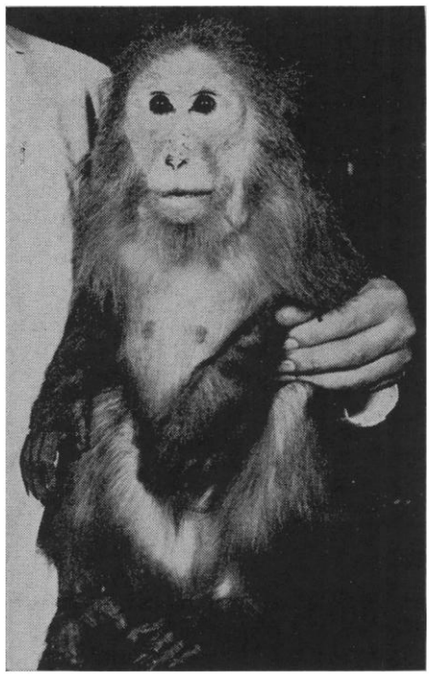

Fig. 1. *Macaca speciosa,* young adult female. Note the placid expression.

essential maneuver when one is catching the adult *M. mulatta,* is never required since, in our experience, *M. speciosa* will not bite. When transported, the 4 year old clings tightly to the handler, but it can be walked and led by the hand. It should be especially noted that the docility is not the result of extensive handling, as is the case when the immature *M. mulatta* is tamed, as it occasionally is.

Despite its unusual docility, *M. speciosa* displays many of the emotional responses characteristic of *M. mulatta,* unaccompanied however by the belligerence and aggression. If suddenly attacked, it will typically show lipsmacking, defecation, and other autonomic responses and a startle-like jerking of the body, but it will not attack or bite in retaliation. In this respect *M. speciosa* behaves very much like individuals of *M. mulatta* that have undergone bitemporal lobectomy. Thus, when frustrated in the learning test situation, *M. speciosa* may shriek and sulk but quickly composes itself. In our observation of monkeys up to 4 years old we have noted no obvious sex differences with respect to docility.

Young adults as well as immature stump-tailed macaques seem to get along well together in group enclosures. Five immature females were housed together over a period of several months. Dominance patterns were established and maintained through nipping, grooming, and other social behavior. No serious biting was observed. The animals were occasionally seen to huddle together, sometimes hugging and rocking in the manner of young chimpanzees. In fact, the similarity to group and individual behavior of chimpanzees was often striking.

The behavior of seven stump-tailed macaques on a number of standard discrimination tests—color, brightness, pattern, and delayed response—was studied. Five of the monkeys were immature; the other two were young adults. All were naive with respect to the experiment. The familiar version of the two-choice discrimination apparatus was used.

The discrimination stimuli consisted of painted plaques. In this preliminary study we were concerned with the docility and tractability of the animals and made no effort to define the limits of learning and memory capacity. Hence we chose problems that were well within the normal abilities of the animals.

The monkeys adapted rapidly to the two-choice situation and learned, usually within one session, to displace one of two plaques to obtain food. No coaxing was needed. All the animals were carried in the arms of the handler, and they experienced little trauma in the process. Acquisition scores did not differ from those previously obtained for a comparable group of *M. mulatta* (*7*). Even the most difficult pattern discrimination (plus symbol versus square) was acquired in no more than 210 trials, with a median for the seven subjects of 110 trials. Delayed response, with 5-second delays, was acquired in one session by five of the seven monkeys. The other two required two and three sessions to reach the criterion of learning. The monkeys worked consistently at the tasks. When there was a run of errors they sometimes shrieked and sulked, but they would quickly recover and return to the task. In all other respects the monkeys behaved in much the same way as *M. mulatta.* The position habit occasionally had to be overcome (*8*).

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28 November 1962

Anticholinergic Drugs and the Central Control of Thirst

Abstract. *Atropine and scopolamine were compared with their centrally inactive quaternary analogs, atropine methyl nitrate and scopolamine methyl nitrate, for effects on water and food intake in rats. All drugs inhibited eating, but only the centrally active compounds inhibited drinking. Anticholinergic drugs evidently block drinking by a central effect and eating by a peripheral effect.*

Attempts to test Cannon's "dry throat" theory of thirst by use of anticholinergic drugs such as atropine date back more than 30 years (*1*). Atropine dries the mouth by blocking salivation and therefore should increase the intake of water if the theory is correct. But the results of these drug tests have been more perplexing than enlightening. Instead of increasing the intake of water, atropine decreases it.

