Table 3. Effect of locally intradermally injected (twice daily from day 2 to day 10) actinomycin D (AMD; sterile isoosmotic saline for the control group) on the growth of chick comb under the influence of topically and daily applied testoterone propionate (TSP; ethanol for the control group). The number of birds in each test follows (in parentheses) the test designation.

Treatment	Comb (mean ± S.E.)	
	Length + height (mm)	Weight/body wt (mg/g)
*Experiment 1**		
(*a*, 8) 0.05 ml ethanol		0.32 ± .01817
(*b*, 8) 0.04 ml saline		.305 ± .0179
(*c*, 8) 1.14 μg TSP		.68 ± .0648
(*d*, 8) 0.2 μg AMD, 1.14 μg TSP		.68 ± .01
Experiment 2†		
(*e*, 7) 0.02 ml ethanol	11.4 ± 0.689	.364 ± .037
(*f*, 10) 0.02 ml saline	12.8 ± 0.6632	.379 ± .022
(*g*, 8) 1.14 μg TSP	16.7 ± 1.31	1.547 ± .0212
(*h*, 8) 0.2 μg AMD, 1.14 μg TSP	15.3 ± 0.65	1.348 ± .114

Statistical significance

Length + height: $e \sim g$, $P < .001$; $f \sim h$, $< .001$; $g \sim h$, $< .7$

Comb wt/body wt: $a \sim c$, $P < .001$; $b \sim d$, $< .001$; $c \sim d$, not significant; $e \sim g$, $< .001$; $f \sim h$, $< .001$; $g \sim h$, $< .5$

* TSP supplied by L. Light and Co., Ltd., Colnbrook, England. † TSP supplied by Sigma Chemical Co., St. Louis, Mo.

that the system has been proposed as a method for bioassay of androgens (*9*).

We now report experiments designed to determine whether the stimulation of synthesis of RNA is essential for the growth of the comb under the influence of androgens. One-day-old, male, White Leghorn chicks (*10*) were divided into three groups. Group 1 received topical application of 95-percent ethanol (0.05 ml) daily. Group 2 received androsterone or testosterone propionate, dissolved in 0.05 ml of ethanol, topically on the comb; the daily dose was 3 μg of androsterone or 1.14 μg of testosterone propionate. Group 3 received actinomycin D topically in addition to the androgen steroid; actinomycin D dissolved in isoosmotic saline was applied twice daily: 1 hour before application of the androgen and 8 hours after. All chicks were thus variously treated from day 2 to day 10 (*9*) when the chicks were weighed. Combs were measured (*11*) before removal and individual weighing (Table 1).

Obviously the size and weight of the combs of birds treated with androgens were significantly greater than those of the control group. Application of actinomycin D along with androgens did not influence the response of the comb to the androgens. In order to rule out possible interaction of actinomycin D and externally applied steroid, another series of experiments was performed in which the growth of the comb was measured at an age at which the endogeneous hormone is active. Here again actinomycin D did not prevent normal growth of the comb (Table 2).

It may be argued that actinomycin D is not absorbed into the tissue when applied topically on the comb. Control experiments were performed, in which actinomycin D was injected subcutaneously into the comb with a microsyringe. The results (Table 3) were essentially unchanged.

A point that remains open is the possibility of nonsaturating levels of actinomycin in the tissue. No precise data are available for calculation of the optimal concentrations of actinomycin to be used for such studies. The investigator is faced with the problem of stepping in ranges which may be toxic and at which aspecific effects may begin to appear. In our experiments we applied daily and individually as much as 0.3 μg of actinomycin D, which amount did not noticeably inhibit stimulation by testosterone of the growth of the comb. Amounts as great as 0.6 μg have been tried on certain animals with no effect. Much smaller amounts of the antibiotic effectively prevent the action of estradiol on vagina or of human chorionic gonadotrophin on immature testis (*3*).

Our results suggest that development of the chick comb under the influence of androsterone and testosterone may not require the prior stimulation of synthesis in the comb of DNA-dependent RNA. If this is so, the chick comb differs in this respect from epithelial-tissue organs such as seminal vesicles and prostate.

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Color Vision in the Antelope Ground Squirrel

Abstract. *Antelope ground squirrels* (Citellus leucurus) *were able, after conditioning, to respond correctly to a port illuminated with light at a wavelength of 460 nanometers. This color, randomly presented at one of two positions, was correctly selected in reference to a second port illuminated with light at 500, 520, 569, and 600 nanometers, or with white light of varying intensity. Luminosity was not a factor in the discrimination.*

The study of color vision in animals is fraught with numerous difficulties which have limited development of the subject. One problem, and not an unimportant one, is the choice of the most

appropriate animal. From the biological point of view, ground squirrels offer many advantages. These animals have retinas of the all-cone type, they are diurnal and their visual system appears to have evolved for the function of high visual acuity. Moreover, they are active animals and can readily be trained to perform simple tasks. There are claims in the literature that some ground squirrels have the ability to discriminate colors (*1*); but Dowling suggested, on the basis of the sensitivity curve and the finding that there is only one visual pigment, that the 13-lined ground squirrel may not have color vision (*2*). For the past 2 years we have been investigating this problem with the antelope ground squirrel (*Citellus leucurus leucurus*), and we have come to the conclusion that this rodent does in fact possess some mechanism for color discrimination.

