Behavioral Pharmacology Staff

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ACCOMPLISHMENTS

1. NICOTINE SELF-ADMINISTRATION

Our first efforts concerning nicotine self-administration were directed at: 1. clearly establishing its reinforcing properties, 2. generating dose response functions, and 3. examining the effects of a central (mecamylamine) and peripherial (hexamethonium) nicotinic-cholinergic blocking agents on nicotine self-administration. The results of these efforts has been previously reported (See Progress Report to Dr. W. Dunn, August 24, 1981 from Victor J. DeNoble). The following are our accomplishments for the period of March 1, 1981 to March 1, 1982.

1.A. NICOTINE SELF-ADMINISTRATION: EFFECTS OF FIXED RATIO SIZE

Under ratio schedules of self-administration, the reinforcer (in this case nicotine) is administered when the animal completes a required number of responses. An important feature of the ratio schedule is the direct relation between the rate of responding and the frequency of reinforcer presentation. Behavior maintained under fixed ratio schedules is characterized by a brief pause in responding at the beginning of the fixed ratio followed by an abrupt transition to a high steady rate of responding that ends in reinforcement. When the ratio size is increased, the response output first increases and then decreases as the ratio size becomes progressively longer. These characteristic patterns occur reliably where responding is maintained by a variety of events (presentation of food, electrical brain stimulation, water, heat, drugs) and in a variety of

species (human, non-human, primates, rodents, etc.). In our attempt to expand the understanding of the behavioral pharmacology of nicotine, we examined the effect of ratio size on nicotine self-administration in several animals. The results show that as the ~~ratio size was increased~~, the response rate first increased, then decreased (Figure 1). This pattern is characteristic of behavior maintained by other reinforcers. Most important is that the number of nicotine infusions (and the resulting blood level) remained fairly constant across several ratio schedules. These data, combined with data previously reported (memo to Dr. W. Dunn, August 24, 1981 from Victor J. DeNoble, Page 2), strongly suggest that responding for intravenously delivered nicotine is being maintained by the nicotine blood level. At present, we are using ratio schedules to determine the relative reinforcing properties of d and dl nicotine (See Section 1.C.).

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1.B. BLOCKING OF NEUROTRANSMITTERS: EFFECTS ON NICOTINE SELF-ADMINISTRATION

To gain some information about the neurochemical correlates of nicotine self-administration, we produced blockade in three neurochemical systems and observed the effect upon self-administration. The animals were maintained under standard experimental conditions and were trained to lever press for an infusion of nicotine (32 ug/kg/infusion). Control procedures were used to establish that nicotine self-administration was being maintained by the response-nicotine contingency, rather than by other behavioral effects of nicotine. Subsequently, the rats (N=9) were injected with: 1. Mecamylamine HCL (1.5 mg/kg/s.c.), 2. Hexamethonium

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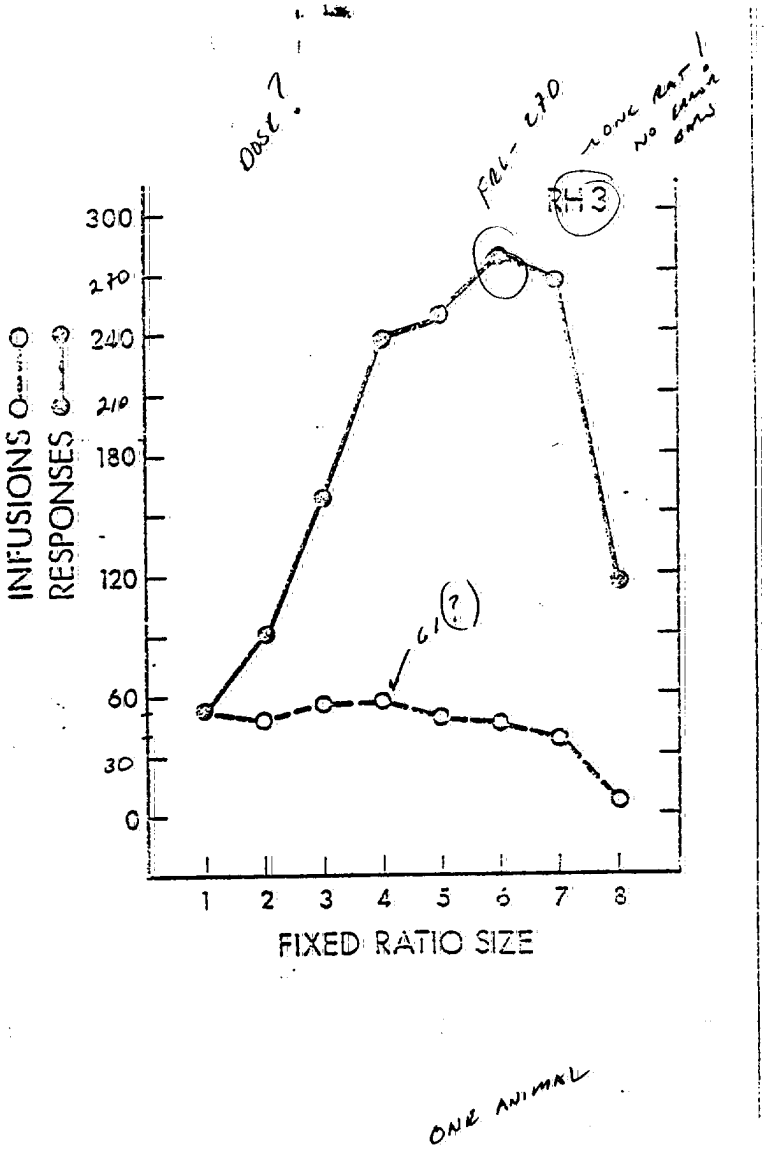

Figure 1. Number of responses and infusions is presented as a function of fixed ratio size. The ratio was presented in ascending order and each data point is a mean of 3 days.

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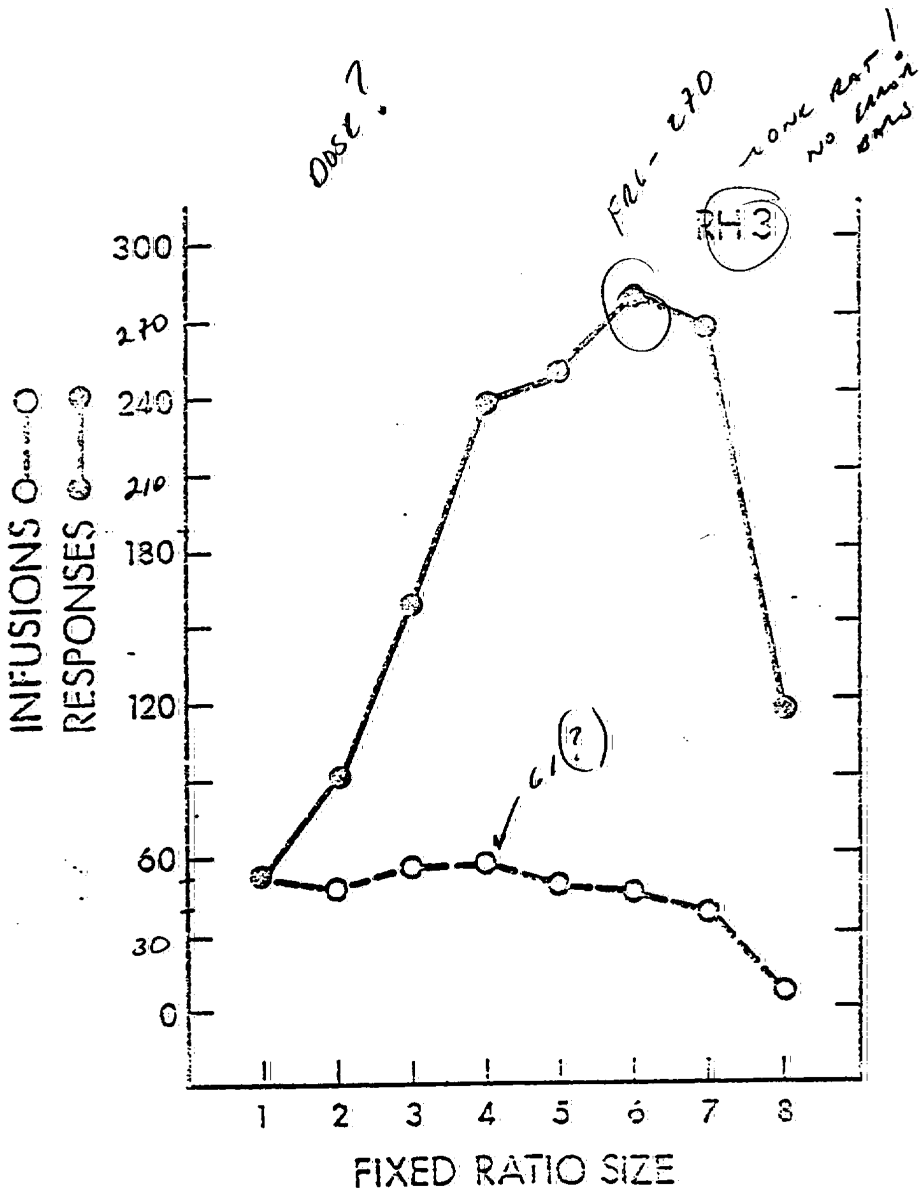

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(1.0 mg/kg/s.c.), 3. Naloxone (0.5 and 1.5 mg/kg/i.p.), and 4. Haloperidol (0.5 mg/kg/i.p.). All injections were separated by a minimum of 10 days and not all animals were tested with all blockers.

The effects of mecamylamine and hexamethonium have been previously reported (See memo to Dr. W. Dunn, August 24, 1981 from Victor J. DeNoble). Pre-injections of naloxone had no effect on nicotine self-administration. This was not too surprising since we have shown that naloxone injections do not alter nicotine induced prostration or nicotine induced discrimination cues. These combined observations suggest that nicotine's effects on central nervous system functioning are not mediated through an endogenous opioid system.

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The results with haloperidol were somewhat surprising. The pre-injection of haloperidol decreased the number of nicotine infusions by approximately 35% for one day. There are several possible explanations for this result. Haloperidol is a dopaminergic antagonist, and dopamine has been implicated in the central nervous system's response to reward. The possibility that the reinforcing properties of nicotine may be partially mediated via the dopaminergic system is intriguing. We have already ruled out a general suppressive effect of haloperidol and a general attenuation of the reinforcement process. We accomplished this by determining that the dose of haloperidol that reduced the nicotine-maintained behavior did not alter food-maintained behavior.

Future experiments utilizing more specific blocking agents are in progress.

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i. We are investigating the relative reinforcing ability of (±)-nicotine as compared to (-)-nicotine as a first attempt to delineate the steryospecificity of the receptor mediating the reinforcing actions of (-)-nicotine. The results show that (±)-nicotine does maintain lever pressing in rats (N=2). Substitution of saline for (±)-nicotine failed to maintain responding. When (±)-nicotine was reintroduced, the number of infusions increased to pre-saline levels (Figure 2). These data show that (±)-nicotine functions as a positive reinforcer for rats.

ii. Effects of infusion dose on the number of infusions and (±)-nicotine intake (mg/kg/day).

Preliminary data to date suggest that as the dose of (±)-nicotine was increased (16, 32, 64, 178, 256 µg/kg/infusion) the number of infusions first increased, then decreased. However, the intake (mg/kg/day) was directly related to the dose of (±)-nicotine. It is important to note that the dose response curve for (-)-nicotine and (±)-nicotine overlap, with the curve for the (±)-nicotine shifted to the right. This suggests that (-)-nicotine is a more potent reinforcer than (±)-nicotine. This potency effect is consistent with other data generated in our laboratory (prostration and discrimination). At present, we cannot provide a potency ratio but expect to be able to do so within four months.

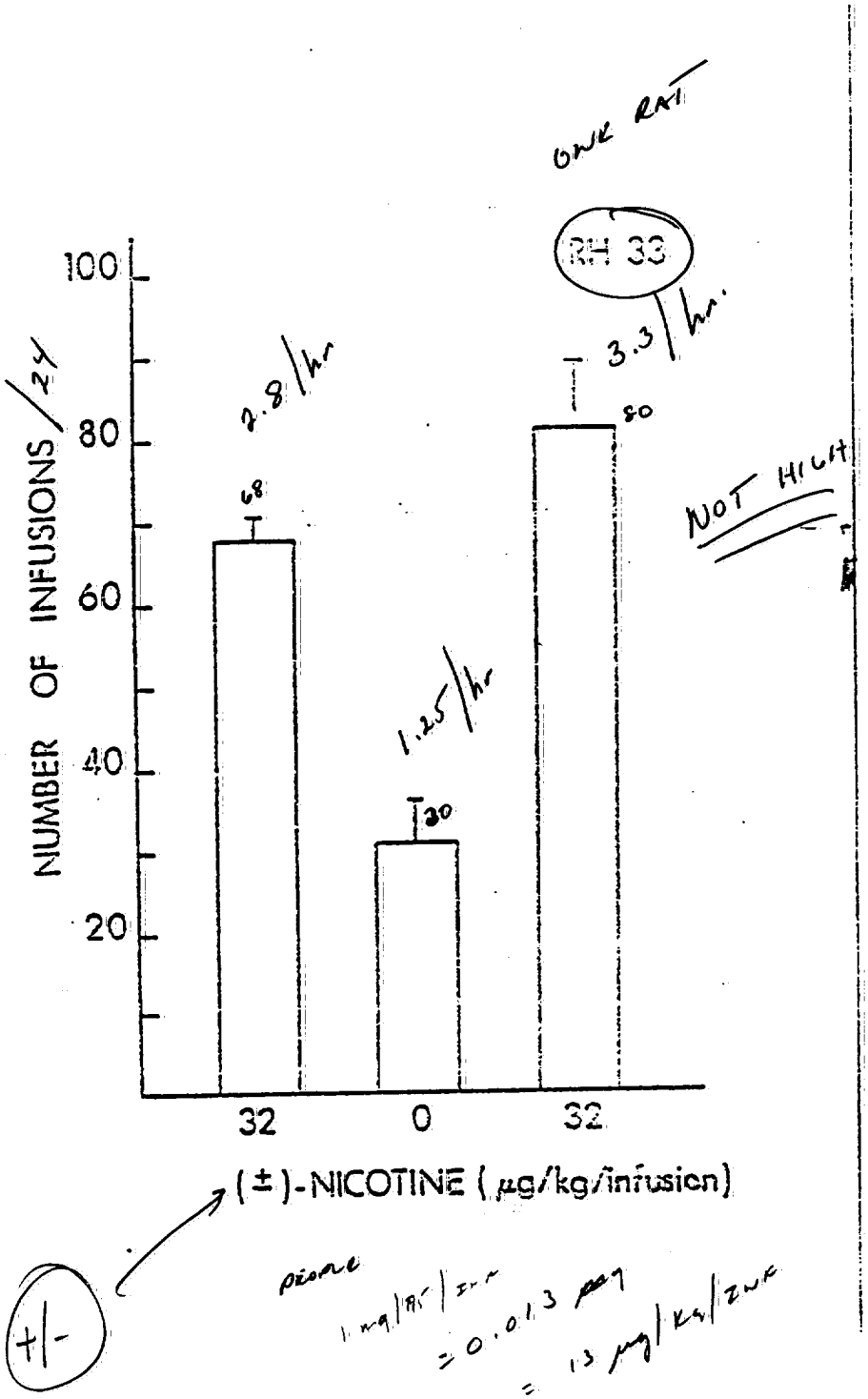

Figure 2. Number of infusions of (±)-nicotine as a function of nicotine or saline access conditions. Each bar is a mean of 5 consecutive days. Vertical lines show the standard error.

iii. Effects of fixed ratio size on (±)-nicotine self-administration.

