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SUMMARY

Several studies were conducted to characterize the behavioral effects of nicotine and acetaldehyde in rats. Nicotine and acetaldehyde were both shown to be positive reinforcers and the self-administration of compounds was shown to be sensitive to both pharmacological and environmental manipulations. The reinforcing effects of nicotine under the present conditions are relatively weak compared to other reinforcers. Nicotine self-administration was shown to be blocked by mecamylamine but not by hexamethonium or naloxone. Preliminary investigation into the interaction of nicotine and acetaldehyde showed that there were several combinations of these compounds that had enhanced reinforcing effects relative to either compound alone. The interaction between nicotine and acetaldehyde in self administration studies was not evident in discrimination tests. Preliminary results using electroencephalographic recording techniques indicate that nicotine and acetaldehyde are interacting in the central nervous system.

The development of tolerance to nicotine was demonstrated to be highly dependent on behavioral variables rather than solely on metabolic or receptor sensitivity factors. Behavioral comparisons between the stereoisomers of nicotine were made; initial results suggest that differences are quantitative rather than qualitative, with (-)-nicotine being six to nine times more potent than (+)-nicotine. The behavioral response to centrally administered nicotine was not altered following chronic blockade of nicotinic receptors. Since this is in contrast to the behavioral supersensitivity found in the classic neurotransmitter systems following functional blockade, it may suggest that the dynamics of central nicotinic receptors are different from those involved in traditional neurotransmitter pathways.

In other studies we have examined the inhibition of several brain sites on nicotine induced prostration. The vestibular nucleus appears to be at least

one brain site involved in the prostration response. Preliminary investigations (in collaboration with Project 6902) examining the transport of acetaldehyde into the brain following vascular administration have begun. The early results show that acetaldehyde readily crosses the blood brain barrier. Additional studies to identify regional distribution of acetaldehyde are now in progress. Substantial progress has been made towards establishing a behavioral profile of the effects of both nicotine and acetaldehyde.

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## INTRODUCTION

The general goal of the Behavioral Pharmacology Laboratory is to develop a behavioral profile of the effects of selected smoke components. This is accomplished by developing a battery of behavioral tests which can compare the effects of these smoke components to other agents with known behavioral properties. This approach affords us qualitative and quantitative estimates of possible interactions between individual smoke components or of the effects of structural modifications of these components. Some specific objectives addressed in this report are:

1. Develop a behavioral profile of the reinforcing effects of nicotine and acetaldehyde.
    a. To find a ratio(s) of acetaldehyde to nicotine that will have optimal reinforcing effects.
    b. To examine the potential physical dependence producing properties of acetaldehyde.
    c. To determine if congeners of acetaldehyde have reinforcing effects.
2. Examine other behavioral interactions between nicotine and acetaldehyde using drug discriminating techniques and intraventricular administration techniques.
3. Develop methods for differentiating between the physical (physiological) dependence producing properties and the behavioral dependence producing properties of reinforcing agents.
4. Identify neuroanatomical sites that mediate the behavioral disruption induced by intraventricular nicotine administration.
5. Examine the interaction of behavioral tolerance and metabolic tolerance induced by chronic nicotine administration.
6. Develop a behavioral profile of nicotine induced tolerance using cross tolerance methods.
7. Initiate neurochemical research projects relevant to the behavioral effects of nicotine and acetaldehyde (in collaboration with Project 6902).

Most of these objectives are long term and are continuously being expanded. However, substantial progress has been made.

I. NICOTINE SELF-ADMINISTRATION SUMMARY

Nicotine is one of the most widely used compounds, however, it is only recently that the effects of nicotine on schedule-controlled behavior have been systematically studied.[1,2,3,4] 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 electrical shock postponement.[5,6] Nicotine decreases responding under fixed ratio (FR) schedules of food or water presentation. 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.[7] In addition, it has been shown that intravenous injections of nicotine will maintain high rates of lever-pressing by squirrel monkeys under a second order schedule. Under this schedule responding results in the presentation of a visual stimulus that is intermittently associated with response contingent nicotine injections.[8]

Many compounds from different pharmacological classes can increase and maintain behavior that leads to self-administration of those compounds.[9] However, there is little evidence that rats will intravenously self-administer nicotine unless self-administration is induced by a food delivery schedule[10] or they are given programmed nicotine infusions for several days.[11] The levels of responding maintained by intravenous nicotine following programmed infusions have been low. The present study demonstrates that intravenously delivered nicotine functions as a positive reinforcer in the absence of food inducement or programmed infusion conditions. Nicotine self-administration was studied under different FR values and across a range of nicotine infusion doses. In addition, the present results extend previous findings[2] by showing that termi-

nation of prolonged access to nicotine under conditions in which it functions as a positive reinforcer does not result in a withdrawal syndrome.

Ten male hooded rats each implanted with a venous catheter[12] were maintained in standard operant conditioning chambers with food (20-30 g/day) and water always available. Each chamber was enclosed in a sound-attenuating box. Responding on one lever activated an infusion pump for 4-5 seconds, delivering an infusion of 0.13 ml of solution. Responses on the other lever (activity lever) were recorded but had no programmed consequence. The rate of activity lever responding was recorded throughout all experimental manipulations and was compared to the rate of responding recorded from the lever resulting in nicotine infusions. The houselight provided illumination and blinked at a rate of 10 Hz during an infusion. First, nicotine self-administration was established in the rats at 32 ug/kg/infusion (all doses are expressed as the free base). Access to nicotine was unlimited (24 hours), with one response required for each infusion (FR 1). Then changes were made in the nicotine delivery procedure to determine if lever pressing was being maintained by the contingency established between lever pressing and nicotine delivery.[9] Changes included substitution of saline for nicotine, systematic changes in nicotine dose and programmed nicotine infusions at intervals of 30, 45, 60 and 90 minutes. All rats were tested with the saline substitution procedure and three rats were given programmed nicotine infusions. In the seven rats not receiving programmed infusions the effect of nicotine infusion dose was determined on the number of infusions delivered and the total intake (mg/kg/24 hour) under an FR 1 schedule. Nicotine doses (64.0, 32.0, 16.0, and 8.0 ug/kg/infusion) were presented in descending order for a minimum of 7 days each. Under each infusion dose, lever pressing was allowed to stabilize before changes were made. In the three rats that received programmed nicotine

infusions the effects of FR size (1-9 responses/32 µg/kg/infusion) of nicotine on the number of lever presses and the number of infusions were studied. Ratios were presented in ascending order and the rats were maintained under each ratio for a minimum of 7-10 sessions.

All rats initiated and maintained nicotine self-administration (Figure 1, left panel). Generally, 10-20 sessions were necessary for the acquisition and stablilization of responding on the nicotine lever. Stability was defined as 3-5 sessions with no increasing or decreasing trends in the number of infusions. The within session pattern of nicotine-reinforced responding under the FR 1 schedule was typically a series of closely spaced infusions (2-4/minute), followed by a pause (30-90 minutes) during which time no infusions were taken. Nicotine self-administration was shown to be maintained by the response-nicotine contingency, rather than by other behavioral effects of nicotine. Substitution of saline for nicotine solution failed to maintain lever pressing (Figure 1). Saline substitution produced a temporary (3-6 hours) increase in lever pressing which rapidly declined to less than 12 infusions during the following 24 hour session. When nicotine was reintroduced (32.0 µg/kg/infusion) the number of nicotine infusions increased to previous levels (Figure 1). Periodic observation of the rats when nicotine was available and during the saline substitution failed to reveal any signs of physical dependence.[13] When nicotine was available lever pressing occurred almost entirely on the lever delivering nicotine infusions. Activity-lever responses were less than 10% of the total number of responses for all rats.

Table I shows the effect of programmed nicotine infusions delivered independently of responding on nicotine maintained lever pressing. The percent decrease in the number of response contingent infusions was inversely related to the programmed interinfusion interval. The sum of response contingent

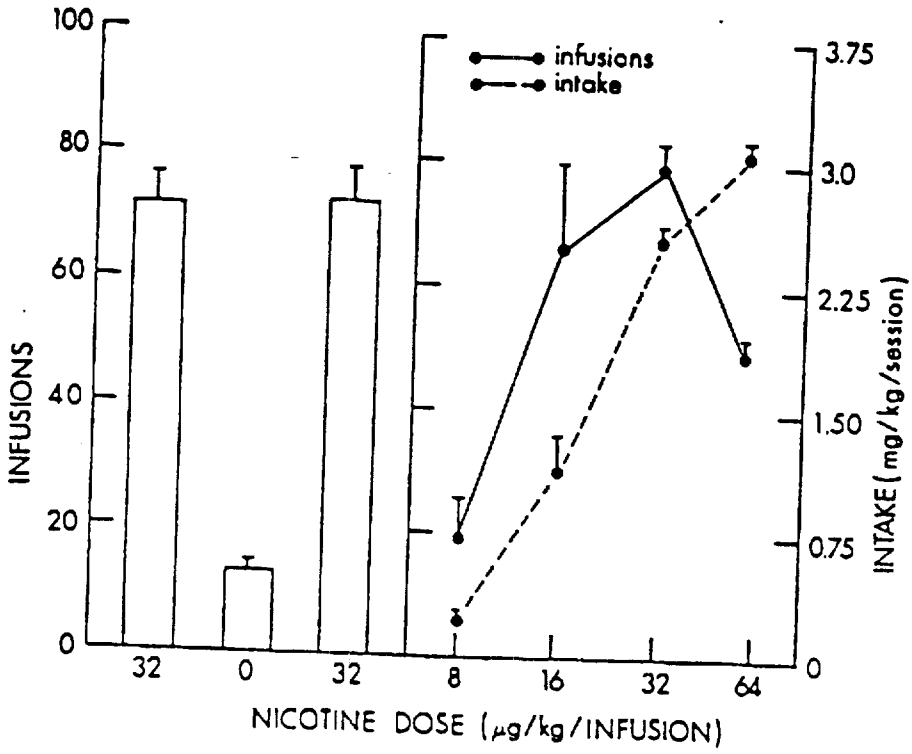



Figure 1. Effects of substituting saline for nicotine on the number of infusions (left panel). Each bar represents a mean of 30 sessions (10 rats x 3, 24 hour sessions). The vertical lines show the standard error. The right side of the figure shows the effect of varying the dose of nicotine on both the number of infusions (solid lines) and session intake (mg/kg/session, dashed lines) under an FR 1 schedule. Each point is a mean of 21 sessions (7 rats x 3 sessions each) and the vertical lines show the standard error.

infusions plus response independent programmed infusions was stable across sessions (Table 1), suggesting that the daily level of nicotine self-administration is at least in part under control of some circulating blood level.

The effect of varying nicotine dose on the number of infusions under an FR 1 schedule is shown in the right panel of Figure 1. As the dose of nicotine was decreased the number of infusions first increased then decreased. In contrast, session intake (mg/kg of body weight) increased as a function of nicotine dose (Figure 1). Similar functional relationships have been found with other reinforcers.[9] The 8 µg/kg/infusion dose of nicotine did not maintain lever pressing above saline levels.

These results demonstrate that intravenously delivered nicotine can increase and maintain lever pressing that results in its delivery. The changes in the nicotine delivery procedure showed that lever pressing was maintained by the nicotine-response contingency. There were four indications of the positive reinforcing effects of nicotine: 1) a greater number of lever presses when nicotine was response-contingent than when saline was response-contingent; 2) a greater number of responses on the nicotine lever than on the activity lever; 3) a systematic decrease in the number of contingent infusions when nicotine was delivered noncontingently; and 4) systematic changes in lever pressing as a function of the nicotine dose.

The effect of increasing the ratio size on the number of lever presses and infusions is shown in Figure 2. Increases in FR size up to FR 5 resulted in substantial increases in the number of lever presses. At a ratio of 6 and 7 the number of lever presses remained relatively stable. A further increase in ratio size to FR 8 resulted in a decrease in the number of lever presses. The number of infusions remained relatively stable across several ratios (1-6), then decreased at ratios of 7 and 8. Although intravenously delivered nicotine

Table 1

The percent decrease in the number of self-administered nicotine infusions (32 µg/Kg) and the total number of infusions as a function of the interval between response independent programmed nicotine infusions.

| Interinfusion Interval (minutes) | Mean (± standard error) % | Mean Total Infusions (± standard error) Programmed Plus Response contingent |
|---|---|---|
| Control | | 76 (± 3.8) |
| 30 | 59 (± 4.3) | 81 (± 3.0) |
| 45 | 45 (± 4.0) | 75 (± 4.3) |
| 60 | 30 (± 3.4) | 79 (± 8.1) |
| 90 | 21 (± 1.5) | 74 (± 2.6) |

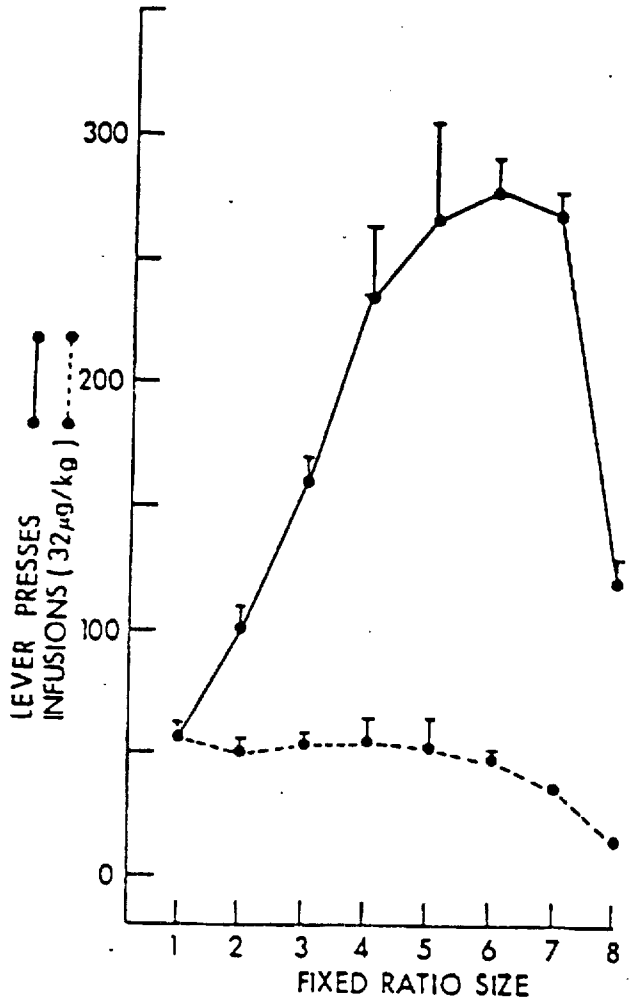



Figure 2. The number of lever presses and nicotine infusions (32 μg/kg) is shown as a function of the FR size. The ratios were presented in an ascending order. Each point is a mean of 9 observations (3 rats x 3 sessions) and the vertical lines show the standard error.

maintained ratio performance, these overall rates of responding compared to other intravenously delivered reinforcers are low, suggesting that nicotine may be a weak reinforcing agent.