The spectral sensitivity curve for this species was first determined by use of the electroretinogram. This curve, similar to those reported by Tansley, Copenhaver, and Gunkel (*3*) for other ground squirrels, was employed to adjust colored stimulus lights to have equal luminosity for this animal. With equality established, the squirrels were trained to go to an illuminated colored port, to press a bar beneath the port, and then to return to a central station where they were rewarded with sunflower seeds. Testing for color vision was carried out by use of a three-cage technique, the cages being next to each other in tandem. The central cage contained the reward station to which the animal returned after making a bar-press in one of the end cages. The two end cages, separated from the central cage by removable gates, were each provided with a diffusely illuminated, round port, the color and luminosity of which were varied by means of interference and neutral filters. Presentation of stimuli at the two end cages was entirely random with respect to both the order of presentation of the several colors and luminosities and as to location. For each trial the animal was required, at the sound of a buzzer and the opening of the two gates, to go into one of the end cages, approach the colored port, press the bar, and finally to return to the central cage for reward if the response was made to the correctly illuminated port.

We have had success in conditioning both a male and a female squirrel to discriminate correctly a port illuminated with blue light (460 nm) from a comparison port illuminated with one of four other colors (500, 520, 569, 600 nm). Table 1 gives the results of 560 trials made in ten daily sessions with the male. In this case all colors were of equal luminosity. The squirrel succeeded in making 90.4 percent correct responses.

Table 1. Discrimination of blue light by a male squirrel with equal luminances at all ports. Results are expressed in numbers of responses. Each row consists of data obtained in one daily session.

Test port, 460 nm	Comparison port			
	500 nm	520 nm	569 nm	600 nm
47	1	1	3	4
49	2	2	1	2
49	2	0	3	2
54	0	1	1	0
53	1	1	0	1
53	0	2	1	0
52	0	2	1	1
49	1	4	2	0
48	3	1	1	3
52	2	2	0	0
		Totals		
506	12	16	13	13
		Percentages		
90.4	2.1	2.9	2.3	2.3

For correct responses it was not necessary to equalize the luminosity of the test (460 nm) port and the comparison port. In another experiment, the luminosity of the test port was made equal to that of the comparison port, brighter (by use of a 0.3 neutral filter in the light path to the comparison port), or dimmer (by use of the 0.3 filter in the light path to the test port). For the three conditions, which were presented randomly to the animal in each of the daily sessions, the percentage of correct responses was about the same. For equal luminances, results were similar to those shown in Table 1 (94.4 percent correct responses). Results obtained under the other two conditions are shown in Table 2.

The squirrel could also discriminate correctly the blue port from the comparison port when the latter was illuminated with white light at any level

Table 2. Discrimination of blue light with luminance different at the test port and the comparison port. Results are expressed in number of responses; each row consists of data obtained in one daily session. In the original experiment, data were also gathered for the condition of equal luminance; these data are omitted because the results were similar to those shown in Table 1.

Test port brighter					Test port dimmer				
Test port, 460 nm	Comparison port				Test port, 460 nm	Comparison port			
	500 nm	520 nm	569 nm	600 nm		500 nm	520 nm	569 nm	600 nm
16	0	0	0	0	12	1	1	0	2
14	0	1	0	1	11	0	3	2	0
13	1	2	0	0	15	0	0	0	1
16	0	0	0	0	14	1	1	0	0
15	0	1	0	0	14	1	0	0	1
15	0	0	0	1	14	1	0	0	1
15	0	0	0	1	13	0	1	0	2
13	1	2	0	0	12	2	0	1	1
14	0	2	0	0	15	0	0	0	1
13	1	0	1	1	14	1	0	0	1
					Totals				
144	3	8	1	4	134	7	6	3	10
					Percentages				
90.0	1.9	5.0	0.6	2.5	83.8	4.4	3.7	1.9	6.2

Table 3. Discrimination of blue light with comparison port illuminated with white light. Responses are expressed in number of responses; each row consists of data obtained in one daily session.

Test, port, 460 nm	Comparison port						
	0*	1.0*	1.5*	2.0*	2.5*	3.0*	Dark
24	4	1	†	1	†	0	0
53	7	†	†	†	†	†	†
51	3	2	†	2	†	1	1
57	†	1	1	†	0	1	†
55	†	0	1	0	0	0	†
			Totals				
240	14	4	2	3	0	2	1
			Percentages				
90.2	5.3	1.5	0.8	1.0	0	0.8	0.4

* Density of neutral filter placed in the light path. † No determination.

of intensity from maximum to complete darkness. This is shown by the figures in Table 3; 90.2 percent correct responses were recorded in five daily sessions involving 266 trials. One would not expect such a performance if the squirrel saw the blue port as gray. With performances such as these the more interesting question almost becomes: what is the cause of the incorrect responses?

