

Cholinergic blockade and tonic immobility in chickens

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To investigate the effects of cholinergic blockade on duration of tonic immobility (TI) and habituation to handling and the TI testing procedure, two groups of cockerel chicks were tested following injection with scopolamine or saline. One week later half of each group was given a second test for TI following injection with saline or scopolamine. Results indicated that scopolamine significantly reduces the duration of TI and suggested some interference with the process of habituation, although the evidence for the latter was not conclusive.

In a series of experiments, Thompson, Piroch, Fallen, and Hatton (1974) have shown that chickens treated with scopolamine hydrobromide, which competes with acetylcholine for receptor sites, showed reduced durations of tonic immobility (TI) compared to noninjected birds or those injected with saline or methylscopolamine (an anticholinergic which does not cross the blood-brain barrier readily). In their initial experiment, cockerel chicks were given two test sessions 1 week apart, each bird being given the same drug or nondrug treatment prior to each test for TI. On the first test day the three control groups (noninjected and saline- and methylscopolamine-injected birds) did not differ among themselves and all had significantly longer durations of TI than the scopolamine-treated group. There were significant decreases in TI durations for the three control groups from the first to the second test day, but not for the scopolamine group; the four groups did not differ on the second test. Thompson et al. attributed the decrease across days for the control groups to habituation to handling and the TI induction procedure, a result that has been noted by others (Ratner & Thompson, 1960). The point of interest for the present experiment is the failure of the scopolamine-treated animals to show a corresponding decrease across test sessions. Thompson et al. suggested that this failure may have been a result of the blockade of cholinergic systems which Carlton (1969) has hypothesized to mediate the process of habituation.

The present experiment was designed to partially replicate the Thompson et al. (1974) experiment and to examine further the effects of scopolamine on the duration of TI and the habituation that occurs during the handling, testing, and injection procedures. Two groups of chicks were given either an injection of saline or scopolamine prior to initial testing for duration of TI;

1 week later, half of each of these groups was injected with saline and the other half was injected with scopolamine before testing. If scopolamine prevents habituation to handling and TI induction, then the following pattern of results for TI durations would be expected: The animals given two saline injections should show a decrease from the first to the second test day and the animals given two injections of scopolamine should show no decrease over the 2 test days. Animals first tested with scopolamine and later tested with saline should have the longest duration of TI of any group during Day 2 of testing, durations similar to those shown by saline-injected animals on Day 1 of testing. Animals first tested under saline and later tested following injections of scopolamine should show the shortest durations of TI on the second test day.

METHOD

The source of animals and testing procedures were similar to those of Thompson et al. (1974). Forty-eight White Leghorn cockerel chicks of the H & H strain were obtained from a local hatchery during the first posthatch day. The birds were maintained in a commercial brooder, with food and water constantly available. An artificial day/night cycle with the lights on from 9:00 a.m. to 10:30 p.m. was maintained in the colony room.

Several days before initial testing the animals were leg banded for identification and randomly assigned to one of four test groups. Each bird was given three test trials for TI duration at 12 days of age and again at 19 days of age. Group N-N was injected with saline before both test sessions; Group N-D was injected with saline prior to the first test session and with scopolamine hydrobromide before the second; Group D-D was injected with scopolamine before each test session; and Group D-N was injected with scopolamine before the first test session and saline before the second. All injections were given IP 15 min prior to testing. Scopolamine injections were of .5 mg/kg of scopolamine hydrobromide and saline injections were of an equal volume of physiological saline (1.0 cc/kg of body weight).

Testing consisted of haphazardly selecting two birds from the brooder. The birds were weighed, injected with the proper solution (saline or scopolamine), and placed in a holding box for 15 min; they were then placed in an intertrial-interval holding box and carried to the testing room. Testing for TI consisted of removing each pair of birds from the box, holding them upright for 15 sec, quickly turning them on their sides for 15 sec, and then releasing them. A bird was placed on each side

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of the intertrial-interval holding box facing away from the experimenter. Tonic immobility was timed from release until 180 sec had elapsed or spontaneous righting occurred. A bird failing to stay down at least 5 sec was given a score of zero. Upon righting or at the end of 180 sec, the birds were returned to the intertrial-interval holding box to await the next trial. The intertrial interval was at least 15 sec and no longer than 195 sec. Three such trials were given on each of the 2 days of testing.

RESULTS AND DISCUSSION

Examination of the data from the three trials for each subject on each test day indicated no consistent changes across trials within each day, so the mean duration of TI for each bird for each session was used for analysis. The means for all groups for each test session are presented in Figure 1.