Previous attempts to establish nicotine as an intravenously delivered reinforcer for rats have shown that only under conditions of reduced body weight and/or concurrent fixed time food presentation will nicotine self-administration occur at rates above vehicle control levels.[10] The present results show that nicotine can function as an intravenously delivered positive reinforcer for rats in the absence of such conditions, and that the level of responding can be maintained across several ratio schedules.

A detailed profile of the behavioral effects of nicotine has been emerging from several laboratories; however, there has been a continuing need for a systematic evaluation of the reinforcing effect of nicotine in the rat. In this study the maintenance of lever pressing was unequivocally the result of consequent nicotine infusions. Furthermore, the behavior was shown to be sensitive to both dose and response contingency manipulations.

## II. EFFECTS OF MECAMYLAMINE, HEXAMETHONIUM AND NALOXONE ON NICOTINE SELF-ADMINISTRATION

In a previous report from this laboratory[14] it was found that lever pressing by rats was established and maintained by i.v. nicotine in the absence of other food inducement or weight reduction procedures. The purpose of the present study was to extend these observations on nicotine maintained responding in rats by 1) replicating control procedures used to determine if lever pressing was being maintained by the contingency established between response and nicotine delivery; and 2) assess the effects of treatment with mecamylamine, hexamethonium and naloxone on nicotine maintained responding. The results show that nicotine can maintain lever pressing above vehicle control levels and that the reinforcing effects of nicotine are blocked by mecamylamine but not by hexamethonium or naloxone.

### RESULTS

#### Acquisition and Maintenance of Responding Under the Fixed Ratio 1 Schedule of Nicotine Infusion

Responding under the FR 1 schedule was initiated and maintained by 32 µg/kg infusions of nicotine in all rats. The number of infusions stabilized in all rats within 21 days. The average number of days for stable behavior to be acquired was 16 days (± 1.3). The range and the general pattern of acquisition is shown in Figure 3 for the rat with the shortest acquisition time (solid lines) and the rat with the longest acquisition time (dashed lines). Most rats maintained acquisition patterns similar to those shown in Figure 3 with the major characteristic being 2-5 days of low responding (4-30 infusions/24 hours) followed by a gradual (3.5 days) increase to near stable response rates. The within session pattern of nicotine infusions under the FR 1 was typically a

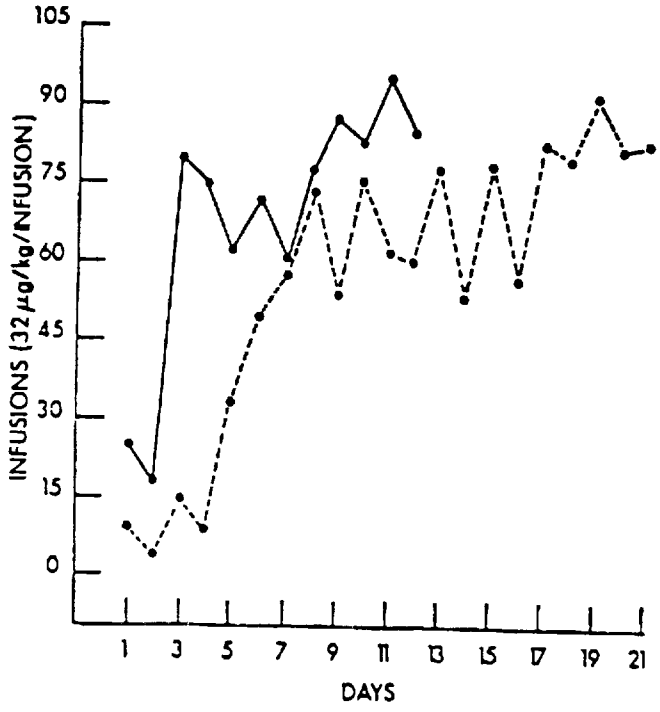



Figure 3. Number of nicotine infusions (32 µg/kg) as a function of the number of days of access to nicotine for two individual rats. The solid line shows the rat with the shortest acquisition time and the dashed line shows the rat with the longest time.

series of closely spaced infusions (2-4/minute), followed by a pause (30-90 minutes) during which time little or no infusions were taken.

Substitution of saline for nicotine solution failed to maintain lever pressing (Figure 4). Saline substitution produced a temporary (4-8 hours) increase in lever pressing which rapidly declined to less than 13 infusions during the following 24 hour session. When nicotine was reintroduced (32 ug/kg) the number of infusions increased to previous levels (Figure 4). The average daily nicotine intake during this condition was 2.84 ± 0.08 mg/kg.

Effects of mecamylamine, hexamethonium or naloxone on nicotine maintained responding.

Before each mecamylamine administration responding by four rats was examined in sessions in which a subcutanous saline injection was given (Figure 5, open bar). When mecamylamine (0.75 mg/kg/sc) was administered there was no change in the total (24 hour) number of infusions. In contrast, mecamylamine at a dose of 1.5 mg/kg decreased the number of infusions by 62%. Increasing the dose of mecamylamine to 3.0 mg/kg decreased responding for nicotine further (Figure 5, shaded bars). Responding for nicotine returned to previous levels the session after the mecamylamine administrations. Presession treatment with hexamethonium (1.5 and 3.0 mg/kg/sc) in the same four rats did not reduce responding (bars with dashed lines).

The within session pattern of responding following saline and mecamylamine treatment are shown in Figure 6. The solid line function shows the pattern of infusions for 24 hour periods in 3 hour blocks beginning at 0830 hours. This pattern was characteristic for all four animals and the largest standard error for any 3 hour period was 2.12 infusions. The number of infusions during the first 3 hours following the 0.75 mecamylamine dose was elevated compared to

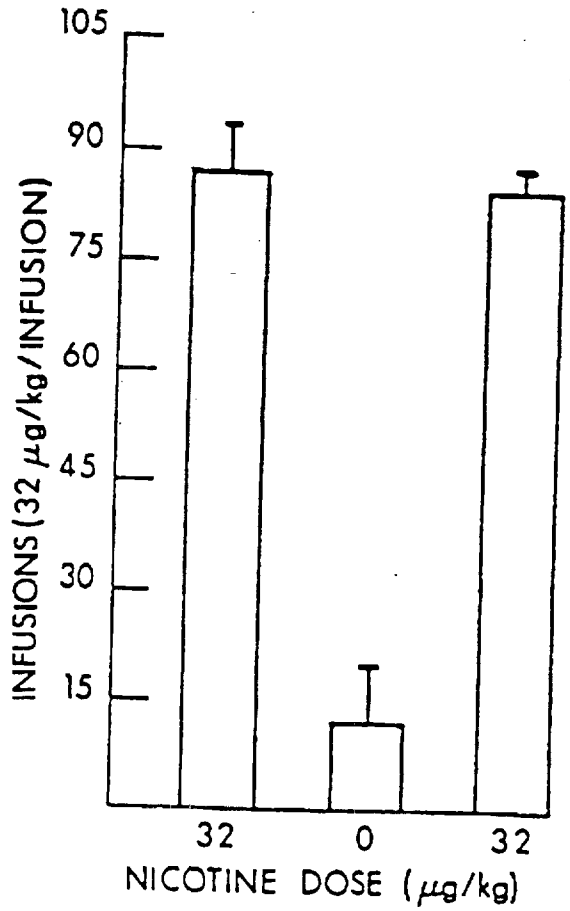



Figure 4. Effect of substituting saline for nicotine on the number of infusions under a FR 1 schedule during 24 hour sessions. Each bar represents an average of 24 sessions (8 rats x 3, 24 hour sessions). The vertical lines show the standard error.

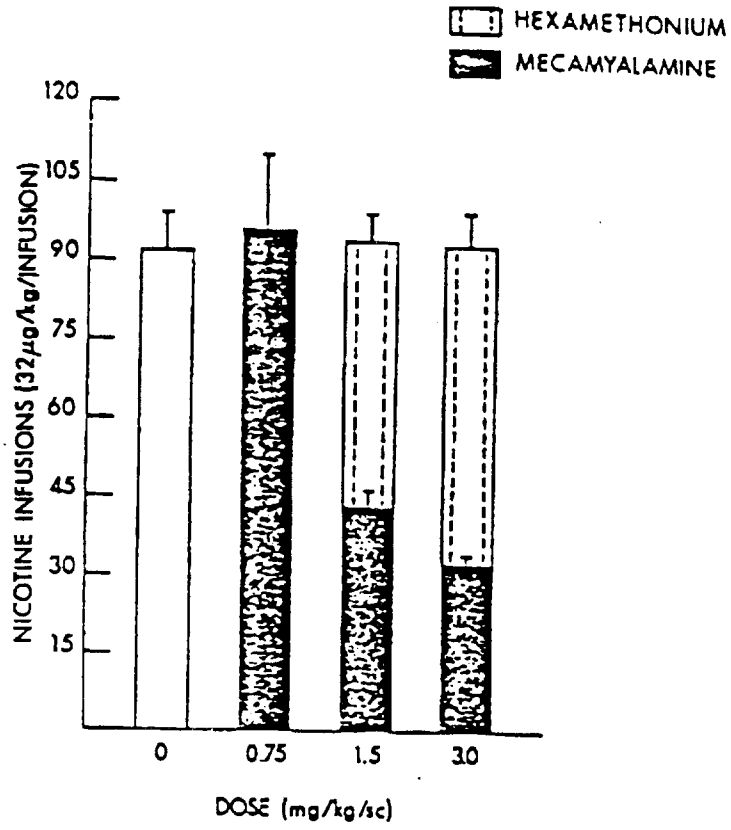



Figure 5. Effects of treatment with mecamylamine or hexamethonium on responding maintained by i.v. nicotine infusions in four rats maintained under a FR 1 schedule. Abscissa: dose of mecamylamine or hexamethonium given s.c. The 0 dose consisted of the saline pretreatments (see methods). Filled bars represent the number of infusions occurring during the 24 hour session immediately following a mecamylamine injection. The number of infusions that occurred during the 24 hours following a hexamethonium injection (1.5 and 3.0 mg/kg/sc) are represented by the dashed lines within the bars. Each bar is an average of 4 rats. The vertical lines show the standard error of the mean.

