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Light-Dark Variation in Response to Chronic Nicotine Treatment and the Density of Hypothalamic α-Bungarotoxin Receptors

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MORLEY, B. J. AND L. L. GARNER. *Light-dark variation in response to chronic nicotine treatment and the density of hypothalamic α-bungarotoxin receptors.* PHARMACOL BIOCHEM BEHAV **37**(2) 239–245, 1990.—Chronic nicotine administration increased locomotor activity during the light, but not the dark, in rats maintained on a 12:12-hr light/dark cycle, but the period and peak of the circadian rhythm (CR) were not affected. In Experiment I, 24 male rats were implanted with battery-operated telemeters and locomotor activity was continuously measured for 10 days before and 10 days after the implantation of osmotic mini-pumps which delivered 0, 0.5, 3.0 or 10 mg/kg/day of (±)-nicotine tartrate. Nicotine increased locomotor activity during the light in a dose-dependent manner. Tolerance to the stimulant effects of nicotine during the light occurred in 5–6 days. To determine if the stimulant properties of nicotine were associated with light as opposed to disruption by the environmental stimuli normally present during the day in our animal facility, a second experiment was conducted in which rats were treated with saline or 10 mg/kg/day (±)-nicotine di(+)hydrate tartrate and maintained on a reversed light/dark cycle. Again nicotine increased activity during the light (21:00–09:00) but not the dark (09:00–21:00). In a third experiment, the density of α-bungarotoxin binding sites was found to be significantly decreased when animals were sacrificed at 06:00 in comparison with animals sacrificed at 10:00 and 14:00

Nicotine Nicotinic receptors Locomotor activity Circadian rhythms Tolerance α-Bungarotoxin binding sites

NICOTINE increases or decreases locomotor activity in rats depending upon several variables, including the dose of nicotine, the experimental situation, habituation to the experimental chamber, previous drug experience, and phase of the light/dark cycle (light versus dark) (1–4, 10–12, 17, 19, 20). The effects of nicotine on behavior are complex, probably because nicotinic cholinergic receptors (nAChRs) are widely distributed throughout the brain (15,16) and behaviors such as locomotor activity are likely regulated by neurons in several brain regions.

The effects of nicotine on many physiological and behavioral characteristics are biphasic. The initial depressant effects of nicotine are followed by excitation. It is hypothesized that tolerance develops rapidly to the depressant effect of nicotine, thus releasing stimulant properties (2). There are few published reports of tolerance to the stimulant properties of nicotine in rats (2,4).

Although an effect of nicotine on the circadian rhythm (CR) of locomotor activity has not been reported, Bovet *et al.* (1) found that nicotine affected locomotor activity during the day not but not during the night and interpreted this observation as indicating that nicotine affected the CR of locomotor activity. High concentrations of binding sites for the putative cholinergic receptor, α-bungarotoxin (BuTX) (16), are localized in areas of the hypothalamus associated with biological rhythms (13,18). The intraventricular infusion of cholinergic agonists and antagonists, including BuTX, alters certain biological rhythms (5, 7–9, 21). Thus, a mechanism exists whereby nicotine might affect circadian rhythms. Most experiments with nicotine have been carried out during the light phase of the light/dark cycle. The effects of nicotine on locomotor activity during light and dark and on circadian rhythmicity have not been systematically studied.

The purposes of the study reported here were to 1) analyze the effects of nicotine on locomotor activity during the active (dark) phase and inactive (light) phases in rats maintained on a 12:12-hr light/dark cycle; 2) determine if chronic nicotine treatment alters the CR of locomotor activity entrained to a light/dark cycle; and 3) determine if the density of α-bungarotoxin binding sites varies throughout the light/dark cycle. Locomotor activity was measured continuously by telemetry for 10 days before and 10 days after surgical implantation of osmotic mini-pumps. In the first experiment, animals were maintained on a 12:12-hr light/dark cycle with lights on at 06:00 and lights off at 18:00. In a second experiment, the light/dark cycle was reversed (lights on 21:00–9:00). The

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density of hypothalamic BuTX sites was measured in animals sacrificed at several times in the light and in the dark.

METHOD

Housing

For the locomotor activity studies, male Sprague-Dawley albino rats (SASCO, Omaha, NE) were adapted to the experimental situation for approximately 3–4 weeks after receipt from the supplier. Six rats were assigned to the control and nicotine-treated groups. Animals were cared for according to NIH animal care standards. Rats were housed individually in stainless mesh bottom cages in a room with a 12:12 light/dark cycle (lights on at 06:00 in Experiment I and at 21:00 in Experiment II) and a temperature of 23°C. Food and water were available ad lib. The room was entered 4 times/week during the light cycle for animal care.