Preliminary data from one rat show that the response rate increases, then decreases as a function of fixed ratio size. Responding was well maintained up to fixed ratio 8, then decreased under fixed ratio 10.

2. ACETALDEHYDE SELF-ADMINISTRATION

In a previous report (Progress Report to Dr. W. Dunn, August 24, 1981 from Victor J. DeNoble) we suggested that acetaldehyde functions as a positive reinforcer when delivered intravenously to rats. We have confirmed these results and have begun to develop a behavioral profile of acetaldehyde.

2.A. CONTROL STUDIES

We previously reported that a within-subject vehicle control procedure was completed on 3 rats and that it appeared that lever pressing was being maintained by the acetaldehyde. We have extended our control studies to include over 15 rats and all show similar patterns. We are convinced that acetaldehyde functions as a positive reinforcer.

2.B. EFFECTS OF INFUSION DOSE ON THE NUMBER OF INFUSIONS AND ACETALDEHYDE INTAKE (mg/kg/day)

Acetaldehyde self-administration was established during 24 hr./day access conditions at 32 µg/kg/infusion under Fixed Ratio 1. After stabilization, the effects of infusion dose were determined on response rate and acetaldehyde intake (mg/kg/day). Infusion doses were presented in ascending and descending order (128, 64, 32, 16, 8 and 4 ug/kg/infusion).

Rats were maintained at each dose for a minimum of seven days. The results show that as the dose of acetaldehyde was decreased, the number of infusions first increased, then decreased (Figure 3). Acetaldehyde intake was directly related to the dose (Figure 4). These relationships between dose, number of infusions and intake are similar to those obtained where other events maintain behavior (food, water, saccharin, etc.).

2.C. EFFECTS OF RATIO SIZE ON ACETALDEHYDE SELF-ADMINISTRATION

In Section 1.A. we explained the importance of developing ratio data to evaluate the relative reinforcer effectiveness of a compound. We have followed the same procedure with the self-administration of acetaldehyde. Figure 5 shows the data collected from one typical rat. The number of lever presses increased as a function of ratio size up to fixed ratio 15. The intake (mg/kg/day) decreased over the first 3 ratios, then remained constant across the next 5 ratio sizes. This data is in contrast to that obtained with (-)-nicotine in that (-)-nicotine did not maintain lever pressing at these high ratios. This indicates that acetaldehyde, at similar doses to (-)-nicotine, is more effective at maintaining behavior.

2.D. EFFECTS OF HALOPERIDOL OR NALOXONE INJECTIONS ON ACETALDEHYDE SELF-ADMINISTRATION

Aldehydes are chemically reactive intermediates that form Schiff bases with amines. If an amine is an aromatic-ethyl amine (Dopamine or Serotonin), the Schiff bases can spontaneously form a cyclic compound. These compounds are called tetrahydroisoquinolines (TIQ). The hypothesis

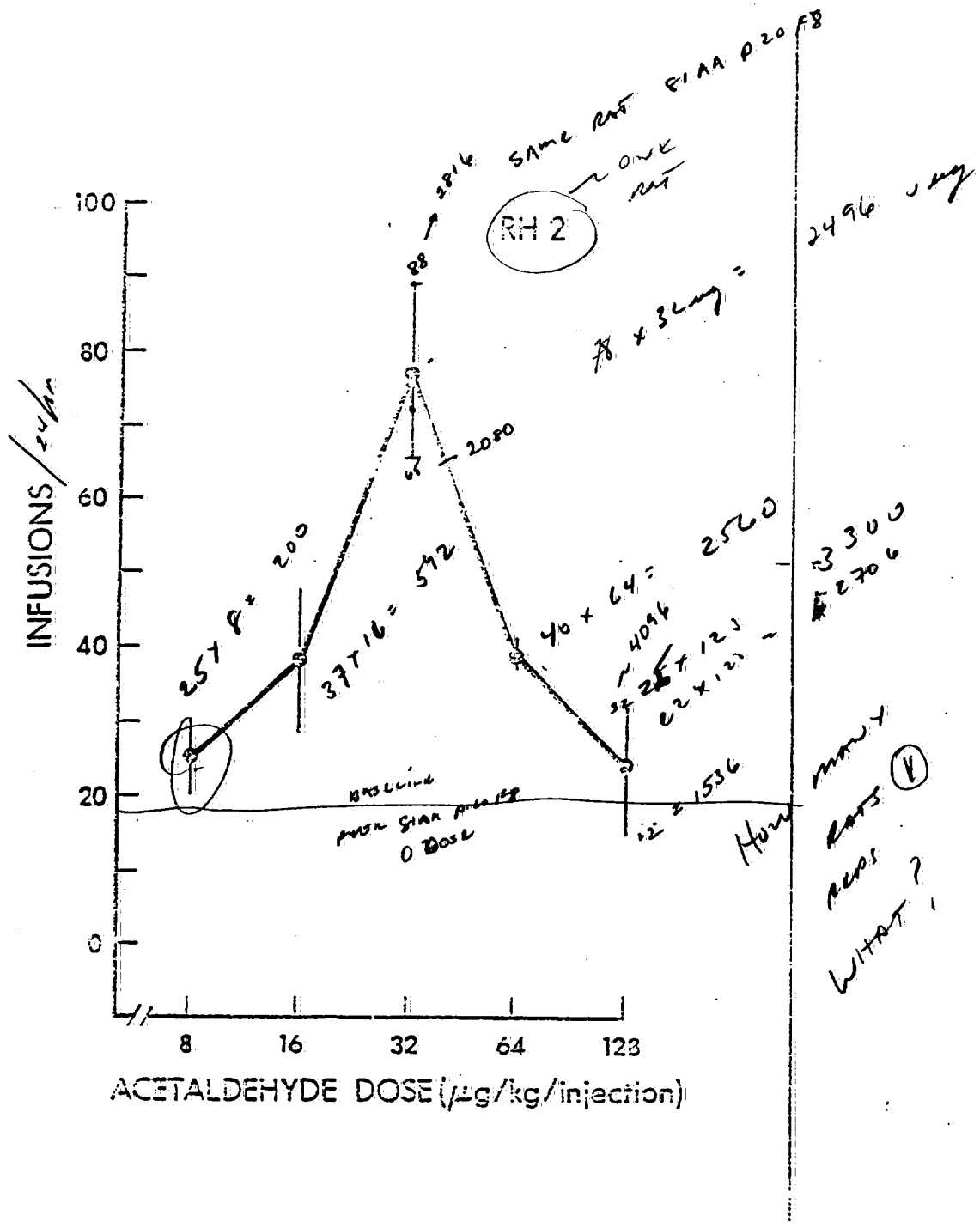

Figure 3. Acetaldehyde infusions during 24 hour sessions as a function of acetaldehyde dose. Each point represents the mean number of infusions over a 3-5 day period with no increasing or decreasing trends. Vertical lines show the standard error. Other rats showed similar functions.

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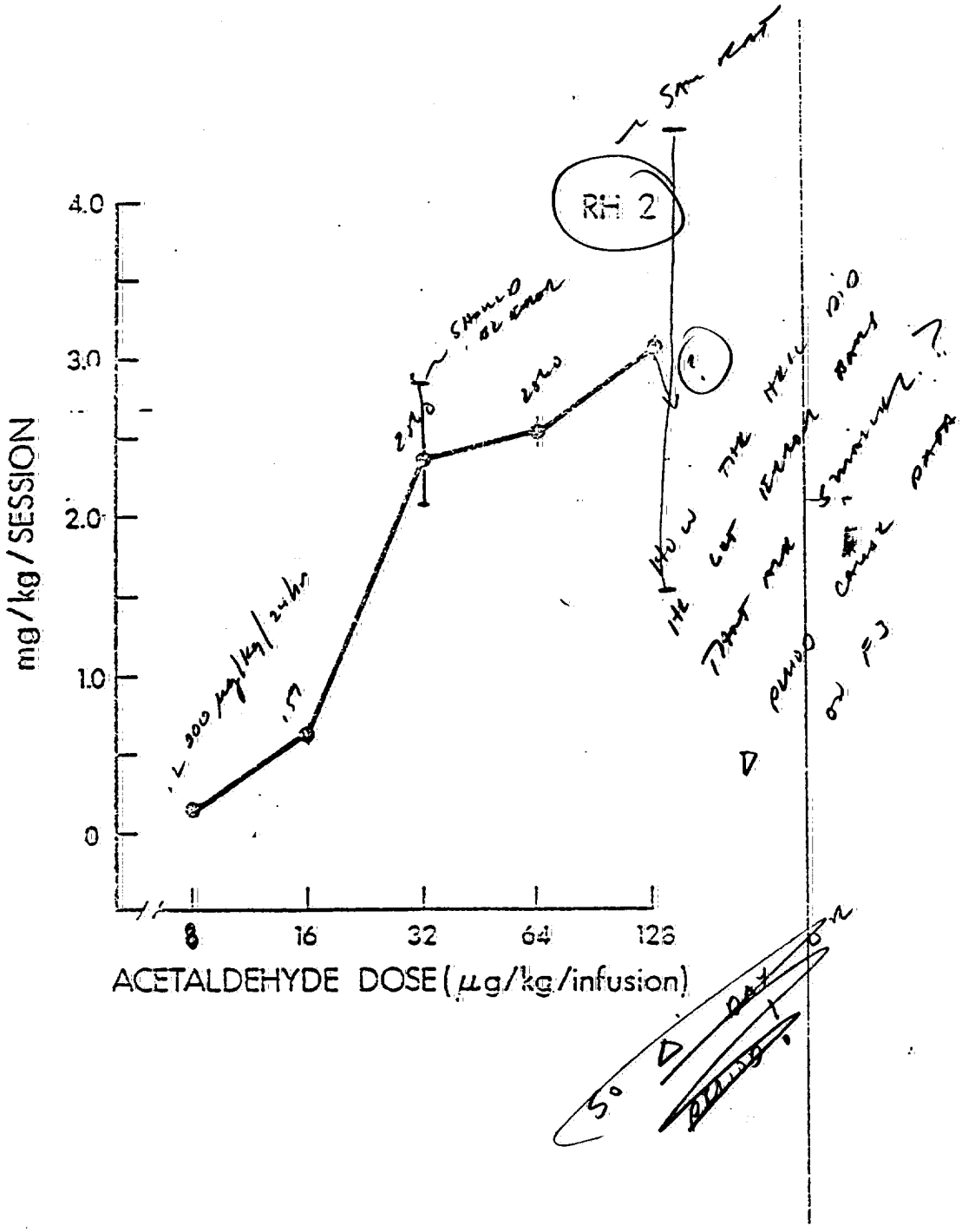


Figure 4. Acetaldehyde intake (mg/kg/24 h) as a function of acetaldehyde dose. Each point is a mean of 3-5 days. Vertical lines show the standard error. Other rats showed similar intakes.

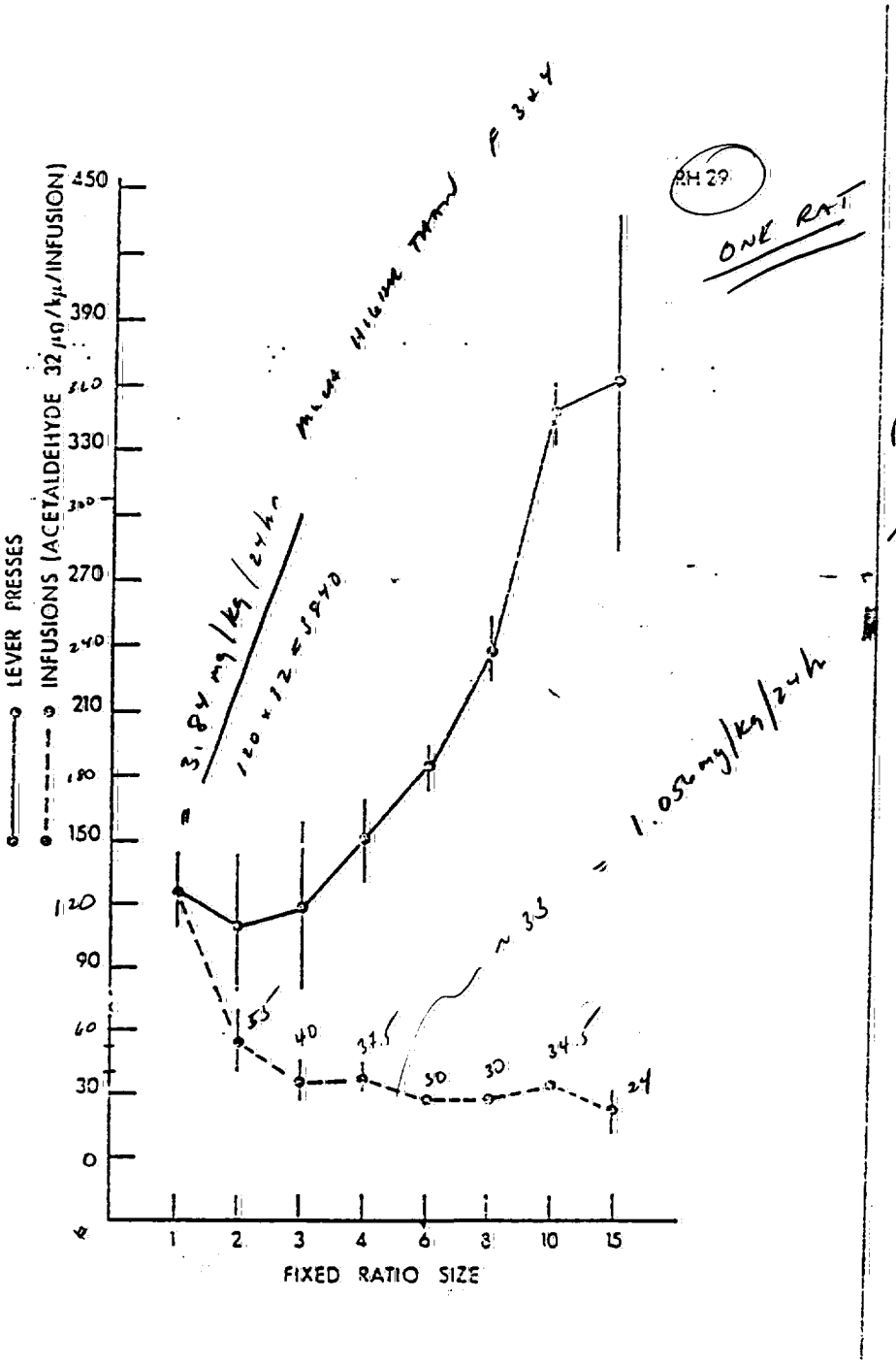


Figure 5. Number of lever presses and infusions as a function of fixed ratio size. Sessions were 24 hours in duration. Each data point represents a mean of 3-5 days of stable data. Vertical lines show the standard error. Other rats showed similar patterns of responding.

that the effect of acetaldehyde on the central nervous system is mediated by the formation of TIQs has been previously postulated. (Ref ?)

Our initial attempt at developing an understanding of the reinforcing effects of acetaldehyde in the central nervous system was to prevent the release of dopamine and to block the endogenous opiate receptors. Four rats were maintained under standard conditions (see Progress Report to Dr. W. Dunn, August 24 1981 from Victor J. DeNoble). Acetaldehyde (64.0 and 32.0 ug/kg/infusion) was established as a reinforcer under fixed ratio 1 conditions. After stabilization of lever pressing, the animals were injected with either naloxone (1.5 and 3.0 mg/kg/ip) or haloperidol (0.5 mg/kg/ip). These doses were chosen because previous work in other laboratories has shown that they are effective in the central nervous system.

