cell. The lumen within such a cell is continuous into the protrusion. (ii) Sometimes when the swarmers become attached the cytoplasmic contents of the nonmotile cell are released into the swarmer, thus indicating confluence of the two membranes. Such cells do not survive.

The thick-walled cells are resistant reproductive cells. A thick suspension of them was placed on watch glasses and allowed to dry thoroughly. The watch glasses were stored 4 months in the dark, culture medium was then added, and the preparations were placed in the light. Within 3 to 4 days *Chodatella* cells appeared, indicating, as did the original isolation of the organism from an old mud sample, the presence of a perennating structure. A thick-walled cell may represent a single resistant aplanospore produced by the protoplast of a vegetative cell, but the presence of flagellated cells which become attached to and apparently fused with nonmotile cells surely favors the interpretation that such a thick-walled cell is a zygospore. *Chodatella longiseta* not only reproduces asexually via autospores, but also is capable of oogamous sexual reproduction.

The classification in the Chlorococcales has long been problematical. Fritsch (*2*) questions the validity of maintaining the Coelastraceae separate from the Hydrodictyaceae for the mere lack of zoospore production by the former. Trainor and Burg's (*3*) recent discovery of flagellated isogametes in *Scenedesmus* emphasizes this recognized artificiality of classification in the Chlorococcales. The results of this investigation of a unicellular member of the Chlorococcales presents similar problems. *Chodatella* certainly cannot be retained in the Oocystaceae as defined by Smith (*4*), yet its relationship to the members of other families is not entirely clear. Hopefully, as more of the genera in the family are studied rigorously, the natural affinities within the group will emerge.

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Neuronal Correlates of Behavior in Freely Moving Rats

Abstract. *Firing patterns of single neurons in the hypothalamus, preoptic area, midbrain reticular system, and hippocampus of awake, freely moving female rats were temporally correlated with exploratory sniffing and vibrissa twitching, feeding, lordosis, locomotion, and (or) arousal. These relationships were remarkably stable during continuous observations lasting many hours. During extended periods when certain of these movements were not performed, the correlated neurons showed no action potentials for minutes at a time. Electrical stimulation at certain recording sites elicited behavior patterns whose spontaneous occurrence was accompanied by neuronal activation. Self-stimulation was elicited from sites spontaneously activated during exploratory behavior.*

Evidence for the regional differentiation of functions in the limbic system-midbrain circuit is based mainly upon studies in which intracranial electrical stimulation, production of lesions, and hormone and drug implantation are used (*1*). Differential sensitivity of individual neurons in this region to neurohumors and to hormones and other normally blood-borne substances (*2*) implies that they function in homeostatic or behavioral mechanisms. We have analyzed the firing activity of limbic and midbrain neurons with respect to a wide variety of species-characteristic behavior patterns in an attempt to determine their functional differentiation (*3*). Other studies have identified neuronal correlates of components of limb movements, fighting, food-getting, and attention (*4*).

Electrodes, made of Diamel-insulated nichrome wire, 0.0025 inch (63.5 μ) in tip diameter, were implanted into the lateral preoptic and hypothalamic areas, dorsal hippocampus, and midbrain reticular formation in each of 13 female rats. In six rats, activity was recorded in a total of 16 sites, as early as 1 day after operation (*5*).

Neuronal firing rate was greater in arousal than in slow-wave sleep in 50 to 100 percent of the units studied. The firing rate of some of these units (for example, Fig. 1, *RF*) was roughly proportional to the degree of arousal; it was lowest during slow-wave sleep (Fig. 1A), higher during quiescence (Fig. 1C), and highest upon startle (Fig. 1D). Activity tended to be high in paradoxical sleep (Fig. 1B), consistent with earlier studies (*6*). Electrical stimulation at this site (Fig. 3, 4160 *RF*) evoked moderate self-stimulation, but no feeding or escape behavior. Other neurons showed greater differentiation of function.

In one differentiated pattern, the neurons fired rapidly during any form of locomotion as well as when the rat was startled, when it pulled away while the observer was holding the tail, when it groomed its face, and when its forepaws were held by the observer but not when the rat scratched with its hind legs. The unit fired very infrequently while the rat was standing very still but alert (Fig. 2, *LH*, F–I). Unit firing decreased dramatically when the rat stood still and started eating after approaching and sniffing food (Fig. 2F). This pattern occurred in 9 of 12 units during feeding and in 9 of 19 units during lordosis induced by manual stimulation of the flanks and perineum. Each of these neurons showed a comparable decline in activity when the rats stood still, but they became activated during locomotion. In contrast, the activity of the former "arousal" neurons was persistently high during all these wakeful states.

