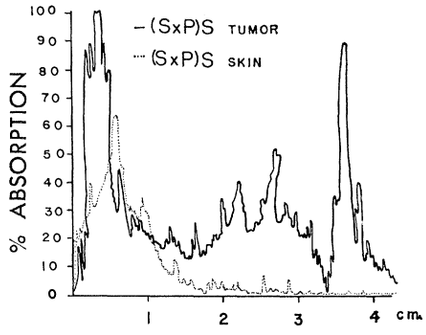

Fig. 2. Densiometric comparison of the soluble proteins of (S × P)S skin and melanoma.

thelial tissue (Fig. 2). The striking difference lies in the changes which have occurred in the relative abundance of the most rapidly migrating proteins and particularly in the occurrence of a very prominent band between 3.8 and 4.0 cm, which appears in the tumor extracts. As can be seen from Fig. 1, very faint bands appear in the 3.8- to 4.0-cm region in the platyfish, swordtail, and hybrid, while in the tumor tissue it is estimated that these bands alone make up 25 percent of the total extractable protein. The function of these protein

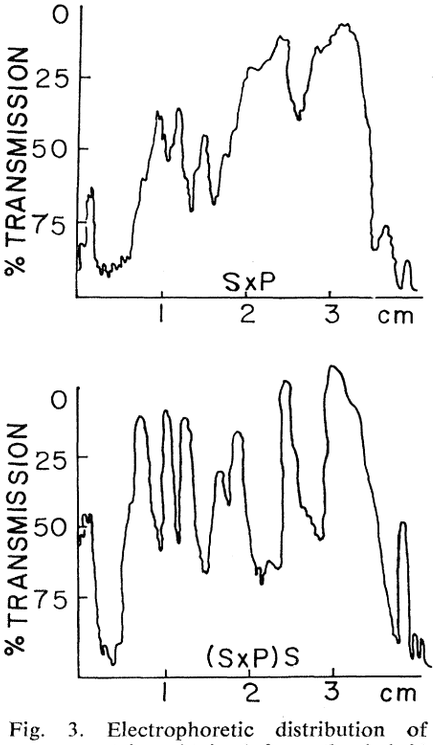

Fig. 3. Electrophoretic distribution of serum proteins obtained from the hybrid (S × P)S. The curve represents a densiometric tracing.

bands is unknown. Analyses so far indicate that these bands have no esterase activity. However, there is in the tumor a new esterase band, migrating about 2.5 cm from the origin.

The possibility existed that these fast-running protein bands represented a considerable incorporation of plasma protein by the tumor tissue. Evidence that such a mechanism exists in several mouse tumors has been presented (*7*). However, the electrophoretic pattern of the backcrossed serum (Fig. 3) shows only a very faint band in the gel in a position corresponding to the tumor protein. It seems more likely to us that the appearance of a small amount of this protein in the serum in this case represents a leakage of the protein into the blood from the tumor, rather than a concentration of the protein from the blood by the tumor.

The changes in the pattern of extractable protein from the pigmented tumor cell, when compared with its normal counterpart, are very striking. That a significant portion of the total protein of the tumor cell runs ahead of the corresponding serum-albumin peak suggests that this protein is not identical with any major component in the serum. Since it is not represented by any similar protein in the normal pigment cell, it is possible that this protein represents a unique product of the tumor. The physiological function of this protein and its relationship to the etiology of malignancy in these cells are not known.