Figure 6 shows the results of naloxone pretreatment on the number of acetaldehyde (64.0 ug/kg) infusions. At both doses tested, there were no major changes in the number of self-administered infusions. These data would suggest that the endogenous opioid system is not involved in the maintenance of acetaldehyde self-administration. However, the effect of haloperidol pretreatment on acetaldehyde (32.0 ug/kg) self-administration was to reduce the number of self-infusions to below saline levels (Figure 7). This is particularly interesting since it has been postulated that the effects of acetaldehyde on the central nervous system are mediated through the formation of TIQs. These represent preliminary results. results. Additional experiments to further characterize the role of acetaldehyde and TIQs in the central nervous system are underway.

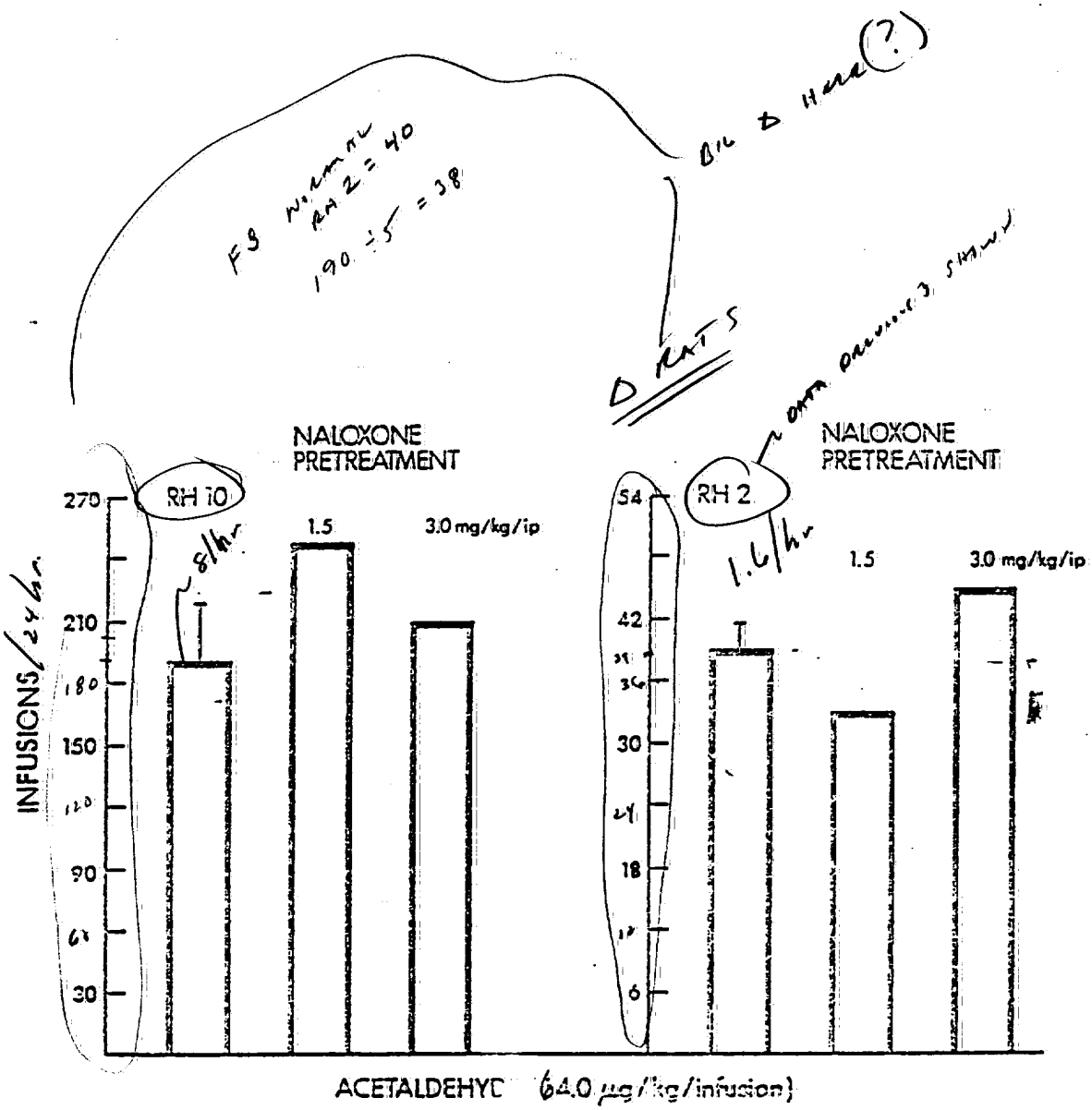


Figure 6. Number of acetaldehyde infusions as a function of the pretreatment dose of naloxone. The first bar in each graph represents a mean of 5 days and the vertical line shows the standard error. Naloxone injections were given at 7 day intervals in descending order immediately prior to a 24 hour test session.

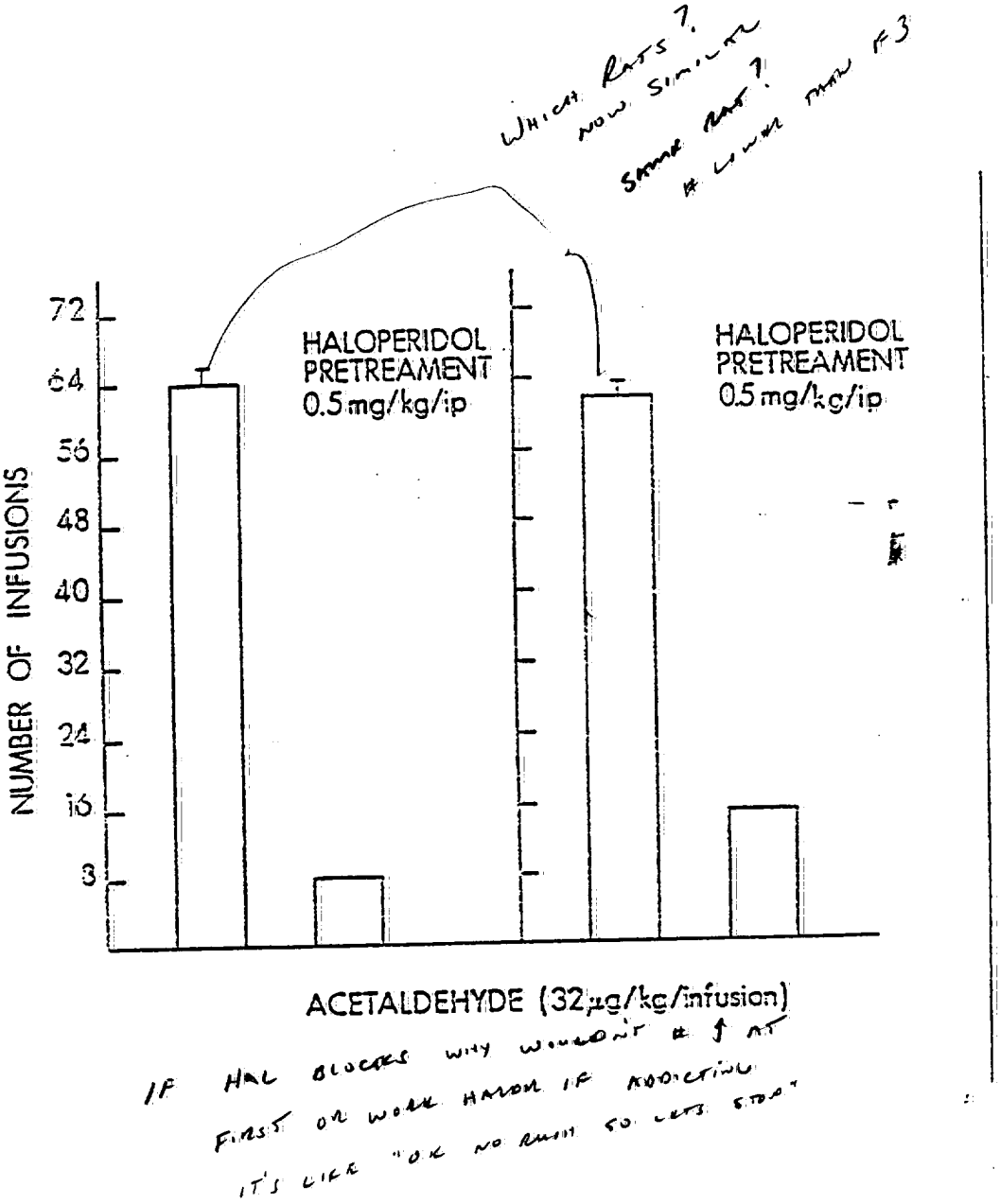


Figure 7. Effects of pretreatment with haloperidol on the number of acetaldehyde infusions during 24 hour sessions. The first bar in each graph shows the mean number of infusions over a 5 day period. The vertical lines show the standard error.

2.E. IN VIVO C^{14} ACETALDEHYDE RESEARCH AT UNIVERSITY OF ROCHESTER

The research was designed to answer basic questions concerning the transport of acetaldehyde from blood to brain. In the first study the following questions were addressed:

1. Does acetaldehyde cross the blood-brain barrier?

2. Is there differential uptake of acetaldehyde by brain through intravenous or intra-arterial injections?

3. What is the ratio of acetaldehyde in blood compared to brain following intravenous or intra-arterial injections?

4. Is there a regional brain distribution of acetaldehyde?

How localized? where metabolism?

PROCEDURE

All tests were performed on male hooded rats weighing approximately 350g. The basic preparation was the exposure of an artery (carotid) or a vein (femoral) in an anesthetized rat. For the intra-arterial preparation, an injection of 10µl of C^{14} acetaldehyde (tracer free) with an activity of 0.25µCi/µl was injected into the carotid artery. For the intravenous preparation, an injection of 10µl or 30µl of C^{14} acetaldehyde (tracer free) with an activity of 0.25µCi/µl was injected into the femoral vein. Five minutes following the injection, a midline incision was made from lower abdomen to clavicle, the rib cage opened, and heart exposed. A blood sample (0.2ml) was collected from the left ventricle of the heart.

Following this the rat was perfused with 0.9% saline for 3 minutes. The brain was quickly removed, dissected into the following three sections: cortex, midbrain and cerebellum (note that the dissection was done by hand and is subject to some variability). Blood and brain sections were placed in individual scintillation tubes to which 5.5ml of scintillation fluid were added. Searle Analytical (Model Delta 300) β Scintillation Counter was used to analyze the samples.

RESULTS

The results show that:

1. Acetaldehyde readily permeates the blood-brain barrier.
2. There is no marked difference between intravenous and intra-arterial injections in the amount of acetaldehyde that gets to brain tissue.
3. The ratio of acetaldehyde in brain compared to blood 5-minutes post injection was approximately 1 to 10.
4. There does not appear to be a gross regional distribution of acetaldehyde in the brain.

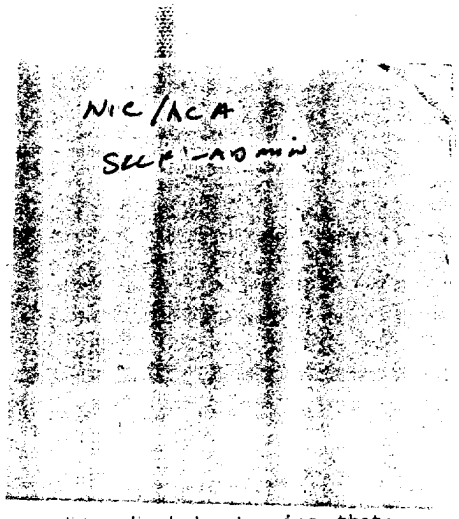
The purpose of the second study was to determine if there was differential uptake of C^{14} acetaldehyde by nerve endings, myelin and mitochondria.

PROCEDURE

Two male hooded rats weighing 300 to 350 grams were utilized in a preliminary study designed to test for cellular acetaldehyde localization in brain following an intravenous injection of the compound. Thirty micrograms (30ug) of C^{14} acetaldehyde with an activity of 0.25 microcuries

per microliter were injected into a femoral vein preparation. Five minutes postinfusion, the animal was sacrificed by a blunt blow to the lumber region, and its brain quickly removed. The following method was performed in duplicate.

The brain was rinsed in 0.9% saline prior to placement in 20ml of 0.32 molar isotonic sucrose. This mixture was homogenized and transferred to two centrifuge tubes. The tubes were placed in an analytical centrifuge and spun for 10 minutes at 1000 gravities (G.). The pellet contained large cellular debris such as unlysed cells and large fragments of cell membrane and it was resuspended in 3ml of 0.32M sucrose to be counted for radioactivity later. The supernatant was spun in an ultracentrifuge at 12,000 Gs for 20 minutes to separate the microsomal fraction (endoplasmic reticulum, vesicles and axonal fragments) from the fraction containing the nerve endings. The supernate from this spin was the microsomal fraction which was set aside for later counting. The pellet or synaptosomal fraction was resuspended in 2 ml of 0.32M isotonic sucrose. At this point sucrose density gradient centrifuge tubes were prepared containing a 5ml bottom layer of 1.2 M sucrose and a top layer of 0.8 M sucrose. The non-resuspended synaptosomal fraction (in 0.32 sucrose) was applied onto these density gradient tubes. These tubes were spun at 100,000 Gs for 1 hour. The three fractions obtained from this spin are myelin, nerve endings, and mitochondria. To 0.5cc of the initial pellet (non-resuspended), the second supernate, and the myelin, nerve ending and mitochondrial fractions of the last spin were added 5.5ml of scintillation fluid. The C^{14} activity of these samples was determined utilizing a Searle Analytic β Scintillation Counter. The amount of



acetaldehyde present in each sample was determined by knowing that:

1) 250μCi/1.0mg

   250μCi/1000μg

   .25μCi = 1μg in 1μl

   30μl was injected; therefore,

2) 30μl = 7.5μCi = 30μg

3) 100cpm = $4x10^{-4}$μg corrected for efficiency

   of the counter with a background of 30cpm

The results of experiment two show that most of the acetaldehyde concentrates in the myelin and nerve endings with a smaller amount located in the mitochondria.

CONCLUSIONS

At present the biochemical data indicate that acetaldehyde readily penetrates the blood-brain barrier and appears to be equally distributed in all brain regions. These data, combined with the behavioral data collected in our laboratory, suggest further investigation of acetaldehyde at both the behavioral and central nervous system levels.

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3. NICOTINE-ACETALDEHYDE SELF-ADMINISTRATION

We have demonstrated in our laboratory that both (-)-nicotine and acetaldehyde have positive reinforcing effects when delivered intravenously to rats. Both of these substances are smoke components that are delivered to the smoker. It is well documented that when two reinforcers are presented to an organism, there can be modification of the behavioral effect of one reinforcer by the other. In the case of (-)-nicotine and acetaldehyde, an interaction between the two compounds can be defined as a modification of the pharmaco-

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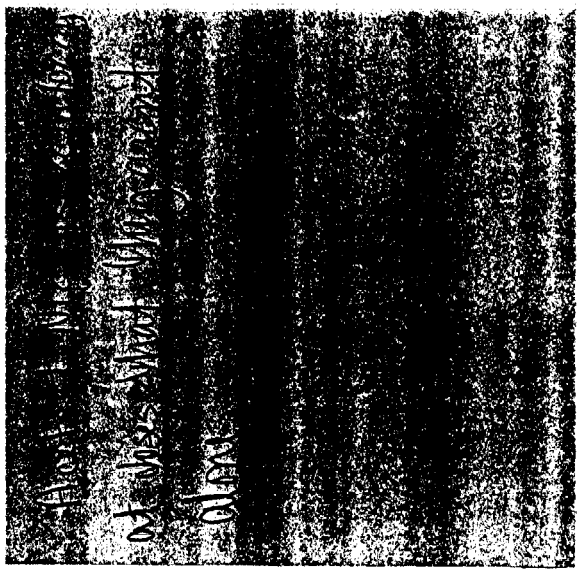
logical effect of one compound by the other. We have used our self-administration technique to evaluate the behavioral interaction between (-)-nicotine and acetaldehyde. Although a behavioral interaction between two compounds can be readily defined as a modification of the behavioral effect of one compound by the other, it can be difficult to demonstrate conclusively that any modification has taken place. Therefore, it becomes necessary to first measure the effect of the two compounds separately, then measure the effect of the two compounds given concurrently. Finally, a decision has to be made on whether or not the joint effects can be predicted from a single additive model, which adds the effect of the first component to that of the second. If the effect of the combination deviates from the prediction of the additive model, then an interaction can be inferred.

