lytic C'1a. As stated previously, we found that approximately 10 percent of the ^{14}C was taken up specifically by EA. Since nearly all detectable hemolytically active C'1a was taken up by 20 mg of precipitate as well as by 2 mg of precipitate, it can be concluded that 17 percent of the radiolabel taken up by 20 mg of precipitate was C'1a and that 29 percent of the radiolabel taken up by 2 mg of precipitate was C'1a. Thus, increasing amounts of immune precipitate took up increasing amounts of protein and label in nearly one to one correspondence, while each level of immune precipitate took up more than 99 percent of the hemolytic C'1a, that is the ratio of C'1a to total protein varied while the ratio of protein to radioactivity remained constant (*17*). These results strongly support the assumption that the specific radioactivity of C'1a is similar to that of other proteins in the partially purified preparation of C'1a. An error of 30 percent in the estimate of specific radioactivity of C'1a would be detected by these methods. Therefore, we concluded that less than 1.4 molecules of C'1a is required to generate a single hemolytic site.

Estimates of the hemolytic efficiency of other complement components have been made with ^{125}I-labeled human C'4 and C'3 (*18*). According to these studies, uptake of 15 to 40 C'4 molecules and several hundred C'3 molecules accompanied the generation of a single effective hemolytic site.

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6. SA, site sensitized with antibody.
7. This is 1 : 50 guinea pig serum in 0.01*M* ethylenediaminetetraacetate.
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10. EA, sheep erythrocytes sensitized with Forssman antibody.
11. Schwarz BioResearch lot No. 6602, specific activity 1500 μc/mg.
12. Determined spectrophotometrically. A comparison of absorption at 280 mμ (light path 1 cm) and determination of nitrogen by nesslerization on partially purified C'1a preparations (approximately 60 percent of protein was active C'1a) indicated that 1.0 optical density unit = 1.03 mg of protein.
13. Estimates of the number of molecules of C'1a by hemolytic activity were slightly less when measured at a later time (see Table 1).
14. The diluent used in these experiments was veronal buffered saline sucrose at an ionic strength of 0.065 with Ca^{++} 0.00015*M*, Mg^{++} 0.0001*M* and 0.1 percent gelatin. Antibody supplied in quantity sufficient to generate 5×10^{12} C'1a fixing sites per milliliter.
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16. Solution of 5 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(5-phenoxazolyl)] benzene in 1 liter of toluene.
17. The nature of the radioactive protein other than C'1a taken up by specific immune precipitates is not known. It may represent nonspecific trapping or specific uptake of the protein. At present we cannot distinguish between these possibilities; however, the mechanism of uptake and nature of the protein do not affect the conclusion that the specific radioactivity of C'1a is similar to that of the other proteins in the partially purified C'1a preparations.
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19. We thank Dr. R. A. Lazzarini for his assistance and helpful advice.

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Olfactory Input to the Hypothalamus: Electrophysiological Evidence

Abstract. *Electrical stimulation of the rat's olfactory bulb or lateral olfactory tract elicited unit discharges in the region of the medial forebrain bundle of the lateral hypothalamus, with latencies of 4 to 25 milliseconds. Unit responses in this area were driven by odors in preparations that were paralyzed to prevent breathing artifacts.*

Anatomic reports point strongly to olfactory influences on the hypothalamus through the medial forebrain bundle (*1*); these connections are probably important in some of the behavioral and endocrinologic functions of the hypothalamus. There is good evidence of hypothalamic function in feeding and in sexual behavior, and it is generally believed that olfaction plays a significant role in guiding these behaviors. The role of olfaction in sex is now receiving particular attention. Male rats prefer the odor of estrous females to nonestrous (*2*), and male rats having their olfactory bulbs removed show reduced ability to mate (*3*). Odors have potent effects also on pregnancy and on endocrine functions in mice (*4*). Experimental evidence of olfactory control of feeding is less complete, but it is known that olfaction can be the basis of aversions to foods (*5*).

One electrophysiologic study of the response of hypothalamic cells to many stimuli showed that a significant portion changed their activity when odors were presented (*6*). As a preliminary step in investigation of the importance of the olfactory hypothalamic connections, our study was planned to extend the observation of olfactory responses in the hypothalamus, particularly in the lateral area, and to ascertain the distribution of neurons responsive to olfaction.

