EFFECTS OF CHRONIC NICOTINE ADMINISTRATION AND ITS TERMINATION ON SCHEDULE-CONTROLLED BEHAVIOR IN RATS

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ABSTRACT

Lever pressing by rats was maintained under a multiple schedule of food presentation. After stable performance was obtained the rats were infused subcutaneously with nicotine (8, 12, or 16 mg/kg/24 hours) for 240 hours. When challenged with mecamylamine, a nicotinic-cholinergic antagonist these animals failed to show disruptions on the scheduled-controlled behavior. These results suggest that chronic nicotine exposure may not result in a physical dependence.

Employing the principles of operant conditioning to demonstrate a physiological dependence on nicotine in animals is of particular interest since, as yet, there have been no demonstrations of either a nicotine (6) or tobacco (7) withdrawal in animals, even following prolonged exposure to these substances. Overall, the effects of nicotine on scheduled-controlled behavior have been studied less extensively than other commonly used compounds. Most studies have investigated the acute behavioral effect of nicotine in rats (1) or monkeys (2). Less is known about the termination of chronic nicotine administration on scheduled-controlled behavior. Chronic administration of a variety of psychoactive agents results in physical (physiological) dependence (3). Physical dependence is generally characterized by abstinence symptoms when drug intake is abruptly terminated or when an antagonist is administered (4). Several laboratories have shown that behavior maintained under various schedules of reinforcement are highly sensitive to the effects of chronic drug administration and withdrawal, and drugs from a number of pharmacological classes have been investigated (5). However, there is a dearth of information about the potential physical dependence producing properties of nicotine.

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

Twenty-four male hooded rats (Blue Spruce Farms, 350-410g) were divided into three groups (8). Rats were reduced to 85% of their free feeding weights and trained to lever press for a 45 mg food pellet (Bio-Serve Inc., N.J.). After lever pressing was established, responding was maintained under a MULT FR 30 FI 120 sec. schedule, with a 60 sec. time out (TO) following the FI

component. A single white light over the response lever was illuminated during the FR component and two red lights were illuminated during the FI component. All lights were extinguished during the TO and responses had no programmed consequence. The components alternated at food delivery and sessions lasted until 11 food deliveries were obtained in the FR component. Rats were trained under the multiple schedule for a minimum of fourteen weeks in order to stabilize responding. The last three days of this training period served as control sessions (phase 1). During phase 2 a baseline consisting of three testing periods obtained within a single day (repetitive runs) was collected. Each run was separated by a 40 minute interval. During phase 3 (10 days after phase 2) data was collected from three repetitive runs as previously described in phase 2, however 20 minutes prior to the first and third runs the rats were injected subcutaneously with mecamylamine HCl (1.5 mg/kg). In phase 4 (10 days later) the rats were anesthetized with ether and an osmotic minipump (9) filled with (-)-nicotine was inserted subcutaneously between the scapulae. Nicotine was infused subcutaneously for 240 hours (0.5 µl/hr) delivering daily doses of 8.0 mg/kg (group 1), 12.0 mg/kg (group 2), and 16 mg/kg (group 3). After 240 hours of continuous (-)-nicotine infusion the rats were challenged (phase 5) with the nicotinic-cholinergic antagonist mecamylamine as described in phase 3. Mecamylamine has been shown to block the behavioral effects of (-)-nicotine in both rats (10) and monkeys (2). Blood samples (450-1000 µl) were collected from the dorsal digital vein in the hind paw under ether anesthesia the day before and the day after the mecamylamine challenge. The animals were tested for an additional ten days (phase 6) after which the pumps were removed and inspected for remaining nicotine. Between phases the animals were tested in single daily sessions.

Characteristic performance was maintained under the MULT FR FI schedule. When the light signalling the FR component was illuminated animals emitted a high rate of responding that was maintained until 30 responses were completed. In the FI component, a period of little or no responding at the beginning of the 120 sec. interval was followed by accelerated responding that was maintained until a response ended the interval. Responding during T.O was less than 1% of the total response emitted during the session.