Animal Surgery and Drug Administration

When the rats for the behavioral studies reached body weights averaging 250–300 g, they were anesthetized with 30 mg/kg ketamine, 14 mg/kg xylazine (Experiment I), or 25 mg/kg ketamine, 6 mg/kg xylazine (Experiment II) and implanted with battery-operated telemeters (Mini-Mitter Co., Inc., Sun River, OR). On the eleventh day of the experiment, the animals were anesthetized with the same anesthetic and doses and implanted with Alzet osmotic mini-pumps (Alza Corp.; Palo Alto, CA). Mini-pump models 2002 and 2ML4 were used in Experiments I and II, respectively. In Experiment I, the pumps were filled with (±)-nicotine tartrate (K & K Biochemicals) dissolved in saline to deliver 0, 0.5, 3.0 or 10 mg/kg/day (0, 0.21, 1.1 and 3.5 mg/kg/day of free base nicotine, respectively). In Experiment II, the pumps were filled with (±)-nicotine di(+)hydrate tartrate (K & K Biochemicals) dissolved in saline to deliver 0 or 10 mg/kg/day (0 and 3.3 mg nicotine free base). In both experiments, the pumps were primed by placing them in saline at 37°C for 8 hr.

Telemetry Recordings

Locomotor activity. Locomotor activity was measured using VM-FH telemeters (Mini-Mitter). Activity was defined as the number of digital pulses (switch closure of >16 msec in duration). The data were sent from receivers (Model RA-1010; Mini-Mitter) positioned directly beneath each cage to a microcomputer which was remotely located. Locomotor activity was collected for each rat continuously and the average of each 10-min period was stored to disk. The data for the light and dark portions of each daily cycle were analyzed by computing a moving average using a 30-min interval. In Experiment I, to determine if increased locomotor activity was associated with a particular time of day after nicotine treatment, the 10-min periods were summed for 1-hr periods beginning at 06:00, 09:00, 12:00, 15:00, and 17:00. In Experiment I, mini-pumps were implanted between 11:00 and 13:00. Data analysis began at 18:00. In Experiment II, mini-pumps were implanted between 21:00 and 22:00. Data analysis began at 09:00.

In both experiments, control activity levels were determined by averaging data for the 10 days prior to the installation of the pumps for each animal. The percent of increase in activity after nicotine treatment was determined for each animal individually and the group average computed. The reason of using percent of pretreatment baseline as the measure of activity is that locomotor activity differs significantly among animals using this telemetry system. Although the measures are reliable from day to day for each animal, there can exist significant differences between groups, as well as large intragroup variation, prior to treatment that would make interpretations of drug effects difficult. The raw data, i.e., pretreatment scores, are provided for each group in Figs. 1 and 3.

Circadian rhythms. Fourier spectrum analysis of 7-day periods pre- and postimplantation of the mini-pumps were performed using DataQuest Software (Mini-Mitter). The power spectrum analysis shows a major peak at the frequency corresponding to the period of the variation in the data, i.e., a circadian rhythm would predominantly exhibit a frequency of approximately 1.00 (1 cycle/day). The Fourier analyses were performed using a 90-min moving average to attenuate random movement and noise in the data. The plots generated by the DataQuest software were replotted using PLT, a plot program written at the Boys Town National Research Hospital. This generated the sets of plots seen in Figs. 3 and 4. They differ from the original plots generated by DataQuest in that the Y axis (Power) is the same for all animals (0.03), allowing a comparison among animals of the amplitude of the peaks. To detect changes in the period, the acrophase of the circadian peak was determined for 3 days pretreatment and for the first 3 days after implantation of the mini-pumps for each animal in both Experiments I and II. The acrophase is the time (in degrees), in relationship to midnight, at which peak activity in the rhythm is observed. Each 24-hr period is 360°.

Receptor Binding

Forty male Sprague-Dawley albino rats (SASCO, Omaha, NE) weighing 200–250 g were housed 4/cage in a 12:12 light/dark cycle with lights on at 06:00. Food and water were available ad lib. The animals were sacrificed at 02:00, 06:00, 10:00, 14:00 and 18:00 (8/group). The times were chosen as representative of light and dark, with 06:00 and 18:00 as normal "lights on" and "lights off" times. The brains were removed and hypothalamic slices, which included most of the basal hypothalamus and weighed approximately 40 mg, were dissected on ice and frozen at −70°C. The tissue samples were assayed 7 days later using ^{125}I-BuTX, as previously described (14). Briefly, samples of hypothalamic tissues were sonicated in 500 µl mM sodium phosphate, pH 7.4, with 1 mM EDTA and 0.32 M sucrose, centrifuged at 100,000 × g for 45 min. The pellet was resuspended in 500 µl of 10 mM sodium phosphate, 1% Triton X-100 with 1 mM EDTA and centrifuged at 100,000 × g for 45 min. The supernatants were retained. One hundred µl aliquots of supernatant were incubated with 1×10^{-9} M ^{125}I-BuTX (sp.act. 17.6 µCi/µg; New England Nuclear) for 60 min and passed through a 5.75-inch Pasteur pipette CM-50 gel column and counted in an LS 5801 scintillation counter. Nonspecific binding was determined by incubating parallel tubes with an equivalent amount of tissue and iodinated toxin in the presence of 5×10^{-4} M unlabeled nicotine. Nonspecific binding averaged 28% and was equivalent in all groups.

