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*).

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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

each coordinate axis and away from the principal diagonal, there is an approximately uniform density of points. This region represents the non-stimulus-related or spontaneous firings of the two observed neurons. A dense concentration of points is visible along the principal diagonal; the two interruptions of this band correspond to the silent intervals between and after the two stimulus-associated bursts of firing. This band is flanked by two diagonal bands of density lower than the uniform background density; these reflect the refractory properties of the neurons. The dense diagonal band of points shows that there is a high probability for the two observed neurons to fire in close coincidence both during the stimulus-induced activity and during the subsequent spontaneous activity. Such closely spaced firing could be caused either by a synaptic connection between the observed neurons or by a strong input to both neurons from a common source (other than the stimulus). The scatter diagram alone usually does not allow unique choice between these alternatives (*8*). However, since our neurons were observed by means of intracellular electrodes, it was possible to determine that a large common synaptic input was responsible for the nearly coincident firing. Both neurons also fired (presumably independently) at instants when the large common input was not seen.

The second scatter diagram of Fig. 1a represents the activity of the two observed neurons during presentation of the same test stimulus immediately after 4 minutes of paired presentation of test and priming stimuli (condition iv). Stimulus-related firing of both neurons is again visible as two bands of high density near and parallel to the coordinate axes, although the second bands for each neuron have become more diffuse. A dense, interrupted diagonal concentration of points is visible in the period 0 to 2 seconds; this feature of the scatter diagram corresponds to the nearly simultaneous firing of the two neurons during the time that each is influenced by the stimulus and is very similar to that observed in the pre-pairing situation. However, in contrast to the pre-pairing situation, the dense diagonal point concentration is essentially absent during the period 3 to 10 seconds. Thus at times that are far from instants of stimulus presentation, there is now only a much reduced, almost

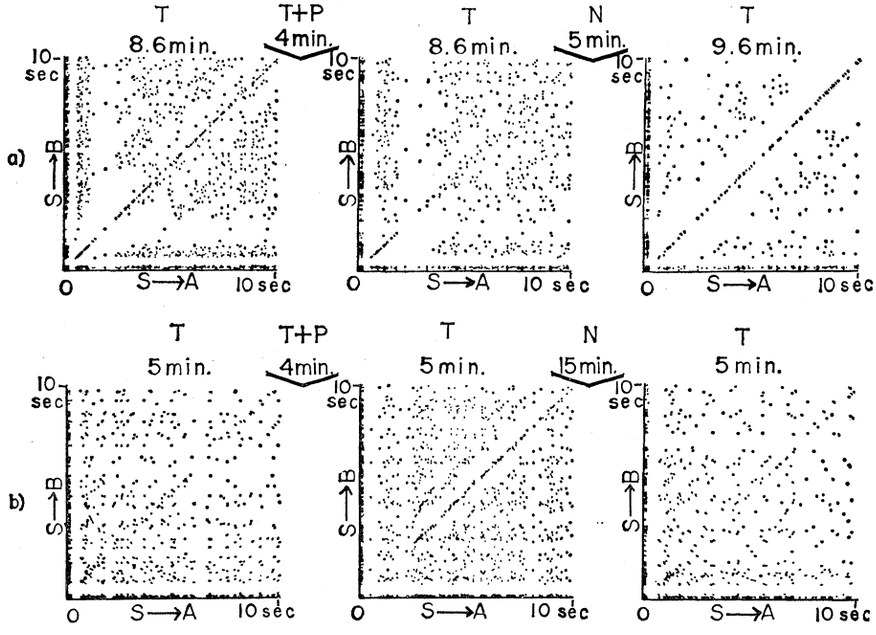

Fig. 1. Joint peri-stimulus-time scatter diagrams for two pairs of neurons. Stimulus conditions and durations of presentation are shown above or between scatter diagrams. T = test stimulus; P = priming stimulus; N = without stimulus. Row (a) shows activity of two neurons for which stimulus pairing produced tonic desynchronization without alteration of phasic synchronization. For each diagram of row (a), the numbers of stimuli, A spikes, and B spikes are: 52, 315, 253; 52, 255, 240; and 58, 234, 203. Row (b) shows activity of two neurons for which stimulus pairing produces tonic synchronization with slight phasic desynchronization. Numbers of stimuli, A spikes, and B spikes: 30, 175, 175; 33, 179, 242; and 26, 144, 145.

chance probability for nearly simultaneous firing of the observed neurons.