Quarter life values (11) for the FI component, response rates in the FR component, and response rates in the last 25% of the FI component were used to examine the effects of chronic nicotine administration and its termination.

The introduction of the osomotic minipump containing (-)-nicotine significantly altered response rates under both component schedules, but there were no differential effects of dose on either FR or FI response rates and no significant dose x day interactions (Figure 1). FI rates significantly decreased on the first day of nicotine exposure but returned to control levels by day 2 and remained stable throughout the remainder of nicotine phase. FR rates also decreased on day 1 of nicotine exposure but this effect failed to achieve statistical significance. Beginning on day 3 FR rates were significantly elevated on 6 out of 8 of the remaining nicotine days. It is unlikely that the change in FR response rates were dependent upon the absolute rate of responding since FI rates were similar and were not changed after day 1 of phase 4. The decrease in rate under both schedules on the first day of phase 4 may be due to the introduction of nicotine and/or osmotic minipump surgery. However, the significant increase in FR rate is most likely due to the direct effect of nicotine. Since FI rates did not change the increase in rate under the FR schedule appears to be schedule dependent. FI quarter life values were not altered by nicotine administration (12).

Figure 2 shows how performance under the multiple schedule varied as a function of phase. Analysis of FR response rate showed a significant effect of phase while the effect of nicotine dose and the nicotine dose x phase interaction were not significant. Subsequent t-tests revealed that chronic nicotine treatment increased FR rates obtained during control sessions from 1.16 ± 0.12 to 1.42 ± 0.13 responses per second. The mecamylamine challenge significantly decreased FR response rate, relative to the last three days of nicotine exposure, to 0.82 ± 0.09 responses per second. Subsequent to the mecamylamine challenge FR response rates were again significantly elevated for the remainder of the experiment (last 10 sessions)(13). Figure 2 shows that FI performance was not altered during any condition including mecamylamine challenge.

Multiple ion detection analysis (14) of nicotine in blood (gas chromatograph/mass spectrometer) was performed on samples collected both before and after the mecamylamine challenge. The data show that level of nicotine present in blood both before and after the mecamylamine challenge were similar, and that the blood levels (ng/ml blood) varied directly with the daily nicotine dose (8 mg/kg/day $\bar{x}$ = 2.28 ± 0.07, 12 mg/kg/day $\bar{x}$ = 4.08 ± 0.81, and 16 mg/kg/day $\bar{x}$ = 6.21 ± 0.63).

The results of this experiment show that blocking nicotine's central nervous system actions following chronic nicotine treatment does not result in a disruption of scheduled-controlled performance, which has been shown to be sensitive to physiological dependence (5). Others have also noted that termination of prolonged exposure to nicotine or tobacco (6,7) does not result in a withdrawal syndrome in animals. However, the available data with human subjects suggests a series of withdrawal signs and symptoms (15). Since the kinds of symptoms reported and the temporal pattern of these symptoms are not

consistent across studies or between individuals within a study it is not necessary that the symptoms reported represent a physiological dependence. Instead, the absence of a withdrawal syndrome in this and other animal studies (6,7), combined with the lack of consistency in the human data suggests a more general interpretation, such as a learning mechanism whereby the interruption of a well learned response that leads to positive reinforcement results in a variety of behavioral and physiological changes which are reported by humans and interpreted as withdrawal symptoms.

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(8) Data from two animals in group 1 was lost due to equipment failure and one animal in group 3 was found to have a wet spot on its back following a mecamylamine injection. Its data was not included in the analysis but did not differ from the other animals in that group. Therefore group 1 had 6 animals, group 2 had 8 animals and group 3 had 7 animals.

(9) Alzet osmotic minipump delivery system, manufactured by Alza Corp., Palo. Alto. CA. Model Number 2002 with a flow rate of 0.5 µl/hr was filled with (-)-nicotine. The concentration of (-)-nicotine in 0.9% saline was adjusted to deliver the three doses.