To establish if acetaldehyde interacts with (-)-nicotine in reinforcing systems, we provided rats (N=4) with access to acetaldehyde-(-)-nicotine combinations. Acetaldehyde (4.0 ug/kg/infusion) mixed with (-)-nicotine (4.0 ug/kg/infusion) was available for self-administration under standard conditions (See Progress Report to Dr. W. Dunn, August 24, 1981 from Victor J. DeNoble). The two graphs in Figure 8 show the results. The combinations maintained responding above either compound when presented alone. In addition, the level of lever pressing maintained by (-)-nicotine or acetaldehyde does not exceed vehicle levels. This suggests that a combination of a dose of acetaldehyde and a dose of nicotine that alone would not be reinforcing, is reinforcing when presented together. Further, the joint effect of the combination is greater than an additive effect, suggesting a synergistic relationship.

Acet. + Nic is reinforcing at doses that aren't alone

To further analyze this relationship, we provided a rat with a mixture of acetaldehyde (8.0 ug/kg/infusion) and nicotine (8.0 ug/kg/infusion) in which



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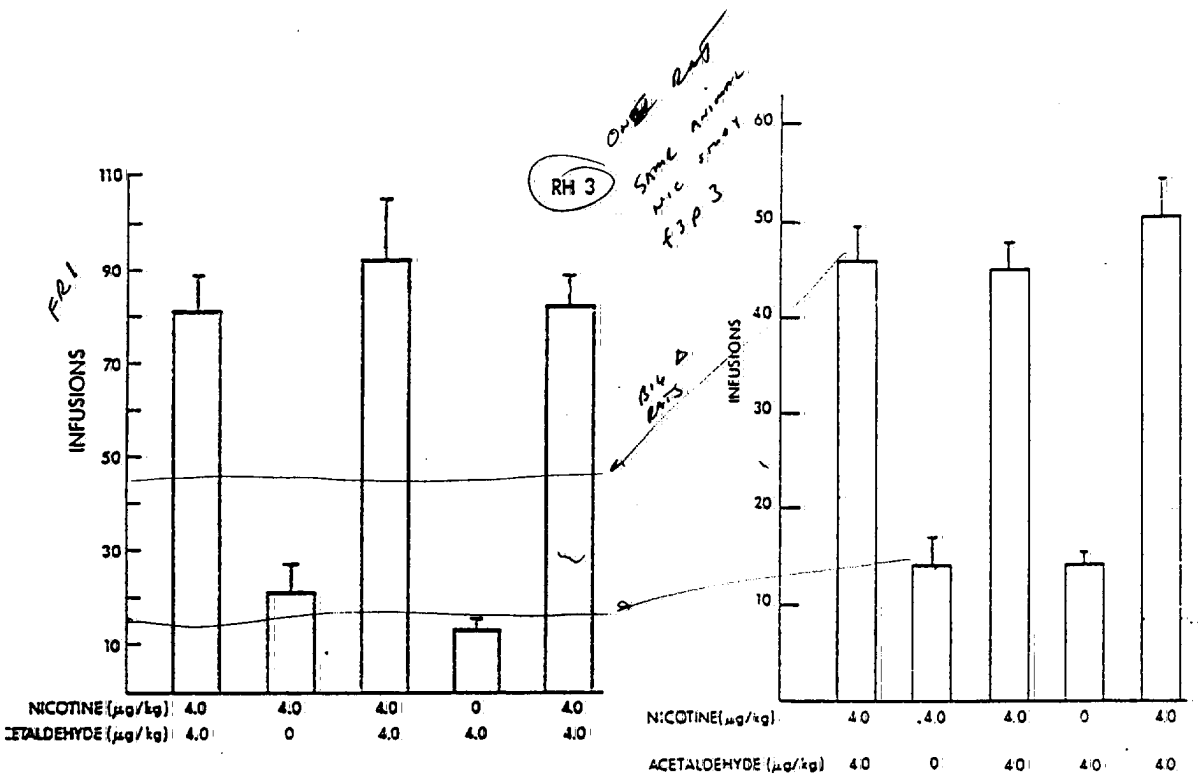

Figure 8. Number of infusions under fixed ratio 1 as a function of (-)-nicotine (4.0 ug) and acetaldehyde (4.0 ug) combinations. Each bar is a mean of 3-5 days of stable data. Vertical lines show the standard error.

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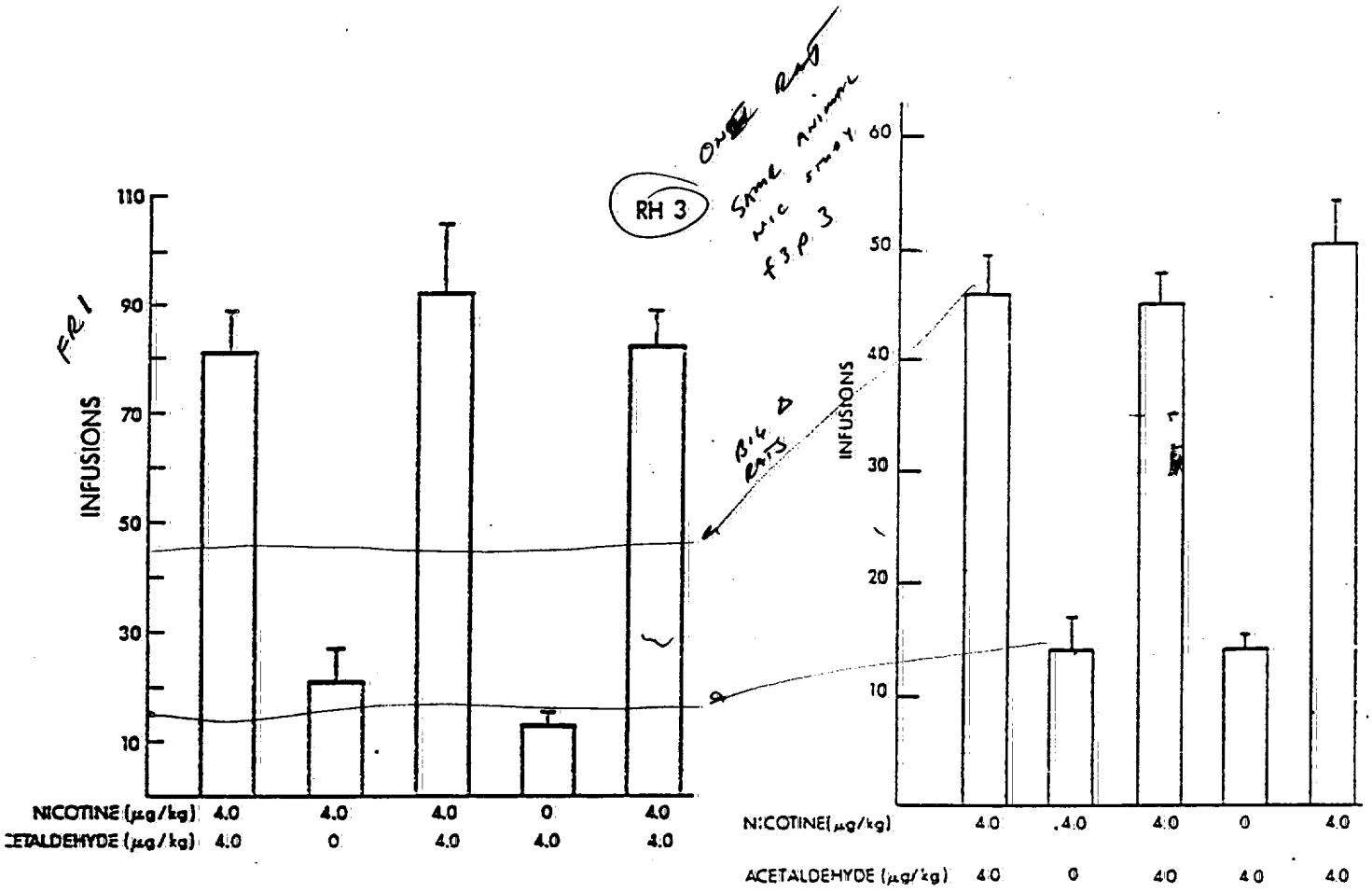

Figure 8. Number of infusions under fixed ratio 1 as a function of (-)-nicotine (4.0 ug) and acetaldehyde (4.0 ug) combinations. Each bar is a mean of 3-5 days of stable data. Vertical lines show the standard error.

each compound would maintain behavior independently. The mixture was available under an FR 6 schedule. Figure 9 shows that when the combination is available, the response rate is very high. When (-)-nicotine or acetaldehyde is removed from the mixture, the number of lever presses decrease. This demonstrates that when both compounds are functioning as reinforcers, they can and do interact.

4. BEHAVIORAL EFFECTS OF INTRAVENTRICULARLY ADMINISTERED (-)-NICOTINE ON FIXED RATIO SCHEDULES OF FOOD PRESENTATION IN RATS

Nicotine is one of the most widely used compounds, but basic research on its mode of action in the brain and on its effects on animal behavior has lagged far behind other research on commonly used substances. Most previous studies have investigated the behavioral effects of systemically administered nicotine in rats or monkeys. In rats nicotine increases responding maintained under fixed-interval (FI), variable-interval, and differential-reinforcement of low rate schedules of food or water presentation and under schedules of electric shock postponement (Bovet and Bovet-Nitti 1965; Morrison and Stephenson 1969; Pradhan 1970; Pradhan and Dutta 1970; Ando 1975) and decreases responding under fixed-ratio (FR) schedules of food or water presentation (Morrison and Stephenson 1969; Pradhan 1970). Qualitatively similar results on responding have been reported in squirrel monkeys maintained under a multiple FI-FR schedule of either presentation of food or termination of a stimulus associated with electric shock (Davis et al. 1973; Spealman et al. 1981).

There are no reports, to our knowledge, of the effects of intraventricular (IVT) administration of nicotine on schedule-controlled behavior. Intraventricular administration is a means of studying nicotine with the relative absence of peripheral effects. Abood and co-workers (1978, 1979) reported that

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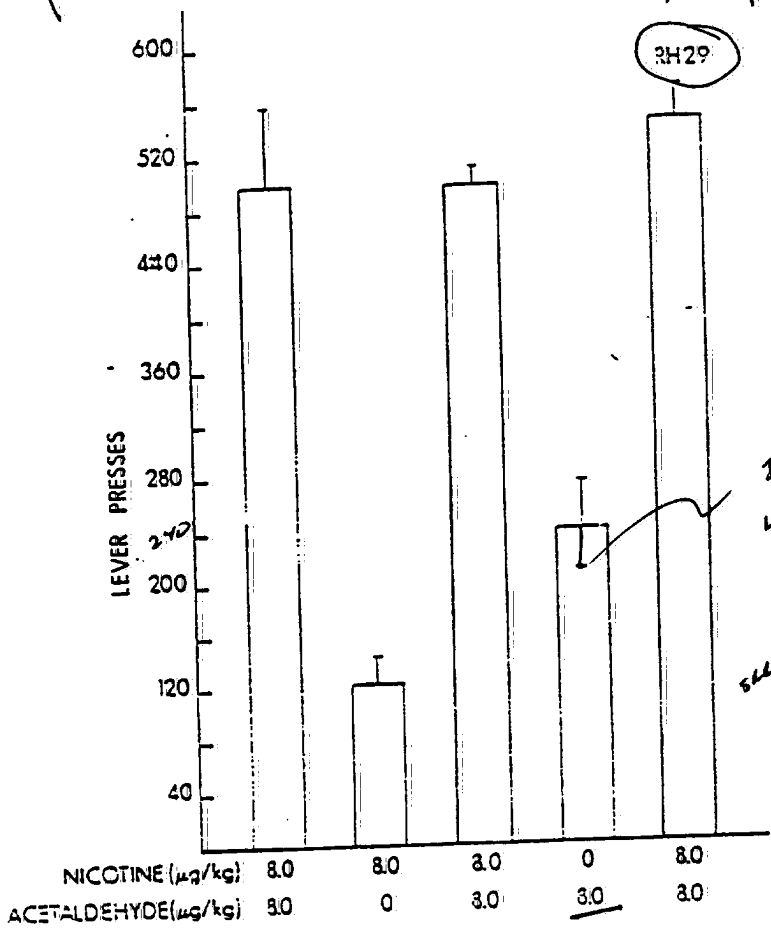

Figure 9. Number of lever presses under fixed ratio 6 as a function of (-)-nicotine (8.0 ug) and acetaldehyde (8.0 ug) combinations. Each bar is a mean of 3-5 days of stable data. Vertical lines show the standard error.

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an IVT infusion of nicotine (2-10 μg) into the lateral ventricle resulted in a prostration-immobilization syndrome in rats. This prostration syndrome was prevented or antagonized by IVT pretreatment with N'-Benzylnornicotine and some piperidine derivatives, but not by a variety of neurotransmitters or psychotropic agents.

The purpose of the present study was to establish a more detailed profile of the behavioral effects of IVT administration of (-)-nicotine. The second purpose of the study was to examine the effects of two nicotinic-cholinergic blocking agents, mecamylamine and hexamethonium on the behavioral changes induced by the IVT infusion of (-)-nicotine.

Experiment 1

FIXED RATIO SCHEDULES OF FOOD PRESENTATION

The effects of IVT infusion of (-)-nicotine were tested on behavior maintained under FR schedules of food presentation.

MATERIALS AND METHODS

Subjects. Eight experimentally naive male albino rats (Holtzman Co., Madison, Wisconsin), between 90 and 120 days old and weighing between 190 and 230 g were used. Animals were housed individually and were allowed food continuously for three weeks, during which time weights were recorded daily. The mean weights were calculated from the last five days of the three week period, after which the rats were reduced to 80% of their free-feeding weights. These weights were periodically adjusted to control for growth rate.

Rats were anesthetized with ketamine (70 mg/kg/im) and sodium pentobarbital (18 mg/kg/ip) and a stainless steel cannula (#220 DK rat cannula, David Kopf Co.) was stereotaxically inserted into the left lateral ventricle (posterior = 1.1 mm from bregma, lateral = 1.7 mm, vertical = 5.1 mm from the skull

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Rats were anesthetized with ketamine (70 mg/kg/im) and sodium pentobarbital (18 mg/kg/ip) and a stainless steel cannula (#220 OK rat cannula, David Kopt Co.) was stereotaxically inserted into the left lateral ventricle (posterior = 1.1 mm from bregma, lateral = 1.7 mm, vertical = 3.1 mm from the skull

surface). The cannula was attached to the skull by acrylic cement and three small set screws.

At the conclusion of all the experiments animals were sacrificed, perfused intracardially with 0.9% saline, 10% formalin and then infused intraventricularly with 2 µl of Blue Evans Dye. Brains were removed and sliced for verification of cannula placement.

Apparatus. Four identical operant conditioning chambers (Lehigh Valley Electronics No. 143-25), each contained in a sound-attenuated cubicle (LVE No. 132-02), were used. Located at one end of the chamber were two levers (LVS No. 121-05), a pellet receptacle, 6 cue lights (lever lights), a speaker, and a house light.

With each operation of the pellet dispenser, a single 45-mg Bio Serve food pellet was delivered to the receptacle. White noise was constantly present and an exhaust fan provided ventilation.