DOUGLAS G. HUMM
AVIS L. SYLVIA
Department of Zoology, University of North Carolina, Chapel Hill

References and Notes

1. M. Gordon, *Growth* **10**, 153 (1951).
2. L. Ornstein and B. Davis, *Disc Electrophoresis* (Distillation Products Industries, Rochester, N.Y., 1962).
3. D. G. Humm and A. L. Sylvia, in preparation.
4. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
5. H. C. McAllister, Jr., Y. C. Wan, J. L. Irvin, *Anal. Biochem.* **5**, 321 (1963).
6. H. Tsuyuki, E. Roberts, R. E. Gadd, *Can. J. Biochem. Physiol.* **40**, 929 (1962); H. Tsuyuki and E. Roberts, *J. Fisheries Res. Board Can.* **20**, 101 (1963).
7. H. Busch and H. S. N. Greene, *Yale J. Biol. Med.* **27**, 339 (1955); J. F. Henderson and G. A. LePage, *Cancer Res.* **19**, 887 (1959).
8. Supported in part by PHS grant CA 1717 from NCI, by the Morris Greenspun Foundation, and the University of North Carolina Cancer Institutional grants 324 CAN 3-11 and 324 AME-6-10 from the American Cancer Society. We thank Dr. K. Kallman for the original stock of fish, and Miss Nancy Hybner and W. Miller for assistance.

20 July 1965

Differential-Approach Tendencies Produced by Injection of RNA from Trained Rats

Abstract. *Two groups of rats were trained in a Skinner box to approach the food cup when a discriminative stimulus (click or blinking light) was presented. Ribonucleic acid was extracted from the brains of these two groups of rats and injected into two groups of untrained rats. The untrained two groups then manifested a significant tendency (as compared with one another) to react differently to the two stimuli. On the average, the response appeared to be specific to the stimulus employed during training.*

When RNA was extracted from the brains of rats trained to approach a food cup and was injected intraperitoneally into untrained rats, the rats so injected made significantly more approaches to the food cup than did controls (rats injected with RNA from brains of untrained rats) (*1*). We now present evidence that this effect is specific rather than general. Our new experiment is similar to our earlier one, except that we used two experimental groups, each trained to respond to a different discriminative stimulus.

Initially, 16 male Sprague-Dawley rats, aged 50 to 60 days, weighing 220 to 240 g, received magazine training in a standard Grason-Stadler Skinner box; that is, they were trained to approach the food cup when a discriminative stimulus was presented. For eight rats, the discriminative stimulus was the distinct click (*1*). For the other eight rats, the discriminative stimulus was a blinking light. The latter stimulus was produced by blinking the 10-watt house light (within the Skinner box) three times in succession, the three blinks taking a total of approximately 1 second.

For the click-trained rats, magazine-training was accomplished in the same fashion as in our earlier study (*1*).

The blinking light proved to be a somewhat more difficult stimulus to establish as a signal, and accordingly, minor modifications in the magazine-training procedure were made. After early training, the rats did not respond to the blinking light but they did run to the cup upon hearing the slight noise produced by the pellet's dropping into the cup. As training progressed, on more and more trials

Table 1. Total number of responses per animal on the 25 test trials with click and on the 25 test trials with light.

Stimulus		Score (C-L)
Click	Light	
Injection with RNA-C		
2	3	−1
3	4	−1
5	2	3
5	2	3
6	1	5
7	1	6
7	0	7
11	2	9
Injection with RNA-L		
0	7	−7
0	7	−7
0	3	−3
1	3	−2
0	2	−2
0	2	−2
0	1	−1
7	5	2

we withheld the pellet until the rat responded to the light alone. Thus, we essentially transferred discriminative control of the approach response from the noise of the descending pellet to the blinking light.

Each of these 16 rats was trained to the stimulus as described (*1*). By the end of training, each rat in both groups approached the food cup promptly and swiftly from any part of the box when the appropriate discriminative stimulus (click or blinking light) was presented, and rarely or never approached the cup in the absence of that stimulus.

Upon completion of the training, each rat was killed with ether, and the brain was taken out as quickly as possible. A cut was made on a line joining the superior colliculus to the rostral end of the pons. The tissue posterior to this cut was discarded, as was the tissue of the olfactory bulbs. RNA was extracted (*1*) from the remaining tissue (1.3 g, average weight) and was dissolved in 2.0 ml of isotonic saline. Approximately 8 hours after extraction, the RNA from each of the rats, light-trained or click-trained, was injected intraperitoneally with a 1.9 cm 22-gauge needle into an adapted untrained rat (1). During adaptation, most animals initially made slight startle responses to the click, but few or no startle responses occurred to the blinking light. By the end of the adaptation series, no animal made any visible response to either the click or the blinking light.