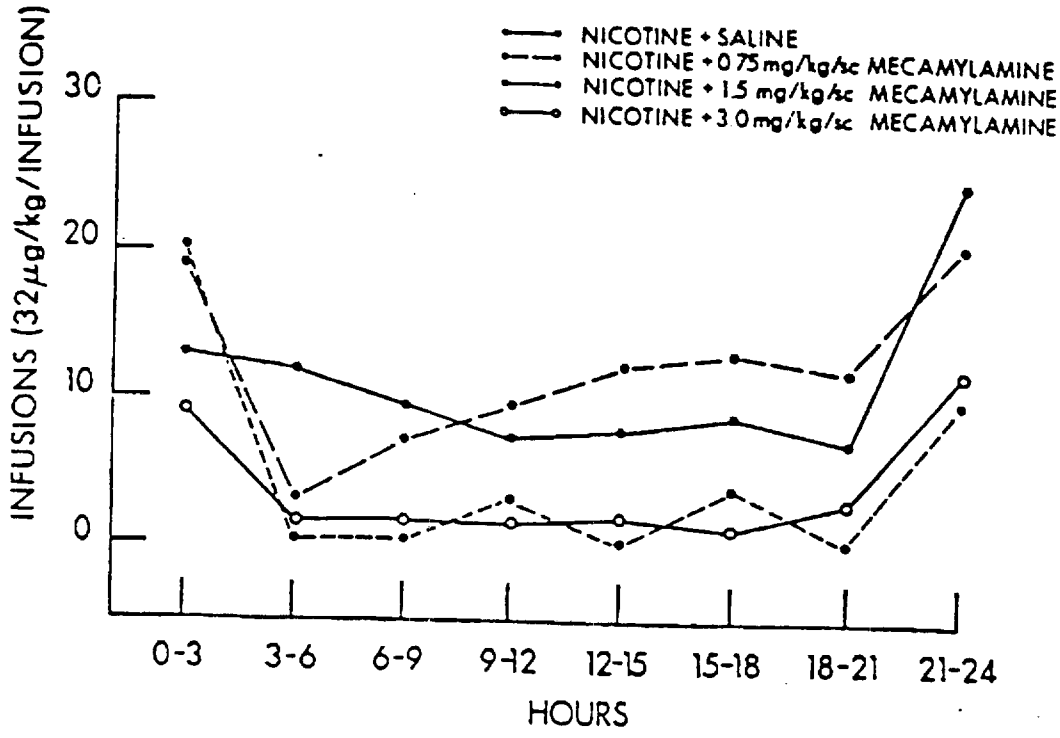



Figure 6. The average number of nicotine infusions in 3 hour blocks is presented for 3 saline (solid lines) and the three mecamylamine pretreatments (0.75 large dashed lines, 1.5 small dashed lines and 3.0 mg/kg/sc open circles) for four rats. The standard error for any 3 hour period during the saline pretreatments was 2.12 infusions.

saline levels. This was followed by a decrease in infusions in the next 3 hours. The number of infusions gradually increased to above control values in 4 of the remaining 6, 3 hour intervals. The number of infusions following the 1.5 mg/kg dose of mecamylamine initially increased (first 3 hours) then decreased to near zero levels for 6 of the remaining 3 hour intervals. During the last 3 hour interval the number of infusions increased but was still below control levels. The 3.0 mg/kg dose decreased the number of infusions in all 3 hour intervals. The number and pattern of infusions during the next 24 hour period did not differ from control values. Hexamethonium pretreatment did not change the within session pattern (Figure 5).

The effect of naloxone pretreatment is shown in Figure 7. Naloxone injections did not produce any within or between session changes in the number or pattern of nicotine infusions.

In the present experiment, mecamylamine was also an effective antagonist of the behavioral effects of nicotine. When mecamylamine was administered at the beginning of a session responding maintained by nicotine infusions was reduced and the amount of reducion was directly related to the mecamylamine dose. In contrast, nicotine maintained responding in the same rats was not altered by presession treatment with hexamethonium.

Naloxone hydrochloride has been shown to reduce the intake of positive reinforcers such as food, water and ethanol. The reduction did not appear to be related to an abstinence syndrome,[15] or a general suppression of behavior,[16] unless high doses (>5.0 mg/kg) of the antagonist were used.[17] Naloxone has also been shown to reduce the amount of cigarettes smoked during a 3 hour test period.[18] In contrast, naloxone has not been shown to be an effective antagonist of nicotine-induced antinociception in rats.[19] The present results

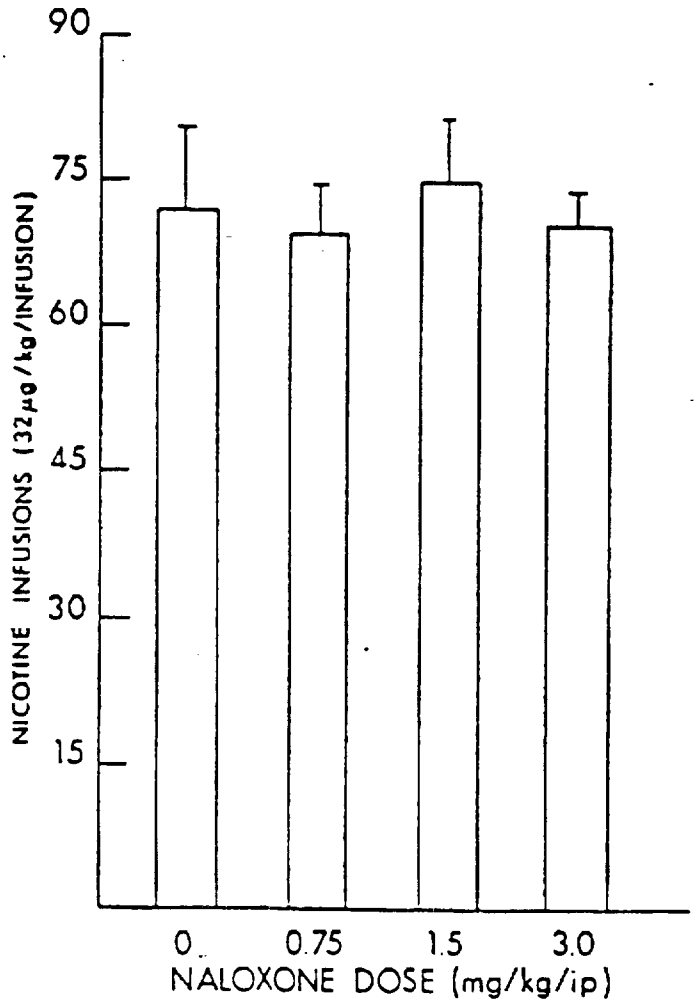



Figure 7. Effects of pretreatment with naloxone on responding maintained by i.v. nicotine infusions for four rats. The 0 dose consisted of the saline pretreatments. Vertical lines show the standard error of the mean.

are consistent with the previous findings in that naloxone across a range of doses was ineffective as an antagonist to the positive reinforcing effects of i.v. nicotine.

In summary, nicotine functioned as an intravenously delivered positive reinforcer for rats in the absence of weight reduction or food inducement procedures. The fact that pretreatment with mecamylamine (a centrally-active nicotine antagonist) but not hexamethonium (a nicotine antagonist that does not readily penetrate the central nervous system) blocked the positive reinforcing effects of nicotine suggests that this effect is centrally mediated. In addition, the failure of large doses (>1.5 mg/kg) of naloxone to alter the reinforcing effects of nicotine suggests that the endogenous opioid system may not mediate the effects.

## III. NICOTINE ACETALDEHYDE INTERACTIONS

### A. Self-Administration Studies

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.[9] In the case of (-)-nicotine and acetaldehyde, an interaction between the two compounds can be defined as a modification of the pharmacological effect of one compound by the other. We have used our self-administration technique to evaluate the behavioral interactions 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 became necessary to first measure the effect of the two compounds separately, then measure the effect of the two compounds given concurrently. It was shown that the joint effects could not be predicted from a simple additive model, which adds the effect of the first component to that of the second. The effect of the combination deviated from the prediction of the additive model, suggesting a behavioral supraadditive interaction.

The purpose of the present experiments was to determine an optimal ratio(s) of nicotine and acetaldehyde combinations that result in the enhanced positive reinforcing effects.

Nicotine was established as an intravenously delivered positive reinforcer as described in the procedure section of Section I. After stabilization in the

number of nicotine infusions (16 µg/kg/infusion) an ascending series of acetaldehyde doses was added to the nicotine solution. Acetaldehyde was increased as follows: 1.0, 2.0, 4.0, 8.0 and 16.0 µg/kg/infusion. Each dose was combined with nicotine (16.0 µg/kg/infusion) and made available to the animal for a minium of 5 days. Acetaldehyde doses were increased when the number of daily infusions stabilized. After the ascending series of acetaldehyde doses were tested nicotine doses were reduced as follows: 16.0, 8.0, 4.0, 2.0 and 1.0 µg/kg/infusion. Table 2 shows the sequence of nicotine and acetaldehyde dosing.

Table 2

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Nicotine µg/kg/infusion | 16 | 16 | 16 | 16 | 16 | 16 | 8 | 4 | 2 | 1 | 0 |
| Acetaldehyde µg/kg/infusion | 0 | 1 | 2 | 4 | 8 | 16 | 16 | 16 | 16 | 16 | 16 |

With one rat the sequence was reversed and nicotine was added to an acetaldehyde dose (reverse the sequence in Table 2).

Data is being presented for four of the eight rats tested. In the other four rats incomplete and minimal data was collected due to catheter malfunctions. In all rats however nicotine (N=7) or acetaldehyde (N=1) maintained lever pressing above vehicle control levels. Figure 8 shows the increase in the number of infusions expressed as the percent of control (nicotine 16.0 µg/kg/infusion N=3 or acetaldehyde 16.0 µg/kg/infusion N=1) as a function of increasing the concentration of either nicotine or acetaldehyde. While the data is based on only four rats and should be considered preliminary some general trends can be identified. Acetaldehyde alone maintained lever pressing at a greater rate than nicotine at equal mg/kg doses. This is consistent with other findings from this laboratory. Second, several combinations of nicotine and acetaldehyde maintains behavior above the levels of either compound when

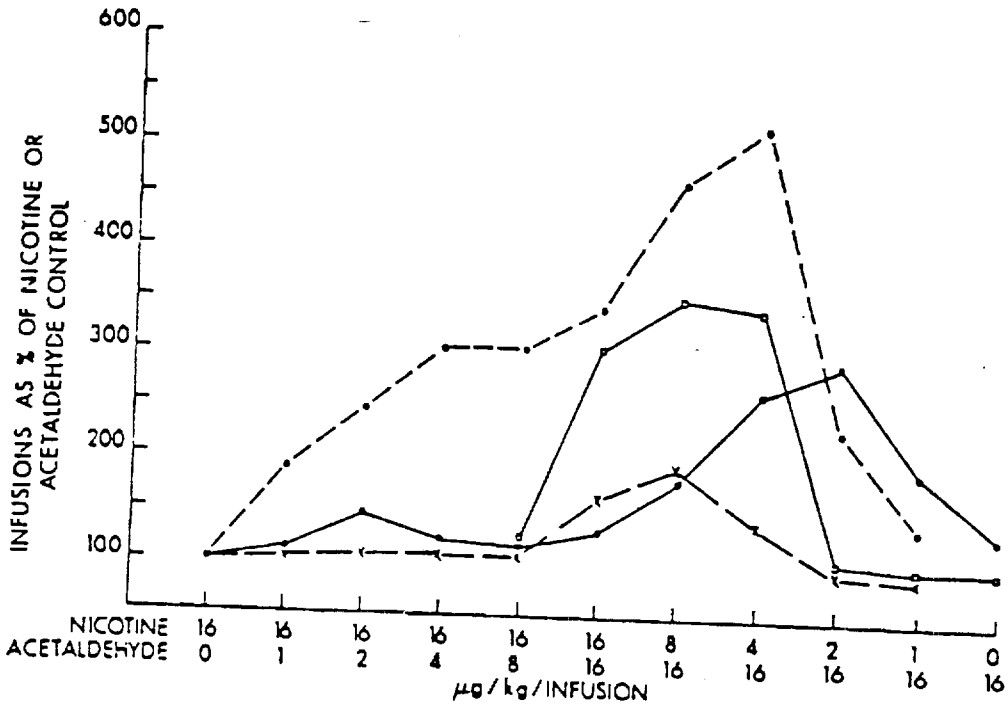



Figure 8. Infusions expressed as percent of nicotine or acetaldehyde control responding as a function of adding acetaldehyde to nicotine solutions (N=3 •----•, •——•, x--x) or adding nicotine to acetaldehyde solutions (N=1 □—□). Each point is an average of 3-5 days of stable responding for individual rats.

presented alone. Finally, at least with these four rats it appears that low doses of nicotine (2-8 µg/kg) added to acetaldehyde (16.0 µg/kg) produce optimal reinforcing effects. Note that the molecular weight of nicotine is 162.23 and acetaldehyde is 44.05.

We are currently refining our techniques and testing several additional animals to increase our confidence in this very interesting finding.

B. Discrimination Studies

The self-administration data clearly shows a behavioral interaction between nicotine and acetaldehyde. The purpose of the present study is to determine if the interaction between nicotine and acetaldehyde is specific to a self-administration situation or extends to other behavioral tests.

Acetaldehyde-Nicotine Combinations

Acetaldehyde at doses of 1.0, 5.0, 10, 15, and 25 mg/kg/sc were administered ten minutes prior to a 0.05 mg/kg nicotine injection. This dose of nicotine was shown to produce 20-30% correct responding in a discrimination paradigm. The percent nicotine correct responding was determined as a function of the acetaldehyde pretreatment dose.

Table 3 shows the effects of varying the nicotine dose on the number of correct responses. At a nicotine dose of 0.57 mg/kg eighty-six percent of the responses were nicotine correct. Nicotine doses of 0.8 mg/kg have been shown to be debilitating in the rat (unpublished observations). As the dose of nicotine was decreased (0.04 - 0.05 mg/kg) the percent correct also decreased.

The effect of the various acetaldehyde pretreatments on the percent correct nicotine responses following a 0.05 mg/kg dose of nicotine is shown in Table 4.

Table 3

Effect of various nicotine doses on the number of nicotine responses as the percent of the total number of responses in test sessions for eight rats.

| NICOTINE DOSE (mg/kg/sc) | % NICOTINE RESPONSES ± SE (Nicotine Responses/Total x 100) |
|---|---|
| 0.57 | 86 ± 6.1 |
| 0.40 | 96 ± 1.0 |
| 0.20 | 95 ± 2.1 |
| 0.10 | 37 ± 4.1 |
| 0.05 | 25 ± 7.1 |

Table 4

Percent Nicotine correct responding as a function of various pretreatments doses of acetaldehyde.

