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To: Dr. W. Dunn

Date: March 27, 1981

From: Victor J. DeNoble and Lisa Carron

Subject: Progress in Behavior Pharmacology Laboratory

The following report outlines the major areas of current research and progress made within these areas by the Behavioral Pharmacology group. At the outset, we would like to gratefully acknowledge the contributions of the Behavioral Research group, the Chemical Research group and Mr. Jim Charles. Jim has been instrumental in the development of the research on other components in smoke which may have positive reinforcing effects.

In addition, we want to thank Drs. W. Dunn and T. Osdene for their advice and support of the research project.

/lw

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Although nicotine is one of the most widely used compounds, basic research on its mode of action in the brain and its effects on animal behavior have lagged far behind until very recently. Further, the dearth of information available about other smoke components and of their effect on brain-behavior relationships is even more striking. With this in mind we have begun to develop a behavioral pharmacology laboratory which has as a major goal the systematic investigation of the brain-behavior relationships induced by smoke components. The following report is divided into two major sections, first there is an outline of the research program as it exists today. Second, there is a brief summary of the major findings.

1. SELF-ADMINISTRATION

*1A. Nicotine

Research Objectives

1. Establish intravenously delivered nicotine as a positive reinforcer.
2. Obtain a dose response function under unlimited nicotine access conditions (24 h per day under fixed-ratio 1).
3. Examine the effects of fixed-ratio size on self administration response rates and nicotine intake (mg/kg/session).
4. Determine the effects of cholinergic agonists and antagonists on nicotine self-administration.

*1B. Acetaldehyde

Research Objectives

1. Establish intravenously delivered acetaldehyde as a positive reinforcer.

2. Obtain a dose response function under unlimited acetaldehyde access conditions (24 h per day under fixed-ratio 1).
3. Examine the effects of fixed-ratio size on response rates and acetaldehyde intake (mg/kg/session).
4. Assess the effects of changes in uptake, storage and release of cholinergic, catecholaminergic, serotonergic and other neurotransmitter systems on self-administration of acetaldehyde.

*1C. Nicotine-Acetaldehyde Combinations

Research objectives

1. Examine nicotine-acetaldehyde interactions on self-administration behavior.
2. Examine the neurochemical correlates of reinforcing properties of nicotine and acetaldehyde.

*1D. Nicotine Analogues

Research Objectives

1. Determine if behaviorally active nicotine analogues can be directly substituted for nicotine in rats for which nicotine is functioning as an intravenously delivered positive reinforcer.
2. Establish nicotine analogues as an intravenously delivered positive reinforcer.
3. Compare the potencies of nicotine analogues to nicotine in producing positive reinforcing effects.

1E. Other Smoke Components

Research Objectives

1. To establish if other components of smoke function as intravenously delivered positive reinforcers.

*1F. Condensation Products of Acetaldehyde with Endogenous Neurotransmitters

Research Objectives

Determine if the condensation products function as intravenously delivered reinforcers.

2. PROSTRATION

*2A. Effects of Intraventricular Administered Nicotine and Nicotine Analogues

Research Objectives

1. The prostration syndrome continues to be used as a screen for behaviorally active nicotine analogues.
2. Establish more sensitive and reliable indices of prostration with which relative potencies of various compounds can be determined.
3. Block activation of the sodium conductance in specific brain sites and examine the effects on the behavioral components of prostration.

3. CHRONIC COMPOUND ADMINISTRATION AND TERMINATION: EFFECTS ON SCHEDULED CONTROLLED BEHAVIOR

*3A. Compare the effect of nicotine and saline administration and termination on behavior maintained under a multiple fixed-ratio-fixed interval schedule of food reinforcement.

3B. Examine the effects of acute and chronic acetaldehyde administration on multiple fixed-ratio-differential reinforcement of low rates schedule.

4. DISCRIMINATION

*4A. Nicotine-Saline Discrimination

Research Objectives

1. This procedure is a routine screen for behaviorally active nicotine analogues.

4B. Acetaldehyde-Saline Discrimination

Research Objectives

1. Establish acetaldehyde-saline discrimination
2. Determine if acetaldehyde-neurotransmitter condensation products show cross discrimination with acetaldehyde.

5. ACUTE COMPOUND ADMINISTRATION: EFFECTS ON MOTIVATION AND EMOTION

Research Objectives

5A. Examine the effects of nicotine, acetaldehyde and combinations of each on behavior elicited by the removal of positive reinforcers.

5B. Utilize techniques developed to induce anxiety and frustration for evaluation of acute compound administration.

6. ELECTROPHYSIOLOGY

Research Objectives

6A. To monitor electrophysiological correlations of self-administration, prostration and the acute and chronic effects of compound administration.

*Ongoing Project

ACCOMPLISHMENTS

(June 1980 - April 1981)*

SELF-ADMINISTRATION

1A. Nicotine Self-Administration

Several reports have shown that rats can be trained to lever press for intravenously delivered nicotine. However, there has been a lack of appropriate control measures to clearly show that nicotine was functioning as a reinforcer. A first step in our laboratory was to demonstrate that nicotine can function as an intravenously delivered positive reinforcer.

Rats were prepared with an indwelling venous catheter made of siliconized rubber. The catheter was anchored in the external jugular vein and passed subcutaneously until it exited through the animals back. This was connected via protective tubing and swivel joints to a remote injection pump. Responding on one arbitrarily selected response lever was automatically programmed to activate the injection pump for 6.0 seconds, delivering an injection of 0.1376 ml of solution directly into the animal's blood stream. Responses on the control lever were recorded but had no programmed consequence.

Control Studies

Nicotine self-administration was initially established at 32 µg/kg/injection. Nicotine was available 24 hours per day, under a fixed ratio 1 schedule. Generally 10-14 sessions were necessary for responding to stabilize. After stable behavior was obtained, changes were made in the nicotine delivery procedure to determine if lever pressing was maintained by the contingency established between lever pressing and nicotine delivery. Changes included

*Approximately five weeks of this period was devoted to laboratory renovation.

substitution of saline for nicotine, reversal of nicotine-lever and control activity lever functions, and automatic response independent nicotine injections. The results show that nicotine self-administration by rats is maintained by the response-nicotine contingency, rather than by other behavioral effects of the nicotine (eg. motor activation). Substitution of saline for nicotine failed to maintain responding. When nicotine (32 µg/kg/injection) was reintroduced, the number of infusions rose to previous levels (Figure 1). During the self-administration, responding occurred almost entirely on the lever delivering nicotine. Control lever responses were less than 10% of the total number of responses. When nicotine injections were delivered response-independently responding decreased as a function of the frequency of the non-contingent infusion (Figure 2).