In the initial experiments we searched the lateral hypothalamus for responses to electrical stimulation of the olfactory bulb or the lateral olfactory tract in 13 Sprague-Dawley albino rats. The anesthesia was a mixture of 75 mg of chloralose and 1.5 mg of urethane, per kilogram of body weight, dissolved in polyethylene glycol–200. Bipolar electrodes and differential amplification were used for recording. The electrodes were constructed of 60-μ nichrome wire, with the tips separated by about 0.2 mm. Square-wave electrical stimulation of 0.1- to 1.0-msec duration and up to 15 volts was delivered with bipolar electrodes either to the olfactory bulb or to the lateral olfactory tract on the same side as the recording electrode. The olfactory bulb was approached dorsally by thinning the overlying skull with a dental burr and removing the remaining bone and meninges with fine forceps. The bipolar stimulation electrode, bared for about 1 mm back from the tip, was then inserted into the bulb. The lateral olfactory tract was exposed in the same manner from the base of the orbit, and the electrode was placed on the tract, under visual control.

Our search centered mainly on the portion of the lateral hypothalamus from the optic chiasma to the mammillary nuclei; it was bounded medially by the fornix and laterally by the internal capsule. Seventy-five penetrations were made in this region with the bipolar recording electrodes. When a responsive area was found we generally halted penetration of the electrode and made a small lesion by passing current between the two electrode tips to mark the responsive areas for histologic identification. After each experiment the rat was perfused with Ringer solution and 10 percent formalin. Alternate frozen sections 50 μ thick were cut and stained with Weil and cresyl violet.

In the lateral ventral portion of the medial forebrain bundle we often found units or groups of units that responded to the electrical stimulus, with latencies ranging from 4 to about 25 msec. Many units that could be driven by electrical stimulation could also be activated with odors. Stimulation con-

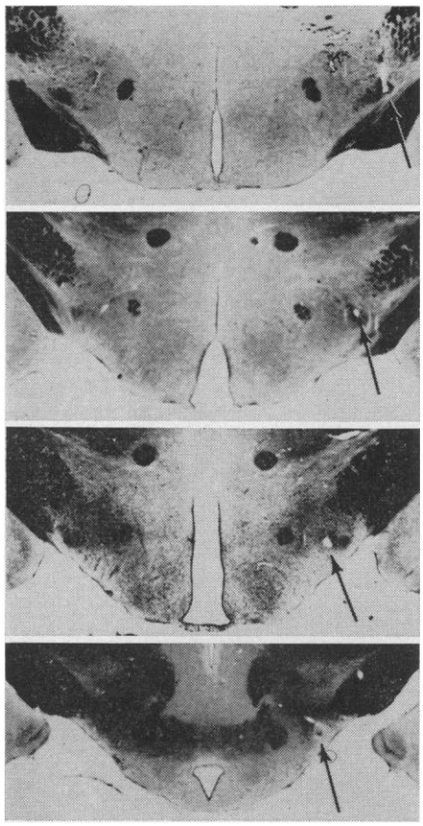

Fig. 1. Weil-stained sections showing sites of short-latency units driven by electrical stimulation of the olfactory system. Each arrow points to the lesion made in a responsive site. The two posterior (bottom) sections show microelectrode recording sites, while the two anterior sections (top) show punctures made with the bipolar electrodes.

sisted of puffs of odor-saturated air from a wash bottle held near the nose of the normally breathing rat. Responses were usually characterized by bursts of activity in synchrony with breathing.

Histologic examination of sections prepared with the Weil stain for myelinated fibers showed that these responses were associated with a specific darkly stained portion of the medial forebrain bundle. This tract is evident where it crosses above the optic tract, but becomes fainter as it passes backward of the ventrolateral portion of the medial forebrain bundle until it is lost in the more darkly stained areas of the posterior hypothalamus, near the mammillary nuclei (Fig. 1). In 35 penetrations in the lateral hypothalamus between the optic chiasma and the mammillary nuclei, no driven spike responses were observed in penetrations that did not pass through the dark bundle, and no responses were seen in the hypothalamus dorsal to this bundle. Of 24 penetrations that were judged from histology to have ended in the bundle, 13 yielded units to stimulation of the olfactory bulb or tract.

