should be tempered with caution. For instance, in the sex attractant pheromones, a far higher degree of stereochemical specificity appears to prevail, and competitive inhibition may occur between close homologs or geometric isomers (*11*). Nevertheless, comprehensive conformational analysis by a fully computerized method is now becoming available (*12*). It is to be expected that further progress will be made into what we have proved to be essentially a stereochemical problem—the specificity of alarm pheromones.

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Unit Activity: Motivation-Dependent Responses from Midbrain Neurons

Abstract. *Single neurons in the midbrain tegmentum of rats showed clear discriminatory responses to three tones announcing either food or water or no reinforcement. Subjects were required to press a single lever and remain motionless for 2 seconds during which time unit activity from several brain sites was recorded. One of the tones, randomly designated, was sounded halfway through this period. Manipulation of drive states revealed that the highest activity was associated with tones announcing the reinforcement for which the animal was most strongly motivated. Thus, from a hungry animal sustained or increased firing rates were elicited by a "food" tone and differentially lowered rates by the other tones.*

The study reported here was directed at understanding neuronal activity changes in the midbrain occurring during states of expectancy. In previous studies of unit responses during anticipatory behavior (*1*), it was found that units recorded from certain areas of the midbrain often exhibited continuous accelerations of firing just prior to the accustomed time of reinforcement, particularly to food rewards. Two main questions were raised: First, were the observed unitary activity changes correlated with the expectant states or with undetected movements and different poses assumed by the subject during waiting for food and water? Second, if expectancies were involved, was it the different reinforcing value between food and water rewards or more specific factors which caused the differences in neuronal firing?

In the present experiment operant and classical conditioning paradigms were combined to decide between these possible interpretations. The aim was to have one standard form of behavior during different anticipatory states. A single, predetermined and highly stabilized motionless operant response was used, and, superimposed upon this response, auditory stimuli were conditioned to induce one of three different expectancies without allowing any change in behavior. In such an experiment there should be no differences in neuronal firing rate based primarily on behavior, but on the organism's anticipatory state.

For this purpose, rats were implanted with four to seven permanently placed microelectrodes made from insulated Nichrome wires (62.5 μ in diameter) bared at the tip. The method of implantation and recording of units by wave-form detectors has been described previously (*1*). Two weeks after surgery rats were selected for preliminary conditioning if there were clearly differentiated unitary spikes observable in recordings from at least three of the four to seven probes. During conditioning the animals were kept in circular plexiglass cages 12 inches in diameter and 11 inches high (30½ by 28 cm). The rats were first trained to press one lever for food and a different lever for water and then to remain relatively motionless for 2 seconds, at the end of which time they received the appropriate reward. During the 2-second waiting periods, movements triggered a movement detector which canceled the trial if the movements were beyond a predetermined level. Animals were kept in this training procedure for 24 hours per day and when they were able to maintain a normal diet, the unitary firing rates during each 2-second waiting period were recorded automatically. On the basis of preliminary unit counts, 12 animals were selected for the test procedure, the criterion of selection being that units recorded from the midbrain probes had to show significantly different rates of firing during responses on the two different levers.

