Evidence That Nicotine Can Acutely Desensitize Central Nicotinic Cholinergic Receptors In Vivo

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"EVIDENCE THAT NICOTINE CAN ACUTELY DESENSITIZE CENTRAL NICOTINIC CHOLINERGIC RECEPTORS IN VIVO"

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

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is producing its effects via an interaction at nicotinic-
cholinergic receptors (nAChRs) which open a membrane cation
channel. Following initial opening of the channel, nicotine
appears to induce a rapid desensitization of the nAChRs,
closing the channel and resulting in a cessation of
nicotine's effects. Research presented here will provide
evidence of this secondary desensitization process in vivo
by demonstrating nicotine's ability to induce acute
tolerance in the discriminative stimulus (DS) paradigm. The
ability of nicotine to elicit DS control of behavior was
significantly reduced via challenge doses of (800, 1200, and
1600 ug/kg, s.c.) of nicotine administered 60-180 minutes
prior to the training dose (400 ug/kg, s.c.). Eight out of
twenty rats demonstrated this phenomena, with time and dose
varying, suggesting that these effects may be contingent upon
the individual rat studied. It appears that we have found a
means of investigating cellular mechanisms in vivo using
operant behavior.
ABSTRACT

Current concepts concerning nicotine’s central nervous system (CNS) mechanism(s) of action suggest that this drug is producing its effects via an interaction at nicotinic-cholinergic receptors (nAChRs) which open a membrane cation channel. Following initial opening of the channel, nicotine appears to induce a rapid desensitization of the nAChRs, closing the channel and resulting in a cessation of nicotine’s effects. Research presented here will provide evidence of this secondary desensitization process in vivo by demonstrating nicotine’s ability to induce acute tolerance in the discriminative stimulus (DS) paradigm. The ability of nicotine to elicit DS control of behavior was significantly reduced via challenge doses of (800, 1200, and 1600 µg/kg, s.c.) of nicotine administered 60-180 minutes prior to the training dose (400 µg/kg, s.c.). Eight out of twenty rats demonstrated this phenomena, with time and dose varying, suggesting that these effect may be contingent upon the individual rat studied. It appears that we have found a means of investigating cellular mechanisms in vivo using operant behavior.
INTRODUCTION

Since the mid 1960’s one of the hottest areas of central nervous system research has dealt with the mechanisms of action of neurotransmitters and their endogenous and/or exogenous ligands. Nicotine has drawn the attention of researchers because of its agonist effect upon the neurotransmitter acetylcholine. Researchers have shown that there are functional receptors for nicotine in both the peripheral and central nervous systems.

The use of in vitro and in vivo studies is reviewed in relation to acute tolerance (desensitization). An attempt is made to integrate these in vitro and in vivo studies into a meaningful analysis of acute tolerance (desensitization) in the brain. The emphasis of this analysis is through the use of time and dose to develop a better understanding of the mechanism(s) of action leading to acute tolerance (desensitization). The discussion will suggest that the mechanism(s) of action is(are) individually specific, and may be useful in the determination of why individual people abuse drugs.
CHAPTER ONE

A BRIEF HISTORY:

Commercial cultivation of tobacco began in the United States in 1612, at Jamestown, Virginia, and soon became the major cash crop in the Colonies. Tobacco was used to pay taxes and often the salaries of public officials (Encyclopedia Americana, 1987). The use of tobacco rapidly spread throughout the world and in 1984 the United States alone produced tobacco products in excess of 17 billion dollars (U.S. Bureau of the Census, 1987).

Research to determine the component(s) of tobacco responsible for its pharmacological effects began in the 1800's. Cerioli and Vanguelin were the first to isolate nicotine from tobacco; they named it "nicotianine". In 1828, Posselt and Reiman purified the compound and renamed it "nikotin", which was first synthesized during the 1890's (U.S. Dept. of Health and Human Services, 1988). Langley and Dickinson (1889) were first to report on the stimulating and "paralysing" effects of nicotine on the autonomic nervous system (ANS), a finding which played a central role in characterizing the physiology of the mammalian autonomic nervous system. Further work led to the realization that large doses of nicotine were able to block the stimulation of ganglion cells (Langley and Anderson, 1895; Langley, 1886, 1911). Langley in 1914 showed that the skeletal muscle effects of nicotine were similar to acetylcholine and could be
blocked by curare. Dale (1914) was further able to distinguish two mechanisms of action. Dale, using a spinal cat also, showed that atropine blocked the action of muscarine, but was unable to block the action of nicotine. Thus, Dale was first to recognize two sites of acetylcholine action, a "muscarinic" and a "nicotinic" site (For a review see Goodman & Gillman, (1975)). Since these early classical studies concerning the ANS, much research has been focused towards learning more about sites of action of drugs such as nicotine and muscarine. From this concentrated effort we now realize that these drugs act on specific receptors which are innervated by neurons which release the neurotransmitter, acetylcholine (ACh). The state of the relationships between ACh and these cholinergic receptors is characterized in Fig. 1.

CHAPTER TWO

MOLECULAR MECHANISMS OF ACETYLCHOLINE AND NICOTINE ACTION:

The overriding hypothesis from earlier work with nicotine suggested that this drug acted at specific sites also sensitive to acetylcholine. At this point, nicotine is thought to mimic acetylcholine, or act as though acetylcholine (ACh) was released onto a specific acetylcholinergic site or receptor. Thus, nicotine is classified as a cholinergic drug, and to better understand nicotine’s effects we need to first look at acetylcholine and how it produces its effects. Katz & Thesleff (1957) proposed, that in addition to acetylcholine’s action as an agonist, it can also act as an antagonist at the same
A schematic comparison of nicotinic and muscarinic Cholinergic neurons.

The n-AChR is viewed as linked to a cation channel, while the m-AChR mediates its effects via a second messenger, IP, or c-AMP. Redrawn from Shephard (1988).
cholinergic sites via a desensitization mechanism. These workers were able to show that acetylcholine could induce a desensitization of the nerve-muscle motor end-plate "when the drug concentration was maintained for a sufficiently long time." It was further shown that desensitization resulted in an extended refractory period that only slowly recovered after the removal of acetylcholine. It was theorized that desensitization of the receptor was due to a change from an active form of the receptor site to an inactive form. Acetylcholine was postulated as having a higher affinity for the inactive state than the active state which was used to explain how small doses of acetylcholine were able to facilitate the desensitization of the receptor. This early analysis of desensitization was supported by the work of Fatt (1950) who showed that the concentration of drug was important in the rate of development of desensitization.

Ochoa et al (1989) have presented a more recent view of nicotinic-cholinergic receptor (n-AChR) desensitization that incorporates the basic concepts put forth earlier by Katz & Thesleff (1957). The model suggests that the synaptic release of acetylcholine first interacts with the n-AChR to form an activated state. The activated state then opens a cation channel on the postsynaptic membrane, which allows the influx of positively charged molecules (Fig. 2). The n-AChR is theorized to be rapidly converted to a deactivated state, thus closing the cation channel.
Ochoa suggested that this process may be important in learning and memory deficits as well as myasthenia gravis. It further seems that the desensitization process has a physiological role as a means of turning off the nAChR if too much ligand is released. On the other hand, this process is utilized each day in our use of insecticides in which the acetylcholinesterase inhibitors (prevent the physiological breakdown of ACh) will elicit an elevation of ACh to a point of desensitization, killing the insect attacked. The increase in ACh induces a paralysis of respiratory and other muscles which kills the animal. It might also be mentioned that this is also the basis for the use of "War Gases" by the military.

