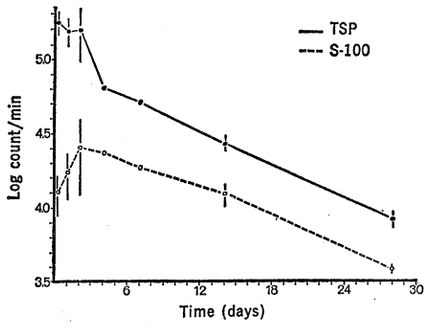

Fig. 2. The log of the specific activity (count/min per milligram of protein) of total soluble brain proteins (TSP) and the S-100 protein for each of the survival periods.

column (*1*). The 15 fractions resulting from this separation were concentrated by pressure dialysis and subjected to electrophoresis on 7.5 percent polyacrylamide gels (*8*) which were then stained with amido black. Figure 1 shows the electrophoretic pattern of the DEAE-cellulose fraction containing the S-100 protein. The S-100 moved faster than any other protein and was, thus, easily identified and isolated. The S-100 band was cut out (Fig. 1), dried in a vacuum overnight, and dissolved in hydrogen peroxide (30 percent) in a 75°C water bath (*9*). To each of these samples was added 0.5 ml of NCS reagent and 10 ml of toluene-base scintillation liquid.

That S-100 was, in fact, the only protein in this fast-moving band (Fig. 1) was established in the following way. First, the amount of S-100 in the DEAE-cellulose fraction applied to the gels was measured by complement fixation (*6*), a method which is both highly sensitive and specific. Second, the S-100 band was cut from the gels (Fig. 1), and the stain was eluted by incubation with 1*N* NaOH for 24 hours. The S-100 content was estimated by comparing the absorbance of the extracted dye solution with that from standards of pure beef S-100 subjected to the same procedure. The amount of S-100 applied to the acrylamide gels was 7.0 μg as determined by complement fixation (for one representative sample), and the dye elution method yielded a value of 6.7 μg of S-100 in the S-100 band cut from the gel. These data, together with the fact that the band presumed to contain S-100 in these experiments moved identically with purified beef S-100, indicate that the S-100 protein, and no other protein, comprised the fast-moving band isolated in our experiments.

As a further control for the validity of our procedure, a known amount of pure beef S-100 was incubated with previously labeled rat liver homogenate, in which no S-100 is found (*1*), and then this mixture was subjected to electrophoresis as described. Total counts in the resulting S-100 band did not exceed background levels. Thus, our results indicate specific incorporation of [^{3}H]leucine into the S-100 protein itself.

The specific activity of total soluble proteins in the brain and the S-100 protein are plotted in Fig. 2 for each of the survival periods. In close agreement with earlier studies of brain protein turnover (*10*) our results confirm that there are at least two populations of soluble proteins in the brain, a small group turning over very rapidly (2 to 4 days) and a larger group with a considerably slower turnover rate of 15 to 16 days. The S-100 protein has a half-life similar to that of the soluble brain proteins that turn over more slowly, that is, approximately 16 days. Of particular interest, is the delay in the incorporation of the radioactive label into the S-100 protein whose specific activity did not reach a peak until nearly 48 hours after the infusion of [^{3}H]leucine. The reason for the delay in the peak labeling of the S-100 protein in these experiments is still unknown.

The results of our experiment are not in agreement with those described in a study by McEwen and Hyden (*4*) in which they reported that the half-life in vivo of the S-100 protein was extremely short. Although the reason for this apparent discrepancy is not immediately clear, it seems likely that the chemical or isotopic purity of the S-100 protein isolated and counted to determine specific activity may be a factor. Detergent extracts were used in the earlier experiments, and these were applied directly to acrylamide gels without prior purification. Under the conditions in our experiment, which may be the first to unequivocally demonstrate the turnover of a single brain specific protein, we conclude that the S-100 protein turns over at approximately the same rate as the average water-soluble brain protein.