In the test experiment there was only one lever available so that there could be only one behavioral response. Whenever the animal required food or water it had to press the lever and remain motionless for 2 seconds before reinforcement, just as during the initial training. During this 2-second interval, 1 second after its commencement, one of three tones was sounded for 1 second, and followed by reinforcement. The tones were 15, 8, and 1 khertz, respectively. Each tone was correlated with one of the three possible outcomes: food, water, or no reward. The three tones were presented on a random basis but with a predesignated probability ratio to one another. Consequently, the rat could not know at the time of lever-pressing whether the reward would be food, water, or nothing; and the rat could not make any posture correlated with the outcome of the lever response. When the tone was presented the animal's uncertainty was resolved in the sense that the type of reward it would receive was then specified. Any change in posture at this point, however, would have triggered the movement detector and canceled the trial without reinforcement, and the data would not have been accepted for computation. There were, nevertheless, subliminal movements which never involved gross changes in posture or orientation, that could not be eliminated without making the task too difficult for the animal. In some cases, these were detected by a "supersensitive" movement detector and counted so that the time histogram they presented could be compared with that of the unit spikes. Four to five units in different brain areas were recorded simultaneously onto paper tapes during the 2-second waiting period, such that the neuronal firing pattern of each unit could be compared. The shapes of the unit were monitored by a PDP-8 computer operating on-line and plotted by an *X-Y* plotter (*2*). This gave a visual confirmation that the unit we were recording was a single one and the same one from day to day. Average response-time histograms were prepared by the computer from the tapes showing changes in the probability of unitary firing during each successive 50-msec interval of the recording periods 1 second before and during the 1-second presentation.

Animals were tested according to this procedure 24 hours a day for several days. Data tapes were analyzed daily.

