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# The Effects of Muscarinic Cholinergic Blockade upon Shock-Elicited Aggression[1]

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POWELL, D. A., W. L. MILLIGAN AND K. WALTERS. *The effects of muscarinic cholinergic blockade upon shock-elicited aggression.* PHARMAC. BIOCHEM. BEHAV. 1(4) 389–394, 1973.–Graded dosages of atropine sulfate, atropine methyl nitrate, scopolamine hydrochloride, and scopolamine methyl nitrate were administered to rats and shock-elicited fighting frequencies determined. Central cholinergic blockade decreased fighting at appropriate dosages, but peripheral cholinergic blockade had little or no effect upon shock-elicited aggression. These results suggest that shock-elicited aggression is similar to other kinds of agonistic behavior (e.g., isolation-induced fighting and muricide) in that a central cholinergic system is apparently involved in its mediation.

Aggression Shock Atropine Scopolamine Central bloackade Peripheral blockade

THERE has been much interest recently in the study of fighting behavior elicited by aversive stimulation [13,24]. Much of this research has been parametric in nature [1,22], or has used the response as a measure of different kinds of "motivational states" [11,16]. There have been few attempts to study the relationship of this kind of aggession to other manifestations of fighting behavior.

In the present experiments shock-elicited aggresssion (SEA) was studied as a function of central cholinergic blockade. Data on several other kinds of animal aggression implicate central cholinergic mechanisms in the mediation of these behaviors. For example, Janssen, Jageneau and Niemegeers [12], and DaVanzo, Daugherty, Ruckart and Kang [8] studied the effects of atropine and scopolamine upon intraspecies fighting induced by isolation in mice. Smith, King and Hoebel [19] as well as Bandler [2,3] studied mouse killing by rats (muricide) as a function of centrally administered cholinergic agonists and blockades. In all these investigations cholinergic blockade decreased fighting or attack, and cholinergic agonists increased aggression. However, the effects of cholinergic agents upon SEA have not been investigated. Both interspecies and intraspecies attack have also been shown to be related to adrenergic and serotonergic drugs [26]. However, SEA is less affected by these manipulations [7, 18, 21]. A question of some interest then concerns the role that the central cholinergic system plays in mediating shock-elicited aggression.

## EXPERIMENT 1: THE EFFECTS OF ATROPINE ADMINISTRATION ON SHOCK-ELICITED FIGHTING

In the present experiment several dosages of atropine sulfate, a central cholinergic blocking agent, were administered to pairs of rats and their shock-elicited fighting rates determined. Saline control injections alternated daily with drug injections.

### *Method*

*Animals.* Thirty-two female Sprague-Dawley rats obtained from Flow Laboratories were utilized. The animals were approximately 100 days old at the beginning of the experiment, and were housed individually with food and water available ad lib.

*Apparatus.* An animal chamber with inside dimensions of 24 x 20 x 30 cm was used. The sides and back of this chamber were aluminum and the top and front were made of Plexiglas. It was housed inside a sound-attenuating compartment with a one-way mirror for observation of the animals. A 12-V bulb at the top of the chamber provided illumination. Scrambled shock was delivered to the 0.6 cm dia. parallel stainless steel grid of the box by a BRS-Foringer shock generator and scrambler. BRS digibits programmed the shock duration, frequency, etc., and BRS counters were used to record the animal's behavior.

*Procedure.* The animals were randomly paired and divided into two groups of eight pairs each. One group of

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animals received 2 mA shocks as eliciting stimuli and the other group shocks of 1.0 mA. All shocks were 0.5-sec in duration with a 3-sec intershock interval. Each daily session consisted of 100 shocks, presented at a frequency of 20 shocks per min. Each animal received seven dosages of atropine sulfate subcutaneously administered in saline vehicle as unit weight per cc, 15-min prior to the experimental session. The dosages were separated by a single fighting session in which a zero-dosage consisting of physiological saline was administered. Dosages used were 0, 0.10, 0.50, 1.0, 2.0, 5.0, and 10.0 mg/kg. Each dose was administered once. The order of administration of the doses was counter-balanced across pairs of animals. An experienced observer rated the behavior of the pairs of rats as fight or no-fight in response to each shock. After the experiment was completed it became apparent that the observer was aware of the fact that drug and placebo conditions alternated. However, he was not aware of the dosage administered on any given day. A fight was defined as a striking, lunging, or biting movement made by either one or both animals of a pair. This stereotyped behavior has been described in more detail by several investigators [17,23].