THEODORE J. CICERO
BLAKE W. MOORE
Department of Psychiatry,
Washington University School of Medicine,
St. Louis, Missouri 63110

References and Notes

1. B. W. Moore and V. J. Perez, in *Physiological and Biochemical Aspects of Nervous Integration*, F. D. Carlson, Ed. (Prentice-Hall, Englewood Cliffs, N.J., 1968), p. 343; B. W. Moore and D. J. McGregor, *J. Biol. Chem.* **240**, 1647 (1965).
2. B. W. Moore, *Biochem. Biophys. Res. Commun.* **19**, 739 (1965).
3. T. J. Cicero, W. M. Cowan, B. W. Moore, V. Suntzeff, *Brain Res.* **18**, 25 (1970); V. J. Perez, J. Olney, T. J. Cicero, B. W. Moore, B. A. Bahn, *J. Neurochem.*, in press.
4. B. McEwen and H. Hyden, *J. Neurochem.* **13**, 823 (1966).
5. R. D. Myers, *J. Appl. Physiol.* **18**, 221 (1963).
6. B. W. Moore, V. J. Perez, M. J. Gehring, *Neurochemistry* **15**, 265 (1968); L. Levine and B. W. Moore, *Neurosci. Res. Progr. Bull.* **3**, 18 (1965); E. Wasserman and L. Levine, *J. Immunol.* **87**, 290 (1961).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
8. P. Calissano, B. W. Moore, A. Friesen, *Biochemistry* **8**, 4318 (1969).
9. J. B. Boyd and H. K. Mitchell, *Anal. Biochem.* **14**, 441 (1966).
10. R. S. Piha, M. Cuenod, H. Waelsch, *J. Biol. Chem.* **241**, 2397 (1966); A. Lajtha and J. Toth, *Biochem. Biophys. Res. Commun.* **23**, 294 (1966).
11. We thank Mrs. L. Mauro for valuable technical assistance. Supported in part by PHS grants MH-13002, MH-07081, NB-05270, and by National Multiple Sclerosis Society grant 513 A. B.W.M. is a recipient of career development award 5-K3-GM-15,543; T.J.C. is a postdoctoral fellow supported by MH training grant MH-07081.

15 July 1970

Pagophagia in the Albino Rat

Abstract. *Pagophagia, or ice-eating, is a common symptom of iron-deficiency anemia in humans. Rats made anemic by withdrawal of blood consume a significantly greater proportion of their daily water in the form of ice than nonanemic controls. Recovery from the anemia eliminates the pagophagia.*

Pica, the consumption of nonnutritive substances, has been correlated with iron-deficiency anemia in humans (*1*). One of the commonest forms of pica associated with iron deficiency is ice-eating, or pagophagia (*2*). People who are severely deficient in iron may consume up to several large glasses of ice a day in preference to cold water (*2*). Ice-eating ceases after recovery from the iron deficiency. However, the evidence suggesting a relation between

the ingestion of ice and iron deficiency has been observational rather than experimental. We now show that the experimental production of anemia in rats increases their tendency to eat ice and that recovery from the anemia eliminates it.

Twelve 90-day-old Wistar rats were housed in individual cages in which food, but not water, was available immediately before experimentation. At the same time of the day on each of three consecutive days, each rat was removed from its home cage and placed in a wooden chamber (8 by 8 by 12 inches long) containing two funnels. The necks of the funnels went through the floors of the chambers and ended inside individual collection jars. On these 3 days, the necks of the funnels were plugged with corks, and each funnel was filled with cold water. After 20 minutes, the rats were returned to their home cages. By the third day, all of the rats approached the water and drank immediately upon being placed in the chambers.

After this 3-day adaptation period, the rats were given three trials in the chambers with ice available in each funnel. The ice was crushed, an average chunk weighing about 1 g. There was one 20-minute trial per day on each of three consecutive days. During these trials, the funnels were not plugged, and any water formed by melting drained into the collection jars beneath the chambers. All of the rats learned to eat the ice over the 3 days.

After the training period, three additional 20-minute trials were given in which ice was present in one funnel and water in the other. The positions of the two substances were randomly varied each day. These three sessions established the relative preference of ice to water for each rat. The dependent variable was the amount of ice consumed, divided by the total amount of ice and water consumed, times 100 to convert it to a percentage score. The range of preferences was from 8 to 73 for the individual subjects on the last of these 3 days, which is typical of all 3 days. A value of 50 means that the rat consumed an equal amount of ice and water. These three 20-minute sessions are referred to as days 1, 2, and 3, respectively, on Fig. 1.