Statistical Analyses

Locomotor activity was analyzed using BMDP repeated measures analyses of variance (ANOVA) with Phase (light/dark), Drug, and Time as the independent variables. Planned comparisons were made between the groups on each day during the Light condition. The acrophase data was analyzed using an ANOVA with Group and Treatment (pre- vs. posttreatment) as the independent variables. Planned matched *t*-tests were used to compare the acrophase the day before surgery with the day after surgery. Both the cortical and the hypothalamic receptor binding data were analyzed with one-way repeated measures ANOVAs with Time as the independent variable (16). Differences between brain area in the density of BuTX receptors was not evaluated since it is known that the cortex and hypothalamus differ with respect to the number

of binding sites. Planned comparisons using the Tukey Standardized Range Method were performed between each time period for the hypothalamic receptor binding data.

RESULTS

Locomotor Activity

Experiment I. The percent of change in locomotor activity from baseline following saline and nicotine treatment during the light and dark phases for each group in Experiment I is plotted in Fig. 1. The results of the ANOVA indicated significant main effects of Phase, $F(1,18)=26$, $p<0.001$, Drug, $F(3,18)=14.6$, $p<0.001$, and Days, $F(9,162)=9.4$, $p<0.001$. There were also significant first order interactions of Phase × Drug, $F(3,18)=4.6$, $p<0.05$, Drug × Days, $F(27,162)=2.8$, $p<0.001$, and Phase × Days, $F(9,162)=30.9$, $p<0.001$. The second-order interaction of Phase × Days × Drug was also significant, $F(27,162)=2.5$, $p<0.001$. Planned comparisons between control and drug groups indicated that differences between the 10.0 mg/kg nicotine-treated group and controls were significantly different on days 1–4 and day 6, and on days 1–6 for the 3.0 mg/kg nicotine-treated group. The 0.5 mg/kg group was not significantly different from the controls on any day.

In order to determine if the increased locomotor activity was associated with "lights on" or the anticipation of "lights off," total activity was computed for several time periods during the day (06:00–07:00, 09:00–10:00, 12:00–13:00, 15:00–16:00, and 17:00–18:00). The percent of change in activity for each animal was calculated and averaged. The data are shown in Fig. 2. No statistical analysis was performed because there is clearly no correlation between increased activity and time across days.

Experiment II. The results of the ANOVA indicated a significant main effect of Drug, $F(1,11)=5.1$, $p<0.05$, and Day, $F(9,99)=4.1$, $p<0.001$. There were also significant interactions between Drug and Phase, $F(1,11)=11.7$, $p<0.01$, and Day and Drug, $F(9,99)=3.0$, $p<0.01$. Planned comparisons between the control and drug group on each day during the light condition indicated that significant differences were obtained on days 1–5 (see Fig. 3).

Circadian Rhythms

Fourier spectrum analyses of the daily activity pre- and posttreatment in all groups in both experiments indicated a period of 1.00 in all animals, indicating that all animals demonstrated circadian (24 hr) locomotor activity rhythms (see Figs. 4 and 5).

The ANOVA on the acrophase data for Experiment I indicated that the effect of nicotine was not significant. The effect of pre-/posttreatment was significant, $F(1,19)=7.1$, $p<0.05$. There was not a significant interaction between Drug and Pre-/Posttreatment. Matched *t*-tests for each group indicated a significant difference between the day before and the day after surgery for all groups (see Table 1), indicating that there was a phase advance attributable to surgery and/or the implantation of the mini-pumps. In Experiment II there was not a significant effect of nicotine nor was there a significant change in the acrophase after surgery (see Table 1).

Receptor Binding

The ANOVA indicated that Time was significant, $F(4,32)=3.44$, $p<0.05$. Planned comparisons between times using the Tukey Standardized Range Test indicated that a significant difference was found for the comparisons between 10:00 and 06:00 and 14:00 and 06:00 (see Fig. 6).