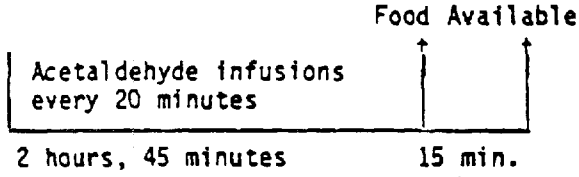
| TREATMENT | % NICOTINE RESPONSES ± SE (Nicotine Responses/Total x 100) |
|---|---|
| Nicotine 0.05 mg/kg/sc + Acetaldehyde | 25 ± 7.1 |
| 1.0 mg/kg/sc | 28 ± 4.1 |
| 5.0 | 27 ± 4.1 |
| 10.0 | 31 ± 6.8 |
| 15.0 | 29 ± 3.0 |
| 20.0 | 29 ± 5.2 |
| 25.0* | 35 ± 5.1 |

*Three animals showed a marked sedation following this dose.

It appears that the pretreatment with the various doses of acetaldehyde did not improve the nicotine discrimination. In addition, it also appears that the pretreatment did not reduce the effectiveness of this nicotine dose (0.05 mg/kg/sc).

mg/kg/day). Following twenty days of chronic acetaldehyde administration saline was substituted for 3 days and the animal observed as previously mentioned.

A second, more sensitive behavioral procedure[2,21,22] was used with four additional rats. First, the rats were trained to lever press under a fixed ratio 32 (FR 32) schedule for a 45 mg food pellet. The rats were then placed in an operant chamber in which sessions lasted 24 hours. Acetaldehyde was infused every 20 minutes. Food was available under an FR 32 schedule for 15 minutes every 2 hours and 45 minutes (see below).




This sequence was repeated 8 times each day. This procedure allows multiple samples of behavior within 24 hour periods that have been previously shown to be sensitive to drug withdrawal. The acetaldehyde dosing schedule is shown in Table 5:

TABLE 5

The number of rats at each condition and the number of days at each dose of acetaldehyde

| Number of Rats | Condition | Number of Days | Dose mg/kg/day |
|---|---|---|---|
| 4 | Saline | 3 | 0 |
| 4 | Acetaldehyde | 10 | 216 |
| 4 | Saline | 3 | 0 |
| 4 | Acetaldehyde | 20 | 216 |
| 4 | Saline | 5 | 0 |
| 2 | Acetaldehyde | 35 | 216 |
| 2 | Saline | 5 | 0 |
| 2 | Acetaldehyde | 10 | 432 |
| 2 | Saline | 5 | 0 |
| 2 | Acetaldehyde | 10 | 664 |
| 2 | Saline | 5 | 0 |

## RESULTS AND DISCUSSION

Table 6 shows the data collected from an individual rat that was chronically infused with acetaldehyde.

Table 6

Food intake and activity scores are shown as a function of chronic acetaldehyde administration and saline substitution.

Acetaldehyde (216/mg/kg)

Food Intake

10 Days — Low 23.1g — High 33.5g — $\bar{X}$ = 28.1g ± 1.42g

Activity

Low 5 — High 38 — $\bar{X}$ = 12.1 ± 2.0

Saline Substitution

| 2 Days | | Day 1 | Day 2 |
|---|---|---|---|
| | Food Intake | 31.0g | 30.1g |
| | Activity | 15 | 5 |

Both food intake and the activity scores obtained during the two saline substitution days were not different from those obtained during the chronic acetaldehyde dosing phase. A common characteristic of a withdrawal syndrome is the loss of appetite, which was not evident in these rats, suggesting that termination of chronic acetaldehyde administration does not result in a withdrawal syndrone. In addition, observations by three individuals failed to detect any overt signs of a withdrawal syndrome.

In the second attempt to produce physiological dependence upon acetaldehyde, the unit dose of this rat was increased from 3.0 to 6.0 mg/kg and the

chronic dosing extended to 20 days. Table 7 shows the data collected from this phase.

Table 7

Food intake and activity scores are shown as a function of chronic acetaldehyde administration and saline substitution.

Acetaldehyde (216/mg/kg)

Food Intake

20 Days Low 28.7g High 38.0g $\bar{X}$ = 39.1g ± 0.5g

Activity

Low 2 High 35 $\bar{X}$ = 7.0 ± 0.88

Saline Substitution

| 2 Days | Food Intake | Day 1 | Day 2 |
|---|---|---|---|
| | | 32.3g | 31.0g |
| | Activity | | |
| | Low 3 | High 20 | |

As with the previous dosing regimen there were no major changes in the food consumed or the activity scores. Further periodic observations failed to reveal any signs of a withdrawal syndrome.

The results from the second procedure are summarized in Table 8. Since we did not find any changes between 15 minute food access periods we have combined the data within 24 hour periods. In addition, the data collected from the individual rats were also combined.

Table 8

Food responses, total responses and food deliveries are shown as a function of saline or acetaldehyde dosing conditions.

| N=4 | CONDITION | NUMBER OF DAYS | FOOD RESPONSES DURING THE 8-15 MINUTE PERIODS | TOTAL RESPONSES IN 24 HOURS | FOOD DELIVERIES |
|---|---|---|---|---|---|
| | Saline | 3 | 11,629 ± 497.8 | 12,175 ± 480.0 | 355.7 ± 15.8 |
| → | Acetaldehyde 216 Mg/Kg/day | 10 | 11,745 ± 335.5 | 12,184 ± 382.5 | 361.0 ± 10.9 |
| | Saline | 3 | 11,243 ± 277.7 | 11,729 ± 225.0 | 351.2 ± 7.5 |
| | Acetaldehyde 216 Mg/Kg/day | 20 | 10,968 ± 166.9 | 11,212 ± 195.5 | 340.0 ± 10.2 |
| | Saline | 5 | 11,323 ± 288.3 | 11,458 ± 230.5 | 347.5 ± 9.1 |
| N=2 | Acetaldehyde 216 Mg/Kg/day | 35 | 13,465 ± 369.0 | 15,569 ± 317.0 | 425.5 ± 7.2 |
| | Saline | 5 | 13,013 ± 337.0 | 13,305 ± 522.0 | 406 ± 11.4 |
| → | Acetaldehyde 432 Mg/Kg/day | 10 | 14,069 ± 311.0 | 14,374 ± 347 | 433.5 ± 8.1 |
| | Saline | .5 | 13,792 ± 530.5 | 14,312 ± 577.5 | 428 ± 16.8 |
| → | Acetaldehyde 664 Mg/Kg/day | 10 | 12,656 ± 386 | 14,035 ± 513 | 424.5 ± 12.5 |
| | Saline | 5 | 13,624 ± 399.5 | 14,634 ± 288 | 424.5 ± 10.9 |

± standard error of the mean

The table shows that there were no consistent changes in the number of lever presses or food intake as a consequence of chronic acetaldehyde infusions or their termination. The failure to find any disruption in food reinforced lever pressing when chronic acetaldehyde was terminated across several doses and across several dosing schedules is strong evidence that chronic acetaldehyde intake does not result in a physiological dependence. In addition, this data, combined with previous data [20] shows that acetaldehyde does not interact with an endogenous opioid system.

## V. TOLERANCE TO CHRONIC NICOTINE ADMINISTRATION: BEHAVIORAL VS. METABOLIC FACTORS (Written by Paul C. Mele)

It is well documented that tolerance develops to many of the behavioral and physiological effects of nicotine following its repeated administration in laboratory animals.[23] At the present time, however, it is unclear whether the development of tolerance to the behavioral effects of nicotine is due to altered concentrations of nicotine at the receptor (pharmacokinetic tolerance), to altered sensitivity of nicotine receptors (pharmacodynamic tolerance), to certain behavioral or environmental factors, or to some combination of the above.[24,25]

In order to examine the development of tolerance to a variety of compounds, several studies have used a procedure designed to separate the influence of behavioral from pharmacokinetic and/or pharmacodynamic factors.[26,27,28,29,31,32,33] This procedure involves the chronic dosing of different groups of subjects either before or after the experimental session. The test performance of the group dosed before the session is altered by the compound, whereas the performance of the group dosed after the session is not altered. Once tolerance develops in the group dosed before testing, the group dosed after testing is administered the compound presession as a test for tolerance. If the before group is found to be more tolerant than the after group, then it is implied that factors arising from the disruption of the test performance by the compound (and not the mere repeated administration of the compound) were instrumental in determining the extent to which tolerance developed.

The present study used the before/after dosing paradigm to investigate whether behavioral factors may be involved in the development of tolerance to nicotine. The fixed ratio 32 (FR 32) schedule of food reinforcement was used

to generate and maintain a behavioral baseline in rats for the investigation of nicotine tolerance. Under this schedule, 32 responses (lever presses) are required for the delivery of one reinforcer (a 45 mg food pellet), and stable day-to-day responding is maintained over several months. The doses of nicotine used in this study were 0.05 - 1.60 mg/kg administered subcutaneously. These doses are higher than those mentioned in previous sections of this report due to differences in the route of administration.

## RESULTS AND DISCUSSION

### Initial Nicotine Dose-Effect Function

Under the FR 32 food schedule all rats showed rates and patterns of responding similar to those reported previously.[46] Determination of the initial nicotine dose-effect functions (0.05-0.8 mg/kg) involved the administration of nicotine before the test session to both groups of rats. Nicotine was administered only once per week during this phase of the study. The initial dose-effect functions were used to compare the effects of nicotine in both groups of rats before the chronic before/after dosing regimen was begun. Figure 9 shows the initial dose-effect functions (filled circles) for the effects of (-)-nicotine on response rates. Nicotine decreased response rates averaged over the total 30 minute session (upper panels) and response rates during the first six minutes of the session (lower panels) in a dose-dependent manner. Reductions in response rates were most pronounced during the first six minutes of the session, indicating that some recovery occurred during the latter portion of the session. The dose-effect functions for the before and after groups were similar.

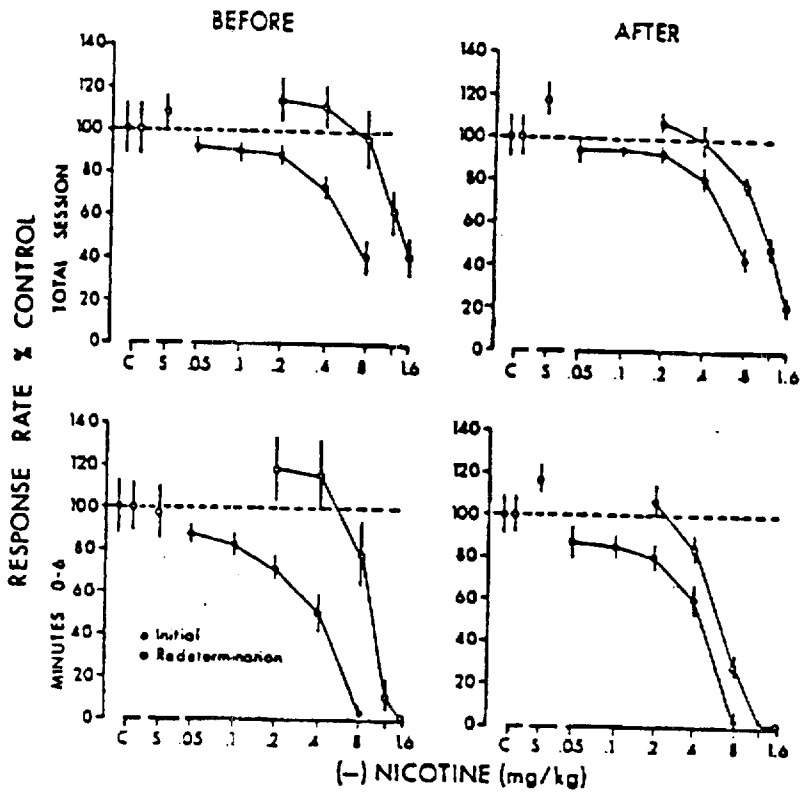



Figure 9. Effects of nicotine on FR 32 response rates during the total 30 minute session (top) and during the first six minutes of the session (bottom). The initial dose-effect functions were determined before chronic nicotine dosing. The redetermined dose-effect functions were determined beginning after 45 days of chronic nicotine dosing. The before group (left side) received nicotine chronically before the session. The after group (right side) received nicotine chronically after the session. Each point represents the mean of seven rats; vertical lines indicate 1 S.E.. Points above C represent control responding; the mean is indicated by 100% on the ordinate. Points above S indicate saline administered twice presession as part of the redetermined dose-effect functions.

Nicotine Tolerance Following Chronic Administration

Figure 10 shows the effects of chronic daily dosing with 0.8 mg/kg of (-)-nicotine. The reductions in response rates in the before group on day 1 of chronic dosing were similar to those obtained initially (c.f. Figure 9). The magnitude of the response rate reductions decreased over sessions of chronic dosing (i.e., tolerance). Responding in the after group was not affected by chronic nicotine. On day 31 when both groups received 0.8 mg/kg before the session, response rates in the after group (both overall session rates and rates during minutes 0-6 of the session) were decreased to a significantly greater degree than in the before group (Student's t-tests, $p<.05$). Thus, after 30 days of chronic dosing the before group was more tolerant to nicotine than the after group.

To determine whether tolerance had also developed to some degree in the after group, the effects of (-)-nicotine on day 31 were compared to those obtained during the initial dose-effect determinations. The reductions in response rates obtained on day 31 of chronic dosing in the after group were slightly though significantly ($p<.05$) smaller than the reductions observed initially. This indicates that some tolerance to (-)-nicotine also developed with chronic postsession administration. As mentioned above, however, significantly less tolerance developed in the after group than in the before group.