The third scatter diagram of Fig. 1a represents the activity of these neurons during later presentation of the same test stimulus after a period of 5 minutes without stimulus presentation (condition vi). Stimulus-related firing of both neurons remains visible as bands of high density near and parallel to the coordinate axes, although the second such band for each neuron has become noticeably diffuse. A dense concentration of points flanked by regions of low density again appears along the diagonal, as in the left scatter diagram. In contrast to the middle scatter diagram, the dense diagonal point concentration is present throughout the period between stimulus instants. Thus, the stimulus sequence applied to the two neurons of Fig. 1a had a desynchronizing effect on their firing only at times remote from stimulus presentation. This effect reverted to the control situation after some time.

The converse situation is depicted for two different neurons in the scatter diagrams of Fig. 1b. All three scatter diagrams show dense bands of points parallel and close to the coordinate axes, corresponding to the direct response of each neuron to the test stimulus. Little, if any, diagonal density is seen in the first scatter diagram, indicating that there is essentially chance probability for nearly simultaneous firing of the two neurons. The second scatter diagram, taken after a period of pairing of the test and priming stimuli, shows a strong diagonal band at times remote from stimulus instants. Thus, after pairing there is a high probability for nearly simultaneous firing except within 2 seconds after the test stimulus presentation. The third scatter diagram corresponds to the activity of these neurons somewhat later after a period without stimulation, and represents a reversion to the properties of the first scatter diagram with essentially chance probability of nearly simultaneous firing.

Similar experiments involving three neurons at a time are shown in Fig. 2. The statistical display was extended by making the scatter diagram in three dimensions (*9*). The origin of each cube is at the center rear, and the three coordinate axes are labeled a, b, c. The edge of the cube represents 10 seconds, the same time scale as used in Fig. 1.

The experiment shown in the left

half of Fig. 2 demonstrates that a stimulus sequence based on a classical conditioning paradigm can reversibly reduce the probability for nearly simultaneous firing of a neuron triplet. The cube pair labeled A represents the activity of three neurons in response to the control presentation of the test stimulus. Bands of increased point density parallel to the three coordinate axes represent the direct effects of the stimulus on the firing of the observed neurons. The lack of symmetry in the scatter diagram shows that the three neurons responded with different temporal patterns. Diagonal bands of increased point density, which are most visible near the ac and ab planes, correspond to the time structure of the probability for nearly simultaneous firing of the corresponding neuron pairs. Finally, a band of increased point density along the principal diagonal of the cube represents the enhanced probability for nearly simultaneous firing of all three neurons.

The cube pair labeled B shows the response of these three neurons to the test stimulus immediately after a period of 20 pairings of the test and priming stimuli. Most of the band structure has faded and the silent period near the origin and near the bc plane has been reduced.

Finally, after 5 minutes without stimulation, the response of the three neurons to the test stimulus is shown in the cube pair C. Most of the features of the point distribution have returned to the pre-pairing situation.

The experiment shown in the right half of Fig. 2 demonstrates conversely that the stimulus sequence can reversibly increase the probability for nearly simultaneous firing of a neuron triplet. Diagonal bands of increased point density occur only in the cubes E, immediately after a period of pairing of priming and test stimuli.

Of the 72 cell pairs in this study that showed alterations in the probability of nearly simultaneous firing during our stimulus sequence, 26 cell pairs were tested for nonspecific effects of the priming stimulus. In half of these cell pairs the priming stimulus alone produced different alterations than had stimulus pairing. Thus, specific effects of stimulus pairing are responsible for at least some of the observations reported here.

