hemiparesis were seen in this animal but have not been observed in such severity in any of the 25 chimpanzees so far affected by experimental kuru.

A 5-percent suspension of brain tissue from the affected animal has been inoculated into another chimpanzee intracerebrally and intravenously (0.2 and 0.5 ml, respectively). A portion of the original human-brain biopsy inoculum, stored at −70°C for more than 1 year, has been reinoculated into another chimpanzee by the same routes and in the same quantities as in the original experiment. Brain tissues obtained at autopsy from a second patient with a severe spongiform encephalopathy (also from W.B.M.) and from two patients with diagnoses of Creutzfeldt-Jakob disease with ataxia (from the United States) have been similarly inoculated into three chimpanzees. Several other species of primates, mice, and primary and stable cell-culture systems have been inoculated.

In summary, inoculation of brain biopsy material from a patient having Creutzfeldt-Jakob disease, with severe status spongiosus, into a chimpanzee was followed after 13 months by the appearance of a subacute, progressive, noninflammatory, degenerative brain disease. The clinical course of the disease was not unlike that in the human patient, and the neuropathological findings were remarkably similar. There is no evidence that the disease was either of spontaneous origin or transmitted by contagion from chimpanzees with kuru. We believe that Creutzfeldt-Jakob disease has been experimentally transmitted to the chimpanzee, and that the disease is caused by a transmissible agent.

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6. Patient R.R., an English male aged 59, was admitted under the care of W.B.M. at the Derbyshire Royal Infirmary on 30 May 1966 because of increasing visual disturbance and confusion. Eight weeks earlier he had first noted that objects looked small or distorted. Ophthalmologic examination at that time was normal, but his vision became increasingly disturbed, and he became confused. One week prior to admission a lumbar puncture in another hospital revealed a spinal fluid protein of 80 mg/100 ml. On admission the patient was conscious, would open eyes on command, and occasionally said "Yes." He moved his legs in response to pain. All four extremities were rigid, with arms flexed and legs extended. Irregular myoclonus was present in the right leg. The optic fundi and pupillary reactions were normal. Lumbar puncture was normal except for a spinal fluid protein of 60 mg/100 ml. After admission the patient's condition continued to deteriorate. His lower extremities became flexed. Myoclonus increased markedly, involving all limbs and head, with violent jerks about 75 times per minute. A right frontal cortical brain biopsy was performed 1 month after admission. Histopathological study of the specimen showed marked astrocytic proliferation and hypertrophy, some reduction in the number of neurons, and status spongiosus of the gray matter—findings interpreted as compatible with a diagnosis of subacute spongiform encephalopathy. After biopsy the patient remained in a vegetative state until his death 5 months later. At necropsy the brain weighed 1030 g and showed very severe cortical and cerebellar atrophy. Histologically this atrophic cortex no longer showed true status spongiosus as in the biopsy, but the gray matter throughout the cortex and basal ganglia appeared collapsed to half of its former width. The cerebellum showed extensive loss of granule cells, gliosis, and many fat-containing microglial cells. In the cerebral cortex there were unusual large cells with pale cytoplasm. Mr. R. H. Shephard performed the brain biopsy and Dr. D. L. Stevens the necropsy.
7. Of the NINDB Laboratory of Slow, Latent, and Temperate Virus Infections, Laurel, Md.
8. This male, weighing 10.6 kg, was estimated to be about 3 years old at the time of inoculation. During the following year he appeared well and gained 700 g in weight. Thirteen months after inoculation he became increasingly somnolent and inactive; he developed moderate ataxia of gait and truncal titubation. Bilateral intention tremor appeared in the upper extremities, with incoordination that was much more striking on the right: he was reluctant to use the right upper limb at all; the right leg bore weight poorly. Passive tone in the extremities was normal bilaterally, and the plantar responses were neutral. The animal ignored stimuli presented in the right visual field. Neurological and general physical examinations were otherwise normal; there was no fever. Routine studies of blood and spinal fluid during the course of the illness showed no difference from normal chimpanzees. Somnolence and lethargy gradually increased; intermittent jerking of the extremities was noted during repose. In spite of supplementary feedings and intravenous fluid the animal lost almost 2 kg of body weight. When death appeared imminent 2 months after illness was first noted, the animal was killed. Cinematographic records were filmed frequently throughout the illness; for these we thank Dr. Edward David—as well as for clinical surveillance.
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Chemosensory Input and Taste Discrimination in the Blowfly

Abstract. *Simultaneous recording of behavioral responses and action potentials from single chemoreceptors in the proboscis of intact blowflies revealed that acceptance of a solution can be mediated either by input from one water receptor or from one salt receptor. Rejection of higher concentrations of salt is mediated by the same salt receptor. A difference of three impulses in the first 100 milliseconds of activity can determine whether the fly accepts or rejects the solution.*