These results show clearly that nicotine can function as a positive reinforcer for rats.

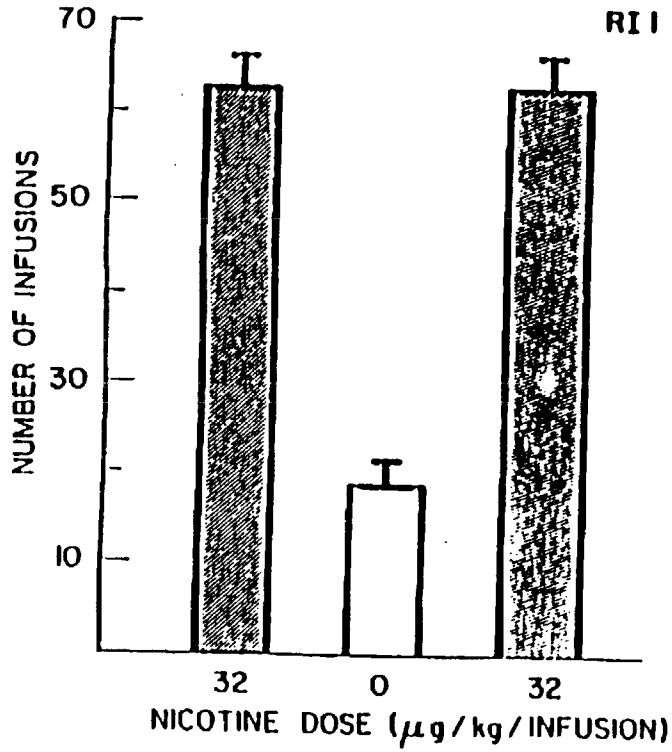
Effect of Infusion Dose on Number of Infusions and Nicotine Intake (mg/kg/session)

Nicotine self-administration was established during 24 hr/day access sessions at 32 µg/kg/infusion under FR 1 conditions. After stabilization, the effects of infusion dose was determined on response rate and nicotine intake (mg/kg/day). Infusions doses were presented in descending order (64,32,16,8.0, 4.0 and 2.0 µg/kg/infusion). Rats were tested for a minimum of 7 days at each dose. The results show that as the dose of nicotine was decreased the number of infusions first increased then decreased (Figure 3). The nicotine intake (mg/kg/session) was directly related to the dose (Figure 4).

FIGURE 1

NUMBER OF INFUSIONS AS A FUNCTION OF NICOTINE OR SALINE ACCESS CONDITIONS. EACH BAR IS A MEAN OF 5 CONSECUTIVE DAYS. VERTICAL LINES SHOW THE STANDARD ERROR.

FIGURE 1



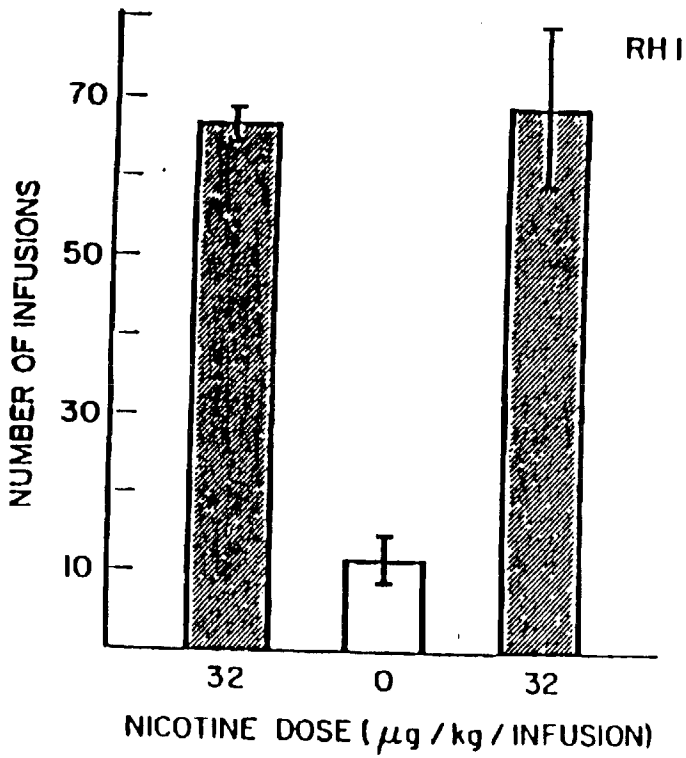


FIGURE 2

PERCENT DECREASE IN THE NUMBER OF CONTINGENT NICOTINE INFUSIONS AS A FUNCTION OF THE TIME INTERVAL BETWEEN PROGRAMMED RESPONSE INDEPENDENT NICOTINE INFUSIONS.