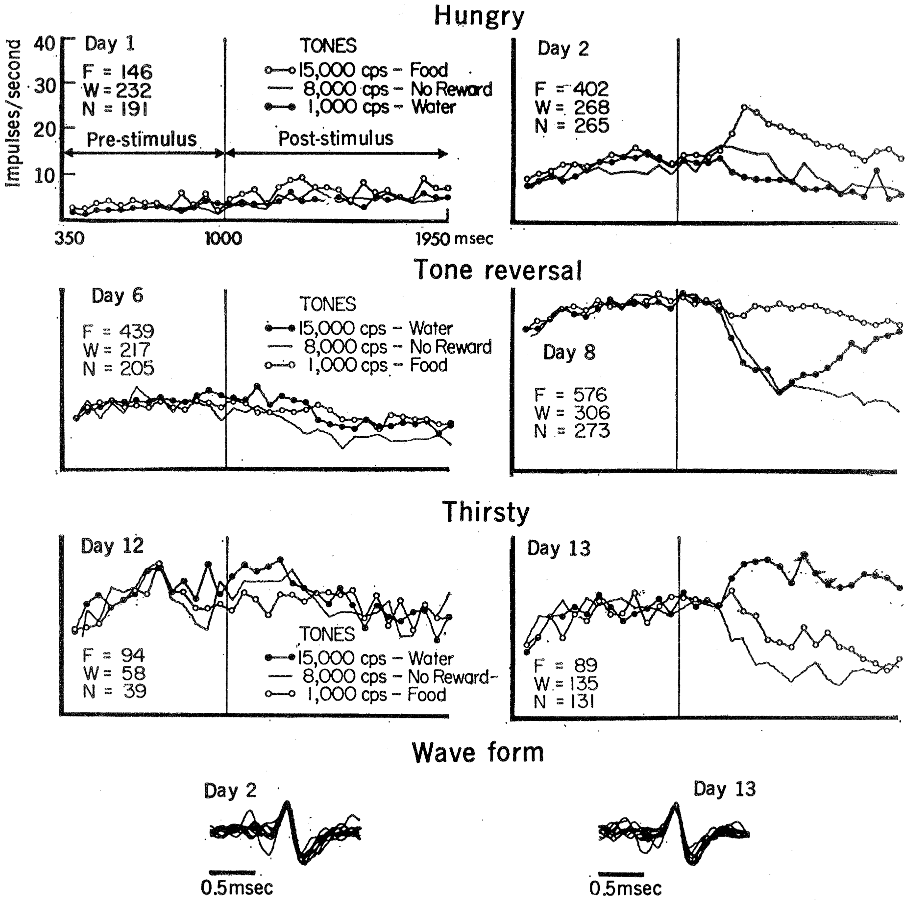

Fig. 1. Representative graphs of the pattern of firing of a neuron in the reticular formation of a rat (5221) during the course of learning and during sensory and drive-state manipulation. Each point on the graphs represents a 50-msec interval, and the ordinate and abscissa for each graph are the same as those shown for day 1. The number of samples taken on each day is also shown. The onset of the conditioned stimulus was 1 second before reinforcement and is indicated by the dividing line in each graph. On day 1, when the rat was hungry (normal schedule), there was little difference in response to the three conditioned stimuli. By day 2 the food tone elicited a high firing rate from the unit. On day 6 the high and low tones were reversed and the previously established discrimination was lost, but by day 8 a higher firing to the new food tone was again elicited. On day 12 the rat was made thirsty and the separations between the response curves disappeared. By day 13 the unit was firing most rapidly in response to the water conditioned stimulus. Below the graphs are examples of the unit wave form monitored on-line throughout the experiment. Each picture consists of ten overlapped sweeps; the full amplitude was approximately 100 μv, and the full duration of each sweep was about 1.5 msec (*3*).

The sites of recording for the group of midbrain neurons were established by histological examination to be in dorsal midbrain tegmentum, in the reticular formation, central gray, and the vicinity of the interstitial nucleus of the posterior commissure. The activity of 22 different midbrain units was recorded from the 12 rats. After 1 day's training some of the units were firing predominantly to one signal. In eight cases this bias was to the water conditioned stimulus and in six cases to the food conditioned stimulus, with no bias apparent in the remaining eight cases. After 3 to 5 days of conditioning, 16 units fired to the food tone an average of 25 percent higher than to the control tone and 5 percent higher to the water tone than to the control tone. The other six units, which were from three animals, showed increments to the water tone. As the subjects' behavior was the same prior to the tone onset for all three tones, the differences could not be ascribed to behavioral changes. The discriminative responses of the units could be based either on disparity in the expectancy states (cognitive-motivational) or on differences of a sensory nature between the quality of the tones.

To rule out the latter possibility a test of "tone reversal" was carried out. Where the food conditioned stimulus had been the high tone and the water conditioned stimulus the low tone, the tones were reversed so that water was associated with the high tone and food with the low tone. The middle tone remained the control conditioned stimulus associated with no reward. All 12 rats underwent this test. A typical effect (Fig. 1, days 6 and 8) was an initial loss of the previously established discriminated responses to the tones, followed by a reestablishment of the differential firing activity. Five of the units showed this relearning to the tones after 24 hours of testing, 11 units required 2 days retraining, one unit 3 days, and four units took 4 to 5 days, with only one unit failing to make the reversal. Units recorded from the auditory pathway (brachium of the inferior colliculus) showed a markedly higher burst of firing to the food tone during the first part of the experiment but after tone reversal this unit still fired rapidly to the onset of the same tone (which was then the water tone), thereby indicating, we assumed, a responsiveness to the stimulus characteristics

(1 khertz in these cases), and not the expectancies. Thus it was surmised that expectancy of reward rather than response to the tones per se accounted for the differing rates of firing in midbrain unit activity.

To find whether it was the motivational aspect of the expectancies, that is, the relative preference for the two rewards, or some factor more specifically linked to food or water, a procedure was used to bias the relative strength of motivation in all animals. For this purpose, animals showing the highest firing rates to food tones were given free food in addition to their food reward, but water was available only by means of the operant response. Ten of the animals were used in this test. After 1 day of this "water-deprivation" the water tone caused an augmentation of firing rates in *all* cases; the average increment was 29.1 percent of the response to the control tone. The food tone still caused an increment in eight of the units recorded, the average increment being 1.5 percent of control. In nine of the cases, however, the increment induced by the water tone was now larger than the increment induced by the food tone. Thirst was accelerated in four of the animals by intraperitoneal injection of 2 ml of $0.1M$ NaCl. Four of the animals were tested for 2 additional days under the same "drive-reversal" conditions; by the 3rd day, the average increment during the water tone amounted to 81.2 percent and that during the food tone was only 2.6 percent above the control tone response. From these data it was concluded that the motivational interest of the animal directed toward the expected goal object very likely determined the firing rate of these midbrain neurons.