Redetermination of Nicotine Dose-Effect Functions Following the Development of Tolerance

The nicotine dose-effect functions determined during chronic dosing are presented in Figure 9 (open circles). Compared to the initial dose-effect functions (filled circles), response rates in both groups of rats were decreased less by all doses of nicotine after chronic dosing. Attenuated rate

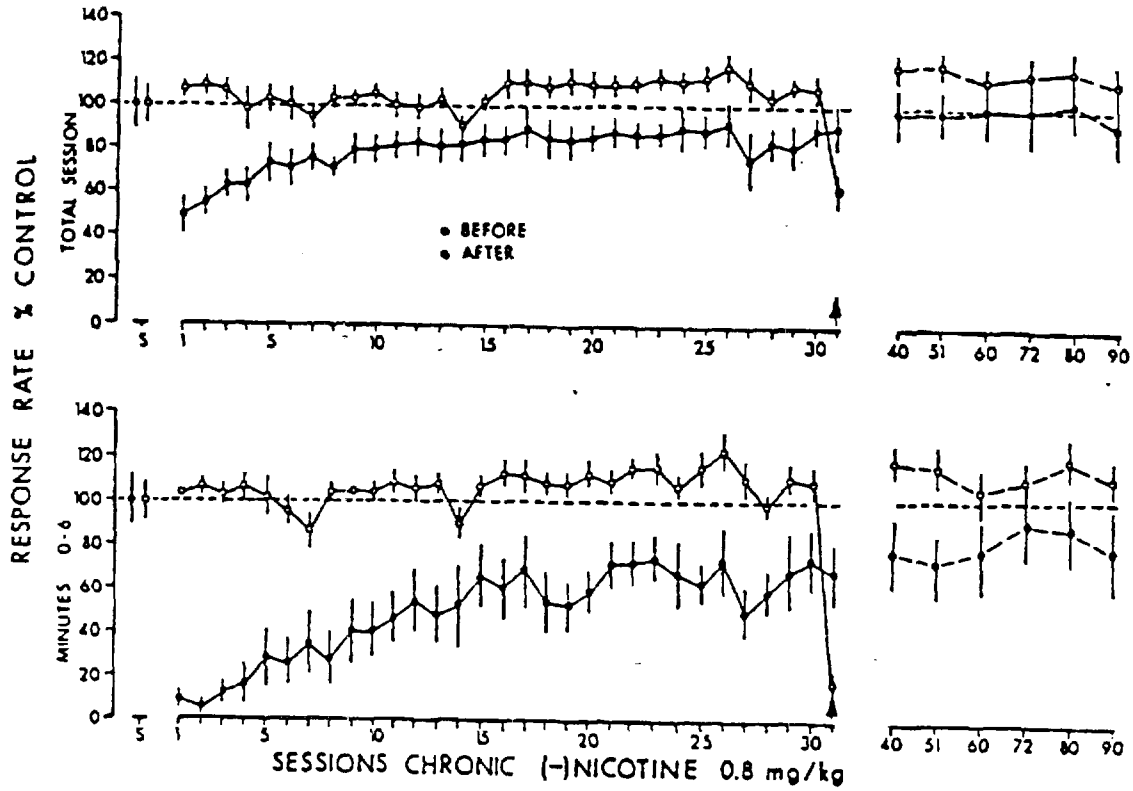



Figure 10. Effects of daily administration of 0.8 mg/kg of nicotine on FR 32 response rates during the total 30 minute session (top) and during the first six minutes of the session (bottom). The before group received nicotine before and saline after the session; the after group received saline before and nicotine after the session. On day 31 both groups received nicotine before and saline after the session. Each point is based upon the data from seven rats; vertical lines indicate 1 S.E.. Points above S represent saline control responding; the mean is indicated by 100% on the ordinate. Saline control points are the mean of five consecutive saline sessions which immediately preceeded chronic nicotine administration.

decreases were greater in the before group than in the after group; this difference was most pronounced at the 0.8 mg/kg dose for rates during minutes 0-6 of the session. Two additional higher doses of nicotine (1.2 and 1.6 mg/kg) decreased overall response rates to a lesser degree in the before group than in the after group. Substituting saline (open circles labelled S) for the usual presession administration of 0.8 mg/kg of nicotine in the before group produced response rates similar to control values. These results indicate that there was a greater shift to the right in the dose-effect functions for the before group than for the after group, further suggesting that the development of tolerance to nicotine was enhanced by factors arising from the nicotine-induced disruption of responding.

Persistence of Tolerance to Nicotine

The persistence of tolerance as determined by weekly administrations of 0.8 mg/kg of nicotine following the cessation of chronic dosing is shown in Figure 11. Response rates in the before group were reduced less than in the after group by nicotine during weeks 1 and 2. By week 3 response rates were reduced to a similar degree in the two groups by nicotine. Since both groups exhibited a small loss of tolerance each week over weeks 1 to 3, it was necessary for the before group to show a greater loss of tolerance than the after group in order for both groups to respond similarly to nicotine during week 3. Response rates were similarly or slightly less affected by nicotine delivery during week 4 compared to week 3, suggesting that a stable level of responding had been achieved with weekly nicotine administrations. Response rates were reduced less by 0.8 mg/kg of nicotine during weeks 3 and 4 than during the initial dose-effect determinations (circles), suggesting that some degree of tolerance persisted in each group.

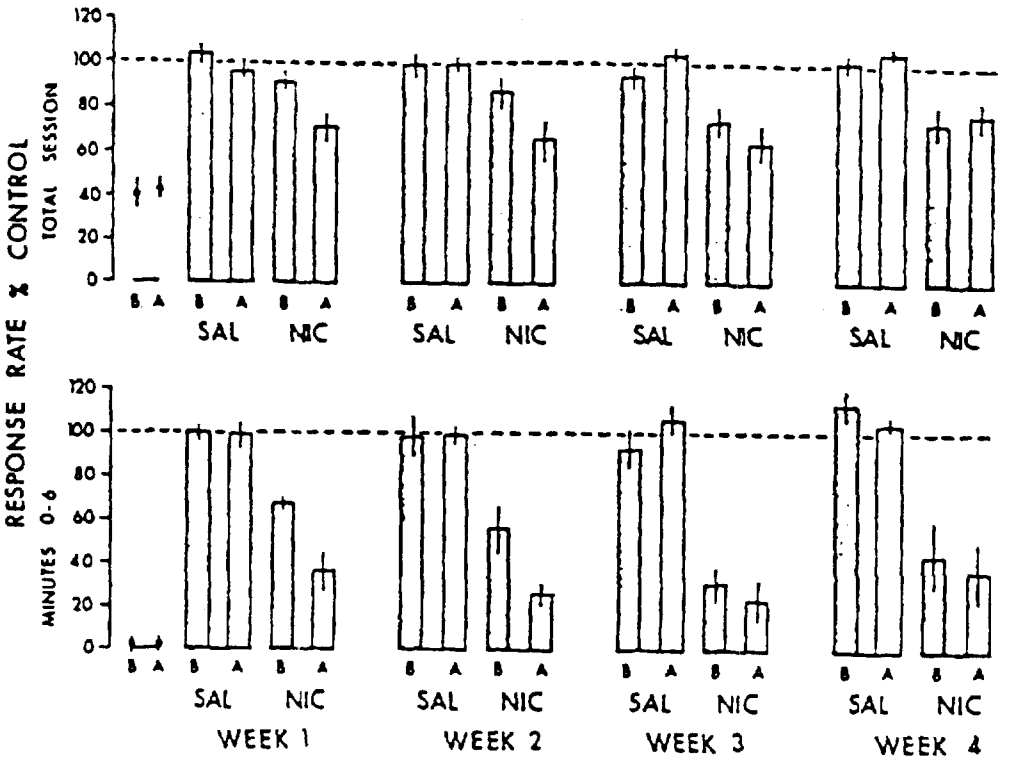



Figure 11. Persistence of tolerance to the effects of 0.8 mg/kg of nicotine on FR 32 response rates following cessation of chronic dosing. Response rates are from the total 30 minute session (top) and from the first six minutes of the session (bottom). The before group (B) had received nicotine chronically before the session; the after group (A) had received nicotine chronically after the session. The weekly testing sequence was baseline (Wednesday), saline (Thursday) and nicotine (Friday). Data are as percentage of respective control mean, indicated by 100% on the ordinate. Control for the saline (SAL) was the preceeding baseline session. Control for nicotine (NIC) was the mean of the preceeding baseline and saline sessions. The points to the left indicate the initial acute effects of 0.8 mg/kg of nicotine. Vertical lines indicate 1 S.E.

The present results confirm the findings of previous studies which demonstrated that tolerance develops to the behavioral effects of nicotine following chronic administration,[34,35,36] including tolerance to the disruption of FR responding.[37,38,39]

The major finding of the present study was that nicotine reduced response rates to a significantly smaller degree following chronic administration in the before group than in the after group. Thus, tolerance to nicotine developed to a significantly greater degree in the before group than in the after group. Since both groups of rats received exactly the same quantity of nicotine on a daily basis, the development of tolerance to nicotine appears to be highly dependent on factors arising from the nicotine-induced disruption of FR responding, rather than on factors resulting from the mere repeated administration of nicotine.

The enhanced tolerance which developed in the before group has been referred to as behavioral tolerance.[24,25] It is presently unclear which specific factors mediate the development of behavioral tolerance. However, the present results are consistent with the hypothesis that tolerance occurs more readily when a compound decreases the number of reinforcers obtained (as in the before group), as opposed to when reinforcement frequency is not altered (as in the after group) by the compound.[25]

The tolerance to nicotine reported here was not only dependent on behavioral factors, however, since tolerance also developed to a small degree in the after group. This finding suggests that the development of tolerance to nicotine under the conditions used here is a two component process. The major component evident only in the before group involves a behavioral adaptation of the organism to the disruptive effects of nicotine on schedule-controlled responding (behavioral tolerance). A secondary and relatively minor component

of tolerance to the effects of nicotine on FR responding arises following the mere repeated administration of nicotine. Although it may be that this secondary component of nicotine tolerance reflects pharmacokinetic and/or pharmacodynamic mechanisms, definitive conclusions must await appropriate experimental support.

VI. CROSS TOLERANCE BETWEEN ISOMERS OF NICOTINE (Written by Paul C. Mele)

Although the naturally occurring (-) isomer of nicotine has received considerable attention in the behavioral pharmacology literature, the unnatural (+) isomer has been infrequently studied.

The data in the present report are from the initial phase of an investigation designed to make qualitative and quantitative comparisons between the optically pure stereoisomers of nicotine on a food-maintained behavioral baseline. The behavior under study is the fixed-ratio 32 (FR 32) schedule performance described in Section V of this report. This study will also provide data on the relative contribution of central and peripheral nicotinic receptors underlying the behavioral effects of the stereoisomers of nicotine. The involvement of muscarinic-cholinergic receptors will also be examined. These data are important because of reports that (-)-and (+)-nicotine differ qualitatively as well as quantitatively in their peripheral effects, and that some of the effects of the (+)-isomer are not mediated via nicotinic receptors.[40] In addition, since the (+)-isomer of nicotine may be viewed as the prototypical analogue of (-)-nicotine, it is of interest to determine if FR performance provides a useful higher-order procedure for evaluation of nicotine analogues.

RESULTS AND DISCUSSION

Responding under the FR 32 food schedule was consistent with the previous studies from this laboratory (see Section V). Both the (-) and (+)-isomers of nicotine failed to alter response rates at lower doses and produced dose-dependent decreases in rates at higher doses (Figure 12). Comparison between overall session response rates (filled circles) and response rates during the first six minutes of the 30 min. session (open circles) revealed that both isomers reduced the latter rates at lower doses; this selective effect was more

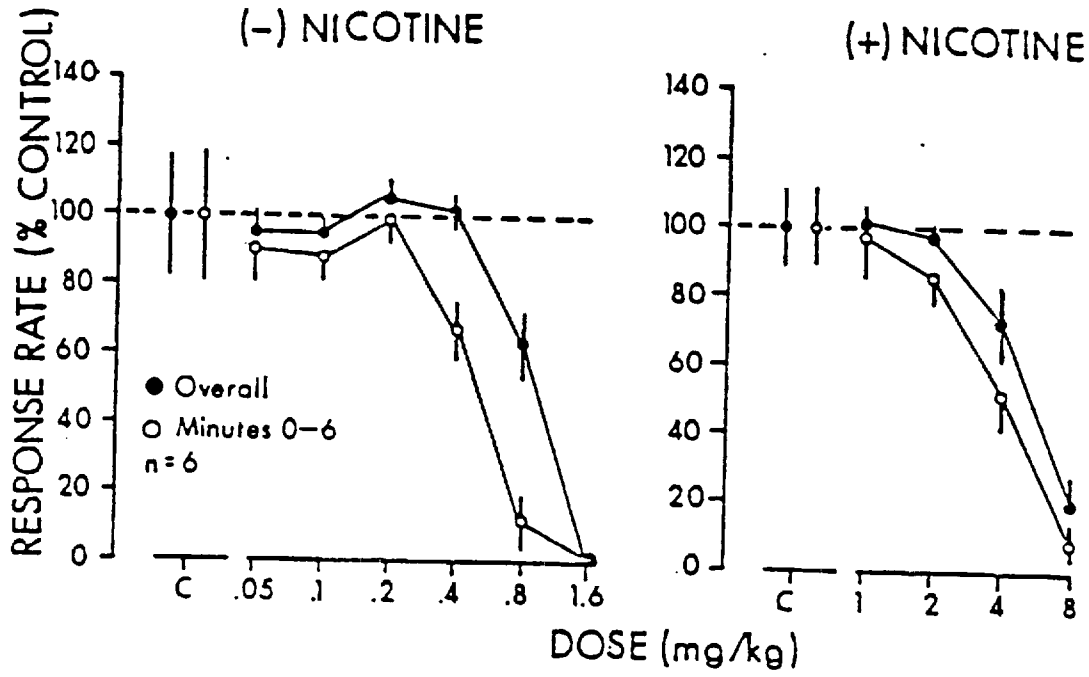



Figure 12. Effects of (-)- and (+)-nicotine on FR 32 responding during the total 30 minute session (filled circles) and during the first six minutes of the session (open circles). Each point represents the mean of six rats; verticle lines indicate 1 S.E. Points above C represent control responding; the mean is indicated by 100% on the ordinate. Each dose of nicotine was administered two to four times to each rat.

pronounced with (-)-nicotine than with (+)-nicotine. Time course data and cumulative records confirmed that minutes 0-6 were the time of maximal effect. The latencies to complete the first ratio are presented in Table 9. Each isomer increased latencies in a dose-related fashion.