The rats were then divided into matched pairs on the basis of preference, and the members of each pair

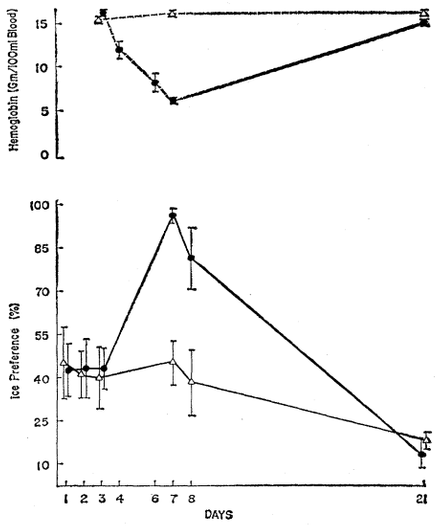

Fig. 1. Upper portion gives the mean grams of hemoglobin per 100 ml of blood for the two groups over days. Lower portion gives the mean ice preferences for the same two groups. Experimental animals are represented by solid circles; controls by open triangles. The brackets represent plus and minus one standard error of the means.

were randomly assigned to two groups. The animals in the experimental group were briefly anesthetized with 5 percent halothane in oxygen, and several milliliters of blood were removed by means of intracardiac puncture. The control animals were similarly anesthetized and punctured, but no blood was removed. This procedure was followed for three consecutive days (days 4, 5, and 6). The amount of blood removed from the experimental animals totaled from 10 to 16 ml over the 3-day period. One rat in the experimental group died during this period, presumably from the blood loss.

After the withdrawal of blood the rats were returned to their home cages where they received a liquid diet which was low in iron. The food was available for 3 hours, and it was the only source of food and water on those 3 days. Its purpose was to allow the experimental rats to regain the lost blood volume and other nutrients without replacement of the lost iron. The basic diet consisted of four egg whites, 250 ml of evaporated milk, 125 g of dextrose, 250 ml of water, and a vitamin supplement.

No trials for preference of ice or water were given on these 3 days, but blood samples were taken and analyzed for hemoglobin content. The hemoglobin content of all animals before treatment averaged about 16 g per 100 ml of blood. In humans, pagophagia is not manifest unless hemoglobin content is as low as 5 or 6 g per 100 ml of blood (*2*). Therefore, no preference tests were given until the hemoglobin content of the experimental animals had reached that level.

On the day after the final withdrawal of blood, the mean amount of hemoglobin in the experimental animals was approximately 6 g per 100 ml of blood, whereas that of the controls was 16 g/100 ml. On this day (day 7), the rats were given a 20-minute preference test. The anemic rats showed a significantly increased preference for ice (Fig. 1). This preference was so great that, as a group, they satisfied 96 percent of their water needs for that day by eating ice, as against 45 percent for the control animals. The total amounts of water ingested by the two groups regardless of phase (solid or liquid) were not different.

The rats were then deprived of food and water for 24 hours, and a second test was given (day 8). This was to ascertain the effect of increased water deprivation on the pagophagia. The anemic animals continued to show a preference for ice over water (Fig. 1). The amounts of water deficit corrected by the two groups were not significantly different. Therefore, a rapid decrease in hemoglobin in the experimental animals was followed by an increase in the consumption of ice relative to water. During the two test trials, the anemic rats actually chewed the ice, rather than licking it.

Further support for a relation between pagophagia and anemia would be the disappearance of pagophagia after recovery from anemia. All rats were given free access to both food (Purina lab chow) and water during the next 2 weeks. A blood sample taken at the end of this time revealed that the hemoglobin of the experimental animals had returned to the normal range, so all animals were deprived of food and water for 24 hours and then given a final preference test (day 21). The preference for ice had decreased in both groups (Fig. 1). The mean of the experimental group was 13, and that of the controls was 18. The difference between them was not significant. The decreased ice-eating in the controls was unexpected and may simply reflect for-

getting, since none of the animals had been offered any ice for 2 weeks.

The rats were made deficient in iron by removal of blood, a process in which many other substances in the body are also reduced. The availability of the iron-poor diet theoretically permitted the restoration of all these substances except iron. Furthermore, an iron-deficient diet given over a long period has been reported to be as effective as blood loss in leading to pagophagia (*1*). Therefore, we attribute the increase in ice-eating in our experiment to the loss of iron.

It is interesting that rats can be trained to eat ice in order to obtain their daily water. Of more interest is the fact that normal rats took a substantial portion of their daily water in the form of ice when water was available (Fig. 1), although this preference appeared to decline over time. The main contribution of the experiment, however, is experimental support for the earlier speculation based upon clinical data that iron deficiency tends to cause ice-eating. Although the experiment suggests no explanation for the phenomenon, other workers have reported that domestic rats suffering from either vitamin or mineral deficiencies show strong preferences for new foods (*3*).

