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Two Types of Habituation in Chicks: Differential Dependence on Cholinergic Activity

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BROWN, C. P. *Two types of habituation in chicks: differential dependence on cholinergic activity.* PHARMAC. BIOCHEM. BEHAV. 4(3) 235–238, 1976. – The role of cholinergic activity in habituation in chickens was tested using spontaneous alternation (SA) and reduction in response suppression to an auditory startle stimulus. While central reduction of cholinergic activity via atropine sulfate administration reduced SA and by inference, habituation, habituation to the tone was unimpaired, suppression decreasing with signal repetition even after a higher dose of atropine than that found effective in disrupting SA-indexed habituation. These findings confirmed an earlier suggested dissociation between habituation to externally produced stimuli e.g. startel stimuli, independent of cholinergic activity, and habituation to self-produced stimulation, e.g. exploration, spontaneous alternation, dependent on cholinergic functioning.

Spontaneous alternation Drinking suppression Atropine Methylatropine Startle stimulus
Chickens Habituation Response inhibition General activity

CARLTON [1, 2, 3] orginally implicated cholinergic activity in a general process of habituation, evident in a gradual reduction in the behavioural impact of a relatively weak and novel stimulus upon its repeated presentation. This process was assumed to be similar in a variety of test situations. The implication of cholinergic activity in such a process gained support in part from the observed disinhibition of irrelevant or nonreinforced responses, attributed to disruption of habituation, following administration of anticholinergics such as atropine and scopolamine in rats [4, 5, 6, 9, 11]. However, on the basis of recent findings [16,17], Williams *et al.* [17] suggest the need to postulate 2 distinct mechanisms of habituation, one of which is independent of cholinergic activity. In particular, these studies report anticholinergic disruption of habituation to stimuli whose presentation is dependent upon the individual's own activity, e.g. those produced by exploration. However in contrast, habituation to externally produced stimuli, e.g. startle responses to loud clicks, appears to be unaffected by anticholinergic administration. Thus it is argued that the process of habituation underlying diminished responsiveness to such externally originating stimuli differs from that to self-produced stimuli. Further, this difference is held to extend to the biochemical correlates of these processes, habituation to self-induced stimulation only depending upon cholinergic activity.

This study examined the role of cholinergic activity in habituation in young chickens, and the possible dissociation in this species, between the 2 types of habituation held to be differentially dependent on the cholinergic system. Spontaneous alternation of choices in a V-maze, and response suppression to a novel and loud auditory stimulus were studied. Spontaneous alternation constituted an index of habituation to the goal of the arm selected on the previous trial. Reduction in response suppression with continued presentation of a novel auditory stimulus provided an index of habituation to a stimulus of external origin, the loud tone.

EXPERIMENT 1

This experiment used spontaneous alternation (SA) as an index of the effect of anticholinergics atropine and atropine methylnitrate on habituation to self-produced stimulation. This response was selected since the capacity to alternate choices depends upon inhibition of immediately preceding responses which are not conventionally reinforced [7]. Such inhibition is effected, according to Carlton [3], via habituation to the stimuli eliciting approach, when that approach is not subsequently reinforced. Further, the dependence of SA on cholinergic activity has been demonstrated in rats [5, 6, 10, 13]. Additionally, in view of previous evidence implicating central effects in the disruption of habituation and thus response suppression [1, 14, 15, 17], the effects of central and peripheral disruption were compared.

Methods

Animals. Sixty White-Leghorn x Black-Orpington chicks were obtained from a commercial hatchery during the first posthatch day, and were housed in laboratory brooders at a temperature of 29° C.

Apparatus and Procedure. Chicks were tested for SA on Day 10, when habituation is well developed [12]. A white

perspex V-maze was used. The 2 arms were set at an angle of 60°, and each arm measured 30 cm long, 12.5 cm wide and 25 cm high. A start compartment located in the junction of the 2 arms was separated from these runways by a white guillotine door. Goal boxes 30 cm long, 12.5 cm wide, and 25 cm high were set at the other end of each runway and were separated from the runways by guillotine doors. A 100 W light was suspended from the lid of each goal box. On each trial these were turned on until the chick had moved into a goal box. These lights were used in the goal boxes to increase the similarity between this test V-maze and a straight runway in which chicks had been pretrained to move from a start box along a runway 2 hours before testing. Such pretraining in a straight runway had been found necessary in chicks, which otherwise remained in the start box during actual SA testing.