In vitro studies have also provided us with abundant evidence that nicotine causes a release of acetylcholine through the opening of a cation channel in close proximity to select nicotinic receptors. The opening of this cation channel allows the influx of one or more of the positively charged components of the extracellular fluid. It is a relatively large channel containing many negatively charged molecules that results in a slow influx. The nicotinic receptor is rapidly desensitized resulting in the channel being in the open state for a very short period of time. Armitage (1969), was one of the first to show that nicotine could cause the release of acetylcholine in the brain, and MacIntosh & Osborn (1953) and Mitchell (1963) have shown that acetylcholine is released from the cerebral cortex which has
Mechanisms of nAChR desensitization.

This figure presents an idealized nicotinic cholinergic synaptic junction containing acetylcholine (black dots) contained within synaptic vesicles (in circles) and postsynaptic membrane (From Ochoa et. al., 1989).

A- Receptor exists in an equilibrium condition of a mixture of two forms: resting (R) and desensitized (D) states.

B- An action potential facilitates the release of ACh. The result is a sudden increase in ACh concentration at the synaptic cleft and occupies one of the receptor states, R and D. This induces a conformational change, which leads to the activated state (A), in which the channel opens allowing cation movements essential for the development of a postsynaptic action potential. As soon as ACh occupies, the affinity of the receptors toward ACh and the D state is promoted, resulting in the termination of the action of ACh.
led to further research suggesting that nicotine might cause the release of acetylcholine at several central nervous system (CNS) sites (Armitage & Hall, 1967; Armitiage, Milton & Morrison, 1966; Morrison, 1968). 

*In vitro* electrophysiological studies using nicotine as a ligand have repeatedly shown that a particular type of cholinergic receptor can be easily and rapidly desensitized (Bertrand, Ballivet, & Rungger, 1990; Zaimis & Heads, 1976; Sumikawa & Miledi, 1989; Adams, 1987; Ogden & Colquhoun, 1985; Adams, 1975; Chabala, Gurney, & Lester, 1986; Neher & Sakmann, 1975). Desensitization has been shown to last from 10 to 20 minutes. The evoked currents generated by nicotine generally are not distinguishable from acetylcholine evoked currents. The *In vivo* work by London (1990) has also shown that nicotine appears to act on select brain area receptors sensitive to acetylcholine (nicotinic-acetylcholinergic receptors; nAChR's). Using the 2-deoxy-D-$[1^{-14}\text{C}]$ glucose method for the identification of central cellular (and brain area activity) activity following the injection of acute systemic (-) nicotine, London showed that a significant stimulation of several brain areas were associated with both $[^3\text{H}]$ nicotine and $[^3\text{H}]$ ACh binding sites. The increased nicotine-induced activity was also attenuated by mecamylamine, a non-competitive nicotine antagonist which is selective for the nAChR.

Evidence that nicotine can elicit a rapid desensitization following its agonist effect at the nAChR *in vivo* has also
been provided by Sharp and Beyer (1986). These workers showed that acute doses of nicotine were able to inhibit the release of adrenocorticotropic and prolactin. Stimulation of the adrenocorticotropic-corticosterone and prolactin axes had threshold i.p. injections ranging from 100 to 250 ug/kg ((-)-nicotine). A single dose of nicotine (500 ug/kg i.p.) caused an acute desensitization of the stimulatory effects, when a second injection (1000 ug/kg i.p.) was administered one hour later. This desensitization lasted for 6 hours. Hulihan-Giblin, Lumpkin and Kellar (1990) replicated the study of Sharp and Beyer using 100 ug/kg i.v. nicotine. The prolactin release had returned to normal within 24 hours. Hulihan-Giblin, Lumpkin and Kellar (1990) using chronic dosing (10 days twice a day) of nicotine (800 ug/kg s.c.) abolished the release of prolactin, when an acute dose of nicotine (60 ug/kg i.v.) was given 2, 6, or 8 days after the last chronic injection. Prolactin release had returned to normal after 14 days from the last chronic injection.

Thus, these studies provide much data that nicotine appears to be acting at neuronal sites sensitive to acetylcholine, suggesting that nicotine ultimately is producing its pharmacological effects by mimicking this endogenous transmitter. In addition, this work also suggests that nicotine is capable of inhibiting the same receptors via a desensitization process similar to that ascribed for the cholinergic endogenous ligand, acetylcholine. Interestingly, this desensitiza-
tion process appears to be specific to the nAChR and does appear to occur at the mAChR (Fig. 1).

CHAPTER THREE

EFFECTS OF NICOTINE ON BEHAVIOR: NICOTINE AS A DISCRIMINATIVE STIMULUS (DS)

Stolerman (1990) has shown that initial dosing of rats with nicotine causes an increase in rates of responding of conditioned behavior to avoid aversive stimuli or to obtain reward. Repeated dosing produces the development of tolerance and a reduction in responding in the same paradigms. Nicotine is also capable of increasing rates of responding in locomotor tasks, with a similar tolerance and reduction of responding developing rapidly. Stolerman (1989) and Rosecrans (1989) have both shown that this tolerance can develop even after only a single dose. Maze running speed and percent correct moves are improved by preinjections of nicotine. It would appear that all of these phenomena are mediated by one receptor type since mecamylamine is capable of blocking all these behavioral tasks. Nicotine is also a drug noted for its ability to induce biphasic effects with a great deal of behavioral and pharmacological variability between subjects (Battig & Shlatter, 1978; Bovet, Bovet-Nitti, & Oliverio, 1967; Domino, 1967; Marks & Collins, 1985; Morrison & Armitage, 1967; Rosecrans, 1971; Clarke & Kumar, 1983; Ksir, Hakan, Hall & Kellar, 1985; Nordberg, Wahstrom, Arnelo, &

The drug discrimination paradigm has had special relevance to research in the nicotine area as it not susceptible to many of nicotine’s variable and individual effects on behavior. This procedure has proved to be an effective methodology for determining an animals ‘subjective’ assessment of a drug. This ‘subjective’ assessment by the animal is created by the development of a relationship between a drug and a particular behavior. The drug then acts as the discriminative stimulus for the behavior. In a typical drug discrimination study the animal is trained to detect the difference between a specific drug and vehicle. Drug discrimination requires the animal, while in an operant chamber, to press one lever when under the influence of the drug and the other lever when in the non-drug state. Thus, the animal emits a behavioral response under the stimulus control of the drug for a reward (Fig. 3).

The discriminative stimulus properties of drugs affecting n-AChR’s is distinct from other AChR’s. Arecoline is an agonist at m-AChR’s and is antagonized by atropine, but is not active at n-AChR’s and is not antagonized by mecamylamine. Nicotine is selective to n-AChR’s and is antagonized by mecamylamine, but not atropine (Rosecrans, 1989; Stolerman, 1987). Meltzer & Rosecrans (1988) showed that physostigmine (an acetylcholinesterase inhibitor) generalized to the m-AChR via an increase of brain ACh at this site, but not to the n-
A description of the drug discrimination paradigm

This figure presents the steps to training a rat to discriminate a specific Nicotine (400 ug/kg, s.c.) from the non-drug (vehicle) state. Once rats learn the specific to discriminated Nicotine from Vehicle they can then be evaluated in relation to mechanisms of drug action. This takes place during Test Sessions in which responding by the rat is recorded on each lever and data is collected in relation to Nicotine-Correct Lever responses. Generalization can be conducted using a variety of Pharmacological and Biobehavioral interventions (From Rosecrans, 1989).
AChR. This result suggests that the mechanism of action of nicotine may have components not directly linked to the cholinergic system.