STEPHEN C. WOODS
RICHARD S. WEISINGER
Departments of Physiology and Biophysics and *Psychology, University of Washington, Seattle 98105*

References and Notes

1. P. Lanzkowsky, *Arch. Dis. Child.* **34**, 140 (1959); C. E. Mengel, W. A. Carter, E. S. Horton, *Arch. Intern. Med.* **114**, 470 (1964).
2. R. D. Reynolds, H. J. Binder, M. B. Miller, W. W. Y. Chang, *Ann. Intern. Med.* **69**, 435 (1968).
3. W. Rodgers, *J. Comp. Physiol. Psychol.* **64**, 49 (1967); P. Rozin and W. Rodgers, *ibid.* **63**, 421 (1967).
4. We thank Profs. Walter Makous and Moncrieff Smith for their assistance. Supported by PHS training grant 5T01 GM 00666.

20 July 1970

Plasticity of Synchronous Activity in a Small Neural Net

Abstract. *Electrical activity from three neurons at a time was recorded in a pleural ganglion of* Aplysia. *Synchonous activity could be temporally patterned by electrical stimulation over a single nerve. After a period of pairing with electrical stimuli over another nerve the degree and time course of the synchrony were altered. Such alterations reverted after 5 to 30 minutes.*

An important problem in neurobiology is identification of mechanisms that underlie plasticity of the nervous system. The multiplicity of such mechanisms has been stressed in a recent review (*1*). In particular, it has been shown that a stimulus sequence based upon a classical conditioning paradigm can increase the size of a postsynaptic potential (*2*) or alter the firing patterns of single neurons (*3*). The purpose of the present study was to examine the effects of such stimulus sequences on the activity of a functionally related group of neurons. This report will show a number of ways in which pairing of stimuli over different afferent paths could reversibly modify the probability of nearly synchronous firings. A more complete report is in preparation (*4*).

Experiments were performed with neurons of the mollusk *Aplysia californica*. The circumesophageal ring of ganglia together with maximal lengths of nerves was dissected free and perfused with oxygenated seawater in a controlled-temperature chamber (15° ± 0.5°C). Connective tissue overlying the pleural ganglion was removed, and glass pipette electrodes (0.5*M* K_2SO_4, 5 to 20 megohms) were inserted into three neurons. Intracellular potentials were amplified, displayed on an oscilloscope, and recorded on an FM instrumentation tape recorder. Suction electrodes were used to deliver electrical stimuli to various nerves or connectives. For each experiment two particular nerves were chosen for delivery of a "test" stimulus and a "priming" stimulus. The test stimulus was a 0.1- to 5.0-msec pulse presented every 10 seconds; its strength was adjusted to slightly above the threshold value for production of postsynaptic potentials in the observed neurons. The priming stimulus consisted of a train of five pulses spaced 100 msec apart, presented every 10 seconds. Its strength was arbitrarily chosen as two to three times that of the test stimulus.

A typical experiment consisted of observation of the three impaled neurons during the following stimulus sequence: (i) 5 minutes without stimulus presentation, (ii) 5 to 10 minutes of test stimulus presentation alone, (iii) 4 minutes of paired presentation of test and priming stimuli (the priming stimulus train began 500 msec after each test stimulus), (iv) 5 to 10 minutes of test stimulus presentation alone, (v) 5 minutes without stimulus presentation, and (vi) repetition of conditions (iv) and (v).

We did not vary the timings or order in the presentation of paired stimuli. However, if the condition of the preparation remained stable, we examined nonspecific effects of the priming stimulus that would be analogous to classical sensitization. This was done by presenting again the entire stimulus sequence outlined above except that the priming stimulus alone was given in part (iii). It should be noted that the experimental design always included periods of "rest" between periods of presentations of the test stimulus. This allowed comparison of spontaneous activity before and after stimulus pairing.

Firings of the observed neurons were analyzed by means of the joint peri-stimulus-time scatter diagram (*5*) (Figs. 1 and 2). This statistical display allows detection of direct stimulus effects on the neurons, as well as of synaptic connections between or of common input to the observed neurons (*6*). Such functional relations of the observed neurons can be inferred from bands of altered density which are often observed in the scatter diagrams. These bands result from clustering of points in the scatter diagram. Bands of altered density that are parallel to the coordinate axes represent the direct effects of the stimulus on each of the observed neurons. Bands that are parallel to the 45° diagonal represent either a direct connection between the observed neurons or a common input from a source other than the stimulus. Variations of density *along* a diagonal band suggest that the stimulus is able to modulate the implied pathway (*5*, *7*).

The effects of our stimulus sequence on the firings of two pairs of neurons are shown in Fig. 1. The leftmost scatter diagram of Fig. 1a represents the activity of the first pair of neurons during presentation of the test stimulus alone (condition ii). Two dense bars surrounded by low density are visible near and parallel to each coordinate axis. These represent two bursts of firing of each neuron in response to the stimulus. In the region far from