Thus eight animals received RNA (RNA-C) from click-trained rats and eight received RNA (RNA-L) from light-trained rats. All were assigned code letters and tested "blind" (*1*).

A session of testing for a given animal consisted of placing that animal in the Skinner box, permitting one minute to elapse, and then delivering a series of ten stimuli (five clicks and five lights in a mixed order, as described below). The stimuli were spaced at least 30 seconds apart. Five such testing sessions were given (*1*). During the first three test sessions, the order of presentation of stimuli was LCCLLCCLLC; during the last two sessions, the order was CLLCCLLCCL. Each test animal thus received a total of 25 click and 25 light trials. At the beginning of testing, all rats had been deprived of food for approximately 24 hours. After the third test session, all rats were fed 4 to 5 g of Purina Lab Chow. The method of testing and the criterion of response were identical to those used in our first experiment.

A comparison of the two judges' tallies revealed that they agreed on 790 out of 800 trials, that is, on 98.7 percent of the judgments.

Each rat received a difference score (C-L) which was obtained by subtracting number of responses to light (L) from number of responses to click (C) for that rat. The Mann-Whitney *U* test (*2*) was performed to test the null hypothesis that the C-L scores of the two groups did not differ from each other. The test indicated that the difference between groups injected with RNA-C and RNA-L was significant ($P<.001$, one-tailed test). The difference in response to click for the two groups was significant by a Mann-Whitney *U* test ($P<.002$, one-tailed test).

We may conclude on the basis of the statistical analysis of C-L scores that the two groups differed in their tendencies to react differentially to click and blinking light. The average difference score for the group injected with RNA-C is positive (3.9), whereas the average difference score for the group injected with RNA-L is negative (−2.8), although the test used compares these scores with each other rather than with zero. A further conclusion is that RNA-C rats responded more to click than did the RNA-L rats. The differences between the groups in response tendencies may be attributed to the RNA preparation injected into the test rats, and hence presumably to the effects which original training produced upon the RNA of the donor animals. Since handling, nutrition, and adaptation to the Skinner box were matched for the two groups, the transfer effect cannot be attributed to these factors. Thus, the new results support our original finding that a response tendency can be transferred by RNA injection, and they further suggest that this effect is to a substantial extent specific rather than general.

ALLAN L. JACOBSON, FRANK R. BABICH
SUZANNE BUBASH, ANN JACOBSON
Department of Psychology, University of California, Los Angeles

References and Notes

1. F. R. Babich, A. L. Jacobson, S. Bubash, A. Jacobson, *Science* **149**, 656 (1965).
2. S. Siegel, *Non-parametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956).

28 July 1965

Nicotine: Effect on the Sleep Cycle of the Cat

Abstract. *Small doses of nicotine (0.005 to 0.01 milligram per kilogram of body weight) given intravenously to sleeping cats with indwelling brain electrodes produce (i) initial electroencephalographic activation which was accompanied by behavioral arousal; (ii) a few minutes later, slow-wave sleep; and (iii) within 15 to 30 minutes, fast-wave sleep. Although peripheral afferent stimulation, release of epinephrine, and arginine vasopressin contribute to the initial arousal effects, the primary action of nicotine appears to be on the central nervous system.*

Small doses of nicotine produce electroencephalographic activation in intact animals and in those whose brainstems had been transected (*1*). Although nicotine has multiple peripheral actions, its primary effects on desynchronization of the electroencephalogram are due to action directly on the central nervous system. Of special significance to pharmacologists, psychologists, sociologists, and others interested in the behavioral consequences of tobacco-smoking is the fact that these actions of nicotine on the central nervous system occur in animals with doses that are fully comparable to the small amounts of nicotine