biological clock behind the sleep rhythm.

Our data are too limited to provide an explanation of this mechanism, but we have drawn certain general conclusions. The neuron and glia form a functional unit with two parts which are energetically coupled and influence each other functionally (*5, 7*). When functional demands increase, inverse changes in enzyme activity occur in the neurons and their glia within hours. The total protein contents per neuron and glia follow these enzyme changes. This indicates that a synthesis of enzyme protein occurs and not only changes in enzyme activities. Furthermore, it seems significant that inverse RNA changes also occur concomitantly. A recent electrophoretic analysis of the RNA fractions which increased in the neuron and decreased in the glia showed an identical base ratio composition of the two RNA fractions. This suggested that RNA molecules or, alternatively, nucleotides (*8*) could be transferred from the glia to the neuron. In summary, such a two-cell collaboration would form a stable functional system from a cybernetic point of view. As a response to functional demands, primary processes in neurons and glia comprising RNA, enzyme, and other types of protein synthesis can evidently vary between two levels.

We suggest that the energy metabolism in the caudal part of the reticular formation oscillates with inverse changes between the neurons and the glia during the circadian sleep rhythm. This recalls the findings of Moruzzi (*2*) that an area within the lower reticular formation of the brainstem has a damping influence on the upper part of the reticular formation and produces the EEG pattern of sleep. The biochemical response of the neurons in the oral part of the reticular formation in the present study may therefore be interpreted as influenced by the caudal part.

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References and Notes

1. H. Hydén and P. Lange, *Life Sci.* **3**, 1215 (1964).
2. G. Moruzzi, *Harvey Lectures Ser. 58* (1963), p. 233.
3. N. Sterner, *Farmacevtisk Revy Sweden* **62**, 121 (1963).
4. H. Hydén, *Nature* **184**, 433 (1959).
5. H. Hydén and A. Pigon, *J. Neurochem.* **6**, 57 (1960).
6. S.-O. Brattgård, J.-E. Edström, H. Hydén, *ibid.* **1**, 316 (1957).
7. H. Hydén and P. Lange, *J. Cell Biol.* **13**, 233 (1962); A. Hamberger and H. Hydén, *ibid.* **16**, 521 (1963); A. Hamberger, *Acta Physiol. Scand.* **58**, suppl. 203 (1963); H. Hydén, in *Recent Advan. Biol. Psychiat.* **6**, 31 (1964); G. Gomirato and H. Hydén, *Brain* **86**, 773 (1963).
8. H. Hydén and P. Lange, *Naturwissenschaften* **52**, 12 (1965).
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Transfer of a Response to Naive Rats by Injection of Ribonucleic Acid Extracted from Trained Rats

Abstract. *Rats were trained in a Skinner box to approach the food cup when a distinct click was sounded. Ribonucleic acid was extracted from the brains of these rats and injected into untrained rats. The untrained rats then manifested a significant tendency (as compared with controls) to approach the food cup when the click, unaccompanied by food, was presented.*

Although there has been abundant theorizing of late which implicates ribonucleic acid (RNA) in the process of memory storage (*1*), direct experimental evidence in support of this contention has been scanty. Hydén and Egyhazi (*2*) reported changes in the ratio of bases in the nuclear RNA of vestibular neurons of rats after the animals had performed a wire-climbing task. In further work (*3*), Hydén and his associates extended their biochemical analyses and studied an additional training paradigm involving reversal of handedness in rats. In the most recent of these experiments, they reported that different types of neural RNA are produced during the early, as compared with the later, stages of learning. Preliminary experiments with planarians (*4*) indicate that, if RNA from trained animals is injected into naive animals, the naive animals respond more than do controls upon subsequent testing. In the present study with rats we used a direct test similar to that used for the planarian experiments.