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(11) Quarter life is the time taken for the first 25% of the total number of responses within the fixed-interval to be emitted (R. J. Hernstein and W. H. Morse, Science 125 (1957). It is a measure of the positively accelerated pattern of responding typically generated by FI schedules and is relatively independent of the absolute rate of responding. The quarter life values are shown as the percent of the FI interval.

(12) E. F. Lindquist, Design and Analysis of Experiments in Psychology and Education. Houghton Mifflin, Boston, 1953, pp. 267-273, Type I design. Separate analyses were made on each measure, follwed by t-tests where appropriate. Analyses combining the control scores and those for each of the 10 nicotine days (Fig 1) showed Fs for dose: Quarterlife <1.0, FR<1.0, FI<1.0 (df = 2,18); for dose x day of nicotine: QL<1.0, FR = 1.13 (NS), FI = 1.09 (NS) (df = 20,180); days: QL = 1.56(NS), FR = 3.38 ($p<.01$), FI = 2.14 ($p<.05$) (df = 10,180). t-tests for the day effects on FR and FI performance showed most FR means higher than control day or days 1 or 2; and the nicotine Day 1 FI mean lower than other days ($p<.05$).

(13) Control and experimental phase (Fig 2) analyses showed Fs for dose: QL<1.0, FR<1.0, FI<1.0 (df = 2,18); dose x phase: QL<1.0, FR<1.0, FI = 1.37 (NS)(df = 5,90); phase: QL<1.0, FR = 7.75 ($p<.01$) FI = 1.35 (NS)(df = 5,90). FR t values: phase 1 vs. phase 4 = 2.29 ($p<.05$); phase 4 vs. phase 5 = 5.03 ($p<.01$); phase 5 vs. phase 6 = 5.29 ($p<.01$), df = 90.

(14) The extraction procedure used to obtain samples for GC/MS analysis was reported by P. Jacob, M. Wilson, and N. L. Benowitz. J. of Chromatography 22, 61 (1981).