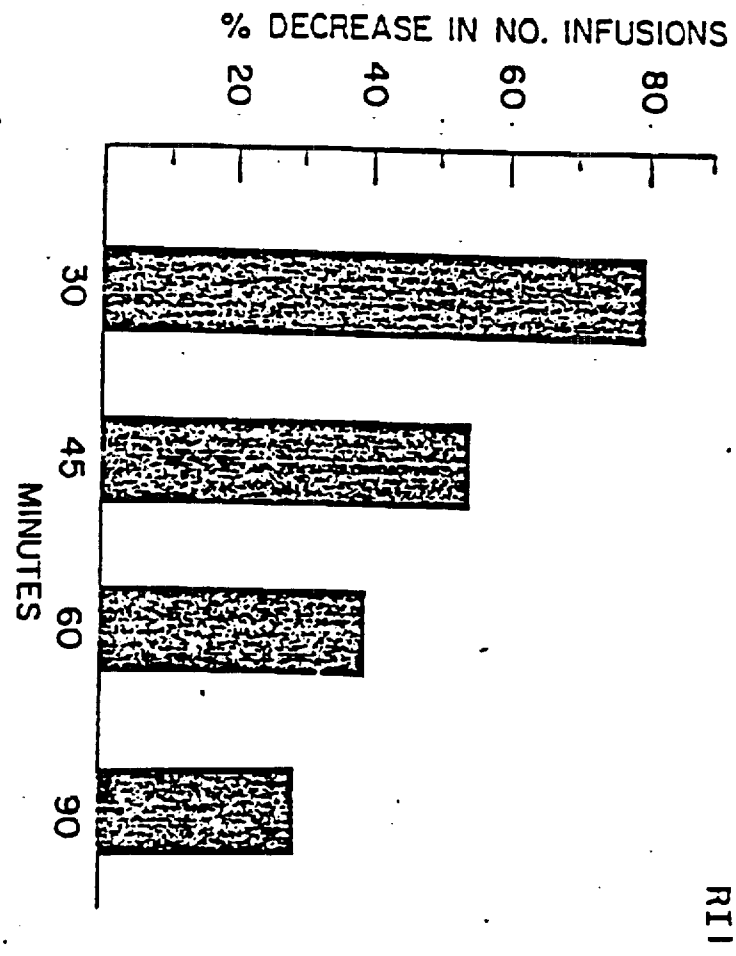


FIGURE 2

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FIGURE 3

NICOTINE INFUSIONS DURING 24 HOUR SESSIONS AS A FUNCTION OF NICOTINE DOSE. EACH POINT REPRESENTS THE MEAN NUMBER OF INFUSIONS FOR A RAT OVER A 3-5 DAY PERIOD WITH NO INCREASING OR DECREASING TRENDS. VERTICAL LINES SHOW THE STANDARD ERROR.

FIGURE 3

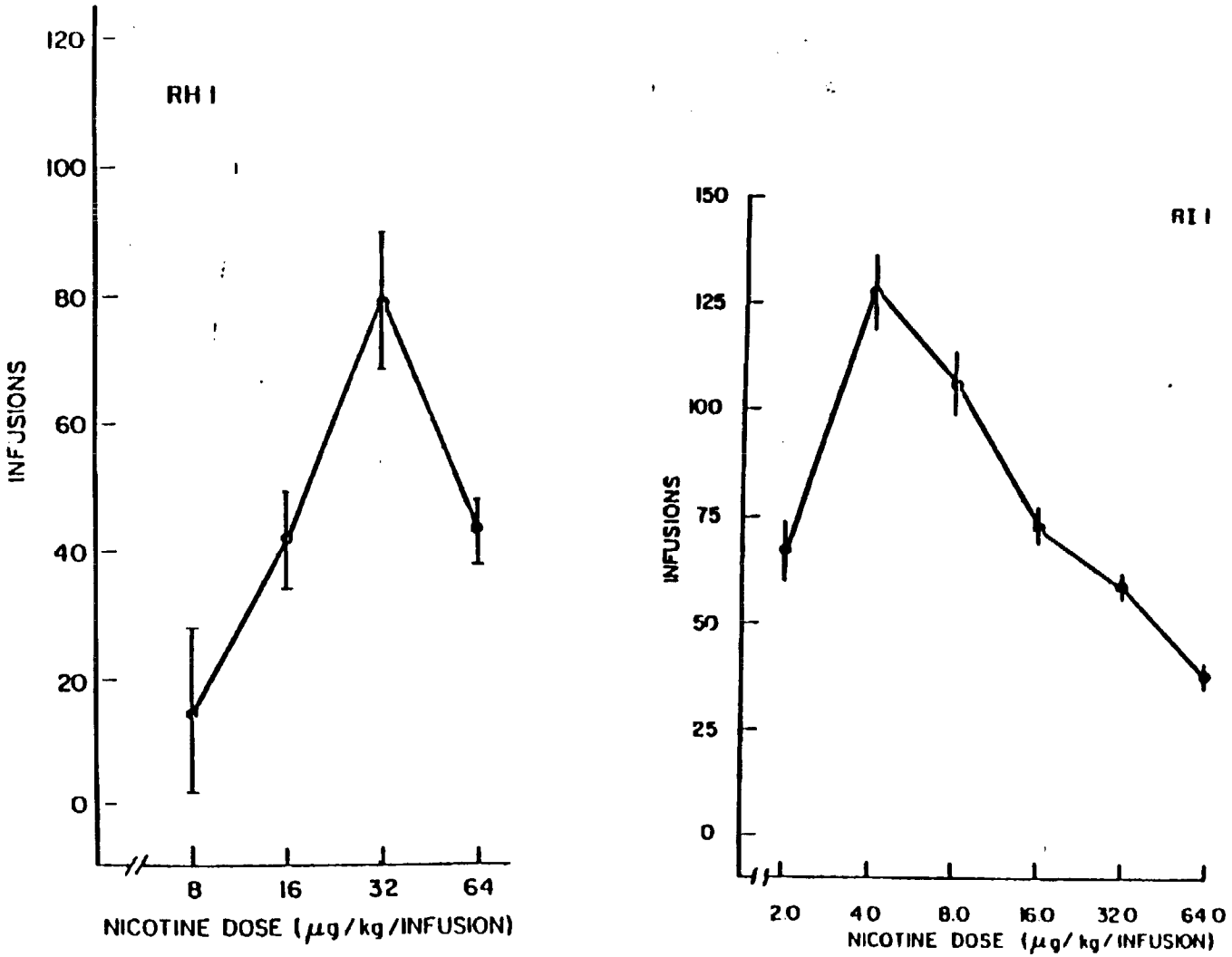

FIGURE 4

NICOTINE INTAKE (MG/KG/24 HOUR SESSIO AS A FUNCTION OF NICOTINE DOSE. EACH POINT REPRESENTS THE MEAN MG/KG INTAKE FOR A RAT IN A 24 HOUR SESSION OVER A 3-5 DAY PERIOD WITH NO INCREASING OR DECREASING TRENDS. VERTICAL LINES SHOW THE STANDARD ERROR.

