against reductions in the plasma T_4, thereby blunting effects of hypothyroidism in this tissue.

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Persistent Behavior at High Rates Maintained by Intravenous Self-Administration of Nicotine

Abstract. *Squirrel monkeys pressed a lever at high rates under a second-order schedule of reinforcement in which lever pressing produced a brief visual stimulus that was occasionally contiguous with an intravenous injection of nicotine. The rate of lever pressing could be markedly reduced either by substituting saline for nicotine injections or by blocking the effects of nicotine with mecamylamine. The rate of lever pressing could also be reduced by eliminating the brief visual stimulus. These results show that nicotine can function as an effective reinforcer under a second-order schedule of drug self-administration and that an environmental stimulus associated with nicotine intake can contribute to the maintenance of persistent drug-seeking behavior.*

The role of nicotine in the maintenance of tobacco smoking has been questioned because of difficulties in demonstrating consistent reinforcing effects of the drug under controlled laboratory situations. Variations in the nicotine content of cigarettes or treatment with agents that block nicotine's actions have occasionally been found to alter human smoking behavior, but the changes have been small, not always reproducible, and open to disparate interpretations (*1*). Moreover, reliable evidence that nicotine can function as a reinforcer of animal drug self-administration is limited. Some investigators, including ourselves, have reported that intravenous injections of nicotine can maintain self-administration behavior of rats or monkeys, but the levels of responding usually have been low (*2*, *3*). Other investigators have found nicotine to be ineffective in maintaining self-administration behavior (*4*).

If nicotine functions as a reinforcer to maintain tobacco smoking, it is likely that its reinforcing effects are magnified by interactions with interoceptive and exteroceptive stimuli associated with smoking, such as taste, tactile sensation, and social setting (*1*). The temporal contiguity of these stimuli with the reinforcing effects of nicotine may result in the stimuli's acquiring conditioned reinforcing properties, which further strengthen smoking behavior. Consequently, nicotine might function more effectively to maintain self-administration behavior by laboratory animals if it were studied under conditions in which responding resulted not only in nicotine injections but also in presentations of environmental stimuli associated with injections.

Previous studies have shown that long and orderly sequences of responding can be maintained by scheduled presentations of environmental stimuli that have been associated with intravenous injections of drugs such as morphine and cocaine (*5*, *6*). The schedules of reinforcement relating responding to consequent presentations of the stimuli and injections of drugs in these studies have been termed second-order schedules (*5*, *6*). We now report that intravenous injections of nicotine can maintain very high rates of lever-press responding by squirrel monkeys under a second-order schedule in which responding results in presentations of a visual stimulus that is intermittently associated with nicotine injection.

Four mature male squirrel monkeys (*Saimiri sciureus*) had venous catheters permanently implanted (*7*) and had unrestricted access to food and water in their individual living cages. During experimental sessions, the monkeys sat in a chair equipped with a response lever and green and amber stimulus lights (*8*); the chair was enclosed in a sound-attenuating chamber. Injections were delivered through the catheters from an infusion pump located outside the chamber (*9*). Before the experiment began, three monkeys (S-151, S-156, and S-200) had been trained to press a lever under a second-order schedule of intravenous cocaine injection (*10*); responding was subsequently extinguished by substituting saline for drug injections. The fourth monkey (S-464) was experimentally naïve at the beginning of the study.

In the cocaine-trained monkeys, responding was established under a second-order schedule of intravenous nicotine injection without preliminary training. In the untrained monkey (S-464), responding was first established under a fixed-interval (FI) schedule of intravenous nicotine injection (*11*), and the schedule then was changed to a second-order schedule. Under the second-order schedule, the green stimulus light was

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turned on at the beginning of each experimental session, and every tenth lever-pressing response [fixed ratio (FR10)] during a 1-minute (S-151) or 2-minute (S-156, S-200, and S-464) fixed interval of time (FI 1 or FI 2 minute) changed the light from green to amber for 1 second; the first FR10 unit completed after the FI elapsed turned off the green light and produced both the 1-second amber light and an intravenous injection of 30 μg of nicotine per kilogram of body weight. A 3-minute time-out period, during which the chamber was dark and responses had no specified consequences, followed the injection. At the end of the time-out, the green light was again turned on and the schedule was restarted. Each session ended after the 12th time-out period or 90 minutes, whichever occurred first.