In addition, Stolerman (1989) has shown that rats trained to 100 ug/kg (-) nicotine in a drug discrimination paradigm generalized to (+) amphetamine (a dopamine receptor agonist). Chance et. al. (1977) has additionally shown only partial generalization to (+) amphetamine in rats trained to discriminate several training doses (100-400 ug/kg (-) nicotine) training under different schedules of reinforcement. These latter studies are extremely important as they suggest that: (1) there may be a dopaminergic component in the discriminative stimulus, and (2) that because of nicotine's known biphasic nature in behavioral studies (see Refs. above) one needs to consider a wide range of doses when examining nicotine's actions.

The basic objective of this research was to further evaluate nicotine's mechanism of action, and to specifically determine whether nicotine can be shown to exhibit in vivo desensitization (acute tolerance) to its DS. The overall design of this research evaluated the ability of nicotine (400 ug/kg, s.c.) to act as a DS when challenged with a different doses of nicotine (800-1600 ug/kg., s.c.). The challenge dose was administered at various times (15-240 min.) prior to testing the ability of a rat to detect the nicotine (400 ug/kg., s.c.) training dose. Two experiments were conducted.
The first experiment was designed to characterize nicotine-induced acute tolerance in the rat in which the tolerance-eliciting dose was held constant over a predetermined time course. The second experiment varied the tolerance-eliciting dose to evaluate the dose-response as well as the time-duration nature of acute tolerance (desensitization).

CHAPTER FOUR

EXPERIMENT ONE: A CHARACTERIZATION OF NICOTINE-ELICITED ACUTE TOLERANCE (DESENSITIZATION) OF THE NICOTINE DISCRIMINATIVE STIMULUS (DS)

INTRODUCTION

The research of Domino (1967) has been central to our quest to determine how nicotine affects behavior. He essentially showed that the behavioral effects of nicotine are elicited by an agonist effect at a central nicotinic-cholinergic receptor. Domino further suggested that the nicotinic-cholinergic receptor (nAChR) significantly differed from the mAChR, but that both receptor types were responsive to the endogenous neurotransmitter, acetylcholine. In vitro studies (Bertrand, Ballivet, & Rungger, 1990; Zaimis & Heads, 1976; Sumikawa & Miledi, 1989; Adams, 1987; Ogden & Colquhoun, 1985; Adams, 1975; Chabala, Gurney, & Lester, 1986; Neher & Sakmann, 1975) have supported this hypothesis and have shown a direct link between acetylcholine and nicotine. In vivo research conducted by Meltzer and Rosecrans (1988), on the other hand, has suggested that the relationship between nicotine and
acetylcholine was not as symbiotic as we would like to think. They showed that rats trained to discriminate (-) nicotine (400 ug/kg) from saline failed to generalize to physostigmine (125 ug/kg-250ug/kg), a cholinesterase inhibitor that retards the metabolism of acetylcholine. Rosecrans (1965) showed that these doses of physostigmine raised acetylcholine levels in the brain. Arecoline (a muscarinic (mAChR agonist) trained rats did generalize to physostigmine and the generalization was blocked by atropine (muscarinic antagonist). The question then becomes why does the increase of acetylcholine trigger the discriminative stimulus cue of a muscarinic drug, but not that of nicotine? Is the nicotinic cue regulated by more than one receptor system, or is the nicotinic cue somehow altered by a receptor transformation?

In vivo studies by Marks and Collins (1990) have shown that there is an up-regulation of nicotinic receptors after chronic administration of nicotine. Unlike the classic receptor theory, that states that chronic exposure to an agonist should result in the down-regulation of receptors, nicotine induces an up-regulation. Wonnacott (1990) has hypothesized that this up-regulation is the result of some form of a functional antagonism which might explain why nicotine does not generalize to physostigmine. Physostigmine causes a rapid increase in extracellular acetylcholine which may result in a rapid desensitization of the nicotinic receptor. Thus, physostigmine might be inadvertently inducing
a "functional antagonist state" recorded by rat subject as a generalization to vehicle, or the non-drug state.

From this, it was hypothesized that the drug discrimination paradigm might be an effective method to test the desensitization hypothesis by assessing acute tolerance involving both nicotine and physostigmine. The time course paradigm would allow us to evaluate in vivo the effects of nicotine over time on the nicotinic acetylcholinergic receptor. Furthermore, the paradigm would allow us to assess the role of physostigmine in the acute tolerance phenomena.

The first phase of the experiment evaluated the ability of (-) nicotine (800 ug/kg) to functionally antagonize the ability of a rat to recognize the training dose of (-) nicotine (400 ug/kg). Acute tolerance (desensitization) was exhibited by 48% of the rats and 52% exhibited no acute tolerance (desensitization). The second phase of the experiment assessed the generalization of physostigmine to nicotine, and physostigmine's ability to antagonize the discriminative stimulus effect of nicotine. In addition nicotine's sensitivity as a DS, and the ability of mecamylamine and the mAChR antagonist, scopolamine, to antagonize the nicotine DS, was also evaluated as function of the ability of each subject to exhibit acute tolerance to nicotine.

METHODS

SUBJECTS:

Twenty-five male Sprague-Dawley rats (175-225 g) obtained
from Dominion Laboratories, Dublin, Virginia served as experimental subjects throughout this investigation. Rats were housed individually under a 12 hour light/dark regime (800–2000 hrs. light) in a temperature controlled colony room (22°C). Animals were maintained on a diet (Purina Rodent Chow) that restricted their body weight to approximately 85% of their free feeding weight. Water was available ad libitum in the home cages.

TRAINING PROCEDURE:

A two lever operant drug discrimination paradigm VI 15 was used in this study. One stainless steel wall of each chamber contained two stainless steel levers separated by a food tray. Illumination was provided by a white light placed directly above the food tray in the BRS chambers used for this research. Illumination in the Lafayette chambers used was provided by a white light directly above each lever. The chambers were housed in sound and light attenuating cubicles. Exhaust fans provided ventilation and white noise during sessions. Data was automatically collected by two Commodore 64 micro-computers. Two 32-line opto-isolated I/O interfaces (Rayfield Equipment Ltd., Waitsfield, VT) each controlled operation of four chambers. Software used to independently control chambers was the Programmers Micro Application Language (PROMAL, Raleigh, NC).

Rats were trained to respond on one lever after a subcutaneous (s.c.) injection of (-) nicotine (400 ug/kg) and
the other lever after a s.c. injection of 0.9% saline. Rats were placed in an operant chamber (4 Lafayette model 80001 chambers and 4 BRS/LVE model sec 002 chambers) 5 minutes after injections. Reinforcement was a Bioserv 45 mg precision dustless pellet.