The dose required to reduce baseline rates of responding by 50% ($ED_{50}$) was estimated for each isomer using linear regression on the descending portions of the dose-effect functions shown in Figure 12. For overall response rates, the estimated $ED_{50}$'s for (-)-nicotine and (+)-nicotine, respectively, were 0.99 and 5.69 mg/kg. These $ED_{50}$'s resulted in a (+)-nicotine/(-)-nicotine potency ratio of 5.75. For response rates during minutes 0-6 of the session, the estimated $ED_{50}$'s were 0.53 and 4.64 mg/kg for (-)-nicotine and (+)-nicotine, respectively. These $ED_{50}$'s resulted in a potency ratio of 8.75. The (+)-nicotine/(-)-nicotine potency ratios determined here are similar to the ratios of 7 and 9 reported for conditioned avoidance[41] and nicotine discrimination tests.[42] Thus, at this time our results indicate that the optically pure isomers of nicotine differ primarily in potency. It may also be, however, that the differences in the dose-effect functions between the isomers for reductions in overall session response rates and for response rates occurring early in the session indicate a true qualitative difference. Further data analysis is required to determine if this is the case. Furthermore, the more specific comparisons involving receptor blockade and tolerance - cross tolerance procedures should be highly informative in this regard.

Table 9

Latencies in Seconds to Complete the First Fixed-Ratio (Mean ± SEM)

(-)-NICOTINE (mg/kg)

| n | Control | 0.05 | 0.10 | 0.20 | 0.40 | 0.80 | 1.60 |
|---|---|---|---|---|---|---|---|
| 6 | 19.1 ±2.4 | 34.1 ±8.7 | 37.4 ±5.3 | 36.5 ±5.5 | 98.5 ±26.2 | 529.8 ±117.2 | 1767.1 ± 32.9 |

(+)-NICOTINE (mg/kg)

| n | Control | 1.00 | 2.00 | 4.00 | 8.00 |
|---|---|---|---|---|---|
| 6 | 19.2 ±5.9 | 19.8 ±3.3 | 29.5 ±5.4 | 266.3 ±149.0 | 1163.76 ±247.2 |

## VII. EXAMINATIONS OF BEHAVIORAL SUPERSENSITIVITY FOLLOWING CHRONIC BLOCKADE OF NICOTINE RECEPTORS IN RATS (Written by Paul C. Mele)

Chronic blockade of postsynaptic receptors may produce an increase in the number of these receptors (up-regulation) as well as an enhanced response to receptor agonists (supersensitivity). Up-regulation and/or supersensitivity have been demonstrated for dopaminergic, beta-adrenergic, serotonergic, muscarinic-cholinergic and gamma-aminobutyric acid receptors in the central nervous system (CNS).[43,44] Peripheral nicotinic-cholinergic receptors at the neuromuscular junction have also been shown to undergo up-regulation and supersensitivity. Following surgical denervation, an increased contractability of muscle to locally applied acetylcholine was noted; a proliferation of nicotinic receptors over the muscle surface was correlated with the development of supersensitivity.[45]

Since there are no published studies which have demonstrated nicotinic receptor up-regulation and supersensitivity in the CNS, a preliminary study was conducted to address this issue. The behavioral baseline used was FR 32 responding (lever pressing maintained by food reinforcement) as described in Section V. The behavioral response to nicotine used to examine supersensitivity was the prostration syndrome produced by nicotine infusion directly into the left lateral ventricle of the brain.[3]

### RESULTS AND DISCUSSION

All rats had rates and patterns of responding characteristic of FR reinforcement schedules.[46] Food pellet delivery was followed by a short pause in lever pressing and then by a high response rate that was sustained until the ratio was completed and the next reinforcer delivered. Latencies to complete

the first ratio under baseline (non-infusion) conditions were similar to those observed previously in this laboratory. Saline control infusions produced no consistent changes in latencies (Tables 10 and 11).

In rats SS3 and SS4, 5 µg of nicotine infused into the lateral ventricle produced large increases in the latency to complete the first ratio (Table 10). Chronic mecamylamine (1.5 mg/kg, administered twice per day for 22 days) did not alter latencies. Twenty-four and 72 hours following the cessation of chronic mecamylamine, 5 µg of nicotine increased latencies. In each rat the latency to complete the first ratio was shorter at 24 hours than observed initially. At 72 hours latencies approximated those obtained with 5 µg of nicotine initially. The baseline sessions occurring 48 hours post mecamylamine revealed latencies similar to initial baseline values.

Chronic mecamylamine dosing (1.5 mg/kg, twice per day) was reinstated three days after the 72 hour nicotine retest; dosing was continued for 17 additional days. Latencies were increased by 5 µg of nicotine 24 hours, 72 hours and 384 hours (16 days) postmecamylamine (the 24 hour data for rat SS3 were lost due to an unsuccessful infusion). Rat SS3 showed replication of the initial response to nicotine, while the latencies of rat SS4 were somewhat shorter than observed initially.

Dose-dependent increases in the latency to complete the first ratio were obtained with 2.5 and 5 µg of nicotine in rats SS1 and SS2 (Table 11). Mecamylamine was administered chronically to these rats at double the previous dose and for a longer duration (3.0 mg/kg administered twice per day for 26 days). In rat SS1, 2.5 µg of nicotine increased the latency to a similar degree as observed initially when tested 24 hours, 72 hours and 96 hours after mecamylamine dosing. In rat SS2, 2.5 µg of nicotine did not increase the latency

Table 10

Latency in Seconds to Complete the First Fixed-Ratio

| Sequential Conditions | RAT SS3 | RAT SS4 |
|---|---|---|
| Baseline[a] | 22.7 ± 2.9 | 22.9 ± 3.6 |
| Saline | 4.9 | 33.8 |
| Nic 5 µg | 394.5 | 508.9 |
| Mecamylamine[b] | 29.8 ± 3.3 | 24.6 ± 8.1 |
| Nic 5 µg - 24 hrs. | 246.9 | 312.9 |
| Baseline - 48 hrs. | 21.5 | 15.9 |
| Nic 5 µg - 72 hrs. | 332.0 | 427.0 |
| mecamylamine[c] | 58.8 ± 7.3 | 32.3 ± 10.7 |
| Nic 5 µg - 24 hrs. | --- | 313.4 |
| Baseline - 48 hrs. | 36.3 | 29.2 |
| Nic 5 µg - 72 hrs. | 423.1 | 355.7 |
| Nic 5 µg - 384 hrs (16 days) | 388.8 | 345.2 |

[a] Mean ± SEM of nine sessions.

[b] Mean ± SEM of last three sessions of chronic mecamylamine dosing: 1.5 mg/kg twice/day for 22 days.

[c] Mean ± SEM of last three sessions of chronic mecamylamine dosing: 1.5 mg/kg twice/day for 17 days.

Table 11

Latency in Seconds to Complete the First Fixed-Ratio

| Sequential Conditions | RAT SS1 | RAT SS2 |
|---|---|---|
| Baseline[a] | 39.2 ± 6.4 | 44.3 ± 7.8 |
| Saline | 34.5 | 35.7 |
| Nic 5 µg | 772.0 | 407.7 |
| Nic 2.5 µg | 499.0 | 150.6 |
| Mecamylamine[b] | 57.0 ± 15.1 | 30.2 ± 7.3 |
| Nic 2.5 µg - 24 hrs. | 566.0 | 19.6 |
| Baseline - 48 hrs. | 25.4 | 30.1 |
| Nic 2.5 µg - 72 hrs. | 371.4 | 52.9 |
| Nic 2.5 µg - 96 hrs. | 471.7 | 20.5 |

[a] Mean ± SEM of nine sessions.

[c] Mean ± SEM of last three sessions of chronic mecamylamine dosing: 3.0 mg/kg twice/day for 26 days.

beyond control values at any time following chronic mecamylamine. Higher doses of nicotine (5 - 20 μg) administered over the next several weeks also failed to increase the latency. It is unclear why there was a complete loss of response to nicotine in this rat. Since similar failures have been observed occasionally in other studies, it may be that this is due to a lack of patency of the cannula that develops several weeks or months after implantation.

Chronic mecamylamine treatment failed to produce an enhanced behavioral response to nicotine. This suggests that under the conditions of the present study, chronic blockade of central nicotinic receptors did not result in an up-regulation that was detected at the behavioral level. This result was unexpected considering that each of the classic neurotransmitter systems have been demonstrated to develop up-regulation and/or supersensitivity following chronic functional inactivation.[43] Nicotine, however, is not naturally present in the CNS and therefore is not a naturally occurring neurotransmitter. Since recent evidence also suggests that the central nicotinic receptor mediating the prostration syndrome may be of a different type than the peripheral nicotinic-cholinergic receptor which has been shown to mediate supersensitivity in muscle,[47] it is premature to predict that the central nicotinic receptor responds to chronic blockade as do established neurotransmitter receptors.

Other data, however, indicate that central nicotinic receptors are modifiable following chronic agonist treatment. Falkeborn et al.[48] reported that tolerance developed to some of the behavioral effects of nicotine in rats following chronic treatment, and that tolerance development was correlated with a decreased number of $^{3}$H-tubocurarine-labelled binding sites (this is down-regulation of receptors resulting in a subsensitivity to receptor agonist). One implication of these data is that the population of nicotinic receptors

involved in tolerance may be different from the population involved in prostration. It may also be that central nicotinic receptors are more susceptible to down-regulation than to up-regulation.

We are currently extending our investigation of the effects of chronic nicotinic receptor blockade on the behavioral response to nicotine. Studies either currently in progress or planned involve the systematic manipulation of the dose, duration and type of receptor blocker administered, the dose and route of administration of nicotine, and the behavioral dependent variable. These studies will aid in the delineation of the functional significance of central nicotinic receptors in a variety of behaviors.

## VIII. BRAIN SITES INVOLVED IN THE MEDIATION OF THE BEHAVIORAL EFFECTS OF INVRAVENTRICULARLY ADMINISTERED (-)-NICOTINE

Physiological studies have shown that nicotine receptors are widely distributed in the brain.[49,50] The distribution or density of those receptors has been shown to be differential and relatively concentrated in the cortex, hypothalamus, hippocampus, and thalamus.[51,52] In addition, neurophysiological studies[53,54] have shown that nicotine has major effects on the reticular formation (RF) and moderate effects on dorsal hippocampus (DH) neurons. Behavioral studies have shown both the DH and RF sites are partially involved in the ability of nicotine to exert stimulus control of behavior.[31] Rats trained to discriminate nicotine from saline showed partial generalization of a peripherally induced cue when nicotine was injected directly into the DH and RF.

Abood and co-workers[47] reported that an intraventricular (IVT) infusion of (-)-nicotine resulted in a prostration-immobilization syndrome in rats. This response was not mimicked by IVT infusions of a variety of neurotransmitters or psychotropic agents. A recent report[3] has shown that IVT administration of various doses of (-)-nicotine (0.625-10.0 μg) produces a reliable dose-related change in behavior maintained under fixed ratio (FR) schedules of food presentations. In contrast to the developing profile of the behavioral effects of IVT nicotine, there is a dearth of information about the brain sites which mediate those effects.

The purpose of the present study was to examine the effects of nicotine infused directly into various brain structures on schedule controlled behavior. A second purpose was to assess the effect of lidocaine inhibition of neural conduction in specific brain sites on the effects of IVT (-)-nicotine infusions.

## RESULTS AND DISCUSSION

### Behavioral

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. Response rates ranged from 1.30 to 2.50 lever presses per second. The latency to complete the first ratio following an IVT saline infusion was less than 48s which was not different from non-infusion values. IVT infusions of 5.0 μg (-)-nicotine increased the latency to complete the first ratio ($\bar{X}$ = 8.2 min ± 1.3, Fig. 13). In contrast to the IVT (-)-nicotine-induced changes in latency, subsequent response rates did not show any systematic changes, indicating that there was no residual effect of (-)-nicotine on the FR schedule.