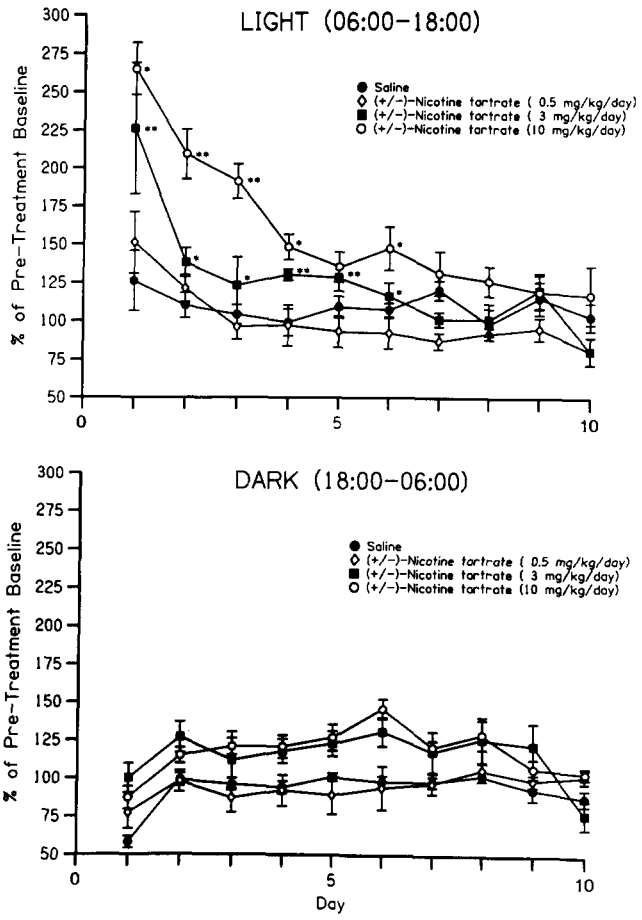

FIG. 1. Chronic nicotine treatment with osmotic mini-pumps increased locomotor activity during the light but not the dark in a dose-dependent manner. Baseline control activity was calculated for each animal by averaging the 10 days (in dark or light) prior to the implantation of the pumps. The percent of change from baseline was calculated for each animal individually. The percent of change in activity for each animal was averaged (N = 6/group, mean percent of change in activity ± SEM). Percent of change rather than raw data were used to correct for differences between animals in pretreatment activity scores. The mean and range of pretreatment activity (30 min moving average, as described in the Method section) scores during the dark for each group were as follows: Control (mean = 97, range = 53–144); 0.05 mg nicotine (mean = 103, range = 88–108); 3.0 mg nicotine (mean = 84, range = 54–106); 10 mg nicotine (mean = 75, range = 57–94). During the light period, the mean scores were as follows: Control (mean = 30, range = 16–26); 0.5 mg nicotine (mean = 29, range = 17–38); 3.0 mg nicotine (mean = 28, range = 21–35); 10 mg nicotine (mean = 24, range = 10–28). Planned comparisons between controls and nicotine-treatment animals were performed on each day, $*p<0.05$, $**p<0.01$.

DISCUSSION

In the experiments reported here, locomotor activity was measured using telemeters in a home-cage environment. Several doses of nicotine were chronically administered with osmotic mini-pumps. The advantage of this procedure is that it allows for the continuous, noninvasive measurement of the effects of nicotine on behavior. The animal is not disrupted by placement in a novel environment or by a drug injection procedure.

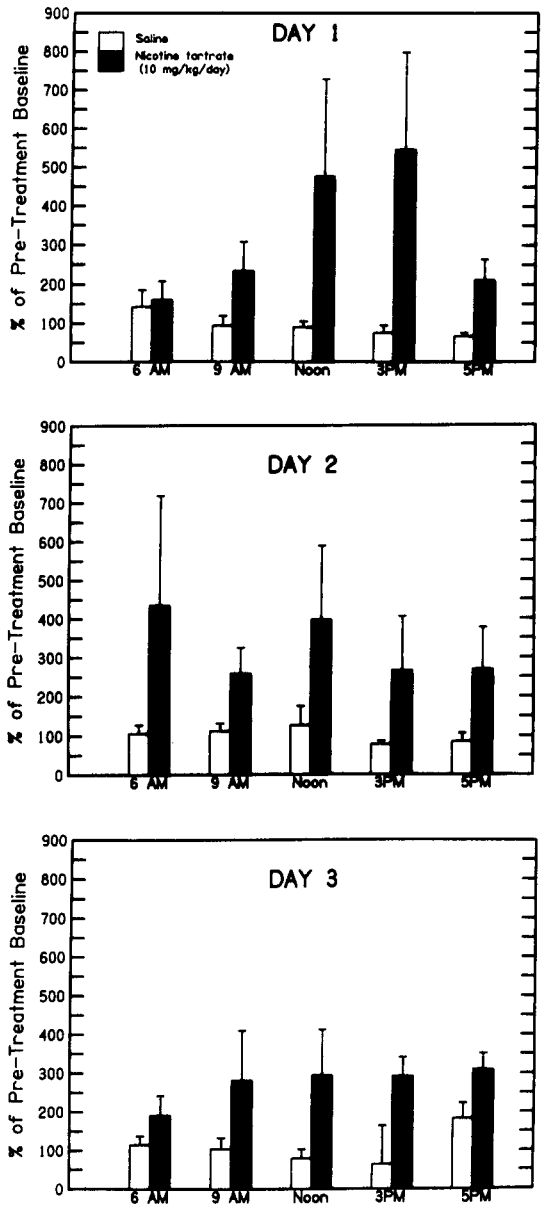

FIG. 2. The total activity scores per hour for the time periods 06:00–07:00, 09:00–10:00, 12:00–13:00, 15:00–16:00 and 17:00–18:00 were calculated for each animal before and after nicotine treatment. The percent of change from baseline was calculated for each animal and the group average taken. The data shown are the mean ± SEM. No statistical analysis was computed because there was clearly no association between increased activity and time over days.