Procedure. Each rat was trained to lever press under an FR 1 schedule for a single delivery of food. Over a two week period the ratio size was increased to 16. Daily sessions (Monday-Friday) consisted of two successive 15-minute periods with a 5-minute time out (TO) after the first 15-minute period. During the TO the rats were placed in a holding cage. When response rates during the two 15-minute periods were stable (less than 10% variance in daily response rate for both 15 minute periods over 5 sessions) IVT infusions were begun. All infusions were given during the last minute of the TO and the rats were immediately placed back in the operant chamber. Data from repetitive 15-min sessions were collected until the response rates were within preinfusion levels. All infusions were separated by 3-5 days of stable response rates. Under FR 16 the rats were infused as follows: 1) 5 µl of 0.9% saline 2) 5 µg of (-)-nicotine in 5 µl of 0.9% saline. Following this the ratio size was increased to 32 and

after stabilization of response rates the rats were infused with: 1) 5 µg of (-)-nicotine in 5 µl of 0.9% saline 2) 5 µl of 0.9% saline and 3) 5 µg of (-)-nicotine in 5µl of saline.

Subsequently, lever pressing was maintained under FR 64 and the rats were infused with 5 µg of (-)-nicotine in 5 µl of 0.9% saline.

Infusion Procedure. The infusion cannula was attached by polyethylene tubing to a 10 µl Hammilton syringe. The tubing and cannula were flushed with 95% ethanol prior to being filled with (-)-nicotine. The microliter syringe was filled with 95% ethanol and was attached to the tubing by an 18-gauge needle. All infusions were given in a volume of 5 µl. Rats were restrained by wrapping them in a cloth towel, leaving their heads exposed. The stylus was removed from the cannula and the infusion cannula inserted. Solutions were infused in less than 1.0 seconds. Following the infusion the stylus was replaced and the rats were immediately placed into the operant chambers.

RESULTS AND DISCUSSION

During non-infusion and saline control sessions, characteristic FR response patterns occurred. That is, a brief pause was followed by an abrupt transition to a high rate of responding that was maintained until the ratio was completed (top panel Fig 10). Response rate varied directly as a function of ratio size (mean ±SE responses per second under FR 16, 1.78 ± 0.11; FR 32, 2.72 ± 0.23; FR 64, 3.23 ± 0.67). The latency to complete the first ratio following an IVT saline infusion under FR 16 and 32 was less than 30s; however, IVT infusions of (-)-nicotine (5 µg/5 µl) increased the latency to complete the first ratio (Fig. 11). The effect of IVT infusions of nicotine on the latency depended primarily on the FR size, which resulted in different response rates. The two nicotine infusions under FR 32 (7 day interinfusion interval) did not

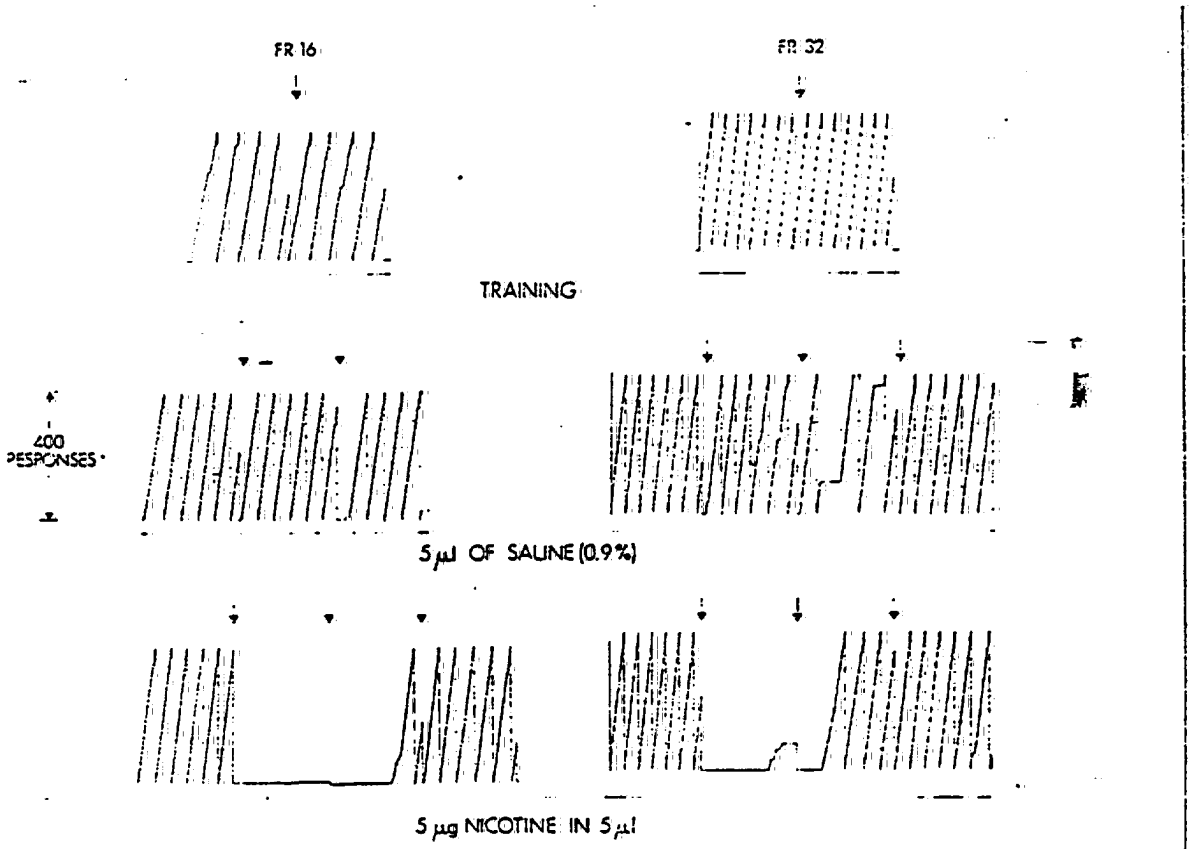


Figure 10. Cumulative records for a single rat maintained under FR 16 and 32. The stepping pen recorded lever presses and each downward deflection of the stepping pen indicated a pellet delivery. The stepping pen reset automatically after 400 responses. Arrows at the top of each record indicate the end of a 15 minute period. Note the difference in latency to complete the first ratio between FR 16 and 32 following a nicotine infusion.

differ significantly from each other (df = 5 t = 0.64 p >0.1) in the latency, suggesting that the decrease in latency with increasing FR sizes was not due to repetitive testing.

Figure 10 contains cumulative records that show the pattern of responding under FR 16 and 32, and the time course of the nicotine-induced response suppression for a single rat. All rats showed similar patterns. Characteristic responding can be seen under both ratios during baseline and saline control conditions. Note the longer latency to the first completed ratio under the FR 16 schedule.

Experiment 2

BEHAVIOR MAINTAINED UNDER AN FR 32 SCHEDULE OF FOOD PRESENTATION

In this experiment the ratio was held constant and various doses of (-)-nicotine were administered.

Subjects and Apparatus. Eight experimentally naive albino rats were maintained under the same conditions and tested in the same apparatus as described in Experiment 1.

Procedure. The rats were trained to lever press for a 45 mg food pellet under an FR 32 schedule. When response rates were stable (less than 10% variance in daily rate for both 15 min periods over 5 sessions) IVT infusions were begun. All infusions were given during the 5 minute TO period and separated by 7 days. Rats were tested with nicotine doses as follows: 2.5, 1.25, 0.625, 0.312, 5.0, and 10 µg of (-)-nicotine in a constant volume of 5 µl of saline.

RESULTS AND DISCUSSION

Increases in nicotine dose led to increases in the latency to complete the first ratio (Fig 12). At the lowest dose tested response latencies were not

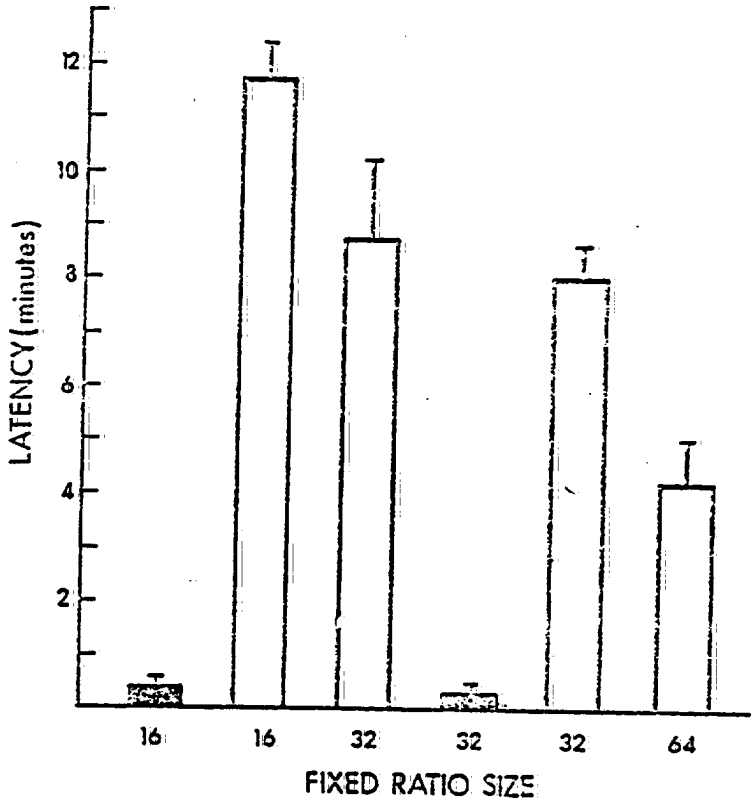


Figure 11. Effects of saline and nicotine (5µg) infusions on the average latency to complete the first ratio under FR 16, 32 and 64 schedules of food presentation. Each bar represents the average latency (N=8) and vertical lines show the standard error. Solid bars show saline infusions and open bars nicotine infusions.

significantly different from saline-infusion values. At the next two doses (0.625 and 1.25 µg) the response latency increased to above saline-infusion levels. When the 2.5 µg dose was infused, the mean latency increased to 6.8 minutes (± 2.2). The two highest doses (5.0 and 10.0 µg) produced the longest latencies. Three animals were not tested at these doses due to blockage in the cannulae.

Experiment 3

NICOTINE IN COMBINATION WITH MECAMYLAMINE OR HEXAMETHONIUM

In a number of studies of discriminative stimulus properties of nicotine in rats pretreatment with mecamylamine consistently blocked the nicotine effect, whereas hexamethonium did not block the effect at any dose tested (Morrison and Stephenson 1969; Schecter and Rosecrans 1971; Hazell et al. 1978). In addition, mecamylamine but not hexamethonium blocked the behavioral effect of nicotine in monkeys maintained under a multiple FI-FR schedule of either termination of a stimulus associated with electric shock or presentation of food (Spealman et al. 1981). Our third experiment compared the behavioral effects of IVT administration of nicotine in combination with either mecamylamine or hexamethonium on responding maintained under an FR 32 schedule of food presentation.

Subjects and Apparatus. Ten experimentally naive rats were maintained under the same conditions and tested in the same apparatus as described in Experiment 1.

Procedure. The rats were trained to lever press for a 45 mg food pellet under an FR 32 schedule. When response rates were stable (less than 10% variance in daily response rate for both 15 minute periods over 5 sessions) IVT infusions

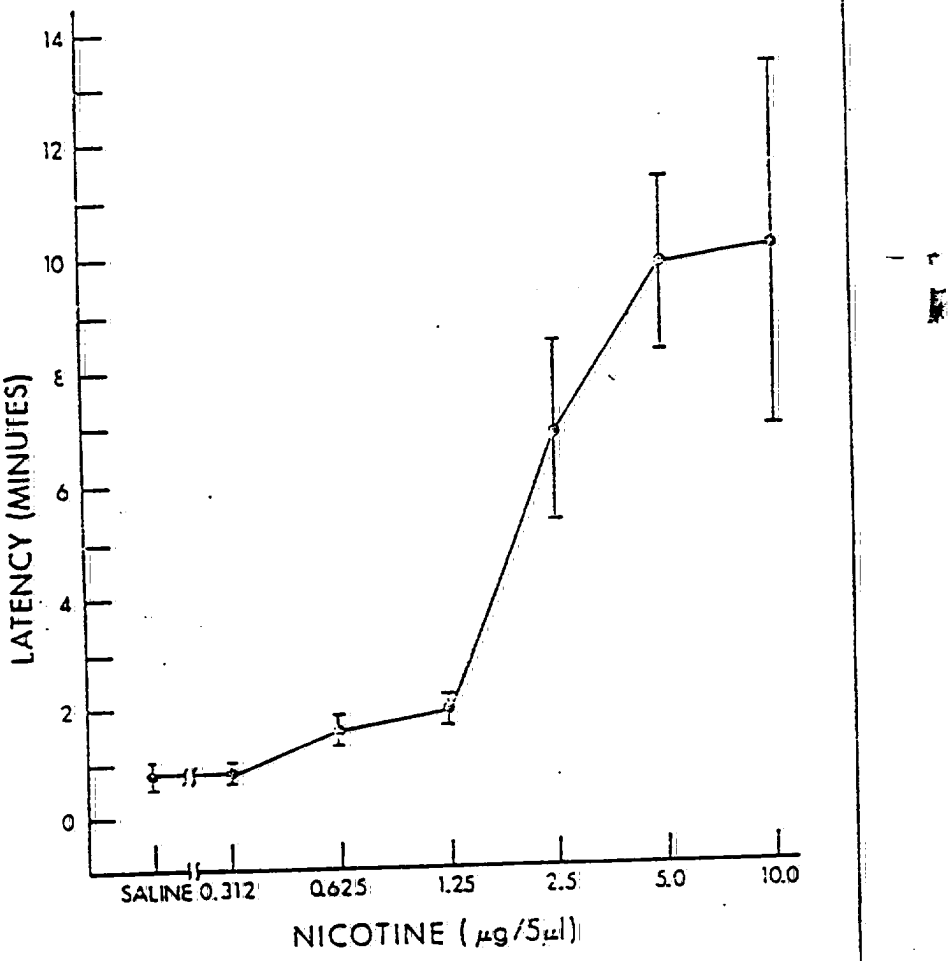


Figure 12. Latency to complete the first ratio as a function of nicotine dose. Each point from 0.312 to 2.5 µg is a mean of 8 animals. At doses of 5.0 and 10.0 the points are means of 5 animals. Brackets indicate the standard error.

were begun. All infusions were given during the 5 minute TO and separated by 7 days. Five rats were administered the following sequence of injections: 1) saline, 5 µl of 0.9%, 2) saline with a presession injection of mecamylamine, 1.5 and 3.0 mg/kg/sc 5 minutes prior to the first 15 minute period, 3) (-)-nicotine, 10 µg in 5 µl, 4) (-)-nicotine, 10 µg in 5 µl with a presession injection of mecamylamine, 0.05, 1.5 and 3.0 mg/kg/sc, and 5) (-)-nicotine in 5 µl of saline.

The remaining five rats were maintained under FR 32 and tested with presession injections of hexamethonium chloride (0.05, 1.5 and 3.0 mg/kg/sc given 10 min prior to a 10 µg infusion of (-)-nicotine. All tests were separated by a 7 day interval.