When (-)-nicotine (0.25 μg) was infused directly into the DH and the locus ceruleus (LC) there was a nonsignificant increase in the latency to complete the first ratio compared to the saline control. There was no effect on the latencies obtained from animals infused with (-)-nicotine into the lateral hypothalamus (LH) and RF; however, when (-)-nicotine was infused into the vestibular nucleus (VN) a complete prostration syndrome appeared and the average latency to complete the first ratio increased to 9.5 min (± 2.0 min). In contrast, lidocaine (5.0 μg) when infused into the brain sites, had no effect on the latency to complete the first ratio nor on the session response rate (Figure 13).

The effect of a brain site infusion of lidocaine on the latency to complete the first ratio following a (-)-nicotine infusion into the lateral ventricle (LV) is shown in Figure 14. Lidocaine infusions into the DH, LC or LH had no effect on the latency. However, lidocaine infused into the RF

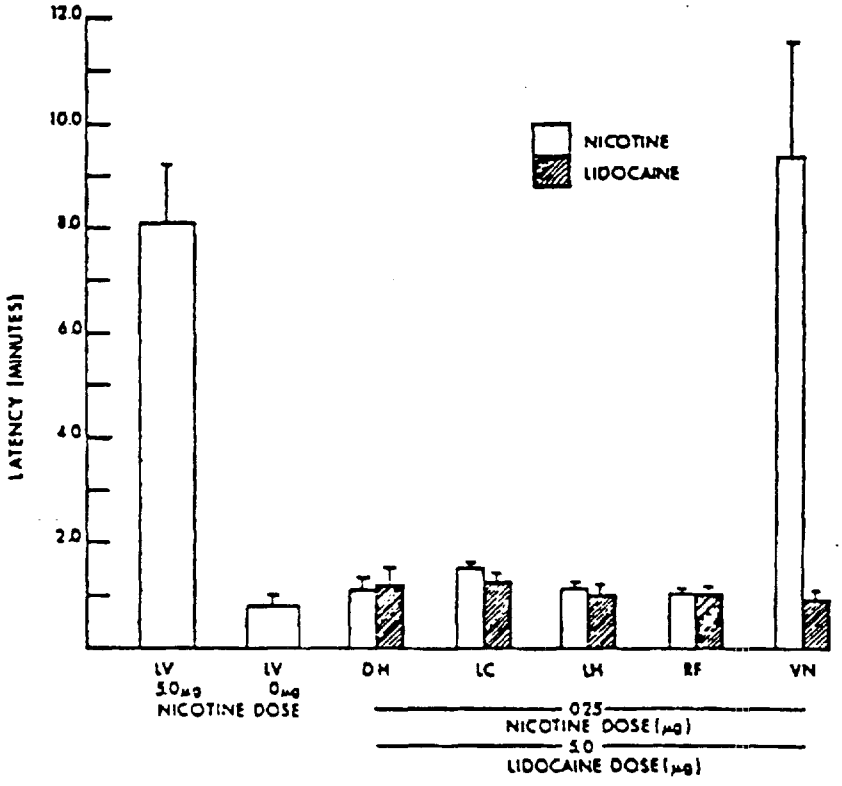



Figure 13. Effects of nicotine and lidocaine infused into the various brain structures on the average latency to complete the first ratio. The first bar represents the average latency (N=15) following a 5.0 µg infusion of nicotine into the LV. The second bar shows the average latency (N=15) following a saline infusion. The remaining bars show the average latency (N=3) following nicotine (0.25 µg, open bars) or lidocaine (5.0 µg, hatched bars) infusions into the brain sites. Vertical lines show the standard error.

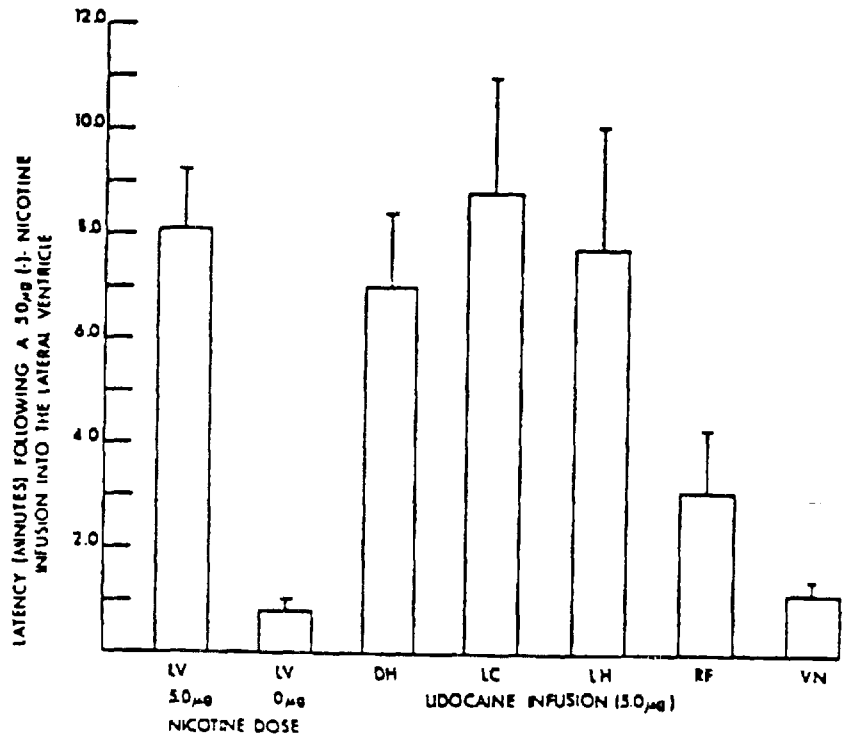



Figure 14. Attenuation by lidocaine of effects of nicotine on the latency to complete the first ratio. The first and second bar shows the average latency (N=15) following a nicotine or saline infusion respectively. The remaining bars represent the average latency (N=3) when nicotine was infused into the LV immediately following a lidocaine infusion into a brain site. Vertical bars show the standard error.

attenuated the effects of (-)-nicotine on the latency. The latency was reduced by 55%. In addition, lidocaine infused into the VN completely blocked the effects of LV (-)-nicotine. Nicotine retest values without lidocaine infusions did not differ from the original (-)-nicotine test values.

Figures 15 and 16 show representative cumulative records of individual rats for control and three infusion conditions. The effect of (-)-nicotine (5.0 μg) infused into the LV on the latency is clearly seen in both figures (top right panel). Nicotine (0.25 μg) infused into the RF had no effect on the latency (Figure 15 lower left panel); however, (-)-nicotine infusions (0.25 μg) into the VN increased the latency to equal to or greater than the values obtained when 5.0 μg of (-)-nicotine was infused into the LV (Figure 16, lower left). The attenuation of the (-)-nicotine induced latency change with inhibition of the RF and the total block following VN inhibition are shown in the bottom right panels of Figures 15 and 16 respectively.

## DISCUSSION

Responding by rats was maintained under a FR 32 schedule of food presentation. Under these conditions the duration of the effect of IVT administration (-)-nicotine was similar to that previously reported.[3] Nicotine infusions into the DH, LC, LH and the RF failed to produce any change in the ratio performance. The absence of any nicotine induced latency changes following an infusion into these brain areas suggest that the previously reported interactions of nicotine with these brain structures are not related to the effect of centrally administered nicotine on FR performance reported here. In contrast, when (-)-nicotine was infused directly into the VN the latency to complete the first ratio was equal to or greater than the latency produced by much larger doses of nicotine infused into the LV.

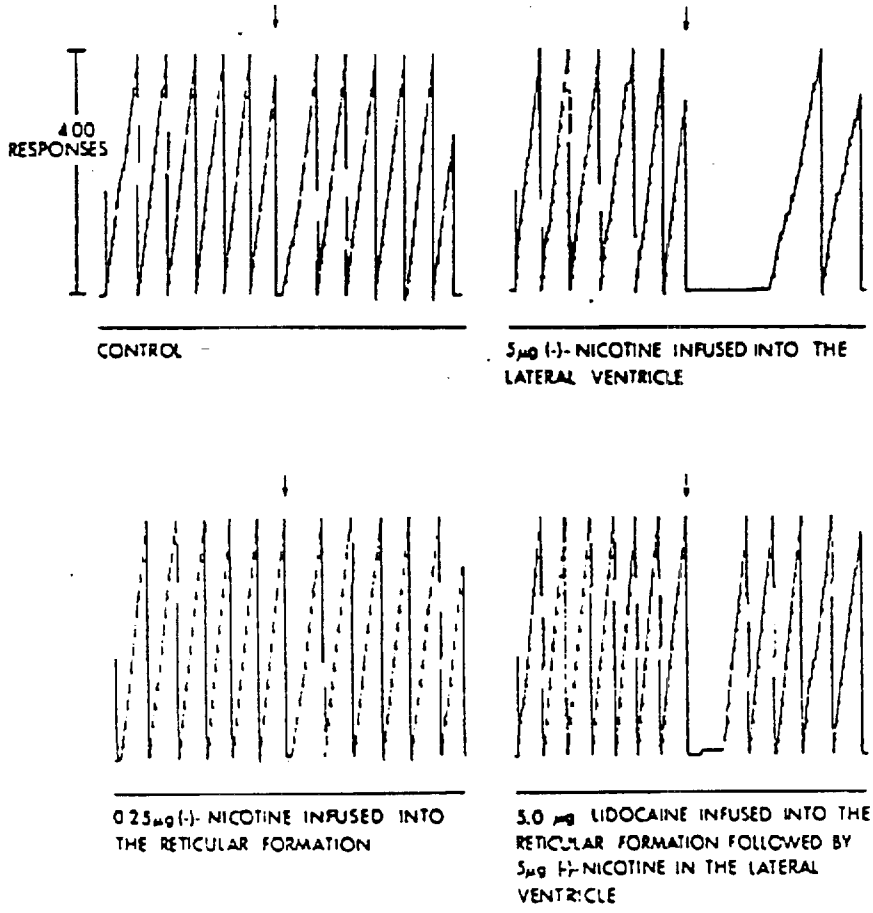



Figure 15. Cumulative records for a single rat showing four infusion conditions. The stepping pen recorded lever presses, and each downward deflection of the stepping pen indicated a pellet delivery. The stepping pen reset after 400 responses. Arrows at the top of each record indicate the end of a 15 min period. Nicotine infused into the RF did not produce a latency change (Top Left vs Bottom Left). Note the attenuation of the latency change when lidocaine was infused into the RF (Top Right vs Bottom Right).

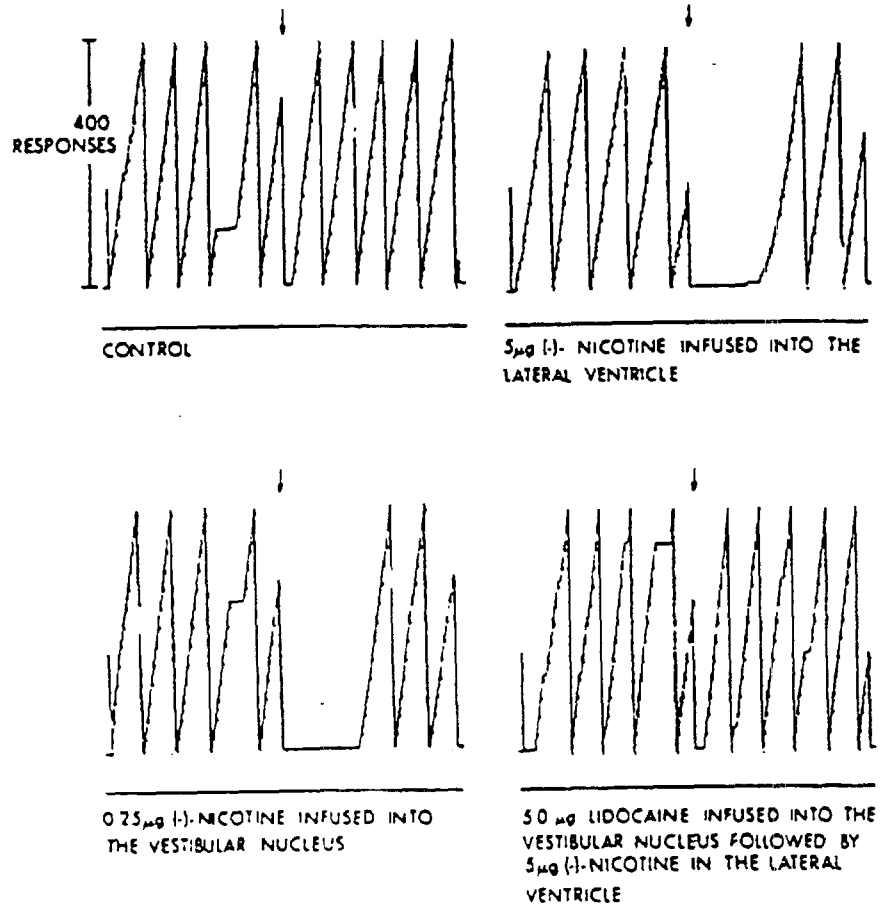



Figure 16. Cumulative records for a single rat showing four infusion conditions. For a description of the recording see Figure 15. An infusion of nicotine into the VN produced a latency change that was equal to or greater than the LV data (Top Right vs Bottom Left). Note the total block of LV nicotine effects with lidocaine infused into VN (Top Right vs Bottom Right).