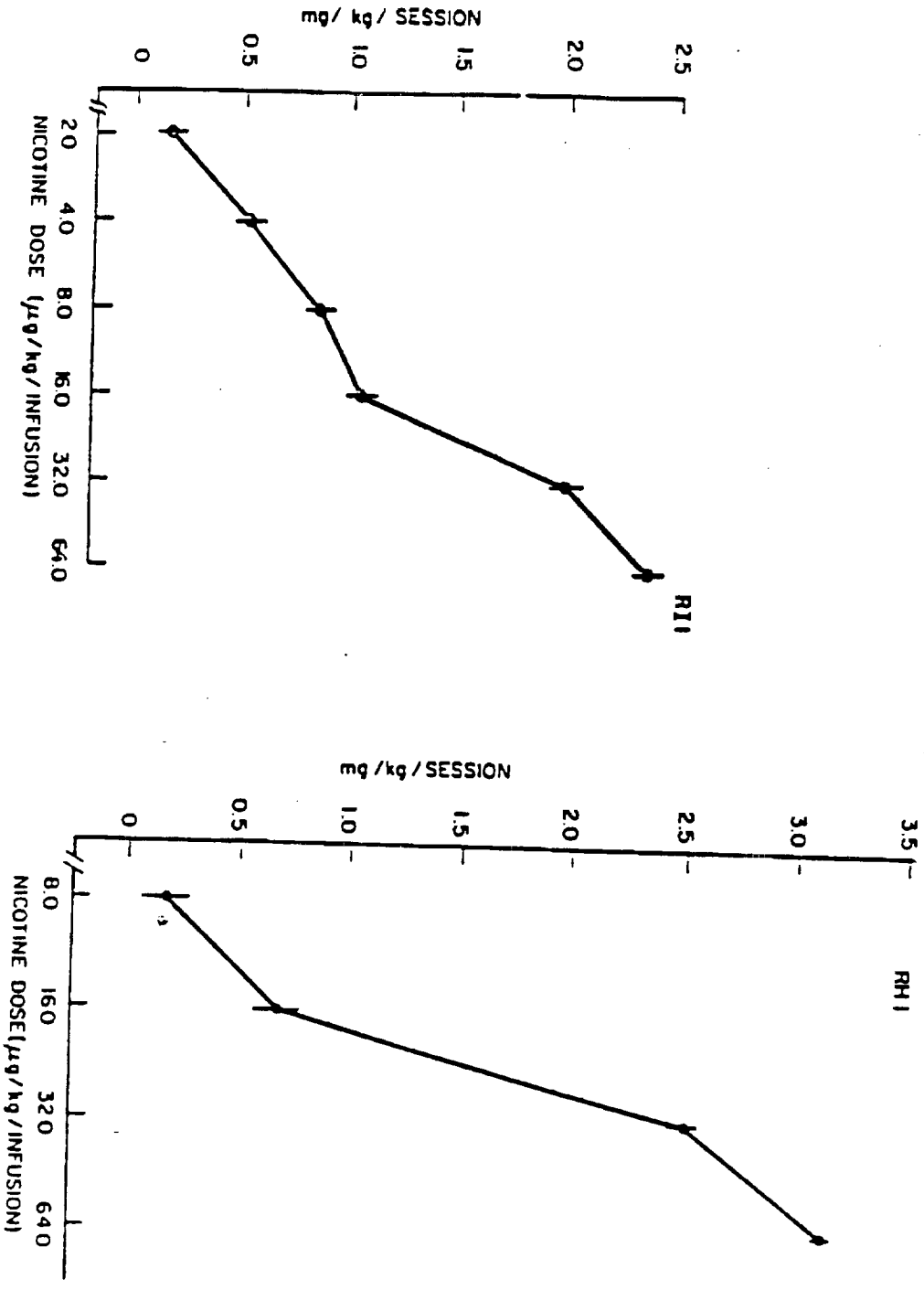

FIGURE 4

Effects of Fixed Ratio Size on Response Rate and Nicotine Intake

Preliminary results indicate that response rate increases as a function of fixed-ratio size. Data is still being collected and a more detailed analysis can be performed within two to three months.

Changes in Nicotine Self-Administration Following Mecamylamine or Hexamethonium Injections

Preliminary data show that presession treatment with the ganglionic blocking agent, mecamylamine HCl (1.5 mg/kg/s.c) completely blocked the nicotine maintained responding. The total number of infusions dropped from a mean of 175.0 per session to 11.0 per session. More interesting is the fact that one day following the mecamylamine injection the number of infusions was within the premecamylamine range, however, the pattern of self-administration was altered. The characteristic pattern of self-administration within a 24-hour period was evenly spaced responses that occurred in bursts followed by pauses. Twenty four hours after the mecamylamine injection the temporal sequence of responding was changed. The animal self-administered at a nigh rate for 6 hours (15 responses/hr), then did not self-administer for 10 hours, followed by a high rate for the remaining 8 hours. This pattern persisted for fourty eight hours. Then the typical pattern was recaptured. We are currently extending our analysis to other rats. Hexamethonium injections had no effect on self-administration.

18. Acetaldehyde Self-Administration

Nicotine is but one of many components in smoke that has been shown to possess behavioral activity, therefore, we began examining other smoke

components for their potential reinforcing effects. Since a substantial amount of work has been done on acetaldehyde as a product of ethanol metabolism, we were interested in determining to what extent, if any, could acetaldehyde function as a reinforcer by itself or as an agonist or antagonist to the positively reinforcing effects of nicotine. Two different access periods were used to determine the positive reinforcing effects of acetaldehyde. With one rat acetaldehyde (1.28 mg/kg/infusion) was available 2 hours each day. Lever pressing for intravenously delivered acetaldehyde was allowed to stabilize (3 days with no increasing or decreasing trends), following which saline was substituted for acetaldehyde. When the saline maintained lever pressing rate stabilized, we re-introduced the acetaldehyde. The reinforcing effect of the acetaldehyde is evident from Figure 5. Acetaldehyde maintained a response rate of 10.1 responses per hour, when saline was substituted, the response rate decreased to 4.75 responses per hour. The re-introduction of acetaldehyde increased the response rate to previous levels (10.0 re-test). This test clearly shows that acetaldehyde can function as a positive reinforcer.