When subliminal overt movements were detected and analyzed alongside the midbrain unit firing patterns, there were certain similarities and important differences between the rates of occurrence of these behavioral responses and the neuronal firing rates. Differences between the two sets of patterns, however, revealed that neither set was directly dependent on the other. First, the midbrain unitary firing rate changes appeared with shorter latencies than the behavioral response changes. Second, with respect to each individual instance of stimulus application there were often behaviorally quiet trials during which highly motivational stimuli were applied and accompanied by the large increments in midbrain neuronal firing. Our conclusion, therefore, was that descending influences of the highly motivational expectancies often influenced gross activity, but that the unitary changes in the midbrain which were observed were prior to and probably independent of these behavioral changes.

Besides these midbrain units, 31 other neurons were recorded from different parts of the brain. Of these, 17 were in the hippocampus and dentate gyrus, 9 in the hypothalamus, 2 in the septum, and 3 in the inferior colliculus. The hippocampal units did not regularly exhibit large differences in firing rates favoring the main-reward tone, and when such differences appeared in the early part of the experiment, they did not quickly reappear after tone reversal. In the case of two of the hypothalamic units there was a pattern quite similar to those observed in the cases of the midbrain units, but the other units in the hypothalamic group did not show such differences. One hypothalamic unit appeared to fire only to the water signal, irrespective of the drive state.

The results of these tests lead us to conclude that there are single cells in the midbrain which can make discriminatory responses to the significance of different sensory signals. These responses reflect an integration of sensory input with the internal state, where the response to tones which signified a reward appropriate to the motivational state of the organism was amplified by the degree of that motivation. The data did not indicate at what level the integration of the stimuli input with the drive occurred. It appeared that units in the auditory pathway were at a preintegrated level because they fired to the stimulus rather than to its significance, whereas the units in the midbrain group were apparently at a postintegrative level because they fired as a function of the relevance of the stimulus input to the actual drive. While these data did not point directly to the "integrative area," they depicted pre- and postintegrative levels, and therefore the method should be useful in the search for the actual integrative level.

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3. The data were analyzed as the probability of a unit firing in a given interval. In Fig. 1, the actual firing rate is given in order to make the ordinates readily comprehensible.
4. The experiment was carried out while we were at the Brain Research Laboratory, Department of Psychology, University of Michigan. Supported by grants from PHS. We thank P. J. Best and J. Rosenberg for computer programming and W. Allan, G. Baldrighi, W. Wetzel, and J. Frey for their technical assistance.

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Retention of Delayed-Alternation: Effect of Selective Lesions of Sulcus Principalis

Abstract. *Monkeys with bilateral lesions of the anterior, middle, or posterior thirds of the principal sulcus, of the periarcuate prefrontal region, or of the inferior parietal lobule were tested for retention of spatial delayed-alternation. Lesions limited to the middle third of sulcus principalis resulted in failure to relearn delayed-alternation within 1000 trials; lesions elsewhere had little effect.*

Since the discovery that ablation of the frontal lobes in monkeys results in severe behavioral deficits on delayed-response and delayed-alternation tasks (*1*), many investigations have assessed the minimum lesion that would produce such deficits. Two behavioral studies (*2*), whose purpose was to localize delayed-alternation deficits within the prefrontal cortex, have focused upon dorsal-ventral comparisons. Lesions involving the entire extent of sulcus principalis result in the severest spatial delayed-alternation (DA) impairments in comparison to lesions dorsal or ventral (or both) to this sulcus. Whereas it is evident that principalis is a focal area for DA performance, the possibility of further localization of function along the anterior-posterior axis of the sulcus and prefrontal region has not been explored. In this study monkeys with bilateral lesions of the anterior, middle, or posterior sectors of