*Results and Discussion*

The results of this experiment are shown in Fig. 1. This figure depicts fighting frequency as a function of atropine dosage for each group of animals. Fighting during the preceeding saline sessions is also shown in Fig. 1. Atropine reduced SEA during sessions in which higher dosages of atropine were administered. The *t*-tests revealed differences between the preceeding saline-day scores and the 10 mg/kg drug-day scores in both the 1 mA and 2mA groups to be significant ($t = 1.94$, $df = 7$, $p<0.05$; $t = 2.64$, $df = 7$, $p<0.05$ for 1 mA and 2 mA conditions, respectively). However, the smallest dosage (0.10 mg/kg) in the 2 mA group also resulted in fighting frequencies significantly less than those obtained during saline control sessions ($t = 2.4$, $df = 7$, $p<0.05$). None of the other saline-drug comparisons were significantly different.

Although these results suggest that atropine decreases SEA, they are contaminated by observer bias, since the observer was aware that drug and saline sessions alternated. This interpretation of the results is strengthened by the finding that the lowest dosage also significantly decreased fighting in one of the shock conditions. Thus a subsequent experiment was performed in which the highest dosage used in the present study was employed in a completely blind experiment in which drug sessions alternated with saline control sessions. In addition to using a completely blind procedure in Experiment 2, the quaternary atropinic analogue, atropine methyl nitrate, was employed to assess the effects of peripheral cholinergic blockade, since it does not cross the blood brain barrier as readily as does atropine sulfate.

## EXPERIMENT 2: THE EFFECTS OF A SINGLE HIGH DOSE OF ATROPINE SULFATE AND ATROPINE METHYL NITRATE UPON SHOCK-ELICITED AGGRESSION

*Method*

*Animals.* The thirty-two Sprague-Dawley rats used in the previous experiment served as experimental animals and were housed in pairs with food and water available ad lib.

*Apparatus and procedure.* The experimental chamber and programming circuitry were identical to that used in the previous experiment. The animals were paired and administered shock sessions with parameters also identical to those used in the prior experiment. A single dosage of each drug was administered twice; physiological saline was administered on alternate days. The order of drugs was randomized with the exception that a saline session separated the drug sessions. Thus over seven consecutive daily sessions either saline, 10 mg/kg atropine sulfate, or 10 mg/kg atropine methyl nitrate dissolved in saline was administered. As in the previous experiment each drug was prepared as unit weight per cc vehicle. The fighting response was also defined and measured as in the previous experiment. However, in the present case the observer was completely blind with respect to the drugs and dosages administered on any given day.

*Results and Discussion*

The results of this experiment are presented in Fig. 2, in which frequency of shock-elicited fighting is shown as a function of daily sessions for each shock intensity. As can be seen, the administration of 10 mg/kg atropine sulfate resulted in decrements in the amount of fighting elicited during drug sessions. Administration of atropine methyl nitrate also resulted in decrements, but they were not as great as those produced by atropine sulfate. Comparing animals which received different shock intensities, it also appears that there is a relatively greater effect of atropine on shock-elicited fighting at lower shock intensities. Analysis of variance of these results revealed that the differences in fighting obtained for groups administered different shock intensities were significant ($F = 14.6$, $df = 1/7$, $p<0.01$); that the saline vs atropine sulfate conditions were significant ($F = 7.1$, $df = 1/14$, $p<0.02$); and that differences related to atropine methyl nitrate and atropine sulfate were also significant ($F = 5.1$, $df = 1/14$, $p<0.05$). However, the methyl atropine scores were not significantly different from the saline scores ($F = 3.1$, $df = 1/14$, $p>0.05$).

The results of the present experiment thus suggest that central cholinergic blockade interferes with the elicitation of fighting by shock, although the decrements obtained with fairly large dosages were not as large as has been previously reported for different kinds of aggression [8,12]. Thus the more potent central blockade, scopolamine, was studied in a third experiment.