RESULTS AND DISCUSSION

Saline infusions with or without mecamylamine pretreatments had little effect on the latency to complete the first ratio (Figure 13). There was no significant difference between saline and saline-mecamylamine combinations (at 1.5 mg/kg/sc, df=4, t=1.29 p>.1; at 3.0 mg/kg/sc, df=4, t=1.97 p>.1). The average latency to the first completed ratio following a 10 µg IVT infusion of (-)-nicotine was 13 minutes (±1.5 min). Presession injections of mecamylamine blocked the effect of nicotine in a dose related fashion (Fig 13). Injections of mecamylamine (0.05 and 1.5 mg/kg/sc) decreased the latency by 40 and 84 percent respectively. In four of the five animals tested, mecamylamine (3.0 mg/kg/SC) completely blocked the effect of IVT nicotine (saline vs 3.0 mg/kg/ mecamylamine and nicotine, df=3, t=1.58 p>.1). For one rat mecamylamine at this dose did not completely block the effect and the latency was 4 min 30 s. No explanation is apparent as to why this rat's behavior differed from the

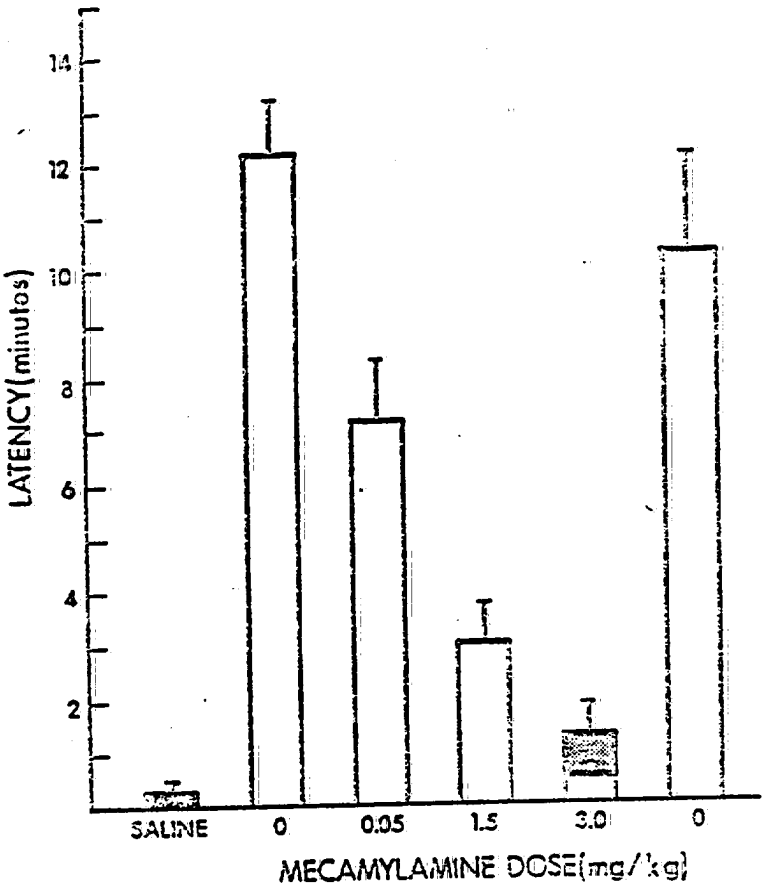


Figure 13. Antagonism by mecamylamine of the effects of nicotine on the latency to complete the first ratio under FR 32 schedule of food presentation. Solid bars show the mean (N=5) latency following a saline infusion with and without mecamylamine pretreatment. Open bars show nicotine infusions. Vertical lines show the standard error. The hatched bar shows the mean latency with aberrant animal included.

others. All rats were given a nicotine retest and their latencies did not differ from the original nicotine test value (Fig 13).

In contrast to the nicotine induced changes in latency, subsequent response rates did not show any systematic changes following any experimental manipulations, suggesting that when recovery occurred it was complete.

Unlike mecamylamine, hexamethonium at doses of 0.5 and 1.0 mg/kg/sc failed to block the latency to lever press. (Fig 14) However, at a dose of 3.0 mg/kg/sc there was a partial antagonism of the nicotine induced latency changes. Penetration of hexamethonium into the brain is limited (Taylor 1980) but not excluded, and it is likely that at the highest dose given amounts sufficient to produce a partial antagonism did cross the blood brain barrier. Nicotine retest values without a preinjection of hexamethonium did not differ from the original nicotine test values.

GENERAL DISCUSSION

Responding by rats was maintained under various FR schedules of food presentation. Under these conditions the duration of the effects of IVT administration of nicotine extended far beyond the observed time course previously reported (Abood et al. 1978, 1979). The latency to complete the first ratio following a nicotine infusion was inversely related to the ratio size. The similarity between the latencies observed from the nicotine infusion under the FR 32 schedule suggests that the effect of ratio size on the nicotine induced latency change was not secondary to repetitive nicotine testing. This finding is similar to that previously reported (Abood et al. 1979). These authors showed that tolerance to the behavioral effects of IVT nicotine would develop after chronic nicotine infusions (i.e., infusions on 6 consecutive days), but that behavior ratings returned to initial levels within 2 days following the

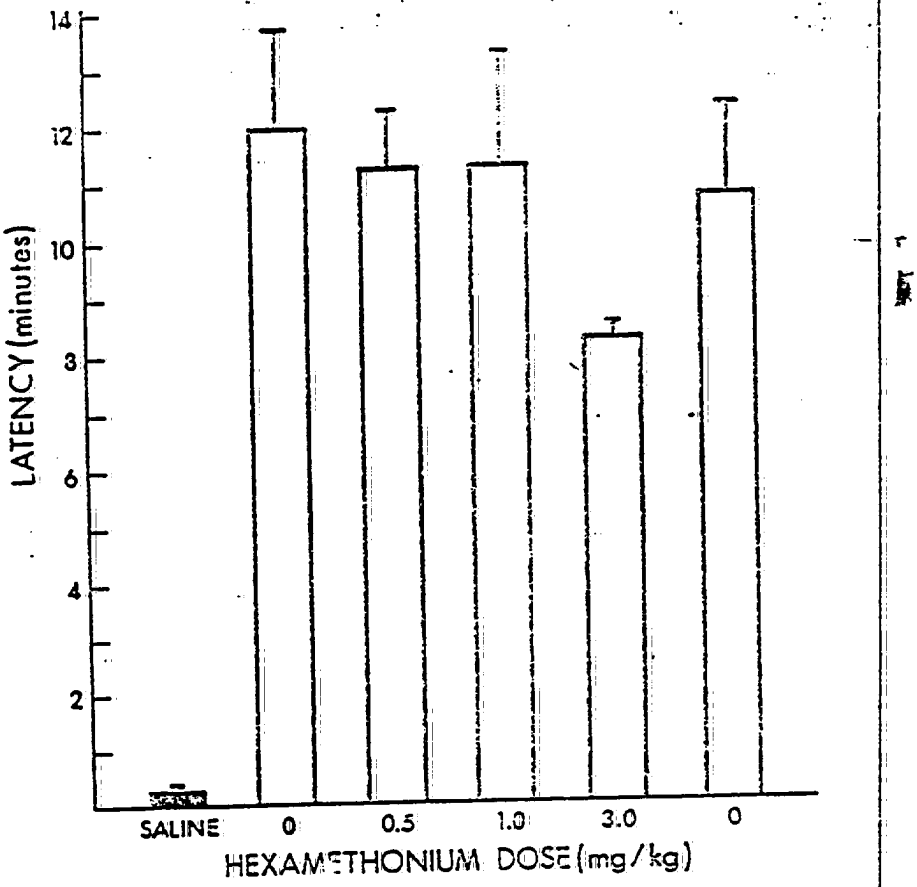


Figure 14. Antagonism by hexamethonium of effects of nicotine on the latency to complete the first ratio under FR 32 schedule of food presentation. Solid bars show the mean (N=5) latency following a saline infusion with and without hexamethonium pretreatment. Open bars show nicotine infusions. Vertical lines show the standard error.

last infusion. The interinfusion interval in the present study was 7 days, and tolerance to the effects of IVT nicotine was not observed. During the non-infusion and saline control sessions, characteristic FR response patterns were obtained. Rates and patterns of responding following recovery from a nicotine infusion did not differ from control values. One major difference between each of the ratio schedules was that the response rate was directly related to the FR size. This would suggest that the effects of IVT nicotine are largely dependent upon the rate of emitted behavior.

As the dose of nicotine was decreased the latency decreased and corresponding changes were noted in the observed prostration to a dose of 1.25 µg. The present data is in contrast with that of Abood and co-workers (1979) which demonstrated a monotonic dose-response function for 2-10 µg of IVT nicotine. An explanation of the difference between the two sets of data is that in the present study a more sensitive measure of behavior was used from which the dose response curve was obtained and doses as low as 0.312 µg were tested.

The behavioral effects of systemically administered nicotine in combination with nicotine antagonists have been examined previously in both monkeys (Spealman et al. 1981) and rodents (Morrison et. al. 1969; Stitzer et al. 1970). In the present study, doses of mecamylamine that had little or no effect on responding when given alone blocked behavioral effects of IVT nicotine. Presession treatment with 0.05 mg/kg of mecamylamine reduced the nicotine induced latency changes by 40%, and as the dose was increased to 1.5 and 3.0 mg/kg latency measures approached control values. When nicotine was again administered alone the latency values returned to the previous high levels. Since mecamylamine produces ganglionic blockade by occupying cholinergic receptors our results suggest that the effects of IVT infusions of nicotine may be mediated by nicotinic-cholinergic mechanisms. These results are not compatible

with previous studies that have examined different behaviors. Abood et al. (1978, 1979) have suggested that the prostration syndrome seen following IVT infusions of nicotine may not be mediated by cholinergic mechanisms. An explanation for the different results between the studies is not apparent from a comparison of the procedure.

Unlike mecamylamine, hexamethonium (0.5, 1.0, 3.0 mg/kg) failed to block the effect of IVT nicotine on the latency changes in the FR schedule. It should be noted that at the highest dose tested (3.0 mg/kg) a partial antagonism of the effects of nicotine on FR responding did occur. Although hexamethoniun does not readily penetrate the central nervous system (McIsaac 1962), it is likely that at this high dose enough is penetrating to produce a partial effect. Since presession treatment with mecamylamine blocked the behavioral effects of nicotine and hexamethonium did not, it would appear that the effects of IVT nicotine infusions on behavior maintained under FR schedules reflect central effects of nicotine on cholinergic sites.

The present findings are compatible with previous reports that mecamylamine is effective in antagonizing the discriminative stimulus effects of nicotine (Morrison and Stephenson 1969; Schecter and Rosecrans 1971; Hazell et al. 1978), and that high doses of hexamethonium (10.0-20.0 mg/kg) can partially block the effects of 0.4 mg/kg of nicotine in rats responding under an FI schedule of water presentation (Stitzer et al. 1970).

5. ANTAGONISM OF CHRONIC NICOTINE ADMINISTRATION: EFFECTS ON SCHEDULE-CONTROLLED BEHAVIOR IN RATS

Employing the principles of operant conditioning in order to evaluate physiological dependence on nicotine in animals is of particular interest since, as yet, there have been no demonstrations of either nicotine Stolerman, et al., 1973) or tobacco (Jarvik, 1967) withdrawal in animals, even following

prolonged exposure to these substances. Overall, the effects of nicotine on scheduled-controlled behavior have been studied less extensively than other commonly used compounds. Most studies have investigated the acute behavioral effect of nicotine in rats (Morrison, 1969; Ando, 1975; DeNoble et al., 1982) or monkeys (Spealman et al., 1981). Less is known about the termination of chronic nicotine administration on scheduled-controlled behavior. Chronic administration of a variety of psychoactive agents results in physical (physiological) dependence (Deneau, et al., 1969). Physical dependence is generally characterized by abstinence signs when drug intake is abruptly terminated or when an antagonist is administered (Martin, 1967). Investigators in several laboratories have shown that behavior maintained under various schedules of reinforcement is highly sensitive to the effects of chronic drug administration and withdrawal, and drugs from a number of pharmacological classes have been investigated (DeNoble and Begleiter, 1976).

In research reported here we investigated the effects of antagonism of chronic nicotine administration on lever pressing by rats maintained under a multiple fixed-ratio fixed-interval (MULT FR FI) schedule of food presentation. Our results show that antagonism of chronically infused nicotine administration does not disrupt scheduled-controlled behavior.

Twenty-four male hooded rats (Blue Spruce Farms, 350-410g) were divided into three groups. Rats were reduced to 85% of their free feeding weights and trained to lever press for a 45 mg food pellet (Bio-Serve Inc., N.J.). After lever pressing was established, responding was maintained under a MULT FR 30 FI 120 sec. schedule, with a 60 sec. time out (TO) following the FI component. A single white light over the response lever was illuminated during the FR component, and two red lights over the response lever were illuminated during the FI component. During the TO all lights were extinguished and responses had

no programmed consequence. The components alternated at food delivery and sessions lasted until 11 food pellets were obtained in the FR component. Rats were trained under the multiple schedule for a minimum of fourteen weeks in order to stabilize responding. The last three days of this training period served as control sessions (phase 1). During phase 2 a baseline was collected which consisted of three testing periods obtained within a single day (repetitive runs). Each run was separated by a 40 minute interval. During phase 3 (10 days after phase 2) data were collected from three repetitive runs as previously described in phase 2; however, 20 minutes prior to the first and third runs the rats were injected subcutaneously with mecamylamine HCl (1.5 mg/kg). In phase 4 (10 days later) the rats were anesthetized with ether, and an osmotic minipump filled with (-)-nicotine (free base diluted in saline) was inserted subcutaneously between the scapulae. Nicotine was infused subcutaneously for 240 hours (0.5 µl/hr) delivering daily doses of 8.0 mg/kg (group 1), 12.0 mg/kg (group 2), and 16 mg/kg (group 3). After 240 hours of continuous (-)-nicotine infusion the rats were challenged (phase 5) with the nicotinic-cholinergic antagonist mecamylamine as described in phase 3. Mecamylamine has been shown to block the behavioral effects of (-)-nicotine in both rats (Stitzer, et al., 1970; DeNoble, et al., 1982) and monkeys (Spealman, et al., 1981). Blood samples (450-1000 µl) were collected from the dorsal digital vein in the hind paw under ether anesthesia the day before and the day after the mecamylamine challenge. The animals were tested for an additional ten days (phase 6) after which the pumps were removed and inspected for remaining nicotine. Between phases the animals were tested in single daily sessions.

Characteristic performance was maintained under the MULT FR FI schedule. When the light signalling the FR component was illuminated, rats emitted a high rate of responding that was maintained until 30 responses were completed. In

the FI component, a period of little or no responding at the beginning of the 120 sec interval was followed by accelerated responding that was maintained until a response ended the interval. Responding during TO was less than 1% of the total responses emitted during the session.

Quarter life values for the FI component, response rates in the FR component, and response rates in the last 25% of the FI component were used to examine the effects of chronic nicotine administration and its termination.

The introduction of the osmotic minipump containing (-)-nicotine significantly altered response rates under both component schedules, but there were no differential effects of dose on either FR or FI response rates and no significant dose x day interactions (Figure 15). FI rates significantly decreased on the first day of nicotine exposure but returned to control levels by day 2 and remained stable throughout the remainder of nicotine phase. FR rates also decreased on day 1 of nicotine exposure but this effect failed to achieve statistical significance. Beginning on day 3, FR rates were significantly elevated on 6 out of 8 of the remaining nicotine days. It is unlikely that the change in FR response rates were dependent upon the absolute rate of responding, since FI rates were similar and were not changed after day 1 of phase 4. The decrease in rate under both schedules on the first day of phase 4 may be due to the introduction of nicotine and/or the surgery to insert the osmotic minipump. However, the significant increase in FR rate is most likely due to nicotine. Since FI rates did not change, the increase in rate under the FR schedule appears to be schedule dependent. FI quarter life values were not altered by nicotine administration.