Few studies exist in which electrophysiological events in sense organs and consequent behavioral responses of the intact animal are monitored simultaneously. Usually the two studies are conducted independently, not infrequently by different investigators working in different laboratories, and causal relations are inferred ex post facto. The difficulty of establishing a meaningful correlation is no more apparent than in studies of the chemical senses where the interpretation of taste preferences in relation to sensory input remains one of the basic unsolved problems (*1*). Exceptional opportunities for attacking this problem are presented by the blowfly *Phormia regina* Meigen because it is possible to record from individual taste receptors without in any way interfering with behavioral responses by the intact animal to various taste stimuli. This report deals with the response to sodium chloride.

One set of taste organs in the fly consists of aboral labellar hairs, each of which is equipped with five bipolar neurons. Their axons pass to the central nervous system without synapsing (*2*). Of the five receptors four have been demonstrated to be chemoreceptors. One responds to water (*3*), one to certain carbohydrates (*4*), and two to salts (*5*). The specificity of receptors and the fact that only one hair need be stimulated to elicit a complete, coordinated, behavioral response means in fact that it is possible to elicit behavior by stimulating a single receptor cell. Thus, since there is only one water cell in each hair, application of water to a single hair is equivalent to application to one receptor. Similarly, since the water cell is inhibited by certain concentrations of sugar and of salt (*3*), it is possible to stimulate a single sugar receptor or a single salt receptor by choosing the appropriate concentration.

The nature of the behavioral response varies according to the stimulus. A fly deprived of food and water responds to water or to sugar by extending its proboscis and, if permitted, by drinking. It also accepts dilute solutions of sodium chloride. High concentrations of salt result in retraction of the proboscis, if it is already extended, or failure to extend if it is in the resting position. The exact value of the respective concentrations depends upon the degree of water satiation of the fly.

It has always been assumed that a fly that accepts dilute salt solutions does so because of the activity of the water receptor. This assumption was reasonable because, as salt concentration is increased, the water cell is progressively inhibited until the only activity remaining is that of the salt cells (*3, 5*). At intermediate concentrations of salt, therefore, both the water cell and the salt cells may be active. Of the two cells that respond to salt, one, the classical salt cell (*4*), is the most invariant and is most responsive to changes in concentration. It has been assumed that the point in an ascending series of salt concentrations at which the behavior of a fly changes from acceptance to rejection is the point at which the frequency of firing of the water cell falls below some critical level. At this level it is too low to counteract, in the central nervous system, the augmented input from the salt cell. Coordinated electrophysiological and behavioral recordings prove that this is not the case.

Two sets of experiments were conducted — one sequential, the other simultaneous. Although the second set is the critical set, results from the first are described because the superior signal-to-noise ratio facilitates counting the spikes and corroborates the other data.

The initial experiments consisted of observing the behavioral responses of an intact fly that was stimulated with a random series of salt solutions of the following molar concentrations: 0.05, 0.1, 0.2, 0.5, and 1.0. A total of ten flies was used, and eight individual hairs were tested on each. The flies were males, 3 to 4 days old, fed freely from emergence on 0.1*M* sucrose until 24 hours prior to testing. At the time of testing they had been deprived of food and water for 24 hours. Each fly was fastened by the wings to a wax block and placed ventral-side up on the stage of a dissecting microscope. Test solutions were applied, in random order, to one hair by means of capillary pipets (tip-diameter, 100 nm). Intertrial intervals of 3 minutes were maintained in order to allow for sensory disadaptation (*6*) and to permit central excitatory state to decay (*7*). As soon as a series of behavioral tests were completed the fly was decapitated. The head was impaled on an electrode, and the same individual hairs that had been stimulated in the behavioral experiments were stimulated with the same solutions applied, one at a time in random order, in pipets that also served as recording electrodes. The action potentials generated by the receptors of the hair were amplified by a neutralized input capacity amplifier, type NFI (*8*), fed into a d-c amplifier, model TA-2 (*9*), set for 0.16- to 10-khz bandpass, and thence to an oscilloscope for display and photography.