In a second, more highly differentiated firing pattern, two neurons were inactive except during vibrissa movement, which occurred during exploratory behavior or paradoxical sleep (for example, Fig. 1, *POA*). Eight additional neurons were activated during exploratory behavior (1 POA, 2 LH, 3 RF, and 2 HPC), but these were also active during any type of locomotion. A preoptic neuron whose activity was correlated with vibrissa movement was completely inactive during slow-wave sleep and quiet wakefulness and was not activated by startle (Fig. 1, A, C, D). It thus appeared to be independent of general degree of arousal. It did not fire when the rat lunged at and chewed on or withdrew from odorous substances presented on a cotton swab (Fig. 1, E–G). Chewing artifacts are seen at the end of Fig. 1, E and F; face-wiping movement artifacts are seen three times in Fig. 1G. Thus, the unit was not necessarily activated by odors to which the rat showed clear and opposite behavioral reactions, nor was it activated by nonspecific arousal. How-

ever, when the vibrissae moved during exploratory behavior (Fig. 1, H and I), the unit was activated. The rat had different reactions to the same substances at different times, and the activity of the neuron was related to the behavior rather than to the stimulus. For example, the rat lunged at and ate a piece of meat on a stick without activation of the neuron, but after the meat was finished, the rat sniffed and moved her vibrissae over the same stick and neuronal activation occurred. When an uncontaminated stick was repeatedly presented within several millimeters of the rat's nose, the neuron was activated only when the rat sniffed at it, moving her vibrissae. Other substances (chloroform, xylene, terpinol, vaginal smear, smegma, bread) were also activating but only if the rat sniffed and moved her vibrissae over them. When an aversive response to chloroform occurred, there was no activation; nor was there activation with aversive responses to ammonia, choline chloride, and benzene. Just prior to I in Fig. 1, the rat had been grooming her face, eating, drinking, and sitting quietly, but the unit fired fewer than ten times during the entire 10-minute period (remaining completely silent for minutes at a time); it never fired in correlation with any particular movement. Passive movement of the vibrissae by manual tugging or during spontaneous facial grooming did not activate the neuron. This preoptic area neuron was involved only in active vibrissa movement, and its activation could be predicted not as a result of the stimuli presented to the rat but as a result of the way in which the rat responded to the stimuli. The only other circumstance under which this neuron was active was paradoxical sleep, and then only when the vibrissae were moving. This description is based on more than 24 hours of almost continuous observation, during which the characteristics of this unit were repeatedly confirmed. This site yielded the highest self-stimulation scores of any of the 20 sites tested in five rats. Feeding behavior, but not escape, was elicited from this site. A different preoptic site which was activated during exploratory behavior or locomotion also yielded self-stimulation and feeding, but not escape, when it was stimulated electrically. Thus, the preoptic area may function in the natural transition from exploratory sniffing to feeding.

The most complex neuron observed was in the midbrain reticular formation and seemed to be activated during behavior directed toward objects in the environment. It was thus different from Fig. 1 (*POA*), becoming active not only in exploratory sniffing but during rejection and approach as well. This RF neuron was also different from