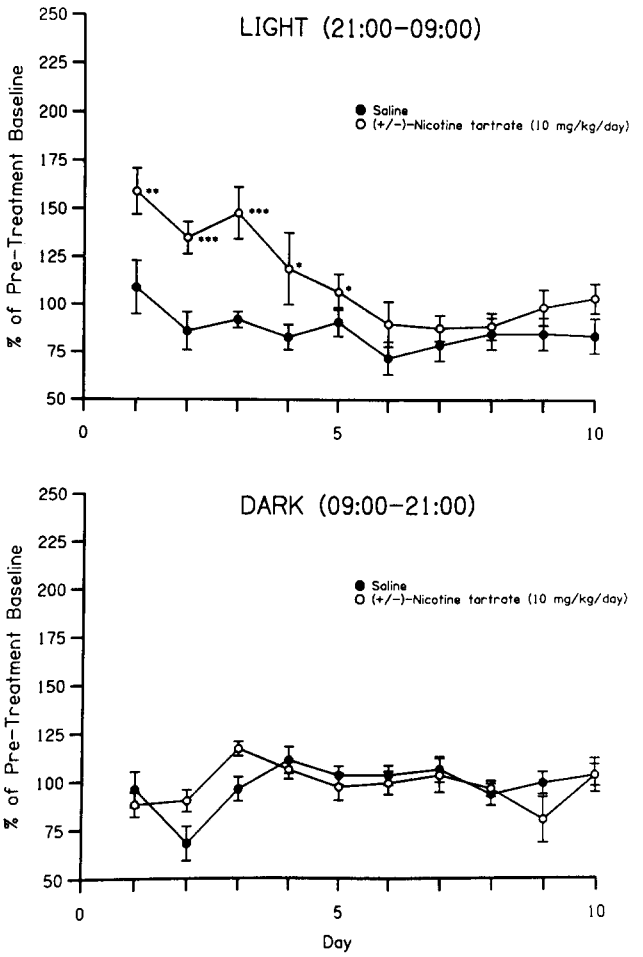

FIG. 3. Chronic nicotine treatment with osmotic mini-pumps increased locomotor activity during the light but not the dark in animals maintained on a reversed light/dark cycle. The data were calculated as described in Fig. 1 (N = 7/group, mean percent of change in activity ± SEM). The pretreatment activity scores during the dark for the groups were: Control (mean = 94, range = 68–111); 10 mg nicotine (mean = 91, range = 52–134). During the light, the pretreatment activity scores were: Control (mean = 25, range = 19–41); 10 mg nicotine (mean = 24, range = 10–32). Planned comparisons between controls and nicotine-treatment animals were performed on each day, $*p<0.05$, $**p<0.01$, $***p<0.001$.

Nicotine stimulated locomotor activity during the light, but not dark, phase of the light/dark cycle. The increased sensitivity to nicotine was not associated with a particular time during the day. Since the effect occurred with a reversed light/dark cycle these results cannot easily be attributed to a greater sensitivity of daily environmental disturbances during the normal day.

The effect of nicotine during the light appears greater during Experiment I than in Experiment II. Although this may be a random occurrence, there are variables that differed between the experiments that may account for the results. The nicotine used in Experiments I and II were from the same supplier, but not from the same lot; the nicotine in Experiment I was monohydrate tartrate, and the nicotine in Experiment II was the dihydrate form. There may be differences in purity between lots of nicotine. Also, a larger mini-pump (2ML4) was used in Experiment II. In several experiments in this lab we have observed that the heavier pump tends to suppress activity. The control rats in Experiment II, for example, have average postsurgical activity levels approximately 20% below those of the pretreatment levels, while the control rats in Experiment I maintained their presurgical baseline activity levels. Alternatively, the effects of nicotine could be diminished in animals maintained on a reversed light/dark cycle.

These results confirm an early study of the effects of nicotine on locomotor activity, in which it was reported that nicotine acted as a stimulant only during the light (1). Those data were inter-