The data for Groups N-N and D-D are essentially a replication of the saline and scopolamine groups of the first experiment in Thompson et al. (1974). To make the appropriate comparison between the two experiments easier, the mean duration of TI for the corresponding groups for both test sessions for the two experiments are presented: Group N-N—109.45 and 81.49, Group D-D—47.22 and 32.80, saline—155.04 and 81.49, and scopolamine—88.54 and 86.75 (Thompson et al., 1974). The slightly overall shorter durations of TI in the present experiment compared to that of Thompson et al. are probably a result of the extra day of handling the birds in the present experiment received during banding for identification. The pattern of results obtained in the present experiment closely replicated those of Thompson et al. On the first test day the birds treated with scopolamine had significantly shorter durations of TI than those injected

with saline: Group D-D vs Group N-N, $t(22) = 2.58$, $p = .017$; Groups D-D and D-N vs N-N, $t(46) = 3.65$, $p = .001$. Group N-N showed a significant decrease in TI duration from the first to the second test session, $[t(11) = 2.45, p = .032]$, but Group D-D did not ($p = .393$). During the second test session, Thompson et al. found no difference between scopolamine- and saline-treated groups, whereas in the present experiment Group D-D had significantly shorter duration of TI than Group N-N $[t(22) = 2.35, p = .028]$. The reason for this difference is not apparent.

Support for the hypothesis that scopolamine may prevent habituation to handling and the TI induction process was demonstrated by Group N-N showing a significant decrease and Group D-D showing no change across test days. The hypothesis predicts that Group N-D will show a significant decrease across test sessions, a result that can be seen in Figure 1 $[t(11) = 2.31, p = .041]$. Since Group N-D combines the reduction of TI duration as a result of habituation effects and drug effects, this group should have the shortest duration of TI of any group during the second test session. As is apparent from Figure 1, this prediction was not supported, although Group N-D did not differ from Group D-D during the second test session $[t(22) = 1.149, p = .26]$. One might hypothesize a state-dependent effect to account for the failure of habituation to transfer from a nondrug state to a drug state (Overton, 1964).

In addition, if scopolamine blocks habituation to handling and TI induction, then Group D-N should show an increase in TI duration from the first to the second test session, a result which can be seen in Figure 1. However, this increase was only marginally significant $[t(11) = 2.17, p = .053]$. The blockage of habituation effects by scopolamine during the first test session and the absence of any drug during the second test session should have resulted in Group D-N having the longest duration of TI of any group. As is apparent from Figure 1, this prediction was not borne out, although Group D-N did not differ significantly from Group N-N during the second test session $[t(22) = .655, p = .52]$. It should also be noted that the Day 2 TI duration of Group D-N did not differ significantly from the first test durations of Groups N-N and N-D combined $[t(34) = 1.646, p = .109]$. For Group D-N, a state-dependency interpretation makes the same prediction as the cholinergic blockade of habituation hypothesis, both hypotheses receiving mixed support. It should be noted that asymmetrical state-dependency effects have been noted previously for scopolamine (Berger & Stein, 1969).

The pattern of results obtained in the present experiment does not clearly support the hypothesis that scopolamine prevents habituation to handling and the TI induction experience, nor does it clearly refute that hypothesis. A state-dependent learning interpretation

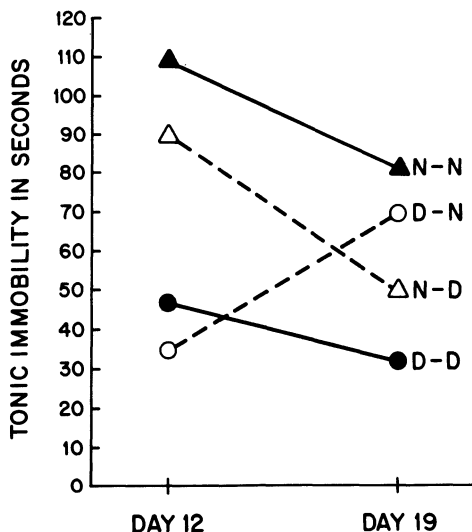


Figure 1. Mean duration of tonic immobility in seconds for all groups on the 2 days of testing. N = nondrug-saline and d = drug-scopolamine.

also is not clearly supported. However, it is clear that treatment with scopolamine hydrobromide decreases the duration of TI in chickens, whether or not the animals have had previous experience with handling and the TI induction procedure.

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(Received for publication December 19, 1976.)