Based on these data we investigated acetaldehyde self-administration in three additional rats under conditions identical to those used with nicotine (Section 1A). While the data is still being collected, one rat has completed the control conditions. The data (Figure 6) show that the lever pressing is being maintained by the acetaldehyde not the vehicle (saline). We are continuing our research in this area and should have dose response curves for acetaldehyde within two months.

FIGURE 5

NUMBER OF INFUSIONS AS A FUNCTION OF ACETALDEHYDE OR SALINE ACCESS CONDITIONS. EACH BAR IS A MEAN OF 3 CONSECUTIVE DAYS. VERTICAL LINES SHOW THE STANDARD ERROR.

FIGURE 5

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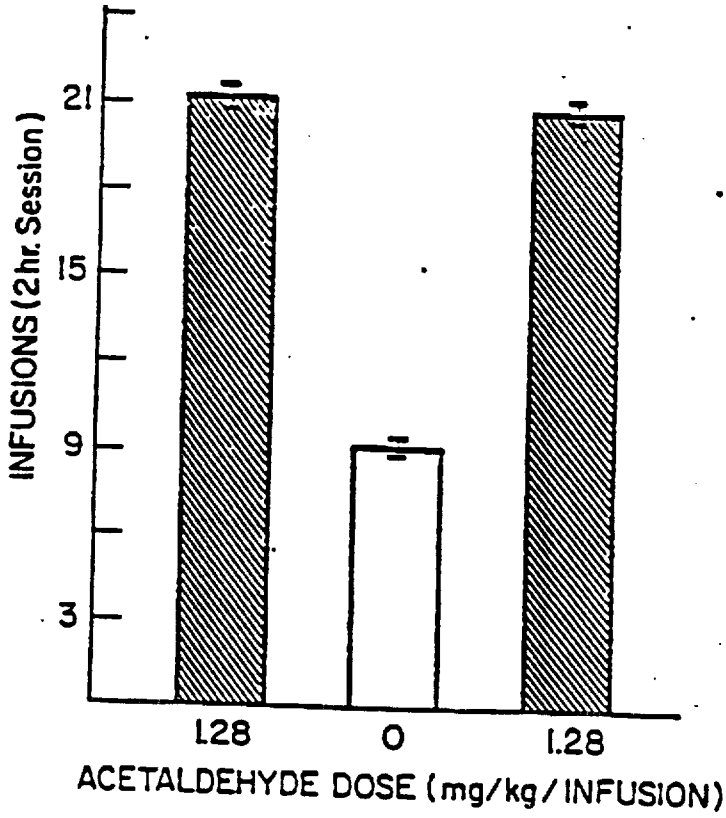
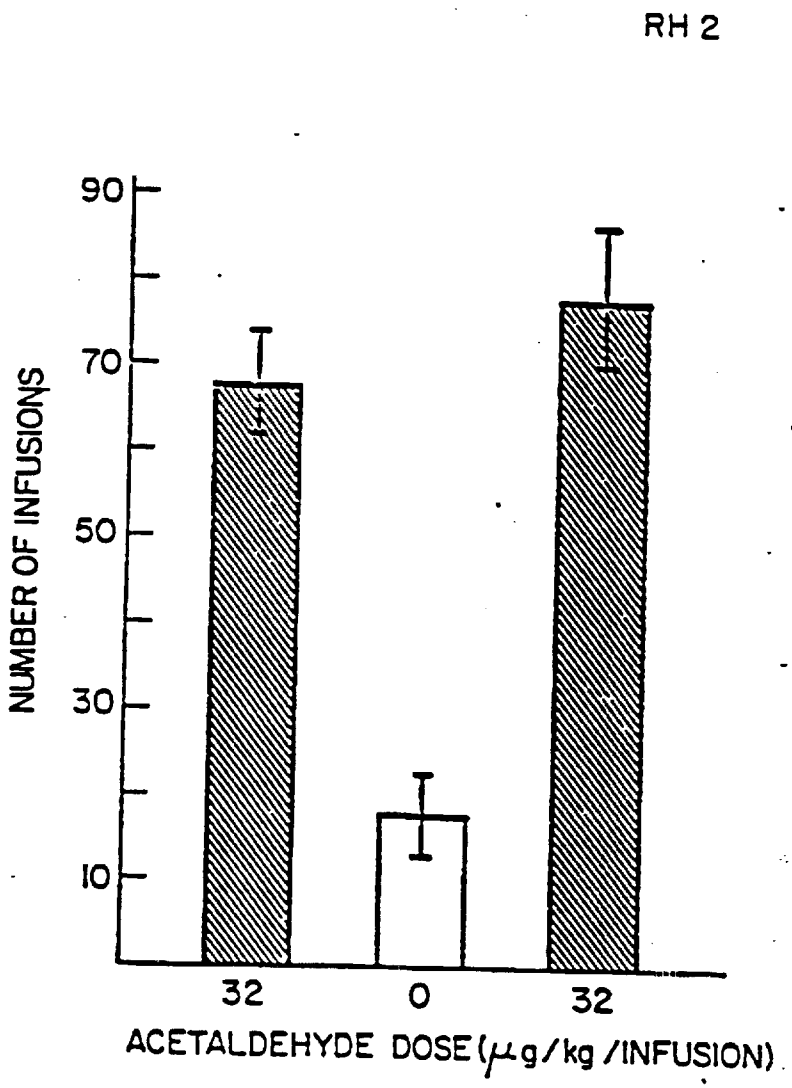


FIGURE 6

NUMBER OF INFUSIONS AS A FUNCTION OF ACETALDEHYDE OR SALINE ACCESS CONDITIONS. EACH BAR IS A MEAN OF 5 CONSECUTIVE SESSIONS. VERTICAL LINES SHOW THE STANDARD ERROR.

FIGURE 6



1C. Self-Administration of Nicotine-Acetaldehyde Combinations

Once we establish a dose response function with acetaldehyde we will investigate the effects of nicotine-acetaldehyde interactions on self administration behavior.