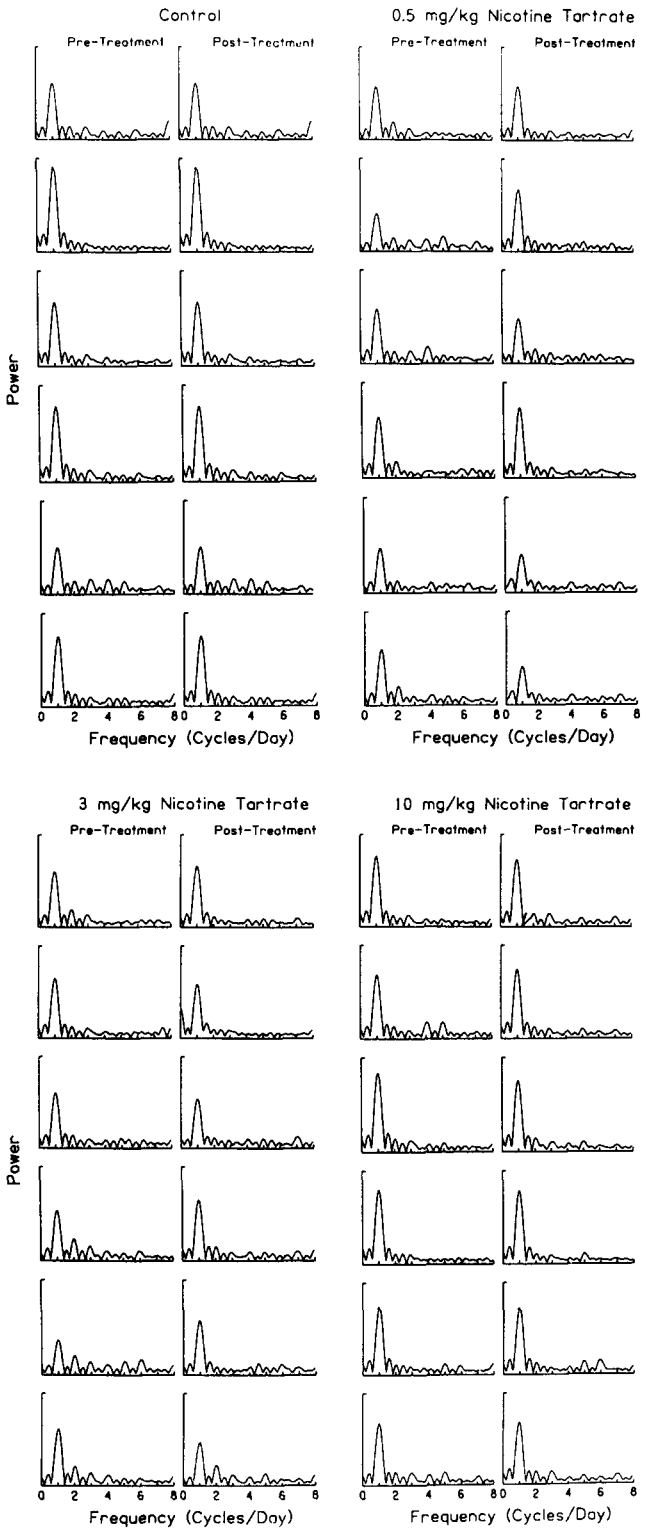

FIG. 4. Fourier analysis was performed on locomotor activity in Experiment I for 7 days before surgery and 7 days after surgery. No differences were observed between the groups. The data for each animal before and after implantation of the pumps is shown (N = 6/group). All animals demonstrated a circadian rhythm of locomotor activity with a peak of 1.00.

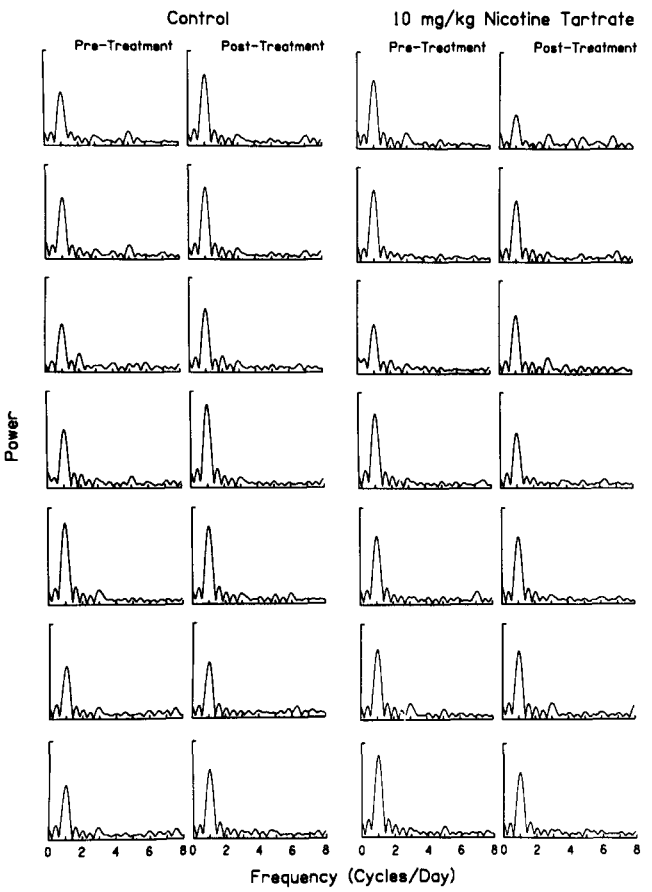

FIG. 5. Fourier analysis was performed on locomotor activity in Experiment II for 7 days before surgery and 7 days after surgery. No differences were observed between the groups. The data for each animal before and after implantation of the pump is shown (N = 7/group). All animals demonstrated a circadian rhythm of locomotor activity with a peak of 1.00.

preted as suggesting that circadian rhythmicity was affected by nicotine. The data presented here, however, provide no evidence to support the hypothesis that chronic nicotine infusion affects circadian rhythmicity.