Animals were initially placed in operant chambers with both levers present. An FR-1 schedule was used until the animal was responding 75 to at least 100 times in a 30 minute session. During the initial training period animals were reinforced only if they responded on the vehicle correct lever in which half of the animals were trained to press the right lever for vehicle and the left lever for drug, and the other half were trained to the reverse pattern. Training progressed from the FR-1 to a VI-3 on the vehicle correct lever. After an animal was responding between 75-100 times during a 30 minute session the schedule was increased to VI-5, 8, 12, and finally VI-15. Training sessions were decreased to 15 minutes once an animal had reached VI-3. Animals were then maintained on the VI-15 schedule for one week. The following week animals were injected daily with vehicle (1ml/kg) 5 minutes before being placed in the operant chamber. Animals were reinforced on the training lever (vehicle lever). The following week animals were injected with 400 ug/kg of (-) nicotine 5 minutes prior to being placed in the operant chamber. These animals were required to switch levers to obtain reinforcement. The following week a double alternation schedule consisting of
vehicle two days and nicotine two days was initiated and were run 5 days a week. Animals learned to discriminate nicotine from vehicle in 40 to 80 training sessions.

CRITERIA TESTING:

Animals were required to meet a criteria of three successive days of 80% or greater correct lever responding during specific Check Sessions before the initiation of specific experiments were begun. The criteria testing sessions were conducted during two minute check sessions, in which neither lever was reinforced for lever pressing (extinction sessions). Check sessions were coupled with training sessions of thirteen minutes. During the training portion of the session, the animal was reinforced for pressing the appropriate drug-correct lever.

Experimental subjects that met criteria, i.e. pressed 80% or greater on the correct lever during three consecutive check sessions were considered ready for testing the following day. This schedule resulted in Wednesday being a training day.

GENERAL TESTING:

All data was collected during specific Test sessions which were identical to the Check Session in duration (two minutes) and were conducted in extinction; however, unlike the check sessions there was no training component.

DOSE RESPONSE EXPERIMENTS:

Initial testing assessed the dose-effects of (-) nicotine (50, 100, 200, 400, 800 ug/kg) under the VI-15 schedule.
Injections were given 5 minutes prior to placing the animal in the operant chamber. The schedule of injections was determined using a Latin Square design.

**NICOTINE ANTAGONISM EXPERIMENTS:**
Rats were then assessed for behavioral effects of 1000 ug/kg of racemic mecamylamine in conjunction with their training dose of (-) nicotine. Mecamylamine was administered 5 minutes prior to the injection of (-) nicotine. Animals were also tested for the behavioral effects of 100 ug/kg scopolamine, using the previous procedure except the animals were injected 10 minutes prior to the (-) nicotine injection.

**NICOTINE-INDUCED ACUTE TOLERANCE EXPERIMENTS:**
Acute tolerance (desensitization) testing required that animals be injected on eight test days at the following time points 15, 30, 60, 90, 120, 150, 180, and 210 minutes, prior to being injected with the training dose of 400 ug/kg of (-) nicotine. A Latin Square design was used to determine the time point order for each test day. Animals were considered to exhibit acute tolerance (desensitization) if % nicotine-correct responding was reduced by 50% or greater.

**RESULTS**
Table 1a presents the overall results for the desensitizing group and table 1b presents the overall results for the non-desensitizing group evaluated in this research: (1) the percent reduction of responding on the nicotine lever induced by the nicotine challenge (800 ug/kg, s.c.), (2)
replication of percent reduction of responding on the nicotine lever, (3) Effective dose 50% (ED-50, 50% nicotine-correct lever responding) of each rat determined via a dose-response evaluation, (4) average response rate, (5) percent antagonism by mecamylamine, and (6) percent antagonism by scopolamine. For a variety of reasons, not all subjects were not tested for either mecamylamine and/or scopolamine antagonism. Twelve of the twenty-five rats exhibited acute tolerance (desensitization) at one or more time point(s). Individual rats were re-tested at their individual peak time point(s) (Figures 4-6). The group that did not show acute tolerance (non-desensitizers) exhibited a potentiation (% correct nicotine responding) from the double dosing (Figure 7). On the other hand, the desensitizing group responded below individual training levels at all time intervals evaluated (Figure 8). Overall response rates were less in the non-desensitizing group when compared to the desensitizing group; this relationship was not statistically significant (Figure 9). The 90 and 120 minute time intervals were the most prominent time periods for desensitization. The median range of acute tolerance (desensitization) at the 90 and 120 minute time points for the desensitization group was 41%. The median range of acute tolerance (desensitization) at the 90 and 120 minute time points for the non-desensitizing group was 8%. The overall difference between the desensitizing and non-desensitizing groups was significant ($F_{1,19}=52.5; p<0.001$).
Seven rats from the first phase of the experiment were tested for acute tolerance (desensitization) following a preinjection of physostigmine (125 ug/kg). Three of the rats were classified as desensitizers and four were classified as non-desensitizers. Rats followed the same patterns of responding after the preinjection of physostigmine as they had following the preinjection of nicotine (Figures 10-13).

**DISCUSSION**

The analysis of variance produced a significant difference in the variable group. This finding supports the contention that acute tolerance (desensitization) to nicotine’s discriminative stimulus can be demonstrated in a subpopulation of rats. All rats in the study received a minimum of sixty injections before acute tolerance (desensitization) testing (400 ug/kg nicotine). Whether these rats were also chronically tolerant is difficult to say. However, these results also suggest there should be some chronic tolerance which may indicate that there are separate mechanisms for acute and chronic tolerance, at least in relation to the nicotine DS.

Chronic tolerance represents a physiological change in an organism over an extended period of time. There is likely a permanent change in receptors; in the case of nicotinic receptors an up-regulation (Marks and Collins, 1985). However, acute tolerance represents a temporary change in receptor activity in response to a specific ligand. Nicotine
appears to be able to rapidly transform an active receptor into an inactive receptor as evidenced in the desensitization group. In the inactive state the discriminative stimulus properties of nicotine appear to be inhibited. Interestingly, this inhibition appears to be specific to individual rats and lasts for varying times. The rats that failed to exhibit acute tolerance (desensitization) actually showed a potentiation of the discriminative stimulus.

One important facet of any behavioral task is the evaluation of response rates. Nicotine historically has shown biphasic locomotor effects (Battig & Shlatter, 1978; Bovet, Bovet-Nitti, & Oliverio, 1967; Domino, 1967; Marks & Collins, 1985; Morrison & Armitage, 1967; Rosecrans, 1971; Clarke & Kumar, 1983; Ksir, Hakan, Hall & Kellar, 1985; Nordberg, Wahstrom, Arnelo, & Larsson, 1985; Rosecrans & Chance, 1977). There were no significant differences between rates of responding for the two groups. The 15 and 30 minute time periods did show reduced responding, these effects were seen in both groups, and were considered an inability of the rats to cope with high blood concentrations of nicotine.

The mecamylamine and scopolamine results were as expected and produced no differences between groups. The failure of physostigmine to generalize to the discriminative stimulus of nicotine has been seen repeatedly in this laboratory and others (Stolerman, 1987), but the ability of physostigmine to mimic nicotine in the acute tolerance (desensitization)
phenomena suggests that this drug is inducing a desensitized nAChR analogous to that induced by nicotine. Katz and Thesleff (1957) hypothesized that nicotine had a higher affinity for the inactive form of the nicotinic acetylcholine-ergic receptor than the active form. This could be an explanation for the ability of physostigmine to mimic nicotine in the acute tolerance (desensitization) experiment, but not generalize significantly to discriminative stimulus properties of nicotine. Physostigmine may cause a rapid desensitization of the nicotinic receptor in vivo resulting in rats generalizing to the vehicle state.