1D. Nicotine Analogue Self-Administration

At present two experiments are in progress. One is an attempt at direct substitution of 2'-methylnicotine for nicotine in rats for which nicotine serves as a positive reinforcer. We have elected to test d,ℓ-2'-methylnicotine first because d,ℓ-2'-methylnicotine is behaviorally active in discrimination and prostration tests and is equally as potent as ℓ-nicotine. Using Dr. L. Abood's rating scale for the prostration syndrome, 2'-methylnicotine did not produce some of the peripheral signs of prostration (see memo from V. DeNoble and L. Carron to W. L. Dunn, dated August 18, 1980, "Research Progress Concerning Discrimination and Prostration Studies") In addition, data collected by Dr. L. Abood show that 2'-methylnicotine is a hypotensive agent when injected in the jugular vein. Direct substitution of 2'-methylnicotine for nicotine will provide information about the ability of the analogue to substitute for nicotine in a reinforcement system. However, 2'-methylnicotine may be a positive reinforcer that does not directly substitute for nicotine. For this reason we will also attempt to establish 2'-methylnicotine as a positive reinforcer using naive animals.

Once an appropriate testing sequence is worked out other analogues will also be tested.

1E. Self-Administration of Other Smoke Components

Expected starting data 1-1-82.

1F. Self-Administration of Condensation Products of Acetaldehyde with Endogenous Neurotransmitters

It is becoming clear to us that acetaldehyde can and does function as an intravenously delivered positive reinforcer. A possible mechanism of the reinforcing actions is the formation of tetrahydroisoquinolines (TIQ). TIQs which are derived from catecholamines (Pictet-Spengler condensation of dopamine with acetaldehyde yields 1-methyl-6,7-dihydroxy-1,2,3,4-Tetrahydroisoquinoline) interact with a variety of membrane systems. Our research objective is to determine if the TIQs can function as intravenously delivered positive reinforcers.

The animals (n = 2) are maintained under the same experimental conditions as described in Section 1A. Following catheter implantations carboxysalsolinol was made available for intravenous infusion under a fixed-ratio-1 schedule. Since this research had never been reported we had no evidence as to effective dose levels. Jim Charles suggested that we deliver an equal molar solution of carboxysalsolinol matched to acetaldehyde. The dose for the carboxysalsolinol was 160 ug/kg/infusion. Both animals readily self-administered carboxysalsolinol at very high response rates (>5 responses/hour). Observation of the animals revealed hyper activity with the major symptom being motor activation. Saline substitution produced a gradual decrease in lever pressing. Re-introduction of carboxysalsolinol (160 ug/kg/infusion) increased the lever pressing to previous

levels (Figure 7). Even though the data is based on only two animals it is important to note that carboxysalsolinol appears to be a very powerful positive reinforcer. We are continuing our efforts to characterize the reinforcing effects of TIQs.

Noteworthy Observations from Self-Administration Studies

During the course of nicotine self-administration we recorded two observations which are worth noting: first, when saline was substituted for nicotine in animals who were self-administering nicotine (2-3 mg/kg/day - I.V.) there did not appear to be a disruption in "normal" feeding, grooming or other routine behaviors. This preliminary observation suggested to us that there may be an absence of behavioral withdrawal symptoms following nicotine termination. We are now investigating this possibility (see Section 3). Second, preliminary results indicate that for albino rats (when compared to hooded rats) nicotine is difficult to establish as a positive reinforcer. The neurochemical differences between these strains may provide some clues as to the physiochemical correlates of nicotine self-administration.

PROSTRATION

2A. Effects of Intraventricularly Administered Nicotine Analogues

The prostration syndrome continues to be used as a screen for behaviorally active nicotine analogues. The following compounds have been tested thus far.

FIGURE 7

NUMBER OF INFUSIONS AS A FUNCTION OF CARBOXYSALSOLINOL OR SALINE ACCESS CONDITIONS. EACH BAR IS A MEAN OF 3 CONSECUTIVE DAYS. VERTICAL LINES SHOW THE STANDARD ERROR.