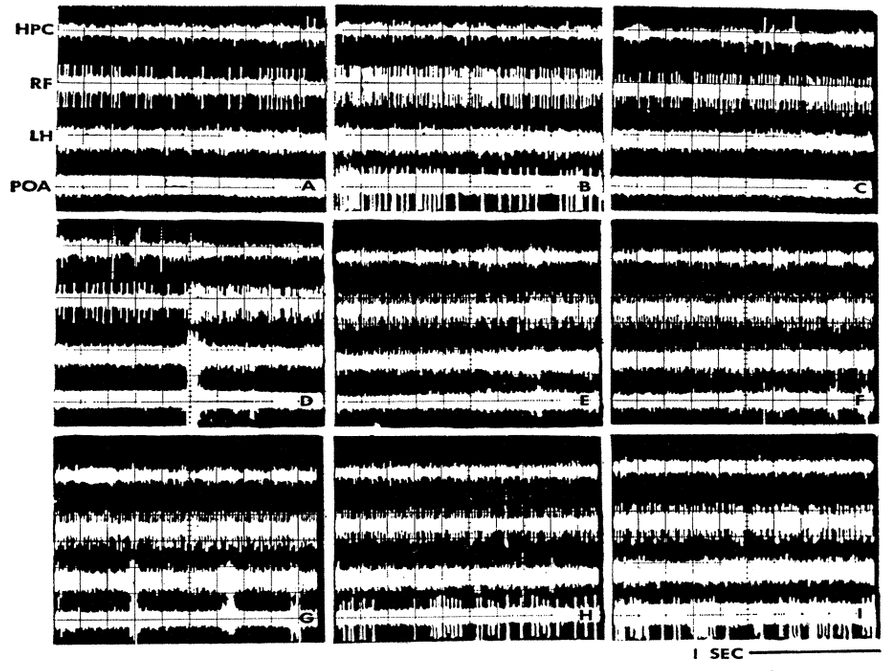

Fig. 1. Rat 4160. (A) Slow-wave sleep; (B) paradoxical sleep; (C) awake, inactive; (D) startle response to puff of air (in center of figure); (E) presentation of rat urine; (F) presentation of acetic acid; (G) presentation of formalin; (H) presentation of rat feces; (I) exploratory sniffing of cage wall. Abbreviations in this and other figures: *HPC*, dorsal hippocampus; *RF*, midbrain reticular formation; *LH*, lateral hypothalamus; *POA*, lateral preoptic area.

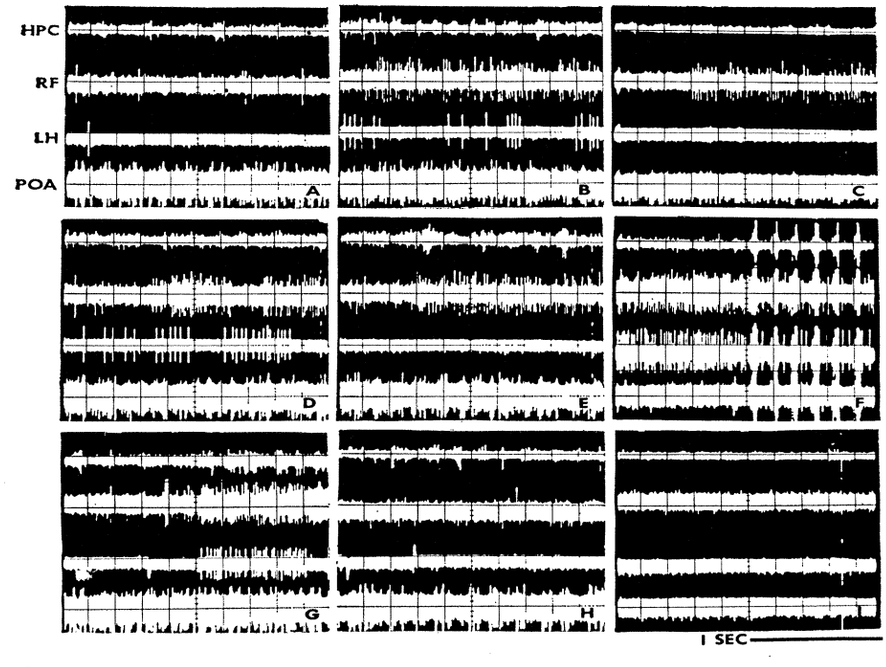

Fig. 2. Rat 4150. (A) Slow-wave sleep; (B) paradoxical sleep; (C) awake, inactive; (D) approach and sniff bare stick; (E) ignoring bare stick; (F) approach and sniff food then (center of figure) eating it; (G) withdraw from benzene (center of figure); (H) lordosis; (I) restraint.