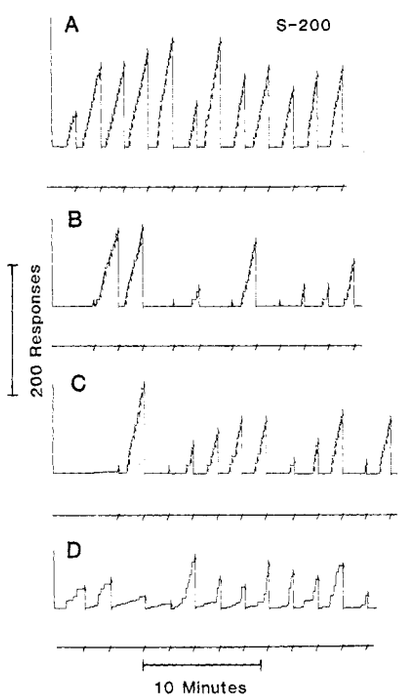

Fig. 1. Cumulative records of lever pressing showing representative performances under the second-order schedule of intravenous drug injection (monkey S-200). Diagonal marks of the response pen indicate 1-second presentations of the amber light; diagonal marks of the event pen and resetting of the response pen indicate injections of nicotine or saline. The recorder did not operate during the 3-minute time-out period, which followed each injection. Each record shows a complete session. (A) The completion of each FR10 unit produced the brief stimulus, and the first FR10 unit completed after the 2-minute FI elapsed produced both the brief stimulus and an intravenous injection of 30 μg of nicotine per kilogram of body weight. (B) Saline was substituted for nicotine injections. (C) Mecamylamine (100 mg/kg) was injected intramuscularly 30 minutes before the session. (D) Brief stimulus presentations were omitted during the FI.

Responding developed and stabilized under the second-order schedule of nicotine injection within 30 sessions in all monkeys. In each case, rates and temporal patterns of responding were characteristic of those observed under second-order schedules involving other consequent events such as presentation of food or intravenous injection of morphine or cocaine (*5*, *12*); an initial pause at the beginning of each FR unit was followed by an abrupt transition to a high steady rate of responding that continued until the FR unit was completed and the brief amber light was presented (Fig. 1A). For individual monkeys, the overall rate of responding (*13*) was consistently high, averaging between 0.81 and 1.58 responses per second; the local rate of responding from the first to last response in each FR unit (*14*) was even higher, averaging between 1.22 and 4.77 responses per second.

The role of nicotine injections in maintaining responding was investigated by substituting saline for nicotine injections and by pharmacologically blocking the effects of nicotine with the nicotinic antagonist mecamylamine. When saline was substituted for nicotine injections in monkeys S-151 and S-200, the overall rate of responding quickly declined to very low levels (Figs. 1B and 2). For monkey S-156, responding fell markedly during the first five sessions of saline substitution, but increased during subsequent sessions. After the tenth session, the brief amber light was omitted during the interval and responding declined further; the rate of responding remained low for this monkey when the brief visual stimulus was reinstated while saline substitution continued. When saline injections were replaced by injections of nicotine (30 μg/kg), responding by all three monkeys recovered to previously high levels. When 1.0 mg of mecamylamine per kilogram was administered intramuscularly to monkeys S-151 and S-200 30 minutes before each session (*15*), the high rates of responding maintained by nicotine injections again fell rapidly to low levels, similar to the levels observed during saline substitution (Figs. 1C and 2). High rates of responding were restored when mecamylamine treatment was discontinued.

Although the frequency of nicotine injection was about the same under the second-order schedules and under an FI schedule used in previous experiments (*3*), overall rates of responding were much higher under the second-order schedules. These higher rates of responding are probably attributable to presentations of the brief visual stimulus, which was intermittently associated with nicotine injection. In monkey S-156, for example, high rates of responding persisted during saline substitution until the brief stimulus was omitted. The role of the brief visual stimulus in maintaining responding was examined further by omitting the stimulus entirely during the FI while responding was maintained by nicotine injection; only the first FR10 unit completed after the FI elapsed produced the 1-second amber light concomitant with an intravenous injection of nicotine (30 μg/kg). With the brief stimulus omitted, the overall rate of responding declined to about half the rate maintained previously for each monkey (Figs. 1D and 2). When the brief stimulus again was presented after the completion of every FR10 unit, the overall rate of responding increased.

In the present experiments, nicotine functioned effectively to maintain lever pressing by squirrel monkeys under a

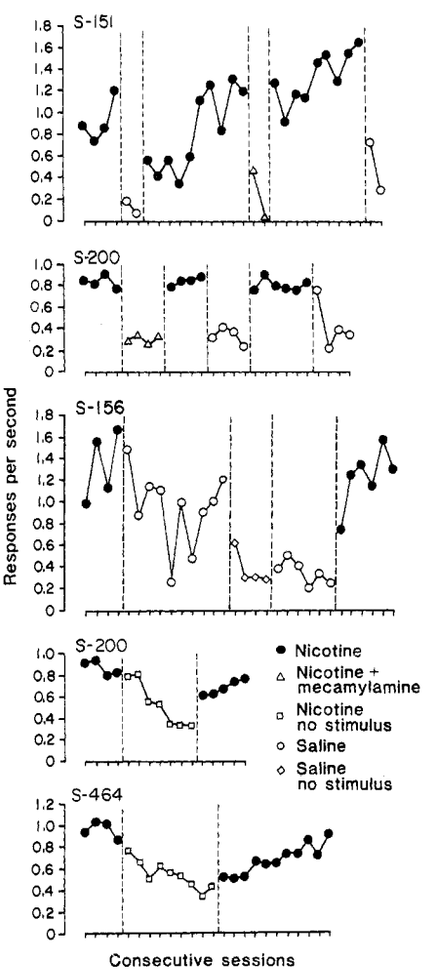

Fig. 2. Effects of substituting saline for nicotine injections (open circles), treatment with mecamylamine before the session (open triangles), or omitting the brief stimulus during the FI (open squares) on responding under the second-order schedule of intravenous nicotine injection for individual monkeys.

second-order schedule of intravenous injection. Average rates of responding often exceeded one response per second, and monkeys pressed the lever as many as 250 times per single injection of nicotine. The maintenance of responding was unequivocally the result of the consequent injections of nicotine, since responding could be extinguished by either saline substitution or mecamylamine treatment. Although responding ultimately depended on injections of nicotine, the brief visual stimulus associated with injections played an important role in the maintenance of persistent responding, since rates of responding were about twice as high when the brief stimulus was presented as when it was not.