The approach pretraining runway and associated end goal box were of white perspex, with dimensions similar to those of the V-maze arms. During approach pretraining, light and heat were provided by a 100 W light globe suspended over the goal box, and 3 stimulus chickens were placed in the goal box. Chicks were pretrained in a darkened room. On the first pretraining trial, the door of the start box was raised and the chick was pushed through to the runway if it had not moved within 30 sec. Once the chick was in the runway, the goal box door was raised. Again, if the chick did not move within 30 sec it was pushed into the goal box from the runway. The test chick was then allowed 30 sec in the goal box. On the second trial, chicks were allowed 60 sec before being similarly pushed through to the runway and then to the goal box. Pretraining continued until the chick moved by itself through to the goal box within 60 sec of the start box door's being lifted.

During the subsequent testing for spontaneous alternation chicks were randomly assigned to 1 of 3 groups. One group was injected IP 30 min before testing with 0.2 ml atropine sulfate (0.4 mg/kg body weight), one with 0.2 ml atropine methylnitrate (0.4 mg/kg body weight) and one with 0.2 ml physiological saline. These doses were selected for initial examination in view of their confirmed effectiveness in previous studies, although with animals other than chickens [5, 14, 15, 17]. Thus further investigation of the effectiveness of larger doses were projected had these dose levels failed to produce any effect. However, significant effects were obtained with these moderate doses. The drugs used were selected on the basis of their blocking actions of CNS acetycholine [1, 2, 3]. A comparison of the effects of the 2 would additionally permit assessment of the relative importance of central and peripheral changes in this respect. While both drugs produce peripheral changes, only atropine passes freely into the brain from the blood stream, thereby producing central reduction of acetylcholine [1, 2, 3, 4, 6]. Since the above mentioned evidence indicates habituation deficits only after such central reduction of acetylcholine, a similar pattern of results was expected in this experiment, with atropine methylnitrate showing no influence on behaviour.

At the start of each trail during testing for spontaneous alternation, the chick was placed in the start box with start and goal box doors down. After 30 sec the start box door was raised. If the chick failed to move within 2 min, it was replaced in a holding box for 10 min and retested. If no response then occurred, it was rejected. The majority of birds moved from the start box on the first such occasion. It was noted that this procedure resulted in a longer injection-test interval in some chicks. However, group differences in this respect were negligible, 1 bird from each of the experimental groups and 2 in the saline group being tested at 42 rather than 30 min postinjection. On each trial when the chick had entered one arm, the start box door was lowered, the goal box door raised and when the chick had entered the goal, the lights in both goal boxes were turned off, to prevent the possibility of any direct reinforcement for that previous choice. After 30 sec in the goal box, the chick was returned to the start box for the next trial. The first 3 trial choices were recorded.

Results and Discussion

Percentage alternation rates are shown in Table 1 and represent the percentage of the 20 chicks tested in each group which showed an alternation in choice of arm entered on the 2 trials specified. Chi-square analyses of such SA rates revealed significant drug-group differences in the percentage alternating between Trials 1 and 2 ($\chi^2 = 8.78$, $df = 2$, $p<0.05$), and Trials 2 and 3 ($\chi^2 = 7.68$, $df = 2$, $p<0.05$). These results confirm the effectiveness of cholinergic inhibition in disrupting habituation to self-produced stimulation. Further, the role of central cholinergic activity in this respect is apparent. It had been anticipated that atropine sulfate, which freely passes from the blood stream to the brain, and therefore acts centrally, would significantly reduce alternation rates. Atropine methylnitrate on the other hand does not cross the blood-brain barrier [1, 2, 3, 4, 6, 13], which develops in chickens before Day 10 [8]. Thus its impact would be purely peripheral. That its administration failed to influence alternation, there clearly being no difference between atropine methylnitrate and saline groups in SA rates between either Trials 1 and 2, or Trials 2 and 3 (Table 1), confirms the involvement of central action in such effects.