The variations of point density along the diagonals of the various scatter diagrams show that the probability of synchronous firing in two or three neurons can itself have a time structure

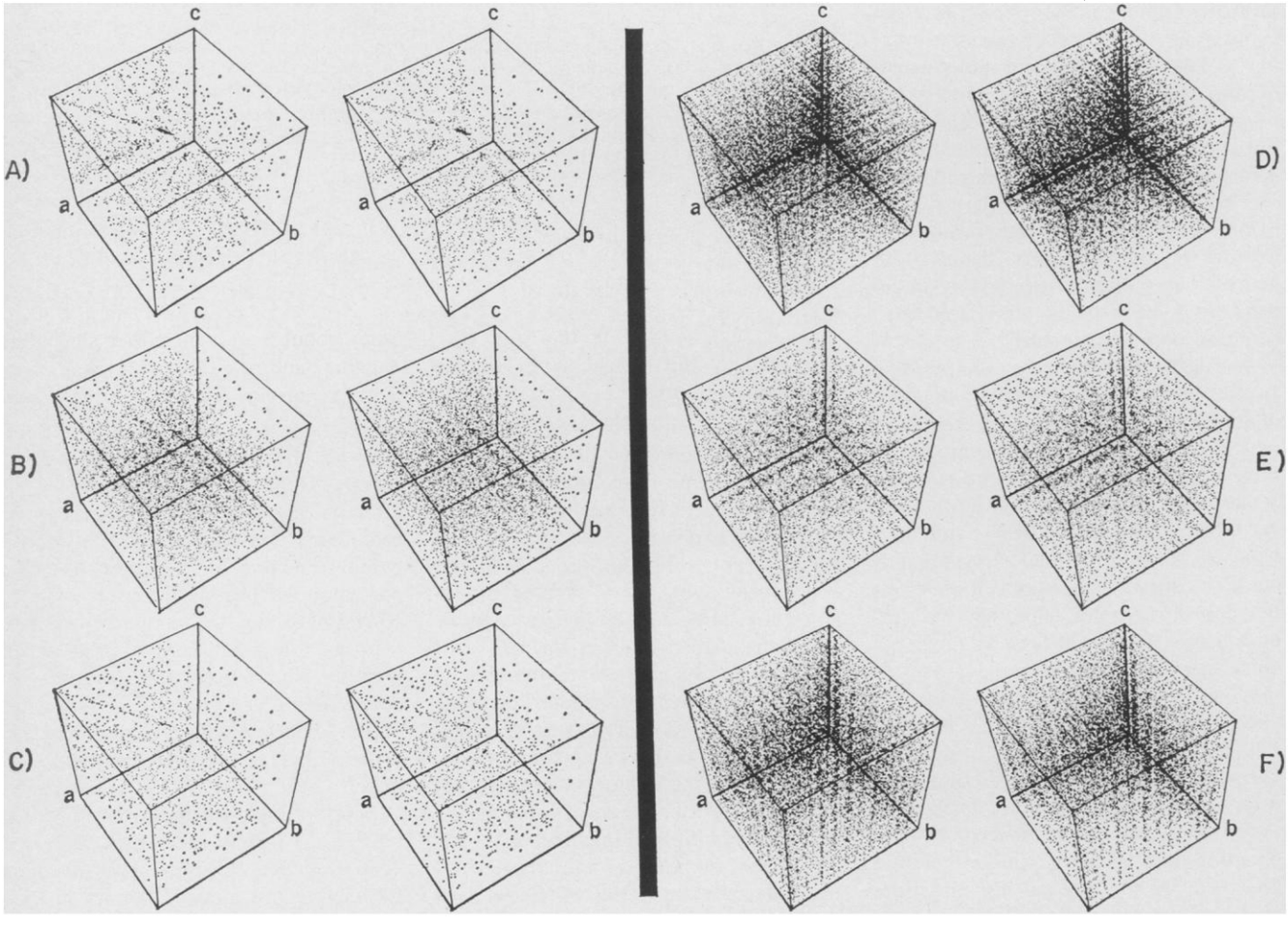

Fig. 2. Joint peri-stimulus-time scatter diagrams for two triplets of neurons. These are stereopairs, and should be merged for optimum viewing. Each scatter diagram is calculated during presentation of test stimulus alone. The test and priming stimuli were paired 20 times between the top and middle scatter diagrams, and a period without any stimulation occurred between the middle and bottom scatter diagrams. The side of each cube represents 10 seconds. Left half represents activity of three neurons that were desynchronized by the stimulus pairing. Numbers of stimuli, A spikes, B spikes, and C spikes: 76, 282, 269, 218; 31, 181, 286, 124; and 41, 168, 165, 148. Right half represents activity of three neurons that were synchronized by the stimulus pairing. Numbers of stimuli, A spikes, B spikes, and C spikes: 32, 329, 310, 273; 39, 278, 253, 192; and 38, 283, 276, 250.

related to the instants of (test) stimulus presentation. We have shown that this time structure can be altered in various ways during presentation of our stimulus sequence. In some cases the alterations of synchronization are not accompanied by alterations of the firing patterns of the individual neurons as measured by the peri-stimulus-time histograms.