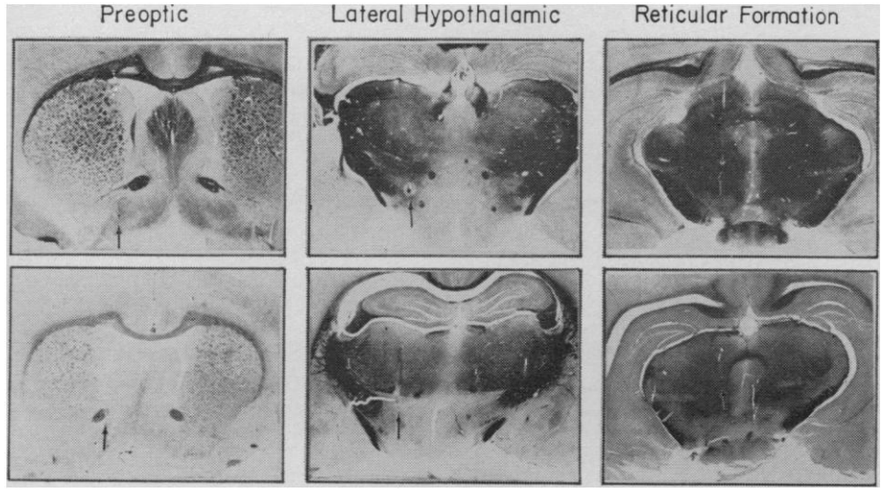

Fig. 3. Locations of electrodes; tip is indicated by arrow. Top row, rat 4160; bottom row, rat 4150.

Fig. 2 (*LH*), for it was active only during certain types of movement. Thus, when the rat was approaching, actively rejecting by pushing away, or investigating a distinct object, the neuron was active. However, when the rat's tail was held and it was pulling away, when the rat rejected food presented to it on a stick by turning its head away, or when it was startled or escaping a strong odor, eating or washing its face, there was no activation. This neuron was also inactive during tooth gnashing, facial grooming, turning the head away from benzene and acetic acid, lordosis, and slow-wave sleep. Electrical stimulation of this site induced an unusual pattern, not of self-stimulating, but of pressing all the available pedals while stimulation was on. This may be considered to be elicitation of "manipulative" behavior, consistent with our finding that this site becomes activated in "directed" behavior.

Neurons in the lateral hypothalamus and preoptic regions thus become activated during exploratory behavior and locomotion, and some neurons in the midbrain reticular formation are related to specific behavioral patterns rather than nonspecific arousal. Consistent with our results are findings that locomotion and exploratory behavior can be elicited by electrical stimulation of the lateral hypothalamus, preoptic area, and midbrain reticular formation (*7, 8*). Although we see clear and reliable correlation between neural activity and behavior, the identification of any neuron's activity with a behavioral movement does not necessarily imply that it initiates the behavior. Indeed, the components of the limbic system involved in related "approach or planned behavior" (*9*), "deliberate movements" (*10*), "attentive, sniffing or searching behavior" (*8*), or "orientation" (*11*) are apparently widespread, complicating the identification of "initiating" neurons. Thus, neural activity underlying a particular behavior pattern could originate elsewhere in the limbic system and only then activate neurons in the path to the motor nerve nuclei. It is also possible that some of these neurons were sensory, since there exist direct projections from olfactory bulbs to lateral preoptic and rostral lateral hypothalamic areas (*12*). What is striking about our findings is the undeniable relationship of these neurons to behavioral movements even though as Nauta (*13*) has pointed out: ". . . there appears to be no fiber system leading from any part of the limbic system-midbrain circuit directly to somatic or visceral motor neurons. It is virtually certain that the transmission of impulses from the circuit to such motor neurons involves relays in lower parts of the brain stem reticular formation."

It is tempting to speculate that physical presence of these neurons in self-stimulation regions (*14*) might result in their activity being reinforced, thus promoting their maintenance in the rats' behavioral repertoire.