There has been a continuing need for a sensitive laboratory method for evaluating the reinforcing effects of nicotine. In this study, persistent behavior was maintained at high rates under a second-order schedule of intravenous nicotine injection. Furthermore, the behavior was highly sensitive to both environmental and pharmacological intervention. Second-order schedules of nicotine injection may therefore provide a useful experimental technique for examining environmental and pharmacological factors that contribute to the maintenance of tobacco use by humans.

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8. The chair was similar to the one described by D. F. Hake and N. H. Azrin [*J. Exp. Anal. Behav.* **6**, 297 (1963)].
9. The volume of each injection was 0.20 ml, infused over 200 msec. Nicotine hydrogen (+)-tartrate was dissolved in 0.9 percent saline; doses are expressed as the salt.
10. Training procedures and apparatus were similar to those described by S. R. Goldberg [*J. Pharmacol. Exp. Ther.* **186**, 18 (1973)].
11. Training procedures were similar to those described by Goldberg and Spealman (*3*).
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13. Overall response rates were computed by dividing total responses in the presence of the green light by total time the green light was on. (Responses and time during 1-second amber lights were not included in computations.)
14. Local response rates were computed as the mean rate from the first to last response in each FR unit. (Pause time before the first response was not included in computations.)
15. This dose was selected on the basis of a previous study, which showed that 1.0 mg of mecamylamine per kilogram of body weight can block the behavioral effects of nicotine in squirrel monkeys but does not alter schedule-controlled behavior when given alone [R. D. Spealman, S. R. Goldberg, M. L. Gardner, *J. Pharmacol. Exp. Ther.* **216**, 484 (1981)].
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Acoustic Communication and Reproductive Isolation in Two Species of Wolf Spiders

Abstract. *Sound production by male wolf spiders during courtship is critical for behavioral reproductive isolation of two sibling species. Females only respond to and copulate with conspecific males, and acoustic signals through a substrate are necessary to induce receptivity. No reproductive barriers that could arise during mating (such as genital or mechanical incompatibility) or after mating (infertility) are in effect between the species, since forced interspecific matings produce viable offspring.*

Sound production in spiders is more common than is generally realized (*1*), although in only a few instances have spider sounds been recorded (*2–4*). Recently, Rovner (*3*) found a substrate-coupled sound-producing apparatus in wolf spiders (family Lycosidae) and demonstrated, by playback techniques, the communicatory function of these sounds during courtship and agonistic display (*4*). Our study provides evidence that communication by substrate-coupled stridulation is critical for the reproductive isolation of two sibling species of wolf spiders.

Schizocosa ocreata (Hentz), common in deciduous forest leaf litter throughout the eastern United States (*5*), is identical to *Schizocosa rovneri* Uetz and Dondale with respect to genital characters, body size, general morphology, and color. These species were previously considered to be a single species (*6*). The females are indistinguishable, but male *S. rovneri* lack the prominent tufts of black bristles present on the tibiae of the first pair of legs in mature male *S. ocreata*. These two species exhibit a high degree of overlap in geographic range, microhabitat, and seasonality (*7*).

Table 1. Behavioral responses of male and female wolf spiders in experimental courtship pairings. Symbols: +, courting by male and receptiveness by female; −, no response, avoidance, or agonistic behavior.

Courtship pairing	Males		Females	
	+	−	+	−
Schizocosa rovneri				
Conspecific	52	1	45	8
Heterospecific	38	3	0	53
χ^2	N.S.*		78.2	
			$P < .005$	
Schizocosa ocreata				
Conspecific	21	1	34	9
Heterospecific	29	1	1	40
χ^2	N.S.		50.71	
			$P < .005$	

*N.S., not significant.

The courtship behavior of mature male *S. ocreata* has been described (*8, 9*) as an active tapping of the first pair of legs in unison, accompanied by movements of the pedipalps. Sounds (vibrations) are produced by a stridulatory organ on opposing segments of the palpal tibiotarsal joint and are conducted through the substrate via stout spines at the distal ends of the palps (*3*). In the later phases of courtship, the display consists of raising and extending the first pair of legs in addition to stridulation.

The courtship behavior of *S. rovneri* differs considerably from this. The male executes a "bounce" several times in succession at 3- to 5-second intervals, then moves to another location. The bounce involves movement of the entire body; the cephalothorax and abdomen are raised up and thrust downward between the legs, sometimes hitting the substrate. Rotating movements of the

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