## IX. ELECTROENCEPHALOGRAPHIC (EEG) ANALYSIS OF DORSAL HIPPOCAMPAL ACTIVITY IN RATS ADMINISTERED NICOTINE AND ACETALDEHYDE ALONE AND IN COMBINATION

(Written by Jennifer Horn and Paul C. Mele)

The behavioral effects of nicotine and acetaldehyde have been examined using a variety of procedures.[2,3,20] Of particular interest are the findings that both nicotine and acetaldehyde function as positive reinforcing stimuli in rats, and that the self-administration of these compounds in combination results in a potentiation of their individual reinforcing efficacies. Neurochemical and electrophysiological mechanisms underlying these phenomena, especially those involving acetaldehyde, are unknown.

To further examine the mode of action of nicotine and acetaldehyde, work was performed at the Center for Brain Research, University of Rochester, New York, February 10th and 11th. Electroencephalograms (EEGs) were recorded from the dorsal hippocampus in rats before and after administration of nicotine and acetaldehyde, singly and in combination.

The dorsal hippocampus (DH) was chosen because: 1) nicotine receptors are relatively concentrated in this brain region; 2) nicotine alters the electrical activity of DH neurons; and 3) the DH is partially involved in the ability of nicotine to exert stimulus control of behavior. Since there are no published data, to our knowledge, concerning the effects of acetaldehyde, alone or in combination with nicotine, on the EEGs of the DH or elsewhere, these studies were conducted to obtain preliminary data on these effects.

### RESULTS AND DISCUSSION

#### 1. Nicotine (0.8 mg/kg)

Rats injected (sc) with nicotine exhibited the characteristic nicotine-induced-response involving gross motor impairment (splayed-out limbs, unsteadiness, muscle flaccidity), defecation and urination. The components that define EEGs

showed a reduction in higher frequency (cycles per second), synchronization, and a decrease in amplitude (voltage) as compared to saline controls.

2. Acetaldehyde (10 mg/kg)

Three of the four rats injected (sc) with acetaldehyde appeared "quieted" as compared with non-injected rats: they sat relatively still in the corner of the test box and exhibited little locomotor/exploratory behavior. EEGs of these rats showed a reduction in higher frequency, synchronization, a decrease in amplitude and enhanced theta as compared to saline controls. Overall, the effects of acetaldehyde on EEGs were similar to those of nicotine.

3. Combination Nicotine/Acetaldehyde

As stated above, rats injected with nicotine exhibited nicotine-induced motor impairments. Five minutes following the nicotine injection, the rats were injected with acetaldehyde. Within approximately three minutes post-acetaldehyde injection, three of the four rats appeared somewhat recovered from the nicotine effect: locomotor activity improved. EEG tracings of the nicotine/acetaldehyde combination resembled the saline control pattern more closely than that of either compound alone: the reduction in higher frequency, synchronization, decrease in amplitude and enhanced theta observed with each compound alone were attenuated in the combination tracings. Visual analysis and interpretations of EEGs are preliminary. A more detailed computer analysis will also be performed.

Self-administration of nicotine and acetaldehyde in combination results in a potentiation of their individual reinforcing efficacies. The present study was designed to examine any interaction between nicotine and acetaldehyde using EEG techniques. Preliminary visual analysis following the combined administra-

tion of nicotine and acetaldehyde revealed a pattern which resembled the saline control pattern more closely than that of either compound administered alone. This indicates that nicotine and acetaldehyde interacted to produce changes in CNS function when administered in combination. This observation needs to be confirmed by computer analysis and directions for future work will depend upon the outcome of the computer analysis.

X. LOCALIZATION OF VASCULARLY ADMINISTERED $^{14}$C-ACETALDEHYDE IN RAT TISSUE
(Written by Bruce Davies, Project 6902)

Introduction

Results from experiments conducted at the University of Rochester[20] indicated that acetaldehyde, a component of smoke, readily crosses the blood-brain barrier. In addition, it was shown that the brain accumulated only 10% as much acetaldehyde as blood and that this accumulation was not dependent upon the route of administration. These results have recently been confirmed and expanded upon through experiments conducted at Philip Morris. Questions which were examined in these experiments include:

1) Does vascularly administered acetaldehyde rapidly cross the blood brain barrier?

2) How do the levels of administered acetaldehyde or its metabolites in the brain compare to those in blood as a function of time?

3) Do different areas of the brain preferentially accumulate higher amounts of acetaldehyde or its metabolites?

4) How do the levels of acetaldehyde or its metabolites in brain compare to liver, the major site of acetaldehyde metabolism?

Procedure

Small (~175 gm) hooded rats were used. At zero time 10 µl of $^{14}$C-acetaldehyde (8.0 mCi/mmol) was injected into the left ventricle of the heart. The animal preparation was then allowed to incubate for various times depending upon the conditions of the experiment. Following incubation, a 0.2 ml blood sample was collected from the right auricle. Animals were then perfused through the left ventricle with 0.9% saline for 3 minutes. The brain was removed and dissected on a petri dish maintained at 4°C, into the following sections: cerebrum, striatum, cerebellum, and brain stem. A small specimen of liver tissue was also excised and washed. In one set of experiments approxi-

mately 100 mg portions of each tissue were homogenized in 1 ml of ice cold 0.9% saline. The homogenate was centrifuged at 9000xg for 30 seconds and a sample of the supernatant removed for the determination of radioactivity. After the remaining supernatant was aspirated off, the pellet was digested overnight at 65°C in 1 ml of Protosol, and 100 µl portions were also removed for the determination of radioactivity. In a second set of experiments, the 100 mg portions of tissue (with the exception of brain stem in which the entire 230-250mg was used) were minced with scissors and the pieces digested as described above.

To determine levels of radioactivity, 100 µl aliquots of supernatant or digest were added to 10 ml of Aquasol scintillation fluid, acidified through the addition of 100 µl of glacial acetic acid, dark adapted, and counted in a Beckman beta scintillation counter (model LS-9800). Blood samples (100 µl) were diluted 1:10 with 0.9% saline and a aliquot (100 µl) digested and counted as described above.

Results

Results in Table 12 and 13 present the levels of radioactive compounds in brain tissues as picomoles of $^{14}C$ per milligram of tissue (pCi/mg). This raw data was normalized and appears in Table 14 as the ratio of $^{14}C$ containing compounds in a particular brain region in each animal compared to its own striatum.

The following summaries can be made of the data in Tables 12 and 13:

1) Vascularly administered acetaldehyde readily penetrates the blood-brain barrier.

2) On the average (animals 4,6,8-10), the brain accumulated only about 2% as much $^{14}C$-containing compounds as the blood three minutes after injection.

Table 12

Accumulation of Vascularly Administered $^{14}C$-Acetaldehyde in Rat Brain Regions and Liver

Amount of $^{14}C$ in Tissue (pCi/mg)

| CONDITION OR TISSUE | ANIMAL # 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Incubation (min.) | 0 | 3 | 7 | 8 | 3 | 3 |
| Date | 1/24/83 ---------- | | | | 2/8/83--------- | |
| Cerebrum | 68.0 | 12.1 | 44.0 | 74.0 | 10.1 | 30.8 |
| Striatum | 101.0 | 10.6 | 64.0 | 104.0 | 15.5 | 47.5 |
| Cerebellum | 80.0 | 9.5 | 37.0 | 67.0 | 11.3 | 37.9 |
| Brain Stem | 129.0 | 6.8 | 42.0 | 76.0 | 17.0 | 29.7 |
| Liver | | | 7.0 | 13.3 | 11.3 | 6.0 |
| Blood (nCi/100 µl) | | | | 19.8 | | 22.0 |

The amount of $^{14}C$-containing compounds was determined as generally described in the Methods section. Specific methodologies included the use of gravity flow perfusion, the use of representative samples of brain stem and the homogenization of tissues. The data represent the total of the amounts of $^{14}C$ found in the resulting supernatant and pellet.

Table 13

Accumulation of Vascularly Administered $^{14}C$-Acetaldehyde in Rat Brain Regions and Liver

Amount of $^{14}C$ in Tissue (pCi/mg)

| CONDITION OR TISSUE | ANIMAL # 7 | 8 | 9 | 10 |
|---|---|---|---|---|
| Cerebrum | 0.22 | 2.48 | 61.0 | 8.6 |
| Striatum | 0.25 | 2.04 | 45.0 | 9.3 |
| Cerebellum | 0.30 | 2.30 | 57.0 | 5.9 |
| Brain Stem | 0.46 | 3.94 | 83.0 | 10.1 |
| Liver | 1.23 | 1.1 | 8.0 | 1.2 |
| Blood (nCi/100 µl) | | 42.4 | 1.3 | 3.2 |

The amount of $^{14}C$-containing compounds was determined as described in the Methods section. The specific portions of the methodology used in these experiments include determination of $^{14}C$ in the entire brain stem and the ;use of an air pressure regulated perfusion apparatus. These data are from whole minced tissue as described in the Methods section.

Table 14

$^{14}$C-Acetaldehyde Experiments
Expressed as Percent ($^{14}$C-Region/$^{14}$C-Striatum)

| TISSUE | RATS 2,5,6 | RATS 7-10 |
|---|---|---|
| Cerebrum | 81 ± 28 | 109 ± 22 |
| Striatum | 100* | 100* |
| Cerebellum | 80 ± 8 | 105 ± 28 |
| Brain Stem | 78 ± 26° | 167 ± 39° |
| Liver | 13 ± 2.5# | |

* = The striatum was defined to be 100%.

# = This mean and standard deviation were calculated from animals 3,4,6,9 and 10.

° - The data are statistically different at the 0.024 significance level.

Data are reduced from Table 12 and 13. They are presented as the mean and standard deviation, for the groups of rats indicated, of the percent ratios of $^{14}$C-containing compounds in a particular brain region in each individual animal compared to its striatum.

4) Although not statistically significant (only 94% confidence level), in studies in which the entire structure was included, the brain stem seems to accumulate the highest levels of $^{14}C$.

5) In studies in which the entire brain stem was not included, the striatum contained the highest levels of $^{14}C$.

In conclusion, while the raw data indicated that problems still exist in the methodology, an analysis of the normalized data suggests that the brain stem accumulates the highest levels of administered $^{14}C$-acetaldehyde. Perhaps more interesting, is the possibility that only certain portions of the brain stem are responsible for this enhanced accumulation. It was also interesting to find that the liver, which is the major site of acetaldehyde metabolism in the body, only accumulates 10% as much $^{14}C$ as the brain.

The possibilities suggested by these observations indicate that further investigation of acetaldehyde at the neurochemical and biochemical levels is required.

III. NICOTINE-ACETALDEHYDE INTERACTIONS

A. Self-Administration Studies

1. Acetaldehyde alone maintains lever pressing in rats at a greater rate than nicotine at equal mg/kg doses.

2. The optimal combinations of nicotine and acetaldehyde that result in enhanced positive reinforcing effects appear to be low doses of nicotine (2-8 μg/kg) added to acetaldehyde (16.0 μg/kg).

B. Discrimination Studies

1. An interaction between nicotine and acetaldehyde was not evident in discrimination tests.

IV. TERMINATION OF CHRONIC ACETALDEHYDE ADMINISTRATION DOES NOT RESULT IN A WITHDRAWAL SYNDROME

1. Termination of chronic exposure to acetaldehyde does not result in a physiological dependence.

2. Acetaldehyde does not interact with an endogenous opioid system.

V. TOLERANCE TO CHRONIC NICOTINE ADMINISTRATION: BEHAVIORAL VS. METABOLIC FACTORS

1. Behavioral factors appear to be critically involved in the mechanisms underlying nicotine tolerance.

2. Physiological factors are minimally involved in the development of tolerance to nicotine.

3. The details of the interactions between behavioral, dispositional and receptor sensitivity factors have not been determined.

VI. CROSS TOLERANCE BETWEEN ISOMERS OF NICOTINE

1. Both the (-) and (+) isomers of nicotine failed to alter response rates at lower doses and produced dose-dependent decreases in rates at higher doses.

2. The doses estimated to reduce baseline rates of responding by 50% indicated that (-)-nicotine is 6 to 9 times more potent than (+)-nicotine in altering response rates.

VII. BEHAVIORAL SUPERSENSITIVITY FOLLOWING CHRONIC ADMINISTRATION OF NICOTINE ANTAGONIST

1. Supersensitivity (enhancement of nicotine-induced suppression following chronic mecamylamine treatment) was not demonstrated following termination of chronic treatment with mecamylamine.

2. Under conditions of the present study chronic blockade of nicotine receptors did not result in an up-regulation (increase in number of receptors) detectable at the behavioral level.

VIII. BRAIN SITES INVOLVED IN THE MEDIATION OF THE BEHAVIORAL EFFECTS OF INTRAVENTRICULARLY ADMINISTERED (-)-NICOTINE

1. The effect of nicotine infused directly into various brain sites was determined on ratio performance.

2. The vestibular nucleus is critically involved in the production of the prostration syndrome.

3. Inhibition of neural conduction in the reticular formation results in attenuation of vestibular-mediated prostration effects.

IX. ELECTROENCEPHALOGRAPHIC ANALYSIS OF DORSAL HIPPOCAMPAL ACTIVITY IN RATS ADMINISTERED NICOTINE AND ACETALDEHYDE ALONE AND IN COMBINATION

1. Electroencephalograms of the dorsal hippocampus indicate that there was an interaction of the individual effects of nicotine and acetaldehyde when these compounds were administered in combination.

X. LOCALIZATION OF VASCULARLY ADMINISTERED $^{14}$C-ACETALDEHYDE IN RAT TISSUES

1. Vascularly administered acetaldehyde readily penetrates the blood-brain barrier.

2. The brain stem seemed to accumulate the highest level of $^{14}$C acetaldehyde or its metabolic products.

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