TABLE 1

EFFECT OF DRUG TREATMENT ON PERCENTAGES OF CHICKS SHOWING ALTERNATION OVER THREE TRIALS

Drug Treatment	Trials 1-2	Trials 2-3
Saline	55	55
Atropine	15	20
Atropine Methylnitrate	55	60

EXPERIMENT 2

This experiment examined the effects of central and peripheral cholinergic inhibition on habituation to an externally produced stimulus, a sudden loud intermittent tone. The extent to which the tone disrupted ongoing behavior, in this case drinking in thirsty chicks, was taken as an index of the strength of the startle or orienting response to that tone. The gradual diminution in such response suppression with repeated presentation of the tone thus provided an index of the extent of habituation to that tone, blockage of such habituation being apparent in continued response suppression during tone presentation.

In order to further confirm that changes in suppression

in 10 day-old chicks with repeated stimulus presentation were in fact due to habituation, chicks were also tested on Day 2 post hatch, previous evidence indicating minimal habituation in chicks of this age [12].

Method

Animals. One hundred White-Leghorn x Black Orpington chicks were obtained and housed as described in Experiment 1. Fifty were tested on Day 2, and 50 on Day 10 posthatch.

Apparatus and Procedure. Chicks were tested individually in a white perspex box 25 cm x 30 cm x 30 cm, over which was suspended a 100 W light globe, providing warmth and light. A hole cut into one side of the box permitted vertical insertion of a water bottle during testing. Water had been similarly available in the home brooders. An intermittent auditory signal, each signal consisting of 5 single 1.0 sec 90 db tones, 1.0 sec apart and produced by a morse key, was used as the startle stimulus during testing.

Twelve hr prior to testing chicks were removed from the home brooder and placed without water in a box similar to the test chamber. Thirty min prior to testing, 10 chicks of each age group were injected IP with 0.2 ml saline, 0.2 ml atropine sulfate (0.4 mg/kg body weight), 0.2 ml atropine methylnitrate (0.4 mg/kg body weight), 0.2 ml atropine sulfate (2.0 mg/kg body weight) or 0.2 ml atropine methylnitrate (2.0 mg/kg body weight). The additional higher dose levels were included on the basis of preliminary findings indicating possibly negligible effects with lower doses. These particular higher doses were selected as comparable with the range of doses used in certain previous experiments [6,17]. It was noted that anticholinergic administration might significantly influence drinking levels independent of its effect on general response suppression via habituation. However, such a general change in rate of drinking would leave unaltered the response suppression ratios based on drinking rates during, compared with rates just preceding and following, the tone. Two min prior to testing, chicks were placed in the test box, and allowed to settle. The water bottle was then inserted and testing began as soon as the chick had taken the first peck at the nipple. The intermittent auditory signal, of 10 sec total duration was presented 5 times at previously randomly determined intervals. The number of pecks toward the nipple (or small beak movements at the nipple of the chick remained at the nipple for longer than 1 sec) was recorded for the 10 sec before, after, and during each 10 sec signal or stimulus presentation. A suppression ratio was then calculated for each of these 5 trials or stimulus presentations, based on number of pecks during the intermittent stimulus, divided by half the total number given in the 10 sec both before and after that stimulus. Thus maximal suppression, i.e. no responding during the signal, would be apparent in a ratio of 0.00, while a ratio of 1.00 or more would indicate zero suppression.

Results and Discussion

Suppression ratios are given in Table 2. The Day 2 and Day 10 results were treated separately in Analyses of Variance.

In the Day 10 chicks, a significant reduction in suppression over the five trials ($F(4,225) = 3.12, p<0.05$) confirmed the effectiveness of habituation in all groups tested at this age, there being no interaction between drug treatment and trials ($F(16,225) = 1.47, p>0.05$). Anticholinergic administration appeared to have no influence on development of habituation during the 5 trials. However, a reduction in suppression generally, following central reduction of cholinergic activity (Table 1) resulted in a significant Treatment effect ($F(4,225) = 2.81, p<0.05$).

