Effects of Nicotine, ed. by A. Rémond and C. Izard. pp. 227-244. Elsevier, New York, 1979.

Myers, R.D. Injection of solutions into cerebral tissue: relation between volume and diffusion. Physiol. Behav. 1: 171-174, 1966.

Nelsen, J.M., Pelley, K., and Goldstein, L. Chronic nicotine treatment in rats. 2. Electroencephalographic amplitude and variability changes occuring within and between structures. Res. Comm. Chem. Path. Pharmacol. 5: 694-704, 1973.

Nieschultz, O. and Schmersahl, P. Zurpharmakologic der wirkstoffe des betels. 2. Mitteilung: Umnoandlung des arecolin in arecaidin. Arzneimittel-Forsch, 18: 222-225, 1968.

Olds, M.E. and Domino, E.F. Comparison of muscarinic and nicotinic cholinergic agonists on self-stimulation behavior. J. Pharmacol. Exp. Ther. 166: 189-204, 1969.

Olivier, A., Parent, A., Simard, H. Poirier, L.J. Cholinesterase striatopallidal and striatonigral efferent in the cat and the monkey. Brain Res. 18: 273-282, 1970.

Overton, D.A. Experimental methods for the study of state-dependent learning. Federation Proc. 33: 1800-1813, 1974.

Overton, D. Influence of shaping procedures and schedules of reinforcement on performance in the two-bar discrmination task: A methodological report. Psychopharmacology (Berl.) 65: 291-298, 1979.

Palkovits, M. and Jacobowitz, D.M. Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. II. Hindbrain (mesencephalon, rhombencephalon). J. Comp. Neurol. 157: 28-42, 1974.

Palkovits, M., Saavedra, J.M., Kobayashi, R.M., and Brownstein, M. Choline acetyltransferase content of limbic nuclei of the rat. Brain Res. 79: 433-450, 1974.

Paul-David, J., Riehl, J.L., and Unna, K.R. The quantification of effects of depressant drugs on EEG activation response. J. Pharmacol. Exp. Ther. 129: 69-74, 1960.

Pellegrino, L.J. and Cushman, A.J. A Stereotaxic Atlas of the Rat Brain. Appleton-Century-Crofts, New York, 1967.

Pepu, G. Mulas, A., Ruff, A., and Sotgiu, P. Brain acetylcholine levels in rats with septal lesions. Life Sci. 10: 181-184, 1971.

Pfeiffer, C.C. and Jenny, E.H. The inhibition of the conditioned reponse and the counteraction of schizophrenia by muscarinic stimulation of the brain. Ann. N.Y. Acad. Sci. 66: 753-764, 1957.

Phillis, J.W. Evidence for cholinergic transmission in the cerebral cortex. In Neurohumoral Coding of Brain Function, ed. by R.D. Myers and R.R. Drucker-Colin, pp. 57-77, Plenum Press, New Yor, 1975.

Pradhan, S.N. Effects of nicotine on several schedules of behavior in rats. Arch. Int. Pharmacodyn. 183: 127-138, 1970.

Pradhan, S.N. and Dutta, S.N. Comparative effects of nicotine and amphetamine on timing behavior in rats. Neuropharmacology 9: 9-16, 1970a.

Pradhan, S.N. and Dutta, S.N. Behavioral effects of arecoline in rats. Psychopharmacologia (Berl.) 17: 49-58, 1970b.

Riehl, J.L., Paul-David, J., and Unna, K.R. Comparison of the effects of arecoline and muscarine on the central nervous system. Int. J. Neuropharmacol. 1: 393-401, 1962.

Rosecrans, J.A. Effects of nicotine on behavioral arousal and brain 5-hydroxytryptamine function in female rats selected for differences in activity. Eur. J. Pharmacol. 14: 29-37, 1971a.

Rosecrans, J.A. Effects of nicotine on behavior and brain 5-hydroxytryptamine function in male and female rats separated for differences in activity. Eur. J. Pharmacol. 16: 123-127, 1971b.