## EXPERIMENT 3: THE EFFECTS OF SCOPOLAMINE HYDROCHLORIDE AND SCOPOLAMINE METHYL NITRATE ON SHOCK-ELICITED AGGRESSION

*Method*

*Animals.* Ninety-six female Sprague-Dawley rats were obtained from Flow Laboratories. They were approximately 100 days old at the beginning of the experiment and were housed in pairs in standard laboratory cages with food and water available ad lib.

*Apparatus and procedure.* The apparatus employed was identical to that used in the previous experiment. Six dosages of scopolamine and scopolamine methyl nitrate, prepared as unit weight per cc saline vehicle were injected subcutaneously, a different dosage being presented to separate groups of animals. The dosages employed were 0.05, 0.10, 0.25, 0.5, 1.0, and 3.0 mg/kg. The eight pairs of animals per drug group were subdivided into four pairs of

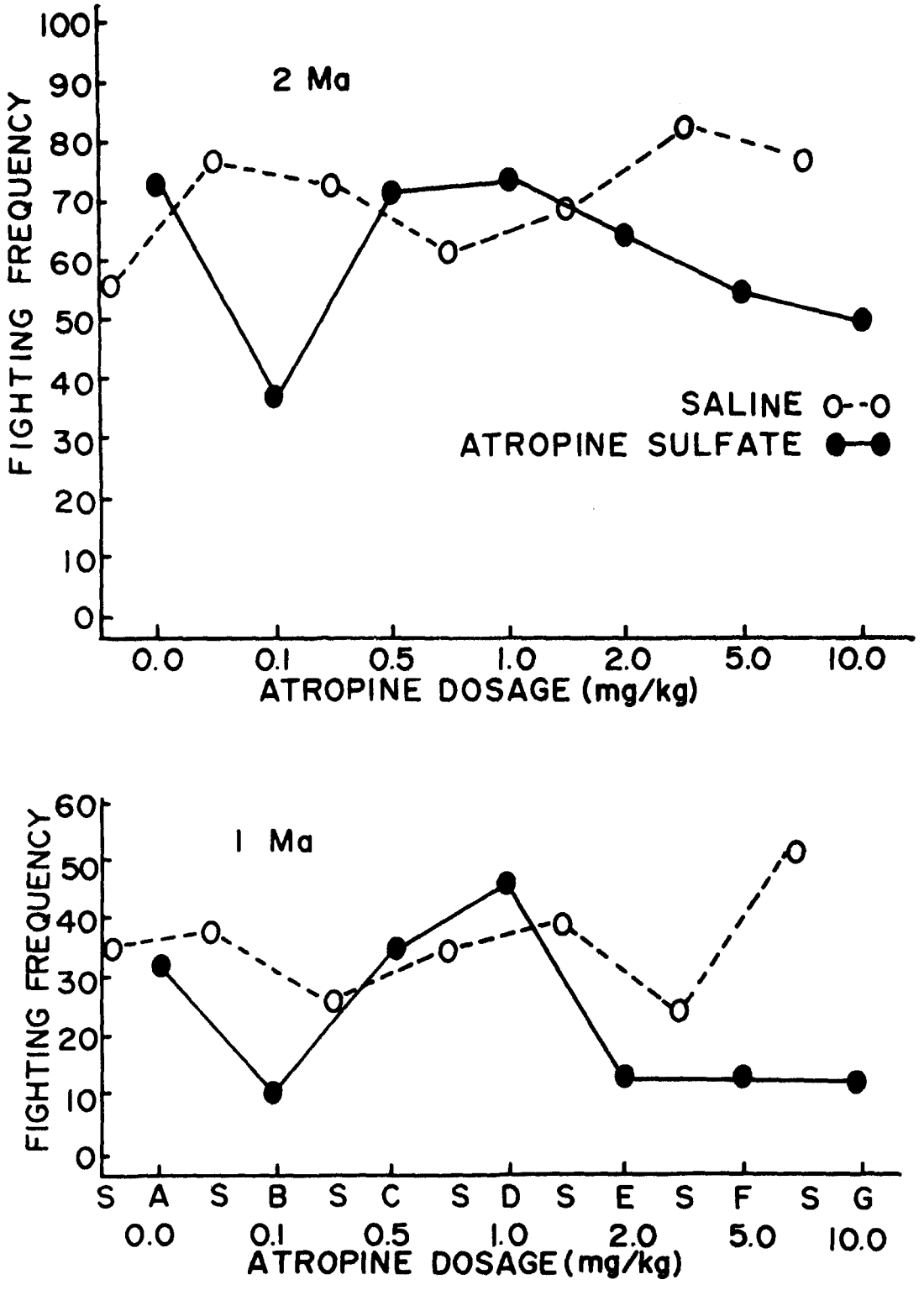


FIG. 1. Frequency of shock-elicited fighting as a function of atropine sulfate dosage (A = 0; B = .1; C = .5; D = 1.0; E = 2.0; F = 5.0; and G = 10.0 mg/kg). Saline injections (S) alternated with drug injections. Doses