The study raised two questions that needed further evaluation. In addition to time being a factor in the demonstration of acute tolerance (desensitization) it seems plausible that the concentration of dose should also be a factor. Furthermore, it is also possible that all rats would show acute tolerance if challenged with a high enough dose suggesting that we may be measuring rate of tolerance development which is not so "all or none". The lack of a dose response effect at the completion of the experiment also raised the question of permanent physiological changes due to the nicotine dosing paradigm. Thus, this research has demonstrated that acute tolerance to nicotine is demonstratable and replicable in the same animal subject, and has many implications in relation as to how nicotine affects behavior. The finding that a select group of rats are not able
to exhibit acute tolerance is extremely important and suggests that some rats may differ in relation to central nAChR machinery.

CHAPTER FIVE
EXPERIMENT TWO: DOSE-RESPONSE RELATIONSHIPS OF NICOTINE-ELICITED ACUTE TOLERANCE (DESENSITIZATION) OF THE NICOTINE DISCRIMINATIVE STIMULUS (DS)

INTRODUCTION

This study was designed as a follow-up to Experiment One, and to determine the dose-response characteristics of nicotine-elicited acute tolerance (desensitization). In the first experiment, acute tolerance (desensitization) was demonstrated after a preinjection dose of 800 ug/kg of nicotine (challenge dose) followed by an injection of 400 ug/kg (training dose) of nicotine. That is, nicotine was capable of inducing acute tolerance, which appears evident in a select population of rats studied. Overall 52 percent of the rats studied in Experiment One were unable to exhibit desensitization to nicotine. Being aware of the acute tolerance effects of nicotine, it was now important to determine if rats that failed to show acute tolerance did so because the challenge dose was too small. If so one should be able to demonstrate acute tolerance (desensitization) if the preinjected doses of nicotine were higher.

The hypothesis presented suggests that rats which failed to demonstrate acute tolerance (desensitization) at 800 ug/kg
of (-) nicotine might show tolerance at either or/both 1200 ug/kg or 1600 ug/kg.

METHODS

The subjects and drug discrimination procedures utilized in this study were essentially identical to that of EXPERIMENT ONE. Significant differences will be specifically discussed where they occur. Acute tolerance (desensitization) testing required that animals be injected on five test days (per dose) at the following time points 60, 90, 120, 150, and 180 minutes, prior to being injected with the training dose of 400 ug/kg of (-) nicotine. A Latin Square design was used to determine the time point order for each test day. Animals were considered to exhibit acute tolerance (desensitization) if their percent nicotine correct lever responding was antagonized by 50% or greater. There were some modifications from Experiment One. First, solutions used in this study were buffered (vehicle, pH 7.4 phosphate solution; nicotine, Ph 7.4); and second the 15, 30, 210 time periods were not used. In addition to acute tolerance determinations at each challenge dose, dose-response nicotine generalization studies were conducted both Before and After completion of the acute tolerance research.

RESULTS

Data were analyzed using the split-plot analysis of variance model on PCA Anova. The overall mean percent correct nicotine responding was 78.5. The mean percent correct
nicotine responding for the desensitized group was 62.0. The mean percent correct nicotine responding for the non-desensitized group was 78.7. The overall mean response rate per second was 0.17. The mean response rate for the desensitized group was 0.12. The mean for the non-desensitized group was 0.19. Table 4 and 5 report results for all conditions.

Figure 14 reports the percent correct nicotine responding for the between subject variable group (F=13.568; p<0.0020), and the response rate for the between subject variable group (F=3.009; p<0.0968). Figure 15 reports the percent correct nicotine responding for the within subject variable dose (F=11.156; p<0.0003), and the response rate for the within subject variable dose (F=3.568; p<0.0377). Figure 16 reports the percent correct nicotine responding for the within subject variable time (F=11.05; p<0.0000), and the response rate for the within subject variable time (F=4.677; p<0.0024). Figure 17 reports the percent correct nicotine responding for the interaction between dose and time (F=27.225; p<0.0000), and the response rate for the interaction between dose and time (F=10.583; p<0.0000). Figure 18 reports the percent correct nicotine responding for the interaction between group and dose (F=6.097; p<0.0055), and the response rate for the interaction of group by dose (F=1.110; p<0.3412). Figure 19 reports the percent correct nicotine responding for the interaction of group by time (F=2.011; p<0.1012), and the response rate for the interaction of group by time (F=0.513). Figure 20 reports
the percent correct nicotine responding for the interaction of group by dose by time ($F=1.706; p<0.1014$), and the response rate for the interaction of group by dose by time ($F=0.975$). Figure 21 reports the dose response data for percent correct nicotine responding by group. Figure 22 reports the dose response data for response rate by group. Figure 23 reports the overall results of the first and second dose response tests.

**DISCUSSION**

The analysis of variance (DV; % correct nicotine responding) produced significant differences for the between subject variable group, and the within subject variables dose and time. The interaction of dose by time was significant suggesting that the mechanism of action of acute tolerance (desensitization) was to some degree dictated by the specific dosing and/or time of injection. The significant interaction of group by dose adds further support for the desensitization model.

The analysis of variance (DV; response rate) produced significant differences for the within subject variables dose and time. The interaction of dose by time was also significant. These results suggest that the acute tolerance (desensitization) phenomena is not related to response rates.

The Tukey test ($p<0.05$) was used to analyze the interactions between group by dose, and dose by time (percent correct nicotine responding).
The group by dose interaction showed that the desensitizing group was significantly less tolerant to the 1200 ug/kg and 1600 ug/kg doses than the non-desensitizing group. The dose by time interaction showed that the 90 minute time period produced a significant reduction in the percent correct nicotine responding for both groups. Over the entire time course there was a downward stairstep effect as the concentration of the doses increased.

The analysis of variance showed that there was no significant difference between groups over time, nor was the triple interaction of group by time by dose significant. The percent correct nicotine responding results suggest that the mechanism(s) of action of acute tolerance (desensitization) are effected by dose (concentration) and time course. The lack of significance for the group by time and the significance of group by dose suggest that the critical factor in the acute tolerance (desensitization) phenomena is the concentration of the dose given at time zero.

The analysis of variance showed that for the dependent variable response rate the within subject variables time and dose were both significant. The interaction of dose by time was also significant suggesting that response rates were not a critical component of acute tolerance (desensitization). The fact that group as a main effect or as an interaction was never significant suggests that response rate is not a factor in the mechanism(s) of action of acute tolerance (desensitiza-
GENERAL DISCUSSION AND CONCLUSIONS

Nicotine and muscarine, both naturally occurring alkaloids, have had a long and rich history in physiology and pharmacology research which has been central to our basic understanding of the physiology of the Autonomic Nervous System (ANS). From this research, a picture has emerged in which we view the parasympathetic division of ANS as having two types of cholinergic receptors, muscarinic and nicotinic. In addition these receptors appear to be sensitive to the endogenous ligand, acetylcholine (ACh) which appears essential for mediation of specific physiological events. The picture which emerged also viewed these receptors as being related serially in that the release of ACh at the nicotinic receptor will initiate a signal to a second neuron eliciting the release of ACh at a muscarinic receptor. This concept is essential to our understanding of the peripheral ANS, but this arrangement appears somewhat different when evaluated from the perspective of the role of these cholinergic neurons to brain function and behavior.

The research of Domino (1967) showed that the behavioral effects of nicotine are elicited by an agonist effect at a select central nicotinic acetylcholinergic receptors (nAChRs). Domino further suggested that the nAChR was physiologically different from the muscarinic acetylcholinergic receptor (mAChR), but that both receptor types were responsive to ACh.
This research also suggested that these receptors had separate independent brain functions which indicated that these receptors may also be innervated by different neuronal pathways, unlike the arrangement in the peripheral ANS.