Figure 16 shows how performance under the multiple schedule varied as a function of phase. Analysis of FR response rate showed a significant effect of phase while the effect of nicotine dose and the nicotine dose x phase inter-

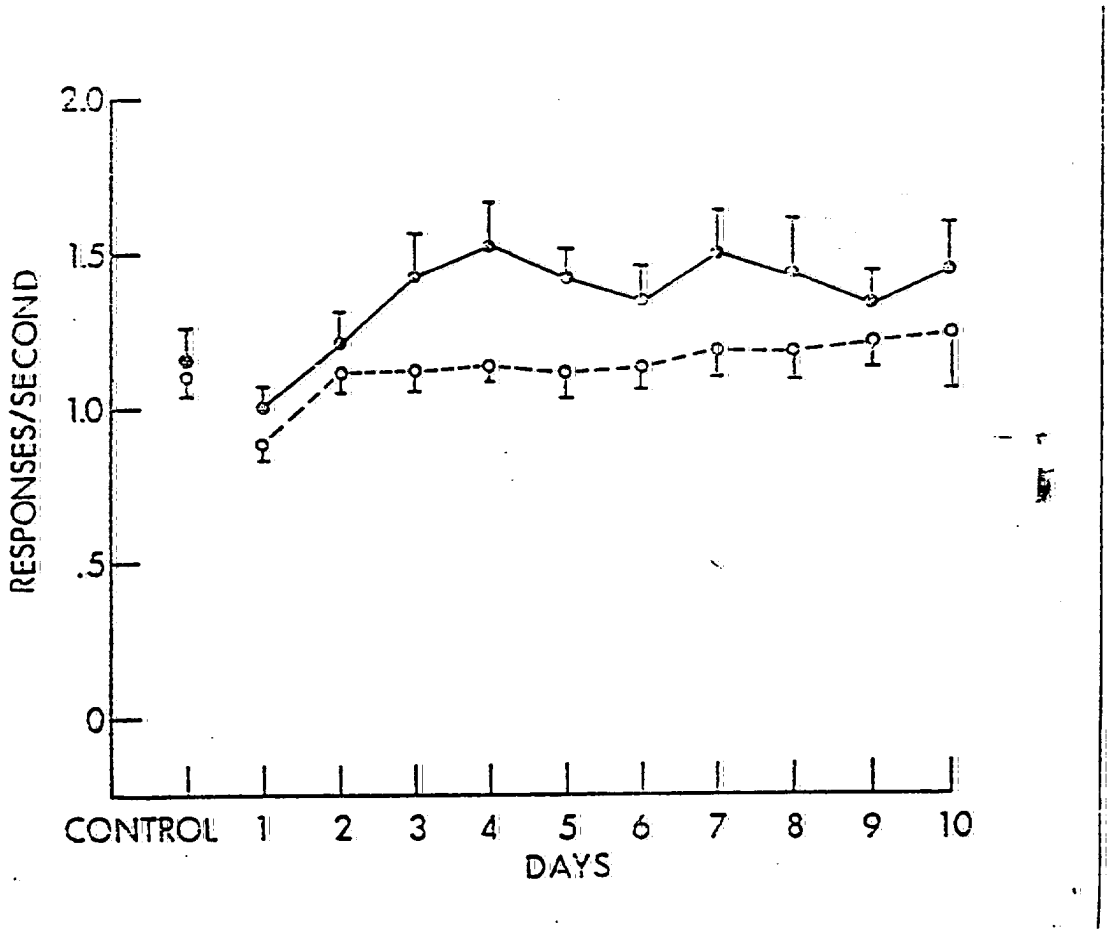


FIGURE 15: Responses per second in the FR (solid symbols) and the last quarter of the FI (open symbols) are shown as a function of the control sessions and the ten days of chronic nicotine treatment. The control point is a mean of 63 data points (3 groups N = 21 x 3 days) and the remaining points represent a mean of 21 data points (3 groups, N = 21 x 1 day). Vertical lines show the standard error.

action were not significant. Subsequent t-tests revealed that chronic nicotine treatment increased FR rates. Rates increased from 1.15 ± 0.12 during control sessions to 1.42 ± 0.13 responses per second during nicotine sessions. The mecamylamine challenge significantly decreased FR response rate, relative to the last three days of nicotine exposure, to 0.82 ± 0.09 responses per second. Subsequent to the mecamylamine challenge FR response rates were again significantly elevated for the remainder of the experiment (last 10 sessions). Figure 16 shows that FI performance was not altered during any condition, including mecamylamine challenge.

Multiple ion detection analysis of nicotine in blood (gas chromatograph/mass spectrometer) was performed on samples collected both before and after the mecamylamine challenge. The data show that levels of nicotine present in blood both before and after the mecamylamine challenge were similar, and that the blood levels (ng/ml blood) varied directly with the daily nicotine dose (8 mg/kg/day: $\bar{x}$ = 2.28 ± 0.07 SE, 12 mg/kg/day: $\bar{x}$ = 4.08 ± 0.81 SE, and 16 mg/kg/day: $\bar{x}$ = 6.21 ± 0.63 SE).

The results of this experiment show that blocking nicotine's central nervous system actions following chronic nicotine treatment does not result in a disruption of scheduled-controlled performance. Such behavior has been shown to be sensitive to physiological dependence (Schuster and Zimmerman, 1961; DeNoble and Begleiter, 1976). Others have also noted that termination of prolonged exposure to nicotine or tobacco (Stolerman, et al., 1972; Sarvik, 1967) does not result in a withdrawal syndrome in animals. However, the available data with human subjects suggests a series of withdrawal signs and symptoms (Shiffman, 1979). Since the kinds of symptoms reported and the temporal pattern of these symptoms are not consistent across studies or between individuals within a study it is not necessary that the symptoms reported

Discusses blood levels *
Ng/ml
8000 µg/kg/24 → 2.28
12000 µg/kg/24 → 4.08
16000 µg/kg/24 → 6.21
Smokers = 40 ng/ml
1.2 mg/hr x 24 = 28800 µg/24h
= 384 µg/kg/24h → 40 ng/ml

Discuss blood levels

	mg/ml
8000 mg/kg/24 →	2.20
12000 mg/kg/24 →	4.08
16000 mg/kg/24 →	6.21

18000

Smoums = 40 mg/ml

1.2 mg/hr x 24 = 28800 mg/24h

= 384 mg/kg/24h → 40 mg/ml

action were not significant. Subsequent t-tests revealed that chronic nicotine treatment increased FR rates. Rates increased from 1.15 ± 0.12 during control sessions to 1.42 ± 0.13 responses per second during nicotine sessions. The mecamylamine challenge significantly decreased FR response rate, relative to the last three days of nicotine exposure, to 0.82 ± 0.09 responses per second. Subsequent to the mecamylamine challenge FR response rates were again significantly elevated for the remainder of the experiment (last 10 sessions). Figure 16 shows that FI performance was not altered during any condition, including mecamylamine challenge.

Multiple ion detection analysis of nicotine in blood (gas chromatograph/mass spectrometer) was performed on samples collected both before and after the mecamylamine challenge. The data show that levels of nicotine present in blood both before and after the mecamylamine challenge were similar, and that the blood levels (ng/ml blood) varied directly with the daily nicotine dose (8 mg/kg/day: $\bar{x}$ = 2.28 ± 0.07 SE, 12 mg/kg/day: $\bar{x}$ = 4.08 ± 0.81 SE, and 16 mg/kg/day: $\bar{x}$ = 6.21 ± 0.63 SE).

The results of this experiment show that blocking nicotine's central nervous system actions following chronic nicotine treatment does not result in a disruption of scheduled-controlled performance. Such behavior has been shown to be sensitive to physiological dependence (Schuster and Zimmerman, 1961; DeNoble and Begleiter, 1976). Others have also noted that termination of prolonged exposure to nicotine or tobacco (Stolerman, et al., 1972; Sarvik, 1967) does not result in a withdrawal syndrome in animals. However, the available data with human subjects suggests a series of withdrawal signs and symptoms (Shiffman, 1979). Since the kinds of symptoms reported and the temporal pattern of these symptoms are not consistent across studies or between individuals within a study it is not necessary that the symptoms reported

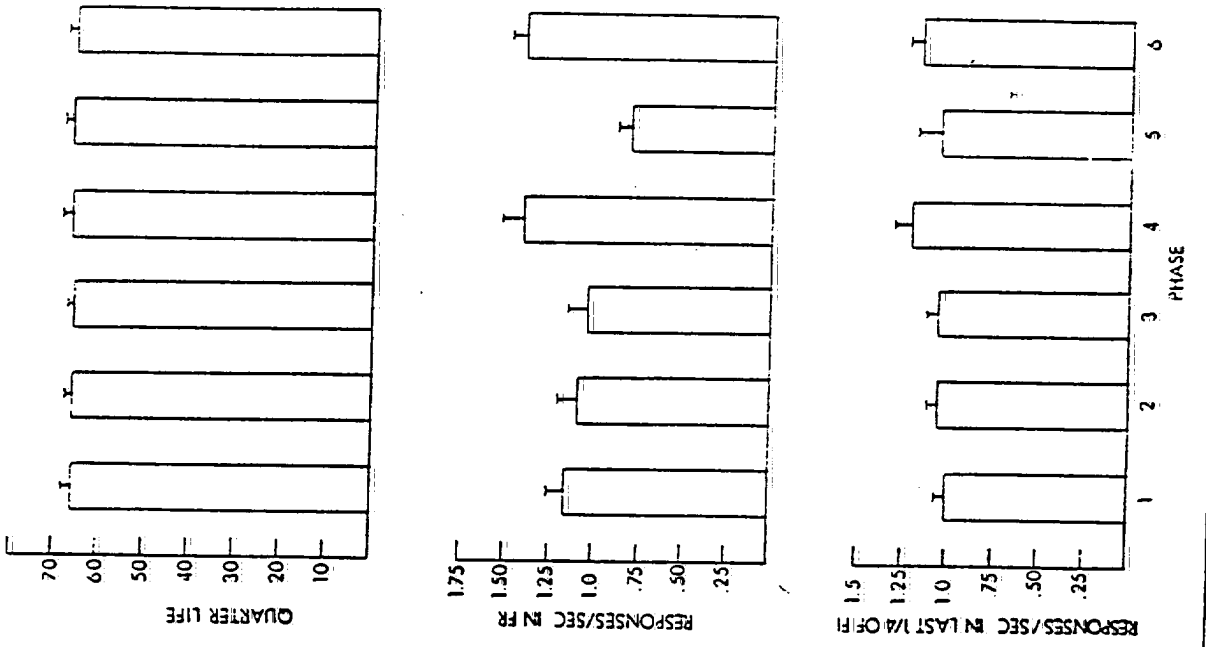
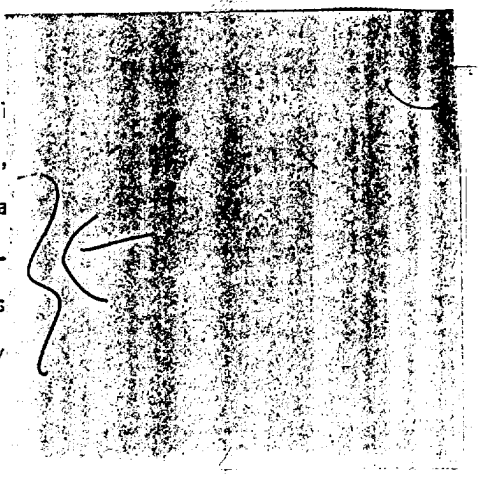


FIGURE 16: Quarter life values, responses per second in the FR, and responses per second in the last quarter of the FI are shown as a function of the six phases. Phase 1 control sessions were the last 3 days of the fourteen week training period. Phase 2 represents 3 repetitive runs on a single day. Phase 3 consisted of 3 repetitive runs on a single day with presession injections of mecamylamine HCl. Phase 4 represents 3 days prior to the mecamylamine challenge of nicotine. Phase 5 represents the mecamylamine challenge and phase 6 the last 3 days of the experiment. Each bar represents a mean of 63 data points (3 groups N = 21 x 3 data points from each animal). The vertical lines show the standard error.

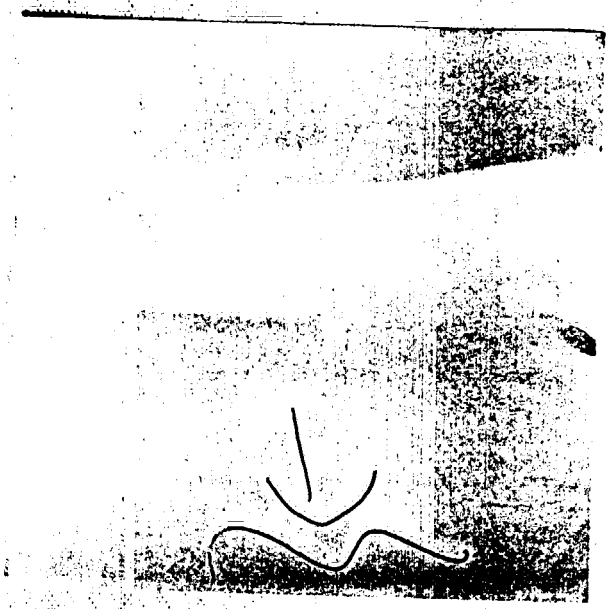
represent a physiological dependence. Instead, the absence of a withdrawal syndrome in this and other animal studies (Stolerman, et al., 1973; Jarvik, 1969), combined with the lack of consistency in the data on humans suggests a more general interpretation, such as a learning mechanism whereby the interruption of a well learned response that leads to positive reinforcement results in a variety of behavioral and physiological changes which are reported by humans and are interpreted as withdrawal symptoms.



6. EFFECTS OF NICOTINE OR ACETALDEHYDE ON BEHAVIOR INDUCED BY REINFORCEMENT OMISSIONS (FRUSTRATIVE NON-REWARD)

Research with humans or smoking and its effects on mood alterations has produced equivocal results. The hypothesis that nicotine ameliorates the effects of a negative emotional experience has been academically entertained, but has not received much experimental attention. Our goal is to develop an animal model from which the putative role of smoke components in mood alteration can be rigorously assessed. The paradigm we chose is modeled after the frustrative non-reward experiments developed by Amsel between 1950-1960s. Studies of the influence of the benzodiazepines on behavioral responses to non-reward are consistent in showing an attenuation of these responses. In other words, they "calm" the animal.

Twelve male hooded rats weighing between 350-400g were used. The animals were housed individually and were gradually reduced to 80% of their free feeding body weights. Each rat was tested in a standard operant conditioning chamber and each chamber was housed in a sound-attenuated cubicle. With each operation of the pellet dispenser, a single 45-mg Bio Serve food pellet was delivered to the receptacle.



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Each rat was trained to lever press under an FR1 schedule for a single delivery of food. After FR1 training, responding was maintained under a multiple FR25 DRL 15 sec. schedule. A single white light over the response lever was illuminated during the FR component, and two red lights over the response lever were illuminated during the DRL component. The components alternated at food delivery and the session lasted until 35 food pellets were obtained in the FR component. The animals were trained for 15 weeks before food omission baselines were obtained. At weekly intervals two correctly emitted responses were not reinforced, that is, the food pellet was not presented but the lights were changed to signal the other schedule was in effect. This procedure induces behavior that is counterproductive to obtaining the next reinforcer, and is very reliable. We examined the effects of presession injections of (-)-nicotine (0.1, 0.2, 0.4, mg/kg/sc) and acetaldehyde (1.0, 5.0, 10.0, 15.0, mg/kg/sc) on the behaviors induced by the reinforcer omission.

The results show that (-)-nicotine, at the doses tested, had no effect on the induced behavioral change. However, acetaldehyde reduced the disruptive effects of food omission. In addition, this effect was dose related.

This experiment is still in progress and additional data will have to be collected before any major conclusions can be stated.