were administered in a random order; the saline scores represent fighting frequencies observed on the day immediately preceding the drug scores. Shock intensities differed for two groups of rats as shown; shock frequency was 20 shocks per min with a constant intershock interval. Train duration = 0.5-sec; total shocks per session = 100.

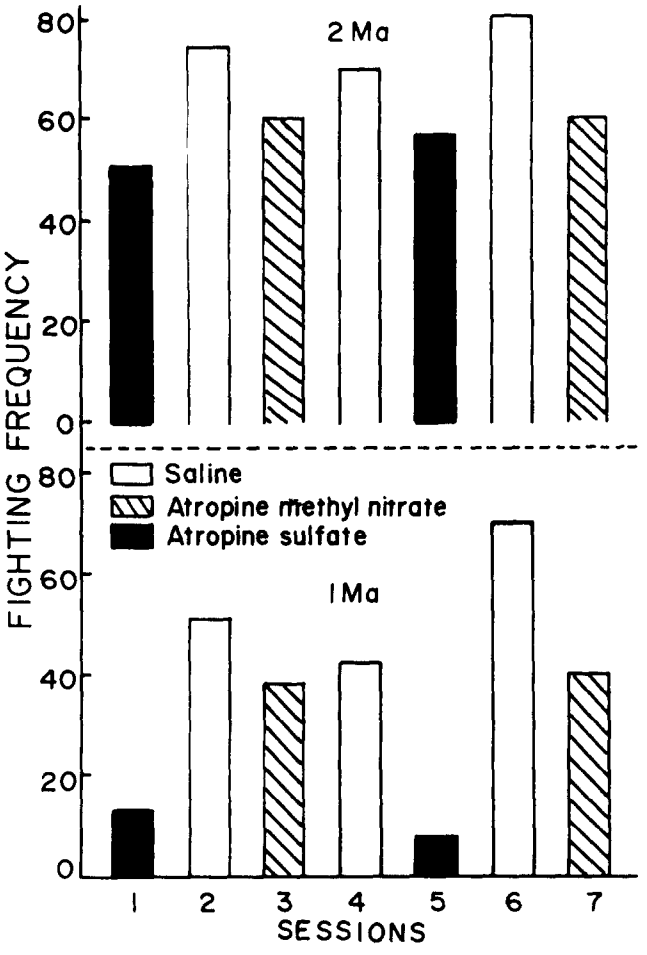


FIG. 2. Frequency of shock-elicited fighting in rats elicited by 1 mA and 2 mA shocks after injection of 10 mg/kg atropine sulfate, atropine methyl nitrate, or saline. Other shock parameters were identical to those described in Fig. 1.

animals each. One subgroup at each dosage was administered scopolamine methyl nitrate 20 min prior to the first session and scopolamine hydrochloride prior to a second session which was run 10 days later. The other groups of animals had these conditions reversed. Only a single shock intensity was used (2 mA). Otherwise the stimulus parameters were identical to that used in Experiment 2, as were the methods of measurement and definition of the fighting response.