Our data indicate the hypothalamic BuTX receptors are significantly decreased in density following exposure to the dark in comparison with midday (10:00 and 14:00). This is in some disagreement with a previous study (6) in which the number of BuTX binding sites in the hypothalamic suprachiasmatic nucleus (SCN) was not found to vary with the light-dark cycle. Our tissue samples included a large portion of the hypothalamus; thus, it is likely that the alterations that we observe in the density of BuTX receptors is in an area of the hypothalamus other than the SCN.

It is not clear why nicotine increased locomotor activity during the light, but it is possible that there is a relationship between available receptors and agonist effectiveness. Although the effect of nicotine on locomotor activity may be attributable to a noncholinergic system, the secondary effects of nicotine are likely mediated by nicotinic receptors on noncholinergic axons (14). BuTX binding sites may not be ion-gated receptors, but they are known to have a cholinergic agonist binding site (15). Therefore, they may be functional. In addition, there is evidence that the LH rhythm in female rats is affected by the ventricular infusion of BuTX (8). Nonetheless, these data should not be interpreted as

TABLE 1

ACROPHASE PRE- AND POSTNICOTINE TREATMENT

	Pretreatment			Posttreatment		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Normal light cycle (lights on 06:00–18:00)						
Control	352 ± 2.1	354 ± 2.5	356 ± 8.0	334 ± 4.9*	349 ± 2.0	348 ± 2.1
0.5 mg/kg Nicotine	344 ± 5.7	338 ± 3.4	345 ± 3.3	334 ± 3.2*	344 ± 5.3	347 ± 4.6
3 mg/kg Nicotine	352 ± 6.4	352 ± 11.0	356 ± 7.7	341 ± 5.4†	356 ± 9.4	346 ± 7.9
10 m/gkg Nicotine	351 ± 7.8	347 ± 2.1	349 ± 2.5	323 ± 2.6†	335 ± 4.6	346 ± 4.4
Reversed light cycle (lights on 21:00–09:00)						
Control	220 ± 9.2	229 ± 7.6	232 ± 3.1	223 ± 3.6	238 ± 6.2	240 ± 9.0
10 mg/kg Nicotine	227 ± 3.0	234 ± 8.4	229 ± 4.6	225 ± 3.9	237 ± 4.8	230 ± 3.0

The acrophase was determined with respect to midnight, as described in the text. Each 24-hour period is 360°. Thus, the peak activity in Experiment I (control, pretreatment, day 1) is approximately 23.5 (5 hr, 30 min after lights off) and in Experiment II (control, pretreatment, day 1) is approximately 14.7 (5 hr, 40 min after lights off). There was not a significant effect of nicotine treatment on the acrophase in either Experiment I or Experiment II. All groups in Experiment I demonstrated a transient phase advance after implantation of the mini-pumps (*$p<0.05$, †$p<0.01$, Matched t-tests), which is attributed to the anesthetic. The surgical procedure did not significantly affect the acrophase in Experiment II. This is attributed to the lower dose of anesthetic in Experiment II.

demonstrating a definitive causal relationship between the density of BuTX receptors and locomotor activity or a circadian sensitivity to nicotine.

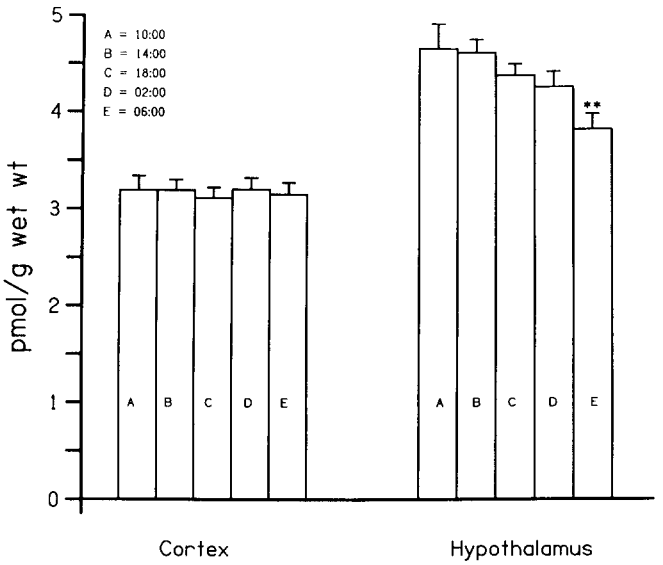

FIG. 6. Animals were sacrificed at 10:00, 14:00, 16:00, 02:00 and 06:00. Planned comparisons using the Tukey Standardized Range Method indicated that the differences between 10:00 and 06:00 and 14:00 and 06:00 were statistically significant (N=8/group; **$p>0.01$ for both comparisons).

In agreement with Cronan *et al.* (4), our data suggest that tolerance develops to the stimulant property of nicotine. We observed stimulation and tolerance only during the light. Cronan *et al.* (4) did not differentially analyze their data with respect to light and dark. The time period for the development of tolerance was different in the two studies. Cronan *et al.* found that tolerance developed within two days while our studies indicate that tolerance develops gradually over a 5–6-day period. This may be attributable to a difference in dose. The authors report that they administered 1 mg/kg daily over a 16-hr period, but they do not indicate the form (free base or salt) or the isomer of nicotine. The doses in our studies yielding tolerance were in the range of 1–3 mg/kg free base.