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

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

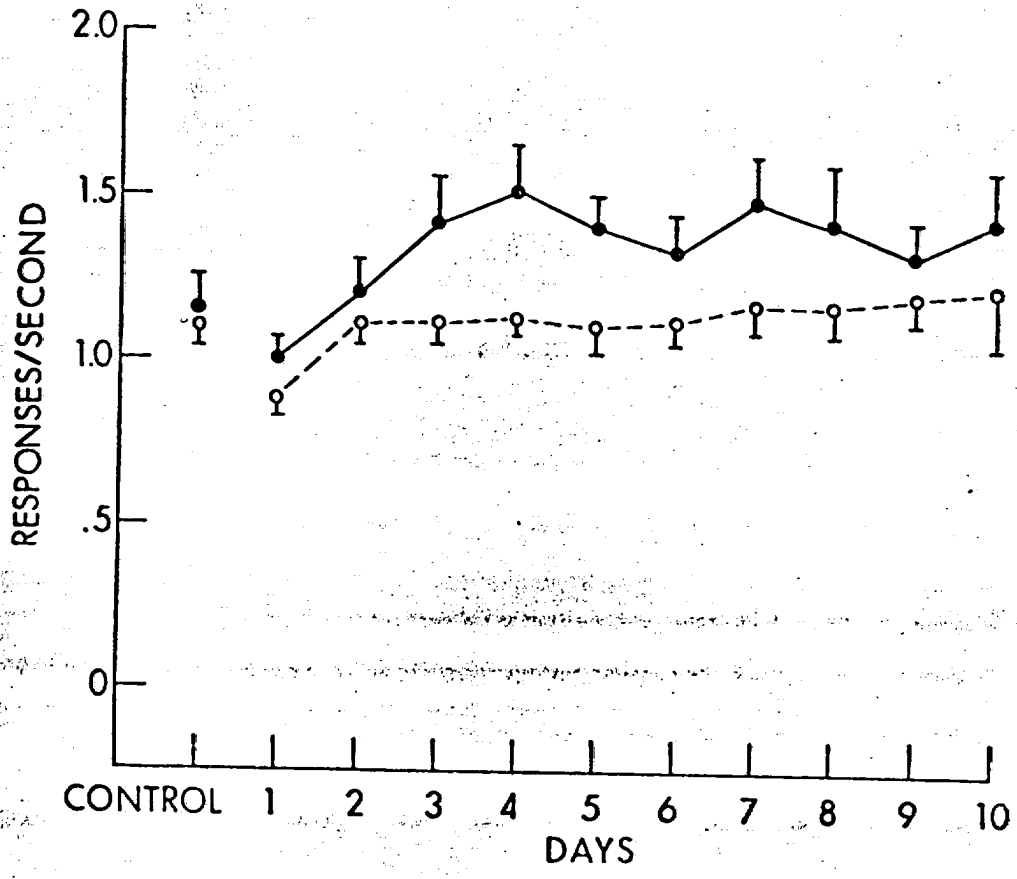

FIGURE 1

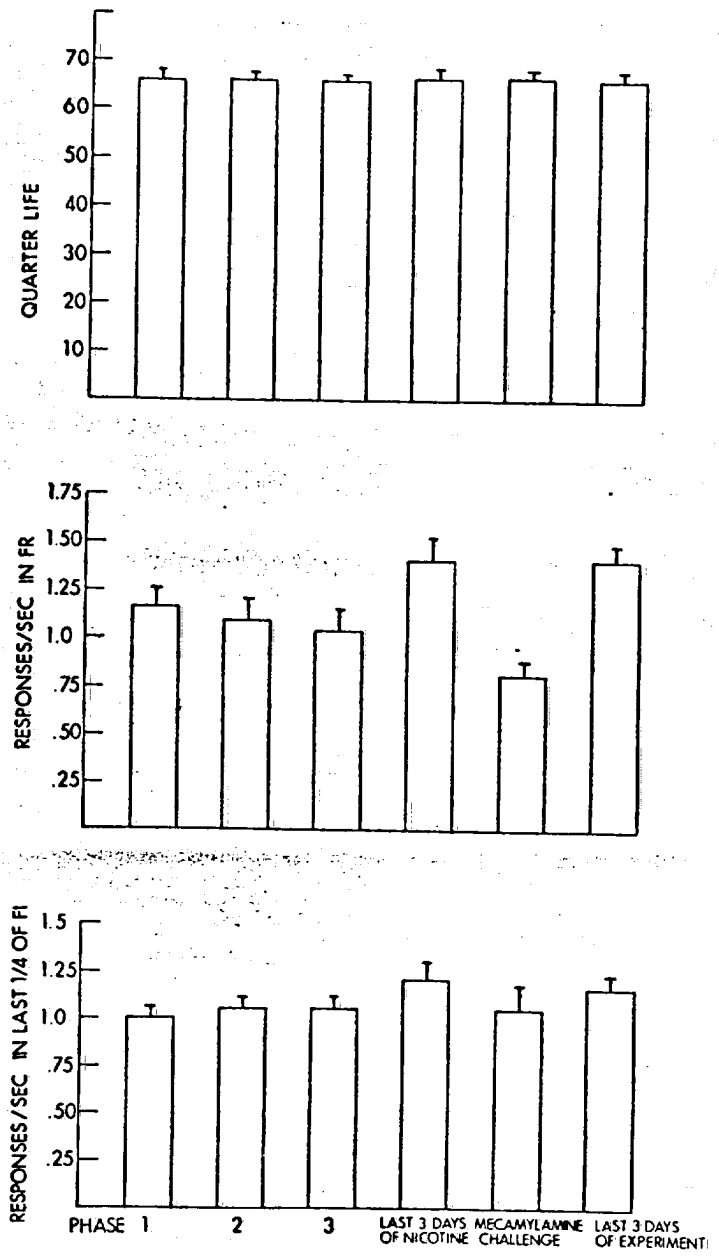

FIGURE 2