*Results*

Figure 3 shows fighting frequency as a function of dosages of scopolamine and scopolamine methyl nitrate. As can be seen, scopolamine dosages greater than 0.25 mg/kg resulted in severe decrements in fighting. However, at dosages less than 0.10 mg/kg fighting rates were similar to those produced by the peripheral analogue scopolamine methyl nitrate. Analysis of variance of these results showed the differences between drugs and dosages to be significant ($F = 11.5$ and 11.6 respectively, $df = 1/42$, $p<0.005$), although the interaction was not ($F = 1.3$, $df = 5/42$, $p>0.10$).

## GENERAL DISCUSSION

The present series of experiments demonstrated that central cholinergic blockade interferes with the development of fighting in response to shock. If one assumes that aggression elicited by electric shock is defensive in nature, then the present results suggest that central cholinergic systems are involved in defensive fighting. These experiments also showed that scopolamine had a greater effect upon SEA than did atropine. Both Janssen, *et al.* [12], as well as DaVanzo, *et al.* [8], similarly found in mice that scopolamine produced a greater reduction in isolation-induced aggression than did atropine. It was noted by these investigators that motoric side effects as well as drug-induced mydriasis could not have produced their results. In the present experiments the responses of the drugged rats to shock also appeared to be unimpaired. These animals vocalized loudly, jumped about, and showed upright behaviors similar to the saline injected rats. They simply did not assume the typical boxing or biting postures so often observed in the SEA situation. Since peripheral blockade should also produce mydriasis, but produced only insignificant decreases in SEA, it can also be assumed that visual impairment was not involved in the present fighting decrements. In addition, visual processes have been previously determined to play a minimal role in SEA [9].

The specific cholinergic mechanisms and neuroanatomical areas involved in mediating aggressive behavior are subject to debate. Cholinergic blockade is known to profoundly depress reticular system activity [5,14]. Also, slow frequency-high amplitude EEG activity is associated with the administration of muscarinic blocking agents [15,27]. Thus, SEA decrements produced by central cholinergic blockade may be mediated by interference with arousal processes. Certainly nondrugged rats in the SEA situation are highly aroused behaviorally. Other studies employing tranquilizers and adrenergic stimulants and depleting agents [19,21] also suggest that interference with SEA by these drugs is via arousal mechanisms. However, the fact that no motor impairment was noted, even at high dosages of scopolamine or atropine suggests that central sedation did not occur in the present study.

Other explanations of the effects of anticholinergic drugs on behavior include (a) possible interference with memory processes [15], and (b) participation in response or drive disinhibitory processes at central levels [6]. It is obvious that the former hypothesis cannot be entertained with regard to the present data since SEA is an unlearned response, and indeed was initially termed reflexive by Ulrich and Azrin [22]. The latter hypothesis, however, may be relevant to SEA phenomena since dosages of scopolamine that depressed SEA in the present experiment overlap at the upper ends with those which produce disinhibitory phenomena [4]. Moreover, Stein [20] has explicitly proposed a medial hypothalamic "punishment system" which he characterized as cholinergic. Since punishment procedures (e.g., extinction of appetitive stimuli and presentation of electric shock) are the same procedures which elicit aggression, such a CNS punishment system may be involved in SEA. Electrical stimulation of medial hypothalamus also elicits defensive rage in the cat [10]. Although it is not clear that such stimulation involves the same cholinergic system as that studied by Stein and colleagues, it is possible that fibers coursing through medial hypothalamus are involved in gating in or out a number of different