In the Day 2 results, absence of any reduction in suppression over trials ($F<1.0$) supported the use of this effect as an index of habituation, and the interpretation of the Day 10 results as due to habituation. This lack of change with repeated trials was equally apparent in all groups, there being no interaction between Treatment and Trials ($F<1.0$). A fruther difference from the Day 10 results was the absence of any effect of treatment on general level of suppression ($F<1.0$). Two day-old control and atropine methylnitrate-injected birds showed none of the suppression apparent particularly on Trial 1, but present throughout testing, in Day 10 control birds relative to the atropine-treated group. In short, at Day 2 there was neither habituation nor significant suppression in normal chicks, and no effect due to anticholinergic administration. By Day 10, habituation was apparent, together with response suppression, central disruption of cholinergic activity having no influence of rate of habituation, but significantly impeding initial and overall suppression of drinking during tone presentation.

GENERAL DISCUSSION

Two features of the results for the 10 day-old birds require discussion. First, the response suppression data show that despite disruption of cholinergic activity, some habituation to the tone developed between Trials 1 and 5. Neither central nor peripheral blocking was effective in eliminating habituation. This pattern of results differed from the effects observed on habituation, as indexed by spontaneous alternation. Central cholinergic activity would appear to be involved in mediating habituation only in those situations where the presentation of the relevant stimulus requires some activity on the part of the animal. Habituation indeed is indexed by the waning of that activity. The proposed [17] differentiation between type of habituation, and the process of habituation to externally originating stimuli is thereby confirmed.

However, the second aspect of the results for the older chicks, the generally reduced suppression following central anticholinergic administration, is perhaps relevant. The ratios observed in these groups are initially similar to those for all 2 day-old birds, although some habituation is apparent in the 10 day-old atropine-treated chicks which is not evident at Day 2. In short, there is incomplete suppression even when no habituation has occurred. The mechanism of complete response suppression would appear to develop between Day 2 and Day 10, and further, to depend upon cholinergic activity. The present findings do not allow any conclusions concerning the nature of such a mechanism. However, the observed perseveration of the dominant response of pecking following anticholinergic administration might be seen as consistent with certain previous reports. Anticholinergics have been found to increase the general level of activity and of ongoing behavior in chickens [14]. Thus a potentially general inhibitory function of cholinergic activity may account for the present finding of reduced suppression to an external stimulus.

TABLE 2

MEAN RESPONSE SUPPRESSION RATIOS ON FIVE STIMULUS PRESENTATIONS AS A FUNCTION OF DRUG, DOSE, AND TEST AGE

Test Age	Drug and Dose level	Stimulus Presentation 1	2	3	4	5	Average Suppression on all Presentations
10 days	Saline	.14	.71	.43	.57	.73	.52
	Atropine (0.4 mg/kg)	.56	.63	.822	.91	.86	.76
	Atropine Methylnitrate (0.4 mg/kg)	.23	.41	.42	.50	.75	.46
	Atropine (2 mg/kg)	.70	.61	.81	.86	.95	.79
	Atropine Methylnitrate 2 mg/kg	.19	.48	.40	.61	.71	.48
	Mean	.36	.57	.60	.69	.80	
2 days	Saline	.75	.73	.77	.85	.83	.79
	Atropine (0.4 mg/kg)	.86	.97	1.06	.88	.72	.90
	Atropine Methylnitrate (0.4 mg/kg)	.71	.89	.62	.80	.73	.75
	Atropine (2 mg/kg)	.60	.78	1.08	.84	.78	.82
	Atropine Methylnitrate 2 mg/kg	.64	.80	.68	.75	.81	.74
	Mean	.71	.83	.84	.82	.77	

Whether this aspect of cholinergic activity is in part responsible for the significant influence of anticholinergic administration on response perseveration for self-produced stimulation, as observed in spontaneous alternation and exploratory behavior, requires further examination also.

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