Thus, these data confirm previous studies that nicotine selectively enhances locomotor activity during the light (1) and that tolerance can develop to the stimulant property of nicotine (4). An increased number of hypothalamic BuTX receptor sites is associated with light, suggesting the possibility that sensitivity to nicotine could be directly or indirectly associated with receptor density. The absence of an effect of nicotine treatment on circadian rhythmicity may relate to the constant infusion of the drug. Acute injections might be more effective in producing a change in the circadian rhythm of locomotor activity.

ACKNOWLEDGEMENTS

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REFERENCES

1. Bovet, D.; Bovet-Nitti, F.; Oliverio, A. Action of nicotine on spontaneous and acquired behavior in rats and mice. Ann. NY Acad. Sci. 142:261–267; 1975.
2. Clarke, P. B. S. Nicotine and smoking: a perspective from animal studies. Psychopharmacology (Berlin) 92:135–143; 1987.
3. Clarke, P. B. S.; Kumar, R. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br. J. Pharmacol. 78: 329–337; 1983.
4. Cronan, T.; Conrad, J.; Bryson, R. Effects of chronically administered nicotine and saline on motor activity in rats. Pharmacol.

Biochem. Behav. 22:897–899; 1985.
5. Earnest, D. J.; Turek, F. W. Neurochemical basis for the photic control of circadian rhythms and seasonal reproductive cycles: role for acetylcholine. Proc. Natl. Acad. Sci. USA 82:4277–4281; 1985.
6. Fuchs, J. L.; Hoppen, K. S. α-Bungarotoxin binding in relation to functional organization of the rat suprachiasmatic nucleus. Brain Res. 407:9–16; 1987.
7. Horseman, N. D.; Pauly, J. R. A detailed analysis of the circadian phase shifting effects of α-bungarotoxin injections into the suprachiasmatic area. Soc. Neurosci. Abstr. 12:550; 1986.
8. Kalash, J.; Romita, V.; Billiar, R. B. Third ventricular injection of α-bungarotoxin decreases pulsatile luteinizing hormone secretion in the ovariectomized rat. Neuroendocrinology 49:462–470; 1989.
9. Keefe, D. L.; Earnest, D. J.; Nelson, D.; Takahasi, J. S.; Turek, F. W. A cholinergic antagonist, mecamylamine, blocks the phase-shifting effects of light on the circadian rhythm of locomotor activity in the golden hamster. Brain Res. 403:308–312; 1987.
10. Ksir, C.; Hakan, R. L.; Kellar, K. J. Chronic nicotine and locomotor activity: influences of exposure dose and test dose. Psychopharmacology (Berlin) 92:25–29; 1987.
11. Ksir, C.; Hakan, R.; Hall, D. P., Jr.; Kellar, K. J. Exposure to nicotine enhances behavioral stimulant effect of nicotine and increases binding of [^{3}H]acetylcholine to nicotinic receptors. Neuropharmacology 24:527–531; 1985.
12. Larsson, C.; Nilsson, L.; Halen, A.; Nordberg, A. Subchronic treatment of rats with nicotine: effects on tolerance and on [^{3}H]acetylcholine and [^{3}H]nicotine binding in the brain. Drug Alcohol Depend. 17:37–45; 1986.
13. Miller, M. M.; Billiar, R. B. A quantitative and morphometric evaluation of ^{125}I-α-bungarotoxin binding in the rat hypothalamus. Brain Res. Bull. 16:681–688; 1986.
14. Morley, B. J. The properties of brain nicotine receptors. Pharmacol. Ther. 15:111–122; 1981.
15. Morley, B. J.; Kemp, G. E. Characterization of a putative nicotinic acetylcholine receptor in mammalian brain. Brain Res. Rev. 217: 81–104; 1981.
16. Morley, B. J.; Lorden, J. F.; Brown, G. B.; Kemp, G. W.; Bradley, R. J. Regional distribution of nicotinic acetylcholine receptors in rat brain. Brain Res. 134:61–166; 1977.
17. Morrison, C. F.; Stephenson, J. A. The occurrence of tolerance to a central depressant effect of nicotine. Br. J. Pharmacol. 45:151–156; 1972.
18. Pauly, J. R.; Horseman, N. D. Autoradiographic localization of α-bungarotoxin binding sites in the suprachiasmatic region of rat brain. Brain Res. 452:105–112; 1977.
19. Stolerman, I. P.; Fink, R.; Jarvik, M. E. Acute and chronic tolerance to nicotine measured by activity in rats. Psychopharmacologia 30: 329–342; 1973.
20. Stolerman, I. P. Bunker, P.; Jarvik, M. E. Nicotine tolerance in rats: role of dose and dose interval. Psychopharmacologia 34:317–325; 1974.
21. Zatz, M.; Herkenham, M. Intraventricular carbachol mimics the phase-shifting effect of light on the circadian rhythm of wheel-running activity. Brain Res. 212:234–238; 1981.