In contrast to the mAChR, nAChR-induced physiological effects do not involve a second messenger system, but are mediated through the opening and closing of a cation channel (Fig. 1). Ochoa (1989) presents a model of ACh action at the nAChr which incorporates many of the basic concepts of the model of Katz and Thesleff (1957). Ochoa’s model suggests that the synaptic release of acetylcholine first interacts with the nAChR to form an active state. The activated state then opens a cation channel at the postsynaptic membrane, which allows the influx of positively charged molecules. The nAChR is theorized to be rapidly converted to a deactivated state, thus closing the cation channel. This process appears to be specific to the nAChR and is essential to its physiological role in the ANS. This secondary process appears to be related to a desensitization of the nAChR as the affinity of the receptor increases (Fig. 2).

Using a drug discrimination paradigm, Meltzer and Rosecrans (1988), on the other hand, provided evidence that the relationship between acetylcholine and nicotine may not be as symbiotic as the research of Domino (1967) suggests. They showed that rats trained to discriminate nicotine (400 ug/kg) from saline failed to generalize to physostigmine (125-250
ug/kg), a cholinesterase inhibitor that retards the metabolism of acetylcholine. A group of rats trained to discriminate arecoline (a muscarinic agonist), however, clearly generalized to the ACh-elicited increase via physostigmine administration, suggesting that the m- and n-AChR were quite different, at least in their responsiveness to ACh and when nicotine is evaluated as a discriminative stimulus. Further evidence that these two cholinergic receptors are different was provided by the ability of mecamylamine, a nicotinic antagonist, to antagonize the discriminative stimulus properties of nicotine but not arecoline. On the other hand, atropine a muscarinic antagonist, antagonized the discriminative stimulus properties of arecoline, but not nicotine (Table 7). The hypothesis which evolved from these investigations was that nicotine may not act on nicotinic cholinergic receptors sensitive to ACh. The suggestion was also made that nicotine may be acting on two cholinergic subtypes, one of which was sensitive to ACh. An explanation of these divergent findings has been difficult, but it has become evident that these data are not as unexpected if one considers the nature of the nAChR-linked cation channel as described by Ochoa et al. (1989). The scenario developed suggests that the physostigmine-elicited increase in ACh centrally, inadvertently desensitized the nAChR, inducing a "physiologically inhibitory state". Rats trained to discriminate nicotine, therefore, generalized to the vehicle state as the nAChR was inactivated. Arecoline
trained rats, on the other hand, were able to generalize to the physostigmine-elicited increase in ACh because the mAChR does not appear to desensitize as easily as is apparent with the nAChR. There are possibly other explanations of these findings, but the "desensitization hypothesis" seems most plausible at this time. From this scenario it would also seem plausible that nicotine should induce a desensitization of the nAChR as well, especially if its actions were tied to ACh. Thus, nicotine should be able to antagonize nicotine, better defined as acute tolerance.

The drug discrimination paradigm presents an ideal paradigm to test and extend this hypothesis. The nature of the nicotine discriminative stimulus (DS) has been well established (Table 7). The DS is selective and sensitive to nicotine, parallels brain nicotine concentrations, and is clearly separable from other cholinergic receptor-acting drugs. In addition, this paradigm is very resistant to other drug effects such as drug-induced behavioral disruption and chronic tolerance. In this paradigm, rats need to become behaviorally tolerant to nicotine to be able to press the lever for food, but can’t become tolerant to the DS if they are to continue to choose the correct-lever for positive reinforcement. Thus, this procedure is extremely reliable which permits one to separate pharmacological mechanisms of drug action (such as in vivo receptor desensitization) from behavioral mechanisms.
The first goal of this research was to demonstrate that nicotine could induce acute tolerance using the drug discrimination paradigm. A time course methodology was chosen in which rats trained to discriminate nicotine were administered a challenge dose of nicotine (800 ug/kg, s.c.) at various time points (15, 30, 60, 90, 120, 150, 180, 210, 240 minutes) prior to testing the ability of these rats to detect the training dose of nicotine (400 ug/kg). Each time point was tested on a different day, and the order was determined using a Latin Square design. Desensitization, or nicotine-induced acute tolerance was defined as a 50% reduction in the ability of a subject to detect nicotine. The data obtained indicated that 40 to 50% of the rats evaluated exhibited acute tolerance (Figures 4-6). Most important was the observation that not all rats exhibited acute tolerance, and we were able to classify these animals as to desensitive-prone and non-desensitizers. Furthermore, desensitive-prone rats exhibited a temporal differential sensitivity which was replicatable in almost every case. Thus, rats showed different times for desensitization between challenge and test dose. The average time for desensitization to develop appeared to be 90 min. This is interpreted to mean that the desensitization process at the nAChR is time-limited for each rat and that not all rats appear able to exhibit desensitization. This is an extremely important observation which suggests that the nAChR-linked channel apparatus varies between rats which may be
essential to nicotine's variable behavioral effects.

The second goal of this research was to learn more about the mechanism of desensitization (acute tolerance) which may also be tied to understanding why nicotine did not generalize to physostigmine. Two possibilities exist as to why physostigmine failed to generalize with nicotine. First, the discriminative stimulus effect of nicotine may not be mediated by a mechanism(s) directly linked to acetylcholine. Secondly, the discriminative stimulus effect is mediated by an nAChR, but the rapid increase of acetylcholine caused by physostigmine desensitized the receptor which induced a vehicle state. If acetylcholine is the primary neurotransmitter associated with the discriminative cue, then physostigmine injected at time zero should be capable of antagonizing nicotine at some later time point. Support for this concept was observed in seven rats challenged with both nicotine and physostigmine (Figures 10-13). This research, therefore, provides presumptive evidence that nicotine is acting at a cholinergic receptor sensitive to ACh which is susceptible to rapid desensitization. Whether nicotine is acting by mimicking this receptor, or inducing release of ACh presynaptically remains unknown. In addition we are not sure as to the location of these nAChR's, and whether they are located on mainly pre- and/or postsynaptic elements of the cholinergic neuron.

The third phase of this research involved answering an additional question concerning the desensitization process in
vivo. A major concern was the apparent "all or none" nature of acute tolerance; some rats did not exhibit tolerance. The question concerns whether we are dealing more with a rate process which is contingent upon dose. Thus, will the non-desensitizer exhibit acute tolerance at some higher dose? Experiment two was designed to evaluate the dose-response characteristics of nicotine-elicited acute tolerance. The results suggest that there is some merit to the concept that dose or nicotine level at the AChR is an important consideration. In fact all such pharmacological processes should be dose-related at some point. In support of this the number of rats exhibiting tolerance increased at each challenge dose from 800, 1200, 1600 µg/kg. (Table 6).

Percent nicotine-correct responding suggests that the mechanism(s) of action of acute tolerance (desensitization) are effected by dose (concentration) and time course. The lack of significance of group by dose suggests that the critical factor is the concentration of the dose given at time zero. The response rate data showed that time and dose were both significant and the interaction of the two was significant. The fact that group as a main effect or as an interaction was never significant suggests that response rate is not a factor in the mechanism(s) of action of acute tolerance (desensitization). This is an extremely important factor as it demonstrates that the ability to detect a drug is independent of behavioral disruption. This also supports the
contention that we are studying pharmacological mechanisms such as desensitization rather than events contingent upon some behavioral effect. Thus, rats are not able to detect nicotine following a large dose of nicotine just because they are also disrupted; the explanation for this antagonism (acute tolerance) is that nicotine induced a desensitization of the nAChR.