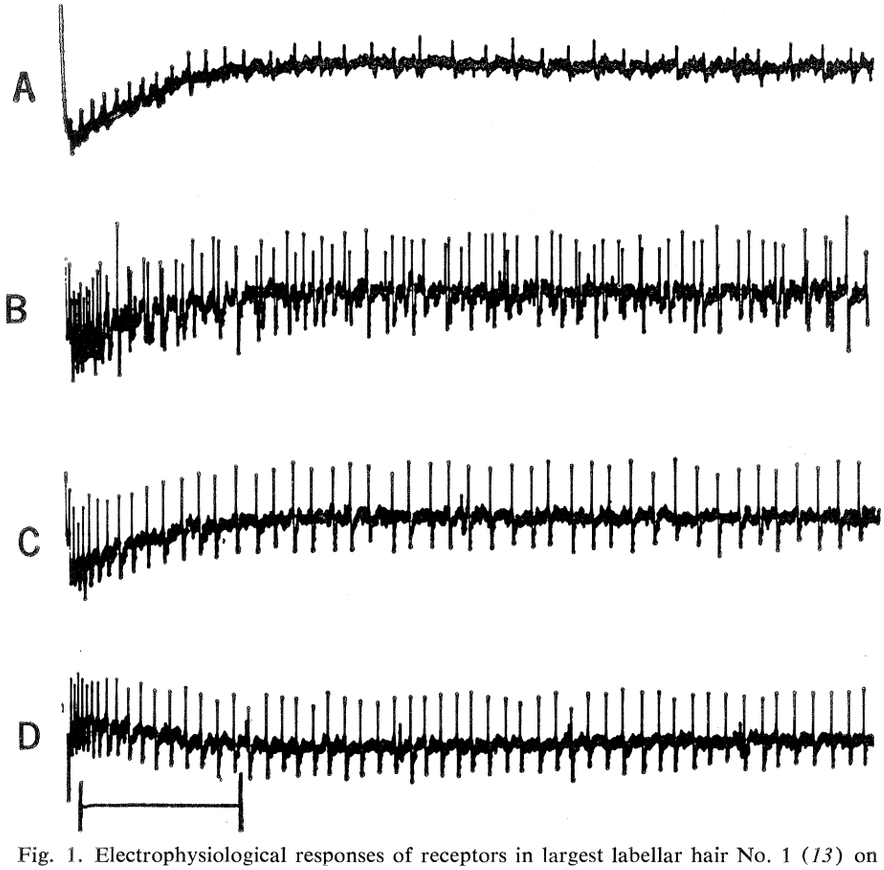

Fig. 1. Electrophysiological responses of receptors in largest labellar hair No. 1 (*13*) on isolated head. All records begin at onset of stimulation. (A) Response of water cell to 0.05*M* NaCl; (B) response of water cell and two salt cells to 0.1*M* NaCl; (C) response of classical salt cell (large spike) to 0.2*M* NaCl; and (D) response of classical salt cell to 0.5*M* NaCl. Solutions in A, B, and C were accepted; solution in D was rejected. Horizontal bar represents 100 msec.

The second set of experiments, designed to provide a direct measure of the relation of input to output, consisted of simultaneous recording of electrophysiological and behavioral events. They were conducted on flies that were attached dorsally to a wax block; ten flies were used. One electrode was inserted into the lateral intersegmental membrane of the last two abdominal segments. The recording electrode was as in the first series of experiments. With these whole mounts both the electrophysiological output of the receptors and the behavioral responses were recorded simultaneously. Behavior was observed as either extension or retraction of the proboscis. Electrophysiologically, its onset was recorded as muscle activity followed by cessation of all activity, when movement of the proboscis caused contact with the recording electrode to be broken. Thus, each record showed which receptors were responding, the number and frequency of action potentials, and the latency of response. The record itself did not indicate whether the response was acceptance or rejection. This was observed visually.

Both sets of experiments agreed in showing that these flies responded to 0.05*M* NaCl, and to all concentrations up to and including 0.2*M*, by extend-

