Table 1. Total number of responses per animal on the 25 test trials with click and on the 25 test trials with light.

Stimulus		Score (C-L)
Click	Light	
Injection with RNA-C		
2	3	−1
3	4	−1
5	2	3
5	2	3
6	1	5
7	1	6
7	0	7
11	2	9
Injection with RNA-L		
0	7	−7
0	7	−7
0	3	−3
1	3	−2
0	2	−2
0	2	−2
0	1	−1
7	5	2

we withheld the pellet until the rat responded to the light alone. Thus, we essentially transferred discriminative control of the approach response from the noise of the descending pellet to the blinking light.

Each of these 16 rats was trained to the stimulus as described (*1*). By the end of training, each rat in both groups approached the food cup promptly and swiftly from any part of the box when the appropriate discriminative stimulus (click or blinking light) was presented, and rarely or never approached the cup in the absence of that stimulus.

Upon completion of the training, each rat was killed with ether, and the brain was taken out as quickly as possible. A cut was made on a line joining the superior colliculus to the rostral end of the pons. The tissue posterior to this cut was discarded, as was the tissue of the olfactory bulbs. RNA was extracted (*1*) from the remaining tissue (1.3 g, average weight) and was dissolved in 2.0 ml of isotonic saline. Approximately 8 hours after extraction, the RNA from each of the rats, light-trained or click-trained, was injected intraperitoneally with a 1.9 cm 22-gauge needle into an adapted untrained rat (1). During adaptation, most animals initially made slight startle responses to the click, but few or no startle responses occurred to the blinking light. By the end of the adaptation series, no animal made any visible response to either the click or the blinking light.

Thus eight animals received RNA (RNA-C) from click-trained rats and eight received RNA (RNA-L) from light-trained rats. All were assigned code letters and tested "blind" (*1*).

A session of testing for a given animal consisted of placing that animal in the Skinner box, permitting one minute to elapse, and then delivering a series of ten stimuli (five clicks and five lights in a mixed order, as described below). The stimuli were spaced at least 30 seconds apart. Five such testing sessions were given (*1*). During the first three test sessions, the order of presentation of stimuli was LCCLLCCLLC; during the last two sessions, the order was CLLCCLLCCL. Each test animal thus received a total of 25 click and 25 light trials. At the beginning of testing, all rats had been deprived of food for approximately 24 hours. After the third test session, all rats were fed 4 to 5 g of Purina Lab Chow. The method of testing and the criterion of response were identical to those used in our first experiment.

A comparison of the two judges' tallies revealed that they agreed on 790 out of 800 trials, that is, on 98.7 percent of the judgments.

Each rat received a difference score (C-L) which was obtained by subtracting number of responses to light (L) from number of responses to click (C) for that rat. The Mann-Whitney *U* test (*2*) was performed to test the null hypothesis that the C-L scores of the two groups did not differ from each other. The test indicated that the difference between groups injected with RNA-C and RNA-L was significant ($P<.001$, one-tailed test). The difference in response to click for the two groups was significant by a Mann-Whitney *U* test ($P<.002$, one-tailed test).

We may conclude on the basis of the statistical analysis of C-L scores that the two groups differed in their tendencies to react differentially to click and blinking light. The average difference score for the group injected with RNA-C is positive (3.9), whereas the average difference score for the group injected with RNA-L is negative (−2.8), although the test used compares these scores with each other rather than with zero. A further conclusion is that RNA-C rats responded more to click than did the RNA-L rats. The differences between the groups in response tendencies may be attributed to the RNA preparation injected into the test rats, and hence presumably to the effects which original training produced upon the RNA of the donor animals. Since handling, nutrition, and adaptation to the Skinner box were matched for the two groups, the transfer effect cannot be attributed to these factors. Thus, the new results support our original finding that a response tendency can be transferred by RNA injection, and they further suggest that this effect is to a substantial extent specific rather than general.

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Nicotine: Effect on the Sleep Cycle of the Cat

Abstract. *Small doses of nicotine (0.005 to 0.01 milligram per kilogram of body weight) given intravenously to sleeping cats with indwelling brain electrodes produce (i) initial electroencephalographic activation which was accompanied by behavioral arousal; (ii) a few minutes later, slow-wave sleep; and (iii) within 15 to 30 minutes, fast-wave sleep. Although peripheral afferent stimulation, release of epinephrine, and arginine vasopressin contribute to the initial arousal effects, the primary action of nicotine appears to be on the central nervous system.*

Small doses of nicotine produce electroencephalographic activation in intact animals and in those whose brainstems had been transected (*1*). Although nicotine has multiple peripheral actions, its primary effects on desynchronization of the electroencephalogram are due to action directly on the central nervous system. Of special significance to pharmacologists, psychologists, sociologists, and others interested in the behavioral consequences of tobacco-smoking is the fact that these actions of nicotine on the central nervous system occur in animals with doses that are fully comparable to the small amounts of nicotine

Table 1. Mean percentage time (± standard error) spent in different electroencephalographic states 5 minutes before and after administration of various treatments. The probabilities (as determined by Student's *t*-test) that the divergence of the various drug groups from the saline groups is due to chance are shown in the footnotes. *n*, Number of animals in each group; DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide.