Rosecrans, J.A. Brain area nicotine levels in male and female rats with different levels of spontaneous activity. Neuropharmacology 11: 863-870, 1972.

Rosecrans, J.A. and Chance, W.T. Cholinergic and non-cholinergic aspects of the discriminative stimulus properties of nicotine. In Discriminative Stimulus Properties of Drugs, ed by H. Lal. pp. 155-186. Plenum Press, New York, 1977

Rosecrans, J.A. and Chance, W.T. The discriminative stimulus properties of N- and M-cholinergic receptor stimulants. In Drug Discrimination and State Dependent Learning, ed. by B.T. Ho, D.L. Chute, and D.W. Richards, pp. 119-130, Academic Press, New York, 1978.

Rosecrans, J.A., Dren, T.A., and Domino, E.F. Effects of physostigmine on rat brain acetylcholine, acetylcholinesterase and conditioned pole jumping. Int. J. Neuropharmacol. 7: 127-134, 1968.

Rossier, J. Immunological properties of rat brain cholineacetyltransferase. J. Neurochem. 26: 549-553, 1976.

Russell, M.A.H. and Feyerbend, C. Cigarette smoking: A dependence on high-nicotine boli. Drug Metabol. Rev. 8: 29-59, 1978.

Schechter, M.D. and Rosecrans, J.A. C.N.S. effect of nicotine as the discriminative stimulus for the rat in a T-maze. Life Sci. 10: 821-832, 1971a.

Schechter, M.D. and Rosecrans, J.A. Behavioral evidence for two types of cholinergic receptors in the C.N.S. Eur. J. Pharmacol. 15: 375-378, 1971b.

Schechter, M.D. and Rosecrans, J.A. Effect of mecamylamine on discrimination between nicotine- and arecoline-produced cues. Eur. J. Pharmacol. 17: 179182, 1972a.

Schechter, M.D. and Rosecrans, J.A. Atropine antagonism of arecoline-cued behavior in the rat. Life Sci. 11: 519-523, 1972b.

Schechter, M.D. and Rosecrans, J.A. Nicotine as a discriminative cue in rats: inability of related drugs to produce a nicotine-like cueing effect. Psychopharmacologia (Berl.) 17: 379-387, 1972c.

Schechter, N., Handy, I.C., Pezzementi, L., and Schmidt, J. Distribution of α-bungarotoxin binding sites in the central nervous system and peripheral organs of the rat. Toxicon 16: 245-251, 1978.

Schuster, C.R. Theoretical basis of behavioral tolerance: Implications of the phenomenon for problems of drug abse. In Behavioral Tolerance: Research and Treatment Implications, ed. by N.A. Krasnegor, pp. 4-17, Natl. Inst. Drug Abuse Res. Monogr. Ser. 18, Washington, D.C., 1978.

Schuster, C.R., Dockens, W.S., and Woods, J.H. Behavioral variables affecting the development of amphetamine tolerance. Psychopharmacologia (Berl.) 9: 170-182, 1966.

Segal, M. The acetylcholine receptor in the rat hippocampus: Nicotinic, muscarinic or both? Neuropharmacology 17: 619-623, 1978.

Segal, M., Dudai, Y., and Amsterdam, A. Distribution of an α-bungarotoxin-binding cholinergic nicotinic receptor in rat brain. Brain Res. 148: 105-119, 1978.

Sheard, M.H. and Aghajanian, G.K. Stimulation of midbrain raphé neurons: behavioral effects of serotonin release. Life Sci. 7: 19-25, 1968.

Shute, C.C.D. and Lewis, P.R. The ascending cholinergic reticular formation: Neocortical, olfactory, and subcortical projections. Brain 90: 497-520, 1967.

Smith, C.M. The release of acetylcholine from rat hippocàmpus. Br. J. Pharmacol. 45: 172P, 1972.

Smith, C.M. Acetylcholine release from the cholinergic septo-hippocampus pathway. Life Sci. 14: 2159-2166, 1974.

Snyder, S.H., Chung, K.J., Kuhar, M.J., and Yamamura, H.I. Biochemical identification of the mammalian muscarinic cholinergic receptor. Federation Proc. 34: 1915-1921, 1975.