In the first phase of this study we noticed that strong odors sometimes caused breathing changes or other movements in the normally breathing rat. The observation that the odor-responsive units could also be driven at latencies as short as 4 msec, by shocks to the olfactory bulb, is a strong indication that these responses were mediated by a relatively short neural pathway, and were not the indirect effect of such factors as movements or changes in blood pressure.

To further rule out the possibility that movements mechanically stimulated the cells as the brain tissue moved relative to the electrodes, several rats in the second set of experiments were paralyzed with Flaxedil (70 mg/kg) in addition to the anesthetic. The anesthetics used were urethane (1.5 g/kg) or sodium pentobarbital (infused intravenously at various rates). Artificial respiration in the paralyzed preparations was provided through a tracheal cannula. A second cannula was inserted into the posterior portion of the nasal passages through the trachea and larynx so that clean air or air mixed with odors could be drawn through the nose. A stream of filtered air was constantly delivered to the rat's nose, and known volumes of odors were injected into this stream of air with a syringe pump. The recordings were made with glass-coated, etched-platinum microelectrodes or NaCl-filled pipette microelectrodes. In many preparations the electroencephalogram (EEG) was simultaneously recorded from screws in the frontal and parietal bones of the skull.

Olfactory responses were recorded with microelectrodes from the ventrolateral dark bundle in 13 preparations under urethane and five under sodium pentobarbital anesthesia; we observed no striking difference in either locus or type of response to olfactory stimuli in this region with different anesthetics. Short-latency responses to electric shocks to the olfactory bulb were recorded from 27 identifiable single units; odor responses were recorded from 15 of these units, ten of these in anesthetized and paralyzed preparations. Figure 2 illustrates single-unit responses to electrical stimulation of the olfactory bulb and to odor.

In most of the paralyzed preparations, other stimuli were tested for ability to arouse these cells. A tail pinch was found the most effective in stimulating other nearby hypothalamic cells, and usually produced a clear EEG arousal. In eight of the nine preparations so tested, odor-responsive cells failed to respond to a strong tail pinch. Responses to odors could be obtained even with very deep pentobarbital anesthesia associated with a flat EEG with occasional barbiturate spikes. These results indicate that the responses of the hypothalamic cells are not the result of changes in arousal produced by odor stimulation.

We have made 12 penetrations into the medial portion of the hypothalamus

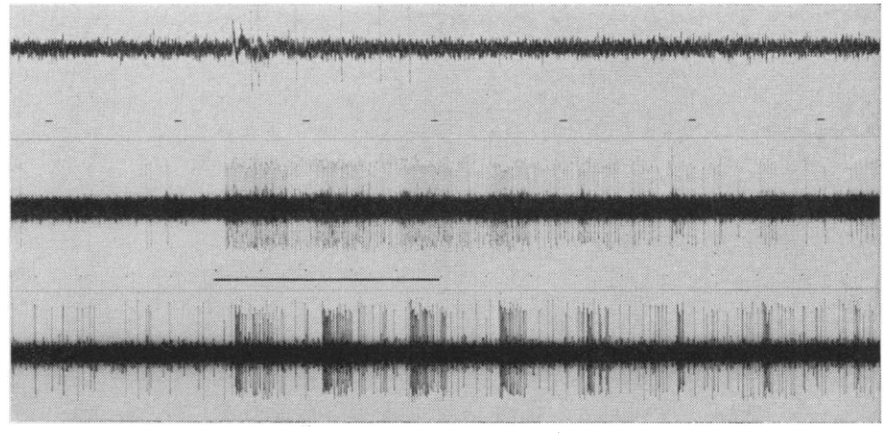

Fig. 2. Single units recorded with microelectrodes in the medial forebrain bundle. The top record is the response of a unit to electrical stimulation of the olfactory bulb; the time marker is a 10-msec bar every 100 msec. The middle record shows the response of the same unit to amyl acetate (an odor like bananas); the heavy line indicates the presentation of odor; the time markers are spaced by 1 second. The bottom record shows the response to amyl acetate of a second unit having higher spontaneous activity and a 4-msec latency to bulb shock. Both units were recorded from rats anesthetized with urethane and paralyzed with Flaxedil.

at the level of the dorsomedial and ventromedial nuclei, but have failed so far to find short-latency responses to stimulation of the olfactory bulb.