Medication and dose	Electroencephalographic state			
	Awake	Drowsy	Slow-wave sleep	Fast-wave sleep
	Before treatment			
None ($n = 41$)	20.7 ± 2.2	19.4 ± 1.9	59.5 ± 2.9	0.4 ± 0.2
	After treatment			
Saline, 1.5 ml ($n = 15$)	20.7 ± 3.9	29.9 ± 3.3	49.3 ± 4.5	0
Nicotine, 0.01 mg/kg ($n = 9$)	79.3 ± 5.1*	8.9 ± 2.5†	11.8 ± 5.5*	0
Epinephrine, 0.002 mg/kg ($n = 5$)	50.7 ± 4.0*	40.0 ± 3.6	9.3 ± 2.7*	0
DMPP, 0.005 mg/kg ($n = 6$)	38.9 ± 4.9‡	32.2 ± 4.0	28.9 ± 7.6‡	0
Arginine vasopressin, 50 mμ/kg ($n = 6$)	53.3 ± 10.0†	8.9 ± 2.2†	37.7 ± 11.1	0

* $P < .001$. † $P < .01$. ‡ $P < .05$.

Table 2. Mean percent time (± standard error) spent in different electroencephalographic states 25 minutes before and after administration of various treatments. The probabilities (as determined by Student's *t*-test) that the divergence of the various drug groups from the saline groups is due to chance are shown in the footnotes. *n*, Number of animals in each group; DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide.

Medication and dose	Electroencephalographic state			
	Awake	Drowsy	Slow-wave sleep	Fast-wave sleep
	Before treatment			
None ($n = 41$)	32.9 ± 2.0	24.7 ± 1.4	38.6 ± 1.9	3.8 ± 1.0
	After treatment			
Saline, 1.5 ml ($n = 15$)	33.1 ± 3.0	23.4 ± 2.6	40.5 ± 3.7	3.0 ± 1.4
Nicotine, 0.01 mg/kg ($n = 9$)	33.8 ± 4.1	13.6 ± 2.2*	40.6 ± 3.6	12.0 ± 3.1†
Epinephrine, 0.002 mg/kg ($n = 5$)	32.5 ± 3.3	34.1 ± 2.2	31.7 ± 2.9	1.6 ± 1.0
DMPP, 0.005 mg/kg ($n = 6$)	31.5 ± 3.2	26.0 ± 2.8	35.8 ± 6.6	6.7 ± 4.6
Arginine vasopressin, 50 mμ/kg ($n = 6$)	30.0 ± 6.1	11.5 ± 2.6*	47.1 ± 6.8	11.3 ± 5.1*

* $P < .05$. † $P < .01$.

absorbed by man upon inhalation of tobacco smoke (*2*). Inasmuch as neocortical electroencephalographic desynchronization has been associated with both behavorial wakefulness and fast-wave or "activated" sleep (*3*), we studied the effects of small doses of nicotine infusion in unanesthetized cats with indwelling electrodes in the brain.

Electrodes were implanted by conventional stereotaxic techniques in the somatosensory cortex, hippocampus, amygdala, posterior hypothalamus, and midbrain reticular formation of 15 cats. Silastic tubing was used for a cannula into the jugular vein for intravenous injections. All surgery was performed under pentobarbital anesthesia. Two weeks after implantation, when the cats had fully recovered, they were placed in a warm, soundproof insulated box which had a one-way window. The natural sleep–awake cycle of the animals was recorded throughout the experimental day. All medications were given in an incomplete Latin square design.

Intravenous infusions of warmed saline for 1 minute had no significant consequences on either the electroencephalogram or behavior in the cats that were in natural slow-wave (deep) sleep. Nicotine in doses of 0.005 to 0.010 mg per kilogram of body weight produced three distinct electroencephalographic and behavioral phenomena. First, there was a brief period (approximately 3 minutes) of neocortical desynchronization and hippocampal theta activity which was accompanied by behavioral wake-up from natural slow-wave sleep. Subsequently the animals again lapsed into slow-wave sleep. Frequently the animals were in a deeper sleep than before nicotine injection. After approximately 15 to 25 minutes, an increased incidence of fast-wave sleep was observed. The effects of nicotine were blocked by mecamylamine, a ganglionic blocking agent which can penetrate into the central nervous system. These actions of nicotine were not blocked by trimethidinium, a quaternary ganglionic blocking drug, which does not easily penetrate the blood-brain barrier. A peripherally acting ganglionic stimulant, 1,1-dimethyl-4-phenylpiperazinium iodide, in equipressor doses (0.005 mg/kg) produced much weaker arousal effects. Nicotine produces a greater percentage of wakefulness than the other agents tested, although these have a similar effect (Table 1).

Continine, a well-known metabolite of nicotine, had no effects when given in doses equal to those of nicotine. Massive doses of continine (25 mg/kg) produced a transient electroencephalographic and behavioral arousal effect which lasted only 1 to 2 minutes.

Nicotine and arginine vasopressin increased the time that the animals spent in activated sleep within a 25-minute period after drug injection (Table 2). The ability of nicotine to promote activated sleep may be related to vasopressin release.

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