Starke, K., Taube, H.A., and Borowski, E. Presynaptic receptor systems in catecholaminergic transmission. Biochem. Pharmacol. 26: 259-268, 1977.

Stitzer, M., Morrison, J., and Domino, E.F. Effects of nicotine on fixed-interval behavior and their modification by cholinergic antagonists. J. Pharmacol. Exp. Ther. 171: 166-177, 1970.

Stümpf, C. and Gogolák. Actions of nicotine upon the limbic system. Ann. N.Y. Acad. Sci. 142: 143-158, 1967.

Stolerman, I.P., Bunker, P., and Jarvik, M.E. Nicotine tolerance in rats; role of dose and dose interval. Psychopharmacologia (Berl.) 34: 317-324, 1974.

Stolerman, I.P., Fink, R., and Jarvik, M.E. Acute and chronic tolerance to nicotine measured by activity in rats. Psychopharmacologia (Berl.) 30: 329-342, 1973.

Tang, M. and Falk, J.L.   Behavioral and pharmacological components of phenobarbital tolerance.   In Behavioral Tolerance: Research and Treatment Implications.   ed. by N.A. Krasnegor.   pp. 142-148. Natl. Inst. Drug Abuse Res. Monogr. Ser. 18, Washington, D.C., 1978.

Todd, G.D. and Dougherty, J.A.   Acute and chronic tolerance to a behavioral effect of nicotine in rats.   Neurosci. Abst. 5: 2271, 1979.

Vaillant, G.E.   Antagonism between physostigmine and atropine on the behavior of the pigeon.   Naunyn-Schmiedebergs Arch. Exp. Path. Pharmcol. 284: 406-416, 1964.

Vaillant, G.E.   A comparison of antagonists of physostigmine-induced suppression of behavior.   J. Pharmacol. Exp. Ther. 157: 636-648, 1967.

Vanderwolf, C.H., Kramis, R., and Robinson, T.E.   Hippocampal electrical activity during waking, behavior and sleep: analysis using centrally acting drugs.   In   Functions of the Septo-Hippocampal System, Ciba Foundation Symposium 58 pp. 119-226. Elsevier/Excerpta Medica, New York, 1978.

Vazquez, A.J. and Toman, J.E.P.   Some interactions of nicotine with other drugs upon central nervous function.   Ann. N.Y. Acad. Sci. 142: 201-215, 1967.

Volle, R.L.   Ganglionic transmission.   Ann. Rev. Pharmacol. 9: 135-146, 1969.

Walaas, I. and Fonnum, F.   The effect of parenteral glutamate treatment on the localization of neurotransmitters in the mediobasal hypothalamus. Brain Res. 153: 549-562, 1978.

Westfall, T.C.   Local regulation of adrenergic transmission.   Physiol. Rev. 57: 659-728, 1977.

WHO scientific group report.   Evaluation of dependence producing drugs. WHO Tech. Rep. Ser. 287, Geneva, 1964.

Woolverton, W.L. and Balster, R.L.   Tolerance to the behavioral effects of phencyclidine: The importance of behavioral and pharmacological variables.   Psychopharmacology (Berl.) 64: 19-24, 1979.

Yamamoto, K.I. and Domino, E.F.   Cholinergic agonist-antagonist interactions on neocortical and limbic EEG activation.   Int. J. Neuropharmacol. 6: 357-373, 1967.

Yamamura, H.I., Kuhar, M.J., Greenberg, D., and Snyder, S.H.   Muscarinic cholinergic receptor binding: regional distribution in monkey brain. Brain Res. 66: 541-546, 1974.

Zetler, G.   Cataleptic state and hypothermia in mice, caused by central cholinergic stimulation and antagonized by anticholinergic and antidepressant drugs.   Int. J. Neurochem. 7: 325-335, 1968.

Zetler, G.   Pharmacological differentiation of "nicotinic" and "muscarinic" catalepsy.   Neuropharmacology 10: 284-246, 1971.