7. GENERALIZATION OF THE INTEROCEPTIVE CUES PRODUCED BY (-)-NICOTINE TO NICOTINE ANALOGUES

The discrimination testing continues to be a routine screen for behaviorally active nicotine analogues. However, several additional tests are now being employed to better characterize the activity of nicotine analogues. The "standard" discrimination test only provides us with a yes or no answer. That is, it is either nicotine like or it's not. With the development of more

sensitive measures, the relative potencies between the nicotine analogues and the duration of the effect in the central nervous system can now be determined.

GENERAL PROCEDURE

ANIMALS

Experimentally naive male albino rats (Holzmann Co., Madison, Wisconsin), between 90 and 120 days old and weighing between 190 and 230 g were used. Animals were housed individually and were allowed food ad lib for 3 weeks during which time weights were recorded daily. The mean weights were calculated from the last 5 days of the 3 week period, after which the rats were reduced to 80% of their free-feeding weights. These weights were periodically adjusted to control for their growth rate.

APPARATUS

Two identical operant conditioning chambers (Lehigh Valley Electronics No. 143-25), each contained in a sound-attenuating cubicle (LVE No. 132-02), were used. On one end of the chamber were two (A and B) levers (LVE No. 121-05), a pellet receptacle, six cue lights (lever lights), a speaker, and a house light. With each operation of the pellet dispenser, a single 45-mg Bio Serve food pellet was delivered to the receptacle. While noise was constantly present, an exhaust fan provided ventilation. Programming and data collection were controlled by electromechanical equipment and a Honeywell CPV computer.

TRAINING PROCEDURE

Rats were trained to lever press for food reinforcement. Half of the rats were initially trained to press lever A with lever B inactive and the other half were initially trained to press lever B with lever A inactive. In

subsequent training sessions, the contingency for reinforcement was switched to the opposite lever. All sessions were 15 minutes and the rats were given equal training on both levers. After the rats were trained to press either lever under a fixed ratio 10 (FR10) schedule, discrimination training was begun. The rats were injected subcutaneously five minutes prior to each session with equal volumes of either 0.4 mg/kg/body weight of (-)-nicotine or saline (0.9%). For half of the animals lever A served as "(-)-nicotine correct" and for the other half lever B served as "(-)-nicotine correct." Injections of (-)-nicotine and saline alternated daily for the first four sessions, then the compounds were injected according to a double alternation schedule. The first completed ratio after placement into the experimental chamber was recorded and determined the response as correct (injection lever coincidence) or incorrect (injection opposite lever). Testing procedures for nicotine analogues were begun after 15 sessions in which "injection correct" responses were 100% of total for the first completed ratio.

TESTING PROCEDURE

Compounds were tested for generalization to nicotine during a session in which no reinforcements were available. These testing sessions lasted until the first ratio was complete on either lever or until 5 minutes lapsed. There was a minimum 3-day intertest interval between tests for generalization to the nicotine cue. In addition, 76% of the total number of responses for the first ratio had to occur on the injection correct lever during these three days.

COMPOUNDS

All doses were calculated as free base and dissolved in saline. All injections were given as equal volumes.

During the period of this report we have tested the compounds listed in Table 1. All of these compounds were tested at a number of dose levels and we are beginning to develop structure - activity relationships.

TABLE 7

DISCRIMINATIVE PROPERTIES OF NICOTINE AND RELATED COMPOUNDS

COMPOUND	N AT EACH DOSE	DOSE (mg/kg)	% NICOTINE LEVER CHOICES
(-)-Nicotine*	20	0.57	90
		0.4	100
		0.2	95
		0.1	35
		0.05	20
(±)-Nicotine*	20	1.14	55
		0.8	95
		0.57	100
		0.4	95
		0.2	25
(+)-Nicotine*	20	16.0	65
		8.0	95
		5.7	40
		3.2	25

TABLE 7 (Continued)

DISCRIMINATIVE PROPERTIES OF NICOTINE AND RELATED COMPOUNDS

COMPOUND	N AT EACH DOSE	DOSE (mg/kg)	% NICOTINE LEVER CHOICES
(-)-5-Isopropylnicotine	5	6.0	0
		4.0	0
		2.0	0
(-)-6-Tbutylnicotine	4	6.0	0
		2.0	0
		1.0	0
(-)-6-Ethylnicotine*	9	4.0	Debilitating
		1.0	Debilitating
		0.4	Debilitating
		0.3	29
		0.25	14
		0.2	43
		0.2	33
(-)-6-Methylnicotine*	27	0.4	Debilitating
		0.2	44
		0.1	21
		0.05	0

TABLE 7 (Continued)

DISCRIMINATIVE PROPERTIES OF NICOTINE AND RELATED COMPOUNDS

COMPOUND	N AT EACH DOSE	DOSE (mg/kg)	% NICOTINE LEVER CHOICES
+)-6-Methylnicotine*	5	8.0	100
		4.0	50
		2.0	25
(-)-6 choromethylnicotine	4	0.4	Debilitating
		0.3	Debilitating
		0.2	0
(-)-3'-4'-Dehydronicotine*	8	0.8	Debilitating
		0.4	100
		0.2	80
		0.1	75
(-)-6-Hydroxymethyl*	8	3.2	Debilitating
		1.2	50
		0.8	57
		0.4	0

*Discrimination is blocked by mecamylamine (1.5 mg/kg/sc) given 5 minutes prior to session but not by hexamethonium (1.0 mg/kg/sc) given 5 minutes prior to session.

TABLE 7 (Continued)

DISCRIMINATIVE PROPERTIES OF NICOTINE AND RELATED COMPOUNDS

COMPOUND	N AT EACH DOSE	DOSE (mg/kg)	% NICOTINE LEVER CHOICES
-)-6-Cyclopropylnicotine*	5	3.2	100
		1.6	0
		0.8	12
		0.4	12
		0.2	0

DOSE RESPONSE CURVES

Twenty rats were tested with (-)-nicotine (0.57, 0.4, 0.2, 0.1, 0.05 mg/kg) (±)-nicotine 16.0, 11.4, 8.0, 5.7, 3.2, 2.8, 1.6, and 1.14 mg/kg). Each dose was administered twice.

Figure 17 shows the dose response curve generated for each compound. Overlaping functions were found for (-)-nicotine and (±)-nicotine. At a dose of 0.4 mg/kg/sc each compound produced maximal nicotine correct responding. The dose of (+)-nicotine that produced similar respending was 20 times higher (8.0 mg/kg). These dose response curves are proving to be very valuable in the assessment of nicotine analogues.

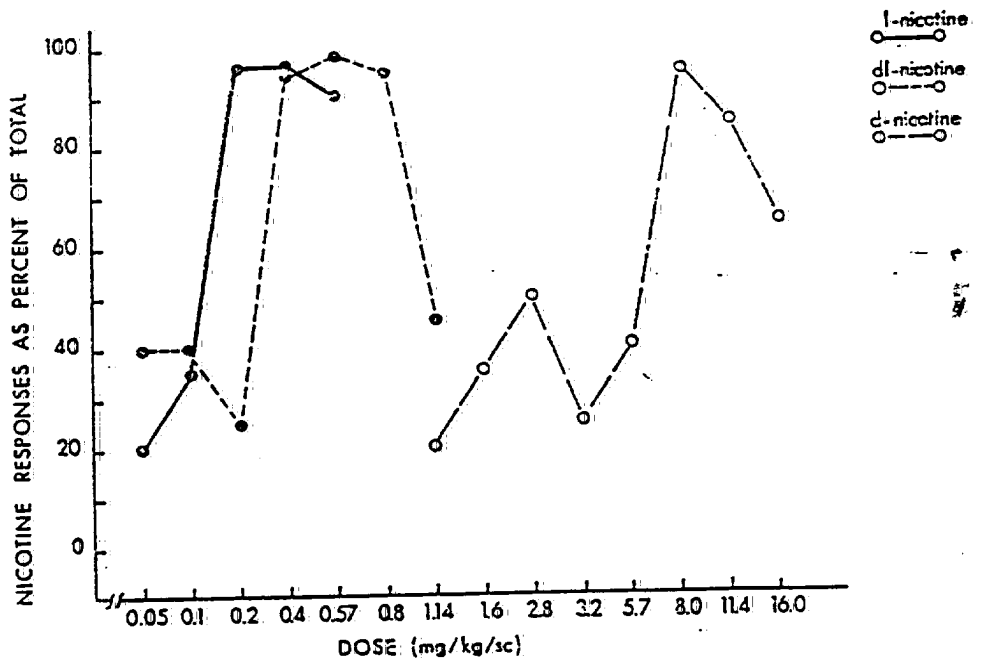

Figure 17: Nicotine responses as percent of control is presented as a function of dose. Each point is a mean of 20 data points (10 rats x 2 tests at each dose). Doses were presented in ascending series and the rats were tested with l, dl and d nicotine respectively.

8. INTERACTION BETWEEN BEHAVIORAL AND METABOLIC TOLERANCE IN RATS FOLLOWING PROLONGED (-)-NICOTINE EXPOSURE

Tolerance to a substance is defined as a diminished effect with repeated administration. Tolerance may be a result of pharmacokinetic factors which alter the effective concentration of the active agent at the receptor. These factors include altered absorption, distribution, metabolic or elimination mechanisms. Tolerance may also result from a decreased sensitivity of the receptor even though the concentration of the active agent at the receptor is unchanged. Together, these two mechanisms comprise what is termed "pharmacological" tolerance.

There is an increasing number of studies in the behavioral pharmacology literature indicating that the development of tolerance to a number of compounds may also be influenced by certain behavioral (i.e. learning or performance) factors. Thus, the development of tolerance to the behavioral effects of a compound may depend on the behavior in question and/or the specific behavioral alterations produced. "Behavioral" tolerance, then, exists when such relationships can be identified. One factor that influences the development of tolerance is whether or not a compound disrupts ongoing behavior in such a way as to alter the frequency or rate of reinforcement delivery. If a compound produces a loss of reinforcement, then tolerance is more likely to occur (or at least occur more rapidly) than when reinforcement frequency is not altered.

That tolerance occurs to some of the behavioral effects of nicotine in animals following repeated administration is well documented. In many studies this appears to be one or more forms of pharmacological tolerance. Whether behavioral fctors are important in the development of tolerance to nicotine is unknown.

To address this question, two groups of rats (n = 7/group) are responding under a fixed-ratio 32 (FR32) schedule of food presentation. Following stable day-to-day performance and the determination of acute (one or two injections per week) &-nicotine dose-effect functions, single daily injections of nicotine will be administered (chronic administration phase). The dose of nicotine to be administered chronically will be determined from the acute dose-effect function as one which produces a marked (> 50%) reduction in the noninjection control frequency of reinforcement. One group of rats will receive the chronic dose of nicotine before daily test sessions (the Before group) and the other group of rats will receive chronic nicotine after daily test sessions (the After group). Daily nicotine administration will continue until the Before group no longer shows behavioral disruption (i.e., tolerance), a period estimated to take from 2-4 weeks. Following the development of tolerance to nicotine in the Before group, the After group will receive the chronically administered dose of nicotine before the experimental session to determine if tolerance has also developed in this group.

With the Before-After parodigm both groups of rats will receive exactly the same quantity of nicotine on a day-to-day basis. What will vary, however, are the nicotine-induced behavioral alterations experienced by the two groups of animals. The Before group will experience nicotine-induced disruption of FR performance, including loss of reinforcement, while the After group will not. If loss of reinforcement is a critical factor in the development of tolerance to nicotine, then the After group is not expected to show tolerance when, or at least to the degree that, the Before group does.

If the After group shows no evidence that tolerance to nicotine developed, then the chronic nicotine dosing regimen will be repeated for this group, only this time nicotine will be administered before the daily test session until

tolerance develops. The time course for tolerance development here can be compared to that obtained with the original Before group to further verify that the experience of nicotine-induced reinforcement loss is an important mediating factor. Additionally, since tolerance implies that a shift in the dose-response function to the right has occurred, additional higher doses of nicotine will be administered to both groups. Finally, to examine the persistence of nicotine tolerance, daily injections will be discontinued and the dose used to establish tolerance initially will be administered at 1 to 2 week intervals to see if the original acute effect is recoverable. At the present time, the initial dose-response functions for acutely administered nicotine are being determined.

9. CROSS TOLERANCE BETWEEN (-)-NICOTINE AND PHARMACOLIGICALLY RELATED COMPOUNDS

Cross tolerance between two substances exists when the establishment of tolerance to one substance results in tolerance to the other substance. We are currently examining whether rats which are tolerant to the behaviorally disrupting effects of &-nicotine on FR32 food-maintained responding exhibit a cross tolerance to a variety of other compounds.

Two groups of rats (n = 5 to 6/group) are being used in this study. One group will be made tolerant to nicotine by receiving daily presession injections as described in the preceeding section, and will be maintained on this treatment regimen. The other group will receive daily presession injections of physiological saline. Once or twice per week a test compound will be substituted for nicotine in the tolerance group and for saline in the nontolerance group in order to determine whether a cross tolerance exists between nicotine and the test compound.

Compounds to be tested include a selection of the nicotine analogues previously examined in the discrimination and self-administration paradigms. It is of particular interest to determine the degree of correspondence among these three paradigms for identifying nicotine-like activity. As one further means of determining the specificity of cross tolerance in nicotine tolerant animals, the use of "reference" compounds which have been widely studied for their behavioral and neuropharmacological effects would be desirable. Reference compounds producing some effects similar to those of nicotine (e.g., CNS stimulants) as well as those with opposite effects (e.g. CNS depressants) would be most informative.

10. EXAMINATION OF BEHAVIORAL SUPERSENSITIVITY FOLLOWING CONTROL CHRONIC NICOTINE-CHOLINERGIC BLOCKADE IN RATS

Chronic inactivation of postsynaptic receptors in the CNS produces an increased sensitivity (supersensitivity) of these receptors to the appropriate agonist. Supersensitivity has been demonstrated for dopaminergic, beta-adrenergic, serotonergic and gamma-aminobutyric acid receptors. Supersensitivity to nicotinic-cholinergic receptors has been demonstrated peripherally at the neuromuscular junction. Following surgical denervation, an increased responsiveness of muscle to locally applied acetylcholine has been noted. A proliferation of nicotinic receptors over the muscle surface was correlated with the development of supersensitivity.

Since there appear to be no studies which have attempted to induce nicotinic receptor supersensitivity in the CNS, preliminary studies are in progress to address this issue. The nicotinic-cholinergic receptor blocker mecamylamine is being used to functionally inactivate central nicotinic receptors. The nicotine-induced prostration syndrome is being used as a behavioral index of supersensitivity.

One rat has completed the initial series of treatments. This animal was treated with 1.0mg/Kg of mecamylamine twice daily for 14 days. On day 15 the prostration produced by a low dose of nicotine (2.5 ug) was enhanced relative to that observed before chronic mecamylamine treatment. We are currently attempting to replicate this effect in additional animals. We also intend to extend our behavioral measures to include the fixed-ratio paradigm, since we have shown that it provides a more sensitive measure of prostration than does direct observation of the animals.

Should mecamylamine-induced supersensitivity to nicotine prove to be a reliable phenomenon, then an examination of changes in the number of nicotinic receptors and the affinity of nicotine for the receptor would be in order to address possible underlying mechanisms. These studies would be conducted in collaboration with Dr. Leo Abood.

Publications

V. J. DeNoble, Y. Dragan and L. Carron. Behavioral Effects of Intraventricularly Administered (-)-Nicotine on Fixed Ratio Schedules of Food Presentation in Rats. Psychopharmacology In Press

This paper was also presented at the Society for Neuroscience, October 17, 1981 Los Angeles, CA.

A technical seminar was also presented during the period of this report.

Manuscripts

V. J. DeNoble, F. J. Ryan, Y. P. Dragan, P. C. Mele, J. Naworal and R. Kornfeld. Antagonism of Chronic Nicotine Administration: Effects on Schedule-Controlled Behavior in Rats.

This paper has been approved by the Manuscript Review Board and is now in New York.