plasma proteins. Keele *et al.* (*1*) demonstrated that such polypeptides were present in blister fluid, in fluid collected from painful joints, and in inflammatory pleural fluid. Ostfeld *et al.* (*2*) demonstrated that a similar substance is present in tissue fluid removed from regions of local tenderness in the scalp during vascular headache of the migraine type. Hilton and Lewis (*3*) described a proteolytic enzyme present in saliva and in saline perfusate of the salivary gland which formed vasodilator polypeptides when incubated with plasma proteins. These authors observed increased amounts of enzyme during heightened metabolic activity of the gland and concluded that the enzyme and polypeptides are responsible for local vasomotor control in the salivary gland. The properties of the polypeptides corresponded to those of "bradykinin," the name given by Rocha é Silva, Beraldo, and Rosenfeld (*4*) to the substance or substances produced on incubation of certain snake venoms or trypsin with serum, plasma, or plasma globulin. These authors reported that when such incubated mixtures were applied to smooth muscle (isolated rat uterus or guinea pig ileum), contractions occurred that were delayed, slow, and sustained.

It therefore was inferred that the specimens which gave type II reactions contained detectable amounts of a proteolytic enzyme capable of acting on plasma proteins (globulin) to form polypeptides of the bradykinin type and that the specimens which gave type I reactions contained detectable amounts not only of the enzyme but of the polypeptides as well