FIGURE 7

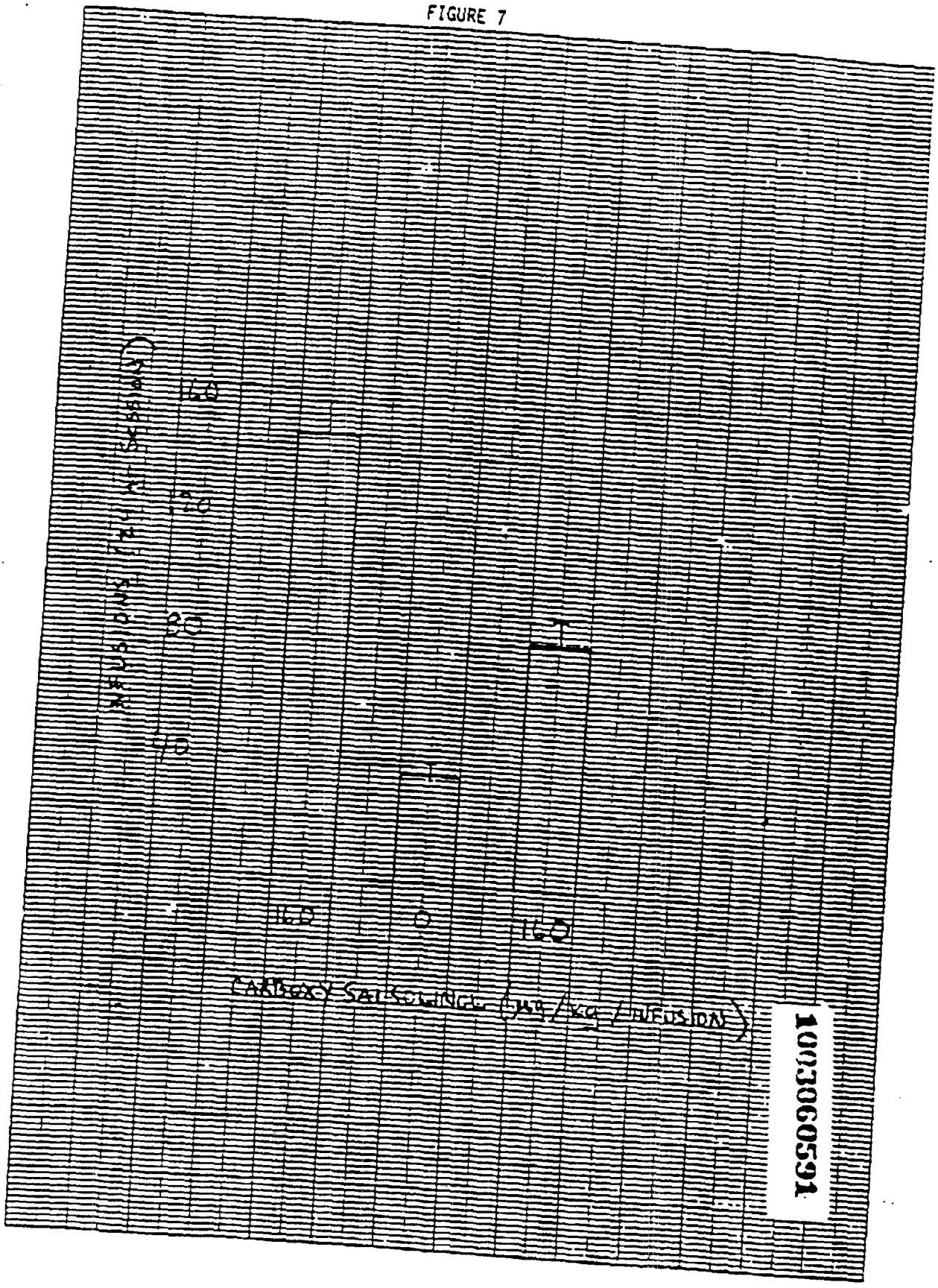


TABLE I

Compound	Dose	Effect
(R,S)-1-Methyl-2-(3-picolyl)pyrrolidine	40µg	1
(R,S)-1-Methyl-2-(2-(3-pyridyl)ethyl) pyrrolidine	40µg	0
6-(2-hydroxyethyl)nicotine	40µg	2
(R,S)-2,3'-Bismethylenenicotine	40µg	0
(R,S)-2'-Methylnicotine	20µg	0
(R,S)-2'-Methylnicotine	5µg	1-2
	10µg	2-3
	20µg	3-4

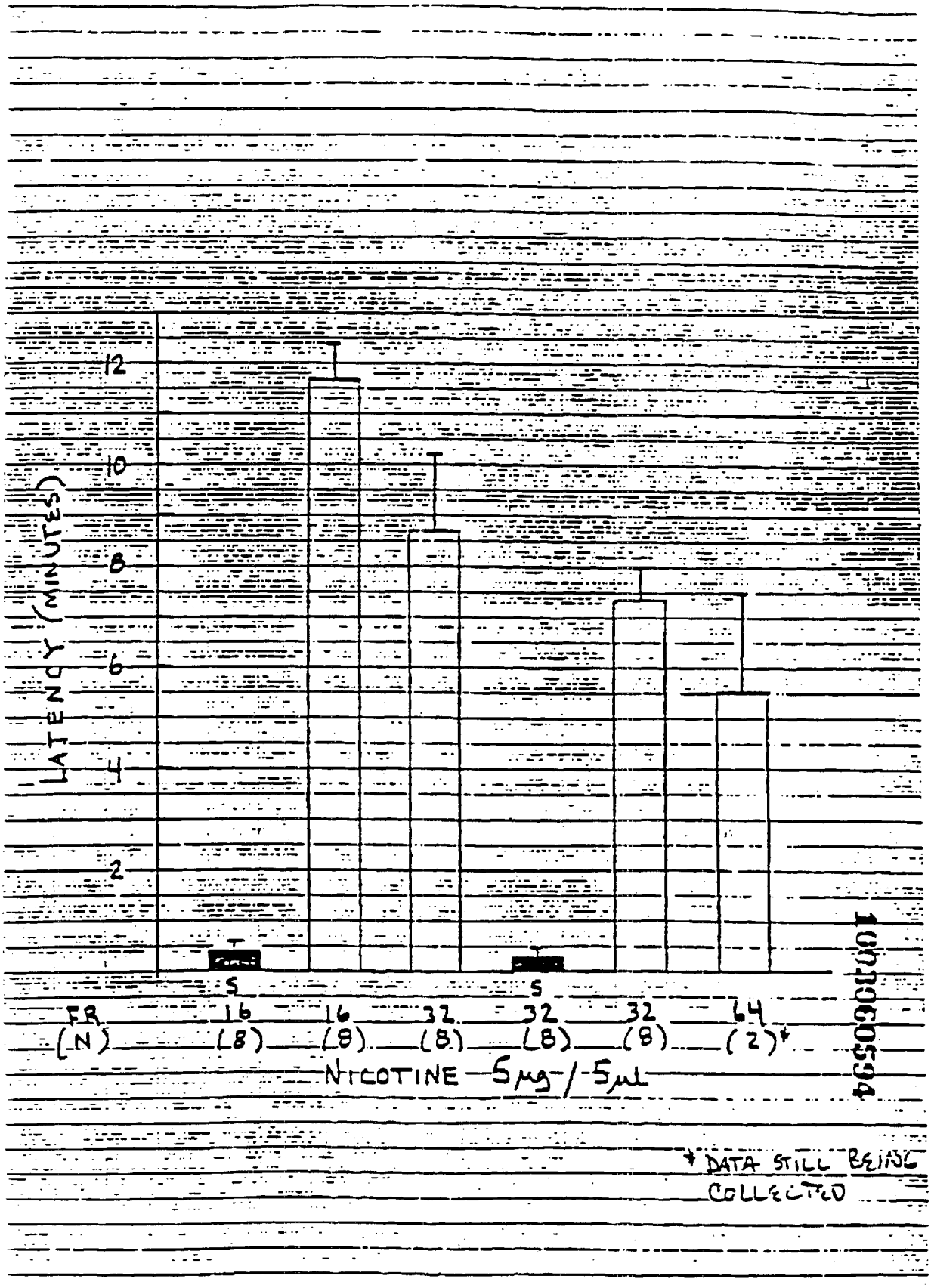
2B. Studies on the Effects of Intraventricular Infusions of (-)-Nicotine on Behavior Maintained Under Fixed Ratio Schedules