This research has provided evidence of the specific nature of the interaction of nicotine with its nAChR. The behavioral paradigm used is reliable and appears to be analogous to what a human perceives when he or she is administered nicotine, usually through some tobacco product. This paradigm also has relevance to studies evaluating neuromolecular mechanisms of drug action. We have provided evidence that nicotine is producing its effects at some nAChR which can be evaluated in vivo. The methodology is now available to evaluate nicotine's acute and chronic effects via the application of drugs centrally with the use of specific brain probes (microdialysis cannula). We can evaluate the effects of drugs on specific structures while rats are in the process of detecting a specific drug state, and we can at the same time, evaluate the effects of the DS on endogenous chemicals through the same probes. Thus, this methodology has many applications for future research employing in vitro approaches with an in vivo paradigm to answer specific questions concerning mechanism's of drug action.
The last point concerns the relevance of this research to the real world. Besides using such approaches in conjunction with more molecular approaches, this research has much relevance to the drug user, in this case those who use tobacco. There are many issues in smoking and the ability of nAChR to desensitize may be extremely important to understanding some of these issues. First, desensitization may be useful in explaining rate of smoking behavior. Thus, one may smoke at specific intervals because the nAChR in that individual has a specific desensitization time. Secondly, an individual who possesses an nAChR which does not desensitize may get a full nicotine dose which is aversive and who may never smoke again because of this initial effect. Some desensitizers, on the other hand, may become heavy nicotine-dependent users because the nicotine effect is extremely positive to which acute tolerance is not apparent. Thirdly, because of predisposition for a high rate of desensitization, an individual may smoke with no aversive effects and become a life time user of tobacco. Thus, there are several implications of this research to human drug dependency on nicotine via tobacco. Interestingly, there have been indications of people becoming dependent on Nicoret gum which is used as a tobacco substitute in tobacco cessation treatment (Roscans, unpublished observations). Thus, would the same principles apply. An important aspect of this hypothesis is that it is testable in humans. Humans can be trained to discriminate
different doses of nicotine, and we can evaluate rates of desensitization via nicotine challenges in individual smokers. Another issue concerns select populations which smoke. It seems that smoking and depression are positively correlated, and schizophrenics can become obsessive smokers (beyond dependent). The question concerns whether nicotine may be affecting other neurochemical systems important to these behavioral disorders. Do people smoke to relieve depression or to control their craziness? Evidence to support such a contention is apparent from studies which indicate that nAChR’s are located on many pre-synaptic non-cholinergic receptors such as dopaminergic and serotonergic neurons. Thus, nicotine can modulate other non-cholinergic neurons, especially if we consider what we have been studying here, nAChR desensitization. It can be envisioned that nicotine is capable of inducing a physiological antagonism of several of these system via its molecular effects at the nAChR. Nicotine, therefore, could modulate a variety of neuron populations in the brain via an agonist and/or antagonist (desensitization) process. Humans who use nicotine chronically appear to have most of their neurons in the desensitized state indicating some long term effects. This work has been used to explain why smoker’s are less likely to develop parkinson’s disease (Rosecrans and Karan, 1992). The nAChR has also been implicated in alzheimer’s as well and several workers are evaluating the long term effects of nicotine on learning and
memory in these individuals. Thus, nicotine, while scorned because of its presence in tobacco, and because of the toxicological effects of tobacco, has many subtle but powerful effects which have the potential to be utilized in several abnormal behavioral and neurological conditions. Its potential as a therapeutic agent is quite great if we consider the numbers of people who have used tobacco products and consider the many reasons why tobacco has been so popular since it was farmed in Virginia.
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**TABLE 1A**
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TABLE 3

SPLIT-PLOT ANOVA:
(DV=number of responses/second; N=20; mean=0.17

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<th>SOURCE</th>
<th>SUM-OF-SQUARES</th>
<th>DF</th>
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<td>3428</td>
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<td>20505</td>
<td>18</td>
<td></td>
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<tr>
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<td>423</td>
<td>2</td>
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<td>132</td>
<td>2</td>
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<tr>
<td></td>
<td>ERROR</td>
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<td>36</td>
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<td>4</td>
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<td>ERROR</td>
<td>5509</td>
<td>72</td>
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<td></td>
<td>DOSE*TIME</td>
<td>7319</td>
<td>8</td>
<td>10.583</td>
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<tr>
<td></td>
<td>GROUP<em>DOSE</em>TIME</td>
<td>674</td>
<td>8</td>
<td>0.975</td>
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</tr>
<tr>
<td></td>
<td>ERROR</td>
<td>12449</td>
<td>144</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 4

**OVERALL MEANS:** (DV= % correct nicotine responding)

<table>
<thead>
<tr>
<th>EFFECT: GROUP</th>
<th>DESENSITIZERS</th>
<th>NON-DESENSITIZERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>61.97</td>
<td>78.67</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>EFFECT: DOSE</th>
<th>800 UG/KG</th>
<th>1200 UG/KG</th>
<th>1600 UG/KG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>65.21</td>
<td>80.39</td>
<td>70.37</td>
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<table>
<thead>
<tr>
<th>EFFECT: TIME</th>
<th>60 MIN</th>
<th>90 MIN</th>
<th>120 MIN</th>
<th>150 MIN</th>
<th>180 MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>54.42</td>
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<td>78.27</td>
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<td>77.23</td>
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<table>
<thead>
<tr>
<th>EFFECT: GROUP*DOSE</th>
<th>D 800</th>
<th>D 1200</th>
<th>D1600</th>
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</thead>
<tbody>
<tr>
<td>62.35</td>
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<td>55.73</td>
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<th>D-90</th>
<th>D-120</th>
<th>D-150</th>
<th>D-180</th>
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<td>50.92</td>
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<table>
<thead>
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<th>EFFECT: DOSE*TIME</th>
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<th>800-150</th>
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<tbody>
<tr>
<td>72.85</td>
<td>76.35</td>
<td>87.75</td>
<td>79.45</td>
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<table>
<thead>
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<th>EFFECT: GROUP*DOSE</th>
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<th>ND 1200</th>
<th>ND 1600</th>
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</thead>
<tbody>
<tr>
<td>67.12</td>
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<th>0-120</th>
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<table>
<thead>
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<th>800-90-90</th>
<th>800-120-120</th>
<th>800-150-150</th>
<th>800-180-180</th>
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<tr>
<td>78.65</td>
<td>68.45</td>
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<tr>
<td>----------------</td>
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<tr>
<td><strong>OVERALL MEANS</strong>: (DV=responses/second)</td>
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<td><strong>EFFECT: GROUP</strong></td>
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<tr>
<td>DESENSITIZERS</td>
<td>NON-DESENSITIZERS</td>
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<td>800 UG/KG</td>
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<td>1600 UG/KG</td>
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<td>0.18</td>
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<td>0.15</td>
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<td>60 MIN</td>
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<td><strong>EFFECT: GROUP*TIME</strong></td>
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<td>D-120</td>
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<td>0.19</td>
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<td><strong>EFFECT: DOSE*TIME</strong></td>
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<td>0.13</td>
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<td>TABLE 6</td>
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GROUP SHIFTS BY DOSE:

<table>
<thead>
<tr>
<th>DOSE</th>
<th>800 ug/kg</th>
<th>1200 ug/kg</th>
<th>1600 ug/kg</th>
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<tbody>
<tr>
<td>DESSENSITIZERS</td>
<td>1</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>NON-DESENSITIZERS</td>
<td>19</td>
<td>16</td>
<td>12</td>
</tr>
</tbody>
</table>

Brain Sites of Action

- Hippocampus
- Reticular Formation

Other Biogenic Amines Involved

- Physostigmine
- Arecamine
- Diamine
- Caffeine
- Lobeline

DS dose-related

Yes

Antagonised by

- Trypanum
- Meacamylamine

All non-cholinergic Receptor Antagonists

- Testicular Antagonists
- Hexamethonium

- 3-PMP
- (+)-Nicotine
- Amphetamine
- Nornicotine
- (Partial)
- Cysteine
- Caffeine
- (Partial)

Does not Generalise to

Brain

- Nicotine
- Amphetamine
- Neostigmine

N.A.
TABLE 7

Summary of the Current Status of the Mechanisms of Action and Specificity of the DS Properties of Arecoline and Nicotine

<table>
<thead>
<tr>
<th>Properties of DS</th>
<th>Nicotine</th>
<th>Arecoline</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS dose-related Antagonized by</td>
<td>Yes Mecamylamine</td>
<td>Yes Atropine</td>
</tr>
<tr>
<td>Note Antagonized by</td>
<td>All non-cholinergic Receptor Antagonists Tested &amp; Hexamethonium</td>
<td>All non-cholinergic Receptor Antagonists Tested &amp; Methyl Atropine</td>
</tr>
<tr>
<td>Generalize to</td>
<td>3-PMP (+)-Nicotine Amphetamine (Partial) Nornicotine (Partial) Cytisine Cotinine (Partial)</td>
<td>Physostigmine Oxotremorine Pilocarpine</td>
</tr>
<tr>
<td>Does not Generalize to</td>
<td>Physostigmine Arecoline Diazepam Caffeine Lobeline</td>
<td>Nicotine Amphetamine Neostigmine</td>
</tr>
<tr>
<td>Brain Sites of Action</td>
<td>Hippocampus Reticular Formation</td>
<td>N.A.</td>
</tr>
<tr>
<td>Other Biogenic Amines involved</td>
<td>Presynaptic Dopamine sites</td>
<td>N.A.</td>
</tr>
</tbody>
</table>
FIGURE 4

% CORRECT NICOTINE RESPONDING

RAT 55

RAT 53

RAT 45

HOURS

MINUTES

0 60 90 120 150 180 210 24 HOURS

0 60 120 150 24 HOURS

0 60 90 120 150 180 24 MINUTES
FIGURE 6

RAT 42

RAT 41

RAT 34

RAT 22

% CORRECT NICOTINE RESPONDING

MINUTES
FIGURE 7

NON-DESENSITIZING RATS

N = 13

% CORRECT NICOTINE RESPONDING

MINUTES

TRAINING DOSE
FIGURE 8
DESENSITIZING RATS
N=12

% CORRECT NICOTINE RESPONDING

MINUTES

TRAINING DOSE

110 130 150 170
0 0.1 0.2 0.3 0.4 0.5
0 20 40 60 80 100
**FIGURE 9**

**DESENSITIZING RATS**

**NON-DESENSITIZING RATS**

MINUTES

- **TRAINING DOSE**

<table>
<thead>
<tr>
<th>MINUTES</th>
<th>RESPONSES/SECOND</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.50</td>
</tr>
<tr>
<td>30</td>
<td>0.40</td>
</tr>
<tr>
<td>60</td>
<td>0.30</td>
</tr>
<tr>
<td>90</td>
<td>0.20</td>
</tr>
<tr>
<td>120</td>
<td>0.10</td>
</tr>
<tr>
<td>150</td>
<td>0.00</td>
</tr>
<tr>
<td>180</td>
<td>0.00</td>
</tr>
</tbody>
</table>

- **TRAINING DOSE**

<table>
<thead>
<tr>
<th>MINUTES</th>
<th>RESPONSES/SECOND</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.50</td>
</tr>
<tr>
<td>30</td>
<td>0.40</td>
</tr>
<tr>
<td>60</td>
<td>0.30</td>
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<td>0.00</td>
</tr>
<tr>
<td>180</td>
<td>0.00</td>
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</table>
FIGURE 11

RAT 57

% CORRECT NICOTINE RESPONDING

NIC TRAIN VEHICLE 60 MIN 90 MIN 120 MIN 24 HOURS

- NO DATA POINT

RAT 58

MINUTES

NIC TRAIN VEHICLE 60 MIN 90 MIN 120 MIN 24 HOURS
FIGURE 12

RAT 52

% CORRECT NICOTINE RESPONDING

NIC TRAIN VEHICLE 60 MIN 90 MIN 120 MIN 24 HOURS

MINUTES
FIGURE 13

RAT 43

% CORRECT NICOTINE RESPONDING

MINUTES

NIC TRAIN VEHICLE 60 MIN 90 MIN 120 MIN 24 HOURS
FIGURE 14

GROUP

% CORRECT NICOTINE RESPONDING

DESENSITIZERS

F 1, 18 = 13.568, p < 0.0020

N=8

NON-DESENSITIZERS

N=12

GROUP

RESPONSES/SECOND

DESENSITIZERS

F 1, 18 = 3.009, p < 0.0968

N=8

NON-DESENSITIZERS

N=12
FIGURE 15

DOSE

% CORRECT NICOTINE RESPONDING

F_{2,36} = 11.15, p < 0.0003

DOSE

RESPONSES/SECOND

F_{2,36} = 3.58, p < 0.0377
**FIGURE 16**

**TIME**

% CORRECT NICOTINE RESPONDING

N = 20

60  90  120  150  180

F, 4, 72 = 11.050 **P < 0.0000**

**MINUTES**

RESPONSES/SECOND

N = 20

60  90  120  150  180

F, 4, 72 = 4.877 **P < 0.0024**
**FIGURE 17**

**TIME*DOSE**

% CORRECT NICOTINE RESPONDING

F \(8, 144\) = 27.22; \(p < 0.0000\)

**TIME*DOSE**

RESPONSES/SECOND

F \(8, 144\) = 10.56; \(p < 0.0000\)
FIGURE 18

GROUP*DOSE

% CORRECT NICOTINE RESPONDING

F 2, 36 = 0.097, p < 0.0055

GROUP*DOSE

RESPONSES/SECOND

F 2, 36 = 1.11, p < 0.3412
FIGURE 20

TIME*DOSE

F_{8,144} = 27.22 \text{, } p < 0.0000

RESPONSES/SECOND

F_{8,144} = 10.58 \text{, } p < 0.0000
FIGURE 21

NON-DESENSITIZERS DOSE RESPONSE

DESENSITIZERS DOSE RESPONSE

% CORRECT NICOTINE RESPONDING

0.0 0.2 0.4 0.6 0.8

0 20 40 60 80 100

1ST

2ND
BIBLIOGRAPHY


Clarke, P.B.S. (1990). Mesolimbic dopamine activation—the key to nicotine reinforcement? In G. Bock, & J. Marsh (Eds.), *The
Biology of Nicotine Dependence (pp. 153-168.) Chichester, Great Britain: John Wiley & Sons.