It is particularly evident in Fig. 1 that the alterations of the probability of nearly simultaneous firing can be either phasic or tonic with respect to the test stimulus. This suggests that there may be two dissociable sources that produce the nearly simultaneous firings. Although the spike train analysis described so far cannot uniquely determine such sources, we had available intracellular recordings. These showed, in addition to individual synaptic activity, that a large excitatory postsynaptic potential appeared almost simultaneously in all the observed neurons and was responsible for much of the nearly simultaneous firing. Various unsynchronized firings occurred at other times due to unshared excitatory postsynaptic potentials or to intrinsic pacemaker potentials.

Thus, the alterations of probability of nearly coincident firing are caused by alterations in the firing pattern of a source common to all the observed neurons. Under the in vitro conditions of these experiments this yet unidentified interneuron is able to partially synchronize the firings of a population of neurons. Synchronous firing in a neural population and plastic alteration of the time structure of such synchronization are of obvious theoretical interest. However, the detailed connectivity and the behavioral significance of these mechanisms remain to be examined, perhaps by methods used in recent studies of gill withdrawal habituation (*10*).

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9. These three-dimensional scatter diagrams are presented as pairs of stereo images. The left image is intended for the left eye. Images may be merged by looking at a distant object and raising the figure into the line of sight. Alternatively, a viewer (model CF-8 Stereoscope, Abrams Instrument Co., Lansing, Mich.) may be used.
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Lack of Coincidence between Neural and Behavioral Manifestations of Cortical Spreading Depression

Abstract. *The presence of cortical spreading depression is typically inferred from the presence of hypesthesia. The electrocorticogram and slow-potential change were recorded during cortical spreading depression and it was found that hypesthesia remained long after the cortex recovered from neural depression. Hypesthesia, therefore, is an unreliable indicant of cortical spreading depression; if cortical spreading depression is used as a research tool, neural activity must be monitored. These data offer a special problem for memory transfer studies.*

Cortical spreading depression (CSD) has been used to investigate learning phenomena such as interhemispheric transfer of training (*1*). The assumption in such studies is that the CSD produced by the topical application of potassium chloride is a "reversible lesion" or a "functional decortication." Recently it has been demonstrated that CSD should not be considered to be a functional ablation during the entire depression period, since the cortex gives evidence of at least partial recovery of electrical activity from time to time during the course of the treatment (*2*).

Typically, the behavior criterion for CSD is the development of hypesthesia (diminished sensibility) of the limbs contralateral to the depressed hemisphere (*3*). It is assumed that this unilateral hypesthesia is the behavioral manifestation of an underlying unilateral neural CSD. The purpose of this study is to question the assumption that the presence of hypesthesia is necessarily indicative of the presence of CSD.

Six male hooded rats of the Long-Evans strain weighing 250 to 350 g were used in the first experiment. Animals were surgically prepared for CSD and for chronic electrocorticographic (ECoG) recording. Two pairs of cortical electrodes (stainless steel screws) were placed bilaterally in the skull about 5 mm on either side of the saggital suture: one pair was about 2 mm anterior to the coronal suture and the other pair was about 2 mm anterior to the lambdoid suture. A polyethylene cannula [0.070 inch (0.178 cm) inside diameter, 0.110 inch (0.279 cm) outside diameter] was inserted (*4*) over one hemisphere in the anterolateral parietal bone. In three rats the cannula was over the left cortex and in the other three it was over the right cortex. The cannula was filled with sterile 0.9 percent saline whenever the rat was in its home cage. The electrical activity of both hemispheres was recorded differentially between ipsilateral pairs of electrodes. This made it possible to compare the activity of the depressed hemisphere with the activity of the nondepressed hemisphere for the same animal. An animal was considered to exhibit CSD when the ECoG amplitude reduced by at least one-third of the baseline amplitude. This was ascertained by making a calibrated template of the baseline amplitude and continuously measuring