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5. The rats were anesthetized with pentobarbital and placed in a stereotaxic instrument; neuronal activity at the electrode tip was monitored as the selected brain region was approached. When activity was encountered near this site, each electrode was permanently fixed to a skull plaque by a stainless-steel screw and dental cement. The connector was fabricated in the laboratory and included a lip which clasped the electrode pedestal and contacts which were spring-loaded against the electrode contacts. Artifacts due to movement of the cable assembly were further reduced by the use of Microdot antistatic cable, in addition to cathode followers and preamplifiers mounted on a counterbalanced arm overhead, approximately 1 foot from the electrodes. The signal was then amplified further and passed into a four-beam Tektronix storage cathode-ray oscilloscope (CRO) and headphone assembly. A slow (2 seconds per trace) CRO sweep speed was usually used to record neuronal activity and EEG from the same electrode, and photographs of the CRO screen were taken with a Polaroid camera. Electrical stimulation tests (parameters, 60 hz and 10 μa root-mean-square in all cases) were performed after unit observations were concluded. In self-stimulation tests, one ¼-second train followed each pedal response; in escape tests, one ¼-second train was applied each second unless the rat pressed the pedal, each response delaying further stimulation for 6 seconds. In assessing self-stimulation and escape, we tested each electrode for 8 minutes six times, and this session was repeated after each other electrode had been tested, one per day. One minute of "shaping" preceded each test; the rats were assisted, if necessary, in depressing the pedal 20 times. In eating and drinking tests, food and water pedals were simultaneously available and stimulation was continuously on 5 minutes, off 5 minutes, for 8 hours on each of 2 days for each electrode. The ratio of food-pedal pressing during *on* to that during *off* periods was the measure of stimulus-induced feeding or drinking. In each of the four types of tests, electrodes were considered positive if they yielded scores more than 3 standard deviations above means previously established with 100 rats in control (no current) self-stimulation and escape tests and 25 rats in control (no current) feeding and drinking tests. The self-stimulation phase of testing was followed by escape, then feeding-drinking tests, each phase taking about 16 days.
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Aldolase and Protease: Unsuspected Structural Homology

Neurath, Walsh, and Winter (*1*) have reviewed the structural homologies between functionally related proteolytic enzymes. Similar sequences were found to be present around one of the functional histidine residues in chymotrypsin, trypsin, elastase, and an α-lytic protease from *Sorangium* sp. (*2*). We now call attention to an unexpected homology between this region of the proteases and a sequence in the Schiff base-forming aldolases. Despite the apparent unrelatedness of the two classes of enzymes, there may be similar roles for histidine residues in the catalytic mechanisms. Histidine appears to participate in the obligatory proton transfer in discharge of the acyl-enzyme intermediate formed in protease catalysis (*3*, *4*); it has also been found essential (although perhaps indirectly) for protonation and discharge of the Schiff base carbanion intermediate formed when aldolase reacts with its substrate (*5*). We therefore examined these proteins for homologous sequences.

The sequences that demonstrate the best homology are shown in Fig. 1. They include the single histidine residue in the α-lytic protease of *Sorangium* (*2*), and a portion of the polypeptide chain in the vicinity of the active site of rabbit liver and rabbit muscle aldolases (*6*). The sequence around the histidine residue in the protease is homologous to those around the "functional" histidine in the mammalian proteases (*1*, *2*); the histidine in the aldolase sequence is eight residues removed from the lysine residue which forms the Schiff base with the substrate.

	1	2	3	4	5	6	7	8
Chymotrypsin:	Val-	Thr-	Ala-	Ala-	His-	Cys-	Gly-	Val
α-Lytic protease:	Val-	Thr-	Ala-	Gly-	His-	Cys-	Gly-	Thr
Aldolase (liver):	Val-	Thr-	Ala-	Gly-	His-	Ala-	Cys-	Thr
Aldolase (muscle):	Val-	Thr-	Pro-	Gly-	His-	Ala-	Cys-	Thr

Fig. 1. Homology of sequences in enzymes.

The degree of correspondence of this section of the protease with liver aldolase (two differences) is the same as that of the protease with chymotrypsin (two differences) and greater (*1*) than that of the protease with trypsin (four differences). The differences at positions 6 and 7 are compatible with mutational changes of one and two nucleotides, respectively (*7*). The apparent homology between the two proteins decreases beyond this region, although relatedness still may be detected if one considers the residues found at corresponding positions in the other proteases (*1*) as alternatives, and evaluates the extent of nucleotide substitution required to account for observed differences.

In addition to the sequences presented in Fig. 1, we have found other regions in the structures of trypsin and chymotrypsin (*8*) which may reflect homologies with elements in the aldolase structure. In order to evaluate the significance of such possible homologies, and to determine whether they represent the result of divergent or convergent evolution, or mere coincidence, it would be desirable to compare the two classes of structures with the aid of a computer program of the type developed for homology detection in other proteins (*9*).

The possibility that the observed homology is a reflection of divergent evolution in which selective pressure has preserved the sequence around the histidine residue might suggest that this residue is essential in aldolase as it is in the proteases. Although the location of the essential histidine residue or residues in aldolase is not yet known, preliminary experiments (*4*) suggest that at least one may be located within ten residues from the active site lysine residue. The mechanism of aldolase action, which shares some elements of base-catalysis and proton transfer with that of the proteases, has been recently reviewed (*10*).

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