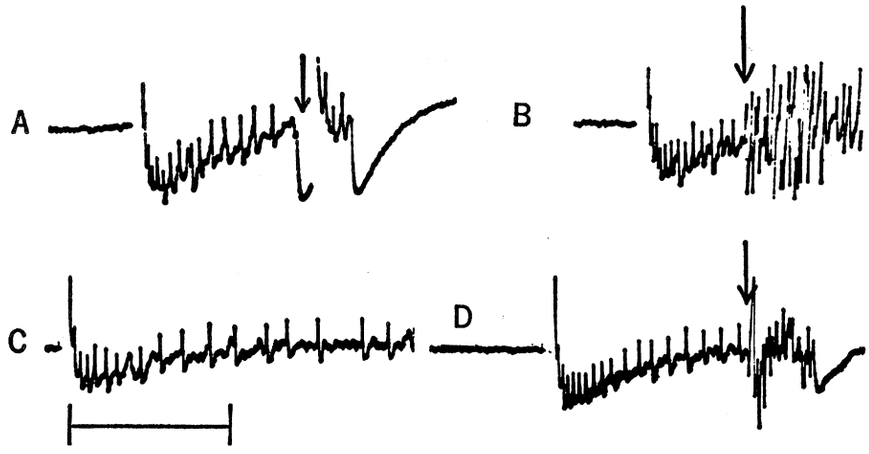

Fig. 2. Electrophysiological responses of receptors in largest labellar hair No. 5 on intact fly. All records begin at onset of stimulation. "On" artifact is followed by action potentials from the salt receptor. Large irregular spikes (beginning at vertical arrow) represent muscle activity of the moving proboscis. (A) Response to 0.2*M* NaCl. After 14 spikes muscles contracted, the proboscis extended, and contact with the electrode was broken. (B) Response to 0.5*M* NaCl with the proboscis in the extended position. The muscle activity after the salt spikes was followed by proboscis retraction. (C) Response of extended proboscis to 0.2*M* NaCl. Salt spikes continued for 5 seconds and no behavioral action occurred. (D) Response of the extended proboscis to 0.5*M* NaCl. The muscle activity was followed by retraction. Horizontal bar represents 100 msec.

ing the proboscis. Concentrations from 0.5 to 1.0*M* caused withdrawal of the proboscis or failure to extend. An examination of record A (Fig. 1A) shows that the only receptor responding to 0.05*M* NaCl was the water cell. At 0.1*M* NaCl the water receptor was responding at a much lower frequency and two salt receptors were active (Fig. 1B); at 0.2*M* NaCl only the salt receptor was firing (Fig. 1C) (after 600 msec of stimulation one other spike appeared); and at 0.5*M* NaCl the salt spike again was the only one firing during the initial 100 msec of stimulation (Fig. 1D). The small spike that appears later originates in another cell that also responds to salt (*5*). It is clear, therefore, that acceptance can be mediated not only by input from the water receptor, as already surmised from behavioral work, but also by the salt receptor. From an electrophysiological point of view the difference between a concentration of salt that is acceptable (Fig. 1C) and one that is unacceptable (Fig. 1D) is solely the different frequency of firing of the salt receptor.

This conclusion is confirmed by records from intact flies. These reveal that the input information utilized by the central nervous system can be contained in the first 100 msec of firing (Fig. 2, A and B). This time agrees with that recorded cinematographically for tarsal sugar receptors (*10*) and for labellar sugar and water receptors measured electrophysiologically (*7*). The difference in frequency that occurs during this period is very small. In record A, Fig. 2, for example, 14 spikes in the first 100 msec (stimulus, 0.2*M* NaCl) resulted in extension of the proboscis (acceptance); 13 spikes in 68 msec (stimulus, 0.5*M* NaCl) resulted in retraction (rejection) (Fig. 2B). This difference is in agreement with that observed in intact animals in which 0.5*M* NaCl in the first 100 msec of stimulation increased the firing of the salt receptor over that produced by 0.2*M* by only three spikes (Fig. 1, C and D). This fact implies that there is in the central nervous system a rate-counter that detects input increases of the order of 20 percent and reacts accordingly, so that one of two outputs is activated which results in either acceptance or rejection. In neurological terms this could mean two neurons of different thresholds connected to the axon of the salt receptor, one being the first step in the network for acceptance; the other, the first for rejection.

Significant rate differences of even lower magnitude have been reported before. In the temperature receptors of drone honey bees a change of 0.5°C causes a change in the frequency of the resting impulse of one impulse per second. The observed behavioral response of worker bees to a change of 0.25°C would, on the basis of the electrophysiological data obtained from drones, be equivalent to a change of 0.5 impulses per second (*11*).

After the mounted flies were permitted to imbibe water and become fully satiated, they no longer responded by extension either to water or salt. A comparison of the input from the water receptor and from the salt receptor during the first 100 msec of stimulation and thereafter revealed that the impulse frequency is not different from that occurring in the thirsty fly. Since the receptors had time to disadapt, the failure to respond to the stimulus represents a central change.

This does not necessarily imply a resetting of the counter. It is possible that at any point neurologically upstream of the rate-counter the switch is blocked. On the other hand, there is evidence that the concentration of the stimulus at which behavior changes from acceptance to rejection is not an absolute one. For flies that were deprived for longer than 24 hours, 0.5*M* NaCl became acceptable and 1.0*M* unacceptable. When these flies were permitted to imbibe some water, but not enough to satiate them, the reversal concentration changed to that already reported. A comparison of the input from the water receptor and from the salt receptor under both sets of conditions revealed no difference in frequency from that already reported. Either the rate-counter has been reset or the units that act upon the information received have had their thresholds altered by feedback from systems known to respond to levels of satiation (*12*).

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