A possible explanation of this unexpected result was suggested by the observation that feeding is also inhibited by atropine. Since water intake is closely related to food intake, it was thought that the depression of drinking resulted indirectly from a primary effect on eating, but this idea was not substantiated by work of Schmidt *et al.* (*2*). These investigators found that atropine depressed drinking even in tests in which no access to food was allowed, and they therefore concluded that the effect was not dependent upon food intake.

In this report I present further support for the idea that anticholinergic drugs inhibit drinking and eating by different mechanisms. Moreover, my data suggest that the depression of drinking is a central effect and that the depression of eating is peripheral.

My experiments are based on the fact that the methyl quaternary analogs of atropine and scopolamine penetrate the blood-brain barrier only with difficulty. Therefore they have relatively weak central anticholinergic effects although each exceeds its centrally active analog in peripheral activity (*3*). If dose levels are chosen judiciously, it is possible to distinguish between central and peripheral anticholinergic effects by testing atropine against methyl-atropine or scopolamine against methyl-scopolamine (*4*). Effects observed only with atropine or scopolamine are presumed to be of central origin. Furthermore, scopolamine is at least 20 times more potent than atropine in central activity, while their peripheral potencies tend to converge (*3, 5*). Hence effects that require much higher doses of atropine than scopolamine are likely to be central.

In a main experiment on water intake, 30 male rats weighing about 450 g each were given access to water for 1 hour each day. Dry food was available at all times except during the 1-hour drinking test. Prior to drug trials, the rats had adapted to the 23-hour water deprivation schedule and their water intakes had stabilized.

On test days, half an hour before the start of the drinking period, each rat was given a 0.5-ml intraperitoneal injection of one of the following: scopolamine hydrochloride (0.4 mg/kg), scopolamine methyl nitrate (0.4 mg/kg), atropine sulfate (5 mg/kg), atropine methyl nitrate (5 mg/kg), or physiological saline. All rats received each drug once and saline twice. The sequences of administration were varied, and at least 4 days elapsed between injections.

Another group of 22 rats was used in a similarly designed experiment on food intake. In the feeding test, free access to dry lab chow pellets was allowed for half an hour. Water was available at all times except during the test. Immediately after the daily test, individual supplemental feedings were given in order to maintain the weight of each rat at 80 percent of its preexperimental weight. Animals were dosed as in the water-intake experiment, except that each rat was tested with only two of the four anticholinergic drugs. Subsidiary experiments were made on small subgroups of the rats before and after the main experiments to sample the effects of other dose levels.

Table 1. Average intake of water or dry food after injections of anticholinergic drugs or physiological saline. Number of rats is shown in parentheses.

Drug	Dose (mg/kg)	Mean intake ± S.E.M.	
		Water (ml)	Food (g)
Main experiments			
Scopolamine	0.4	10.3 ± 1.2 (30)*	2.5 ± 0.7 (11)*
Scopolamine methyl nitrate	0.4	18.8 ± 0.7 (30)	1.6 ± 0.6 (11)*
Atropine	5	12.5 ± 1.1 (30)*	1.5 ± 0.3 (11)*
Atropine methyl nitrate	5	17.0 ± 0.6 (30)*	1.3 ± 0.2 (11)*
Saline (first test)		19.8 ± 0.6 (30)	8.6 ± 0.2 (11)
Saline (second test)		21.6 ± 0.8 (30)	7.8 ± 0.3 (11)
Subsidiary experiments			
Scopolamine	0.8	6.3 ± 3.0 (7)*	Not tested
Scopolamine methyl nitrate	0.8	15.7 ± 1.9 (7)	Not tested
Atropine	1	Not tested	2.4 ± 0.7 (6)*
Atropine	2.5	17.3 ± 1.2 (5)	1.6 ± 0.4 (5)*
Atropine methyl nitrate	1	Not tested	1.3 ± 0.1 (6)*
Atropine methyl nitrate	2.5	Not tested	1.4 ± 0.3 (5)*

* Significantly different from saline at the .001 level or beyond.