We used 50- to 60-day-old male Sprague-Dawley rats, each weighing approximately 250 g. Eight rats received magazine-training in a standard Grason-Stadler Skinner box—that is, they were trained to approach the food cup upon hearing the distinct click produced by operation of the pellet dispenser. Magazine training was accomplished as follows. On the first day, a rat deprived of food for 48 hours was placed in the Skinner box and allowed to eat two 45-mg Noyes pellets which had been placed in the cup. Then, while the rat was investigating the cup, the food magazine was operated a number of times in succession, producing each time a distinct click and delivery of a single 45-mg pellet. As training progressed, the click was withheld until the rat moved first a short, and later a longer distance from the cup. During this time, the rat was permitted a number of interspersed cup investigations which were not preceded by the click and hence were not rewarded with food.

Each of the rats was given 200 food-reinforced approaches to the food cup per day for 4 days and 100 such trials on the 5th day. No additional food was given to these rats. By the end of training, each rat approached the food cup promptly and swiftly from any part of the box when the click was sounded, and rarely or never approached the cup in the absence of the click. Control rats were fed daily an amount of Purina Lab Chow equivalent in weight to the amount of food received by the experimental animals.

On the day of completion of magazine-training, each of the eight experimental rats was killed with ether, and the brain was removed as quickly as possible. A posterior cut was made on a line joining the superior colliculus to the rostral end of the pons. An anterior cut was made which removed the frontal areas and the olfactory bulbs. The tissue posterior to the posterior cut and the tissue anterior to the anterior cut were discarded. The selection of this portion of the brain was based on preliminary work showing that (i) this portion was sufficient to give the effect observed in the present experiment, and (ii) this amount of tissue was convenient for our techniques. The average weight of the tissue retained was 1.0 g. We then extracted RNA from this tissue by the following procedure. The tissue was placed in a cold mortar with 5 ml of phenol (90 percent) and 5 ml of

isotonic saline and was ground with purified sand for approximately 3 minutes. The mixture was then centrifuged at 18,000 rev/min for 30 minutes at 0°C. The aqueous phase was carefully drawn off to avoid contamination with phenol or with the interphase. The aqueous phase was then brought up to a concentration of 0.1*M* $MgCl_2$, and 2 volumes of cold ethanol were added to precipitate the RNA. Precipitation time was 15 minutes. The suspension was centrifuged at 6000 rev/min for 15 minutes, after which the supernatant liquid was poured off. The remaining ethanol was evaporated off, and the RNA was dissolved in 1.0 to 1.5 ml of isotonic saline. The amount of RNA was determined from the optical density at 260 mμ (ϵ^p = 7450 in 0.2*M* NaCl). The yields were in the range of 0.7 to 1.1 mg per gram of tissue. Tests for protein with biuret (*5*) and for DNA with diphenylamine (*6*) were negative. The RNA seemed to be relatively undegraded as measured by Sephadex chromatography.

We also extracted, in the same manner, RNA from nine control (untrained) rats. Approximately 8 hours after extraction, the RNA from each of the rats, experimental or control, was injected intraperitoneally with a 1.8-cm, 22-gauge needle into an untrained rat (the xiphoid process was used as a guide for the injection). Before being injected, each of these untrained rats had been adapted to the Skinner box for 5 days, 15 minutes per day; during each session the magazine had been operated two separate times, producing a distinct click each time. No food was ever given to these animals during the adaptation series, although food powder was sprinkled lightly over the grid floor to keep the animals active and to counteract any tendency on the part of the rats to approach the food cup on the basis of residual odor.

Seventeen rats in all were injected with RNA; eight of them received RNA from trained rats and nine received RNA from untrained rats. These 17 rats were assigned code letters, and all testing from this point on was conducted "blind"; the testers did not know the group to which each rat belonged until the completion of testing.

A session of testing consisted in placing an animal in the Skinner box, permitting 2 minutes to elapse, and then delivering a series of clicks (produced by operation of the empty food magazine) spaced no less than 1 minute apart. Five such testing sessions were given, at 4, 6, 8, 22, and 24 hours after injection. Each test animal thus received a total of 25 trials. At the beginning of testing, all rats had been deprived of food for approximately 28 hours. After the third test session, all rats were fed 4 to 5 g of Purina Lab Chow.