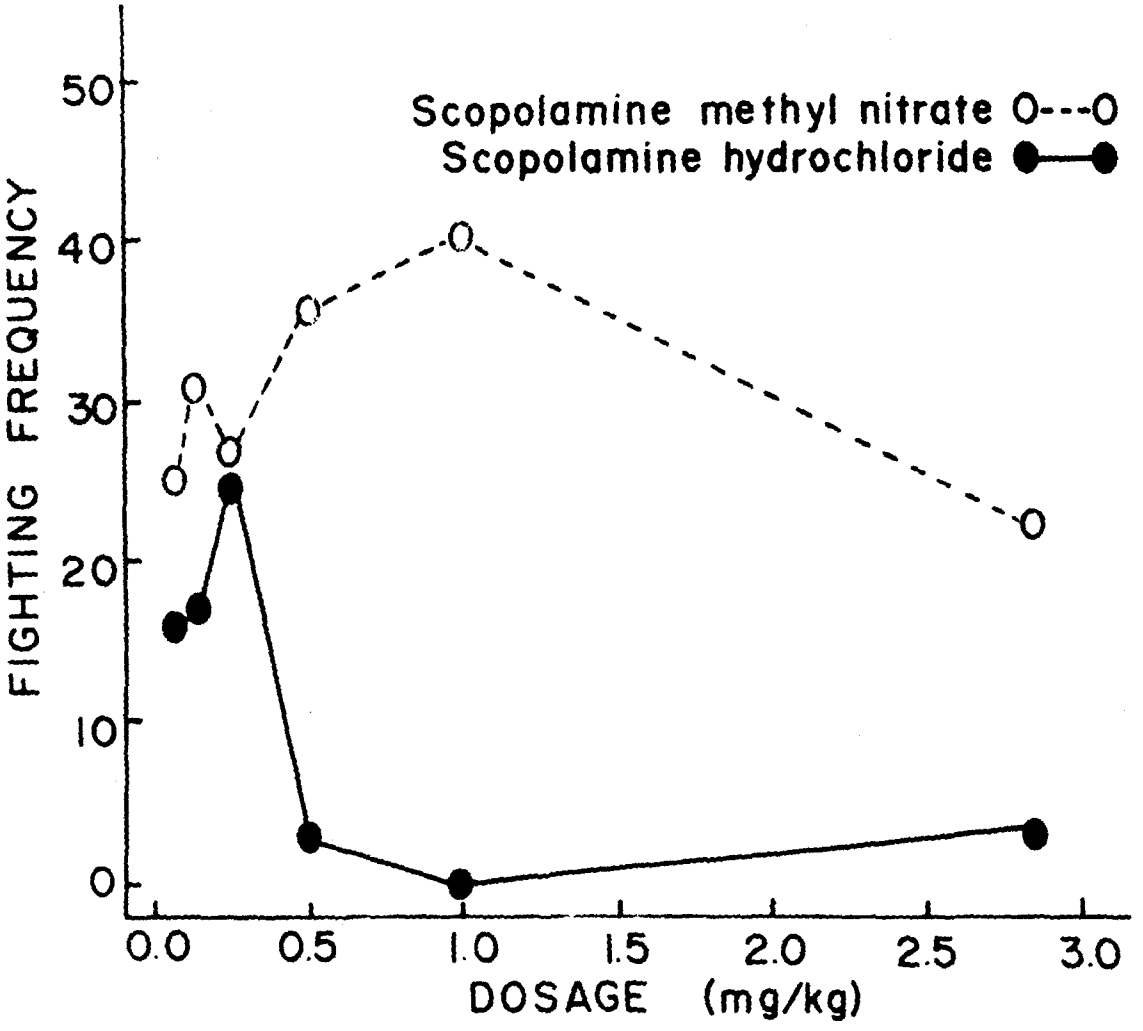


FIG. 3. Frequency of shock-elicited fighting in rats as a function of different dosages of scopolamine hydrochloride or scopolamine methyl nitrate. Shock parameters were as follows: intensity = 2 mA, train duration = 0.5-sec, and frequency = 20 shocks per min with a constant 3-sec intershock interval. Total shocks per session = 100.

response classes as well as the response inhibiting system suggested by Stein and others [6]. In favor of such an interpretation are studies which report that a wide range of behaviors other than aggression (e.g., sex, preening, etc.) are elicited by peripheral shock [18]. It is thus possible that a medial cholinergic system is involved in eliciting these behaviors.

However, other central cholinergic pathways are obviously also involved. Lateral hypothalamic injections of cholinergic drugs produce increases in mouse killing while cholinergic blockade inhibits mouse killing in natural killers [19]. Experiments by Bandler [2,3] have shown that cholinergic pathways in the thalamus, subthalamus and midbrain were also involved in interspecific attack. More recently, Vogel and Leaf [25] demonstrated that systemic injections of the cholinergic agonist, pilocarpine, also increased the muricidal behavior of rats. Thus, cholinergic mechanisms are apparently involved in a variety of agonistic response systems. However, their differential control by CNS mechanisms and relationship to other cholinergic functions is at the present time unknown.

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