The results of the main experiments and the most extensive subsidiary experiments are summarized in Table 1. Interest centers on the observation that atropine and scopolamine depressed water intake extensively while their methyl analogs did not. The difference was somewhat clearer in the case of scopolamine. At the 0.4-mg/kg dose level, scopolamine reduced water intake by 50 percent while methyl-scopolamine was almost without effect; the discrepancy was even greater at the 0.8-mg/kg dose.

Figure 1, which gives individual water-intake scores for each rat, furnishes a striking display of the difference between these drugs. In 27 out of 30 cases, intake was lower under scopolamine. Frequently, the scores for scopolamine were only a small fraction of the scores for methyl-scopolamine.

Atropine had an important effect on drinking only at the 5-mg/kg dose.

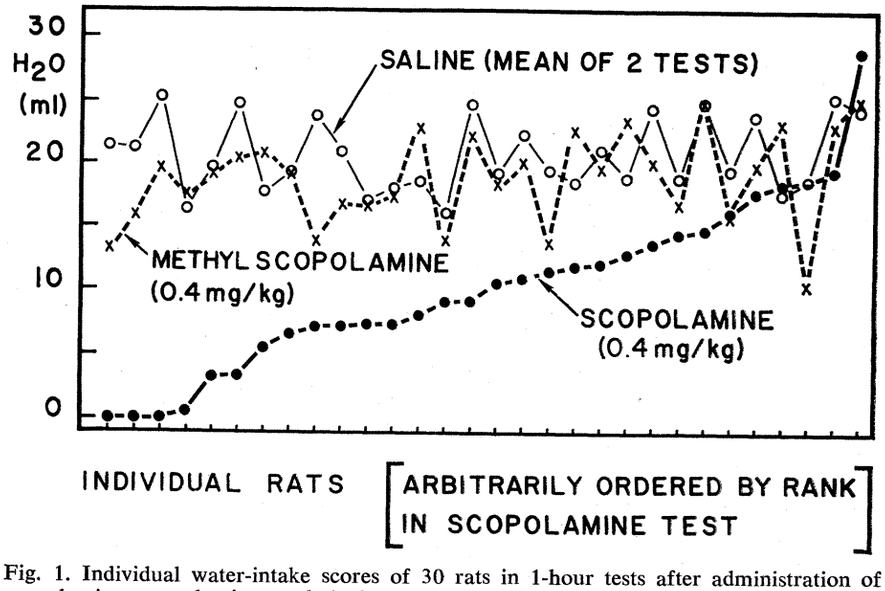

Fig. 1. Individual water-intake scores of 30 rats in 1-hour tests after administration of scopolamine, scopolamine methyl nitrate, or physiological saline (three scores per rat). Note strong depressing effect of scopolamine and virtual inactivity of its quaternary derivative. Ranking the scopolamine scores shows their relationships to other scores. Thus the horizontal slope of the saline (control) curve shows that the inhibiting effect of scopolamine is not a function of normal intake.

Scopolamine was much more potent; a good effect has been observed even at 0.2 mg/kg. This superiority of scopolamine, taken together with the demonstrated (relative) inactivity of the quaternary compounds, is compelling evidence that anticholinergic drugs inhibit drinking by a central effect.

Table 1 also shows that all compounds strongly depressed food intake. In the feeding experiments, however, the quaternary compounds were slightly more potent than their tertiary analogs. In addition, the potencies of atropine and scopolamine tended to converge; note that atropine at 1 mg/kg had about the same effect as scopolamine at 0.4 mg/kg. These observations indicate that anticholinergic drugs inhibit food intake by a peripheral effect. Possibly feeding is inhibited because it is aversive to chew dry food when salivation is blocked.

The results on water intake complement recent findings of Grossman (*6*) and Stein and Seifter (*7*). Grossman induced drinking in water-satiated rats by applying minute quantities of cholinergic substances directly into the hypothalamus. Stein and Seifter confirmed and extended this work by showing that the effect was muscarinic rather than nicotinic (*8*). These studies suggest strongly that, at least in the rat, muscarinic synapses in the hypothalamus are part of a brain system that regulates water intake. Such hypothalamic synapses, and perhaps other muscarinic synapses at different levels, provide likely sites of action for the drug effects reported here (*9*).