Our efforts to develop more reliable and sensitive measures of the prostration syndrome have been quite successful. We are using schedule-controlled behavior to quantify and qualify the prostration syndrome. Specifically, rats are trained to emit a pre-determined number of responses (fixed ratio) on a lever to obtain food. This technique produces a highly stable and reproduceable baseline of behavior which has been shown to be sensitive to changes in central nervous system functioning. In addition, by manipulating the work requirement for food (fixed-ratio size, eg. 16, 32, or 64 responses per food delivery) we can manipulate the rate of emitted behavior. Figure 8 shows the latency to complete the first ratio following saline or (-)-nicotine infusions as a function of the fixed-ratio size. The mean response latency following saline infusions was less

FIGURE 8

THE LATENCY TO COMPLETE THE FIRST RATIO FOLLOWING SALINE OR (-)-NICOTINE INFUSIONS AS A FUNCTION OF THE FIXED-RATIO SIZE. EACH BAR REPRESENTS THE MEAN LATENCY (N=8). VERTICAL LINES SHOW THE STANDARD ERROR.

FIGURE 8



than 30 seconds. However, (-)-nicotine infusions increased the latency substantially. More important the increase in latency to complete the first ratio following a nicotine infusion varied as a function of the ratio size. The ability to establish functional relationships between nicotine infusions and the contingencies controlling behavior will add substantially to our development of structure-activity relationships among nicotine analogues.

2C. Inhibition of Sodium Conductance in Various Brain Regions: Effects on Intraventrically Administered (-)-Nicotine

In an effort to identify the neuroanatomical substrates mediating the prostration syndrome we requested that Dr. ... Abood block the activation of the sodium conductance mechanism in axons within specific brain regions. His results were very impressive! Of all the brain regions investigated only inhibition of the lateral vestibular nucleus blocked the prostration syndrome. We are currently replicating these results and extending the analysis to other brain regions and other behavioral tests.

CHRONIC NICOTINE ADMINISTRATION AND TERMINATION

3A. Multiple Fixed Ratio - Fixed Interval Performance During Chronic Nicotine Administration and Following Termination

It has been previously shown that termination of chronic drug administrations (ethanol, barbituates, opiates, and psychomotor stimulants) result in a withdrawal syndrome. This withdrawal syndrome is measurable as changes in schedule-controlled behavior. The absence of any observable behavioral effects when saline was substituted for nicotine in our self-administration studies prompted us to systematically investigate if

termination of chronically adminstered nicotine disrupts ongoing behavior.

Twelve rats are being trained under a complex schedule of food reinforcement (multiple fixed ratio-fixed interval-time out). At the start of the session the rat is presented with a single white light. When this light is illuminated the rat emits 30 rapid lever press responses. The 30th response results in a food pellet delivery and changes the stimulus conditions form one white light to two red lights. When the two red lights are illuminated, food is delivered after a two-minute interval (fixed interval 2 min). Following a food pellet delivery during the FI component all the lights are extinguished and lever pressing has no programmed consequence for 1 minute (time out). This sequence (FR, FI, time out) is repeated 11 times each day. The total session time is approximately 40 minutes.

After training differential response patterns develop. Under the fixed ratio component the response rate is consistent and high (eg. 1-2 responses per second). Under the fixed interval component the response rate accelerates as the interval approaches its end. These differential patterns engendered by the different schedules are mediated by different brain regions and allow for bi-directional changes in behavior.

Baseline data has been collected and chronic nicotine administrations have begun. An osmotic mini pump was implanted subcutanously (sc) and is delivering 175 µg of nicotine in 0.5 µl every hour 24 hours per day for 10 days (12 mg/kg/day). The animals will be tested daily under the MULT FR-FI-TO schedule. On the tenth day they will be challenged (injected sc) with mecamylamine (1.5 mg/kg). Mecamylamine injections will

occur every 12 hours for 3 days.

**It is important to note that this study will yield information about possible withdrawal effects of nicotine, not cigarette smoking.

DISCRIMINATION

4A. Nicotine Discrimination

The discrimination testing is one of the routine screens for behaviorally active nicotine analogues. Within the period of this report the following compounds have been tested (Table II).

4B. Acetaldehyde Discrimination

Since acetaldehyde functions as an intravenously delivered reinforcer and the data from the rat maintained with intravenous carboxy-salsolinol is promising, it is important to establish if the introspective effects of acetaldehyde are similar to TIQs.

5. ACUTE COMPOUND ADMINISTRATION: EFFECTS ON MOTIVATION AND EMOTION

In planning phase.

6. ELECTROPHYSIOLOGY

In planning phase.

TABLE II

BEHAVIORAL ACTIVITY OF NICOTINE ANALOGUES IN NICOTINE-DISCRIMINATION TASK

Compound	Dose (mg/kg)	% of Rats Emitting Nicotine Responses	% of Rats Emitting Saline Responses	% of Rats Emitting Incomplete Responses
Metanicotine	4.0	50	50	0
	8.0	100	0	0
2'-Ethylnicotine	0.4	0	100	0
	0.8	0	100	0
	1.6	0	100	0
(R,S)-1-Methyl-2-(3-picolyl) pyrrolidine	0.4	0	0	0
	0.8	0	0	0
	1.6	50	50	0
	3.2	75	25	0
(R,S)-1-Methyl-2- α-(3-pyridyl ethyl) pyrrolidine	0.4	0	100	0
	0.8	0	100	0
	1.6	0	100	0
4-Isonicotine	8.0	29	71	0
2-Isonicotine	8.0	0	0	0
4,3'-Isonicotine	8.0	0	0	0
3-dimethylaminomethyl pyridine	4.0	75	25	0
	2.0	40	60	0
2'-Methylnicotine	0.4	100	0	0