Our results confirm prior anatomic indications of an olfactory pathway into the lateral hypothalamus by way of the medial forebrain bundle. The multiunit character of the records with gross electrodes suggests that we were recording from a cluster of cells or fibers responsive to odors, rather than from randomly scattered cells in the area. Since we could usually isolate one or two large spikes with the microelectrodes, we assume that we were often recording from cell bodies observed in our stained sections, lying in or near the bundle of fibers. Medial-forebrain-bundle components are known to arise in the olfactory tubercle, the piriform cortex, and the amygdala, all of which have anatomically demonstrated connections from the olfactory bulb (*1*). The immediate source of the particular bundle that is associated with responses to odors and stimulation of the olfactory bulb remains to be established. The broad range of latencies observed to electrical stimulation may be due to multiple pathways.

Our results are of interest for the study of hypothalamic mechanisms in that they show a strong olfactory input that, at least in the anesthetized rat, is localized in a ventrolateral portion of the medial forebrain bundle. How this sensory input relates to the elicitation of feeding and sexual behavior by hypothalamic stimulation, or to the phenomenon of intracranial self-stimulation, is an intriguing line for future research. The localized character of the system should make it readily amenable for study with small lesions and discrete stimulation.

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Puromycin and Retention in the Goldfish

Abstract. *A first experiment compared the behavior of goldfish injected with puromycin immediately after each of a weekly series of brief discriminative training sessions in the shuttlebox to that of appropriate controls. Discrimination was not prevented, nor was escape from shock impaired, but probability of response to the conditioned stimuli, both positive and negative, was reduced substantially. These results suggest that puromycin interferes with the consolidation of conditioned fear. The null outcome of a second experiment, in which all training was given in a single long session instead of a series of short sessions, suggests (contrary to recent indications) that consolidation begins in the training session. The conditioned-fear hypothesis is supported by the results of a third experiment in which the animals were shocked upon entering a goalbox to which they had previously learned to swim for food; animals injected with puromycin, immediately after the shock, entered the goalbox more readily 1 week later than did appropriate controls.*

Agranoff and his co-workers have found that goldfish injected with puromycin immediately after a brief session of simple avoidance training in the shuttlebox perform less well 3 days later than do control animals injected either with saline immediately after training or with puromycin after some delay (*1*). One may reasonably conclude from these results that the drug somehow interferes with retention, but whether the interference is general or restricted to certain products of shuttlebox training—to fear conditioning, to the selection of the instrumental response, or merely to sensitization—remains to be determined. Although (at higher dosages) the Agranoff effect is large in relative magnitude, it is small in absolute magnitude (because little is learned in a single brief training session) and therefore does not lend itself easily to functional analysis. In our first experiment we used repeated training and injection in an effort to produce a greater effect, and a somewhat more elaborate (discriminative) procedure designed to permit closer inquiry into its cause.

In a set of fully automated shuttleboxes (*2*) we trained 52 goldfish, 7.5 to 10 cm in length (snout to base of caudal peduncle), living in individual tanks, their water being filtered, aerated, and controlled as to temperature; 6 training days were 1 week apart. On each training day, 20 trials were given, with a mean intertrial interval (in darkness) of 1.5 minutes. The conditioned stimulus was light, either amber or green; the interval between conditioned stimulus and unconditioned stimulus was 5 seconds; and the unconditioned stimulus was a train of 0.25-second pulses of low-voltage a-c shock (1.5 seconds between pulses) with a maximum duration of 15 seconds. In each session the lights of the two colors were presented equally often in quasirandom (Gellermann) order. Light of the "positive" color (amber for half the animals and green for the others) was followed by shock after 5 seconds if the animal failed to respond to it; response to the light turned off the light and forestalled the shock (avoidance), while response after the onset of shock turned off both light and shock (escape). Light of the "negative" color (the alternative color in each instance) was terminated either by response or automatically after 5 seconds (if the animal failed to respond within that time). The position of each animal in its shuttlebox was monitored continuously by photocells, and the conditioned stimulus was presented only in the compartment that the animal happened to occupy at the start of any trial. The response required to avoid or to escape the shock was swimming to the opposite compartment.

One group of 16 animals (the O-Puro group) were injected intracranially by Agranoff's technique with 170 μg of puromycin dihydrochloride in 10 μl of saline immediately after training on each day. A control group of 18 animals (the O-Saline group) were injected with saline alone immediately after training, and a second control group of 18 animals (the 24-Puro group) were injected with puromycin 24 hours later.

In Fig. 1 the performance of the three groups is plotted in terms of