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24 October 1962

Isolation and Identification of the Sex Attractant of the American Cockroach

Abstract. *The highly potent sex attractant of the female American cockroach,* Periplaneta americana *(L.), has been isolated in pure form and identified as 2,2-dimethyl-3-isopropylidenecyclopropyl propionate. The hydrogenated form of the attractant has been synthesized.*

The virgin female American cockroach, *Periplaneta americana* (L.), emits a powerful attractant which elicits intense excitement and characteristic wing-raising in the males of this species (*1*). Wharton *et al.* (*2*) showed that filter paper over which these females had crawled was highly attractive to males, and these investigators have very recently reported that they obtained from such paper 28 μg of a pure attractant which they were not able to identify (*3*). As the result of an independent investigation, we report the successful isolation and identification of the natural sex attractant.

Extraction of filter papers with which virgin female roaches had been in contact gave extremely small amounts of an attractive mixture from which the abundant contaminants were very difficult to separate. Much larger amounts of fairly pure attractant were obtained by passing air continuously over many virgin females in metal containers, condensing the vapors in a dry ice cooled flask containing a little 0.1-percent hydrochloric acid (*4*), and extracting the condensate with distilled hexane (*5*). The hexane solution was washed with water, dried over sodium sulfate, and freed of solvent at 20 mm-Hg pressure (bath below 40°C). The residual yellow semisolid was chromatographed on a column of silicic acid [Bio Rad Laboratories, Richmond, Calif., specially treated to conform with that described by Hirsch and Ahrens (*6*)] by successive elution with spectral-grade hexane and 3 percent ether in hexane; elution with 10 percent ether in hexane then removed a highly active yellow liquid from which the pure attractant was obtained by steam distillation. In this way there was obtained, from the equivalent of approximately 10,000 females "milked" continuously over a 9-month period, 12.2 mg of the pure attractant as a yellow liquid with a characteristic odor; it elicits a response from males at levels below 10^{-14} μg.

Gas chromatography of the attractant on a 4-foot Chromosorb W column (80 to 100 mesh) coated with 5-percent Apiezon M, under conditions identical with those reported by Wharton *et al.* (*3*), gave a single peak with an elution time of 6 minutes as contrasted with 105 or 145 minutes reported by them. In a single determination it analyzed for $C_{11}H_{18}O_2$, showed no optical rotation at a concentration of 1.8 percent ($CHCl_3$), and was free of absorption in the ultraviolet range. Its infrared spectrum showed it to be an ester, which was supported by its inactivation through refluxing with dilute alkali or concentrated hydrochloric acid; the presence of an isopropylidene group at 12.5 μ was also indicated (*7*). Catalytic hydrogenation of 2.2 mg of the attractant with platinum oxide catalyst resulted in an uptake of hydrogen sufficient for 1.1 double bonds, giving a colorless, inactive oil whose infrared spectrum lacked isopropylidene absorption and showed splitting at 7.25 μ characteristic of an isopropyl group. Hydrogenolytic chromatography of the attractant by the method of Beroza (*8*) gave ethane and 2,2,4-trimethylpentane. A nuclear magnetic resonance spectrum at 60 mcy/sec on the limited amount of attractant in deuterochloroform showed no hydrogen attached to a double bond and disclosed, among other features, two sharp prominent peaks: one at 75.5 cy/sec from internal tetramethylsilane was roughly equivalent to six hydrogen atoms (two methyl groups) and one at 140.5 cy/sec was equivalent to one hydrogen atom. Alkaline saponification of 2.2 mg of the saturated compound showed a saponification equivalent of 182, yielding 1.5 mg of a liquid alcohol, whose infrared spectrum showed a secondary hydroxyl group, and 0.85 mg of an acid identified by paper chromatography and its infrared spectrum as propionic acid. Oxidation of 4 mg of the attractant with periodate-permanganate reagent (*9*) gave propionic acid (identified by infrared spectrum and paper chromatography), acetone [2,4-dinitrophenylhydrazone, mp 127°C, undepressed by an authentic sample, λ_{max} 349 mμ (EtOH)], and 2.2 mg of a neutral substance that formed colorless crystals, mp 55°C (from ethyl acetate), λ_{max} 277 mμ (EtOH), whose 2,4-dinitrophenylhydrazone melted at 232°C, λ_{max} 355, 358 mμ ($CHCl_3$). The infrared spectrum of this substance indicated the presence of a chelated hydroxyl and showed two carbonyl bands. Further