Table 1. Total number of responses per animal on the 25 test trials.

Experimental rats	Control rats
1	0
3	0
7	0
8	1
9	1
10	1
10	2
	3

A response on a test trial consisted of the rat's placing its nose inside a demarcated area, 63-cm^2, surrounding the food cup, within 5 seconds of the click. The food cup was located in one corner of the box, the floor of which had an area of 670 cm^2. That is, the rat had to approach to within a certain specified distance of the cup in order for a response to be counted. Further restrictions were placed upon the test trials as follows: Two judges scored all trials independently, and a response was counted only if their tallies agreed; and trials were given only when the animal was facing away from the cup by more than 90° and was located at least a body length from the cup. During testing, as during the adaptation period before testing, food powder was sprinkled lightly over the grid floor.

A comparison of the two judges' tallies revealed that they agreed on 370 out of 375 trials—that is, on 98.7 percent of the judgments. Table 1 presents the score for each test animal in terms of the number of cup approaches, as defined earlier, out of the 25 trials during which clicks were presented (*7*). The mean number of responses for the experimental rats was 6.86; the mean for the control rats was 1.00. By a Mann-Whitney *U*-test (*8*), the difference between the groups was significant at $p<.002$ (one-tailed test). Total scores of the seven experimental rats for the separate test sessions, in order, were: 5, 13, 9, 12, 9; corresponding scores for the eight control rats were: 3, 2, 1, 1, 1.

Thus the experimental animals showed a significantly greater tendency than controls to approach the cup area when the click was presented. In order to understand this phenomenon more precisely, further analysis is required to determine, for example, how general or specific the effect is and the range of behavioral situations in which the effect will occur. Moreover, although it appears most reasonable that the observed effect was produced by RNA, the possibility should not be overlooked that other substances in the extract might have been involved.

If the effect can be shown to depend on associative learning rather than on something more general (for example, sheer amount of stimulation), and if this effect can be definitely ascribed to RNA, then the problem arises of what modifications in RNA are produced by the training situation, such that the present result could occur. Several possible coding mechanisms have been proposed, such as changes in the linear sequence of bases, changes in the helical structure, or changes in the overall configuration or composition of the RNA molecule (*3, 9*). Whatever the coding mechanism, an additional problem remains: namely, how the injected material acts to affect the behavior of the recipient animal (*10*).

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References and Notes

1. J. Gaito and A. Zavala, *Psychol. Bull.* **61**, 45 (1964); T. K. Landauer, *Psychol. Rev.* **71**, 167 (1964).
2. H. Hydén and E. Egyhazi, *Proc. Nat. Acad. Sci. U.S.* **48**, 1366 (1962).
3. ———, *ibid.* **49**, 618 (1963); **52**, 1030 (1964); H. Hydén and P. Lange, *ibid.* **53**, 946 (1965).
4. C. Fried and S. Horowitz, *Worm Runner's Digest* **6**, 3 (1964); A. Zelman, L. Kabat, R. Jacobson, J. V. McConnell, *ibid.* **5**, 14 (1963).
5. E. Layne, in *Methods of Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1957), vol. 3, p. 450.
6. W. C. Schneider, *ibid.*, p. 680.
7. Two injected rats were discarded during test trials because they persistently froze in one corner and thus could not be tested. One of these rats was a control and the other an experimental animal. In contrast, all the other 15 injected rats typically roamed around the box freely during test sessions. The discarded control rat was replaced, but the experimental rat was not; hence, *N* was unequal in the two groups.
8. S. Siegel, *Non-parametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956).
9. W. Dingman and M. B. Sporn, *J. Psychiat. Res.* **1**, 1 (1961).
10. Since this research was completed, we have learned of a similar experiment recently conducted in Copenhagen [E. J. Fjerdingstad, Th. Nissen, H. H. Røigaard-Petersen, *Scand. J. Psychol.* **6**, 1 (1965)]. Despite several differences in procedure, the results of the two studies appear to corroborate each other.

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