We believe that the odor of the seeds is ruled out as an explanation, for the reward was always placed symmetrically with respect to the two ports. Unconscious clues by the human attendant seem to be ruled out by virtue of the fact that both of us have tested the male squirrel and have obtained the same results. Noises during the trials were completely random or else were symmetrical to the two ports. The female squirrel was as successful as the male in selecting the blue port. Moreover, attempts to train other animals to select green or orange ports have failed to elicit correct responses. The behavior of these animals toward green or orange light was random except possibly in relation to the blue port.

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Sleep: Changes in Threshold to Electroconvulsive Shock in Rats after Deprivation of "Paradoxical" Phase

Abstract. *Rats were deprived of paradoxical (radid eye movement or REM) sleep for a 6-day period but were allowed substantial non-REM (slow wave) sleep. Thresholds for electroconvulsive shock dropped significantly after deprivation in all these animals, but thresholds in control animals treated in a similar manner, but allowed REM sleep exhibited no change. Deprivation seems to heighten neural excitability.*

While REM sleep invariably occupies a substantial portion of the total sleep time in mammals, and has been closely associated with dreaming in man (*1*), little is known of its physiological function (*2*).

After a cat has been deprived of such sleep for 30 days or more, its first periods of REM sleep are dramatically enhanced by overt manifestations. The animal becomes completely flaccid, but against the background of muscular atonia there are episodes of violent facial and limb twitches interspersed with convulsive movements of the entire body so intense that the animal seems to be in the throes of a myoclonic seizure. The severity of these spasms appears to increase as the animal is increasingly deprived. During the recovery period, when the animal is allowed uninterrupted sleep, there is a rebound or compensation for the lost sleep that is roughly proportional to the length of period of deprivation (*2–4*), and the intensity of the phasic motor activity gradually subsides. In addition, Dewson *et al.* found accelerated auditory recovery in cats deprived of REM sleep for 5 or more days (*5*). The ratio betwen amplitudes of potentials evoked by a pair of clicks was greater in the nondeprived but equally disturbed animals than it was during a prior or subsequent period of deprivation. Hence, at a given time (25 to 75 msec) after neural discharge, more cells seem to be available for a second response in the deprived animal than are available in its nondeprived counterpart. This change was invariably reversed when the animals were allowed to make up the lost sleep.

These findings led us to hypothesize that an effect of the selective deprivation of REM sleep is a generalized increase in neural excitability. We have now tested this hypothesis in a study in which the threshold for electroconvulsive shock was used as a gross measure of change in excitability. The rat was chosen as the experimental animal because it displays the physiological features of REM sleep (*6*), (see Fig. 1), and there is an extensive literature on factors affecting electroconvulsive thresholds in this species (*7–11*).

Base thresholds for electric shock were determined for a group of 48 male Wistar rats, ranging in weight from 150 to 175 g. Thresholds were established with a constant-current stimulator. On-line current measurements were made with a Tektronix oscilloscope and a current-measuring probe. To ensure reliability, three determinations were made, by the method of Schwartzbaum *et al.* (*7*), for each rat; these did not vary by more than 2 percent of their original values.

Twenty-four of these rats (group 1) were then placed on inverted flower pots whose bottom diameter was 7 cm. The pots were placed in large tubs filled with enough water to just cover the pots' bottoms. Wire mesh was attached above the tubs and shaped to hold food and water bottles. The rats were able to rest on the pots and were even able to get non-REM sleep, but at the onset of REM sleep, with its ensuing muscular relaxation, they would either fall into the water and clamber back to their pots or would get their noses wet enough to awaken them. Twenty-four control animals (group 2), matched as closely as possible with those in group 1 for age, weight, and shock threshold, were placed in similar tanks. However, the bottoms of the flower pots in these tanks were wide enough (11.5 cm) to allow the animals to curl up and obtain REM sleep without falling into the water. The method is an adaptation of the method used successfully to deprive cats of REM sleep (*2, 3*).

Four animals in a third group, weighing 210 to 220 g each, were treated in the following manner in order to control nonspecific effects of sleep loss produced by the deprivation technique. These rats were placed in tanks identical with those used for the first group. However, they were removed from the tanks for a 6-hour period each day and allowed to sleep. Electromyographic and electroencephalographic potentials were recorded. Two animals were awakened at the first sign of loss of EMG potential and EEG activation; the remaining pair served as controls and were awakened from non-REM (slow wave) sleep with the same frequency as the deprived pair. Thus the control animals lost an amount of non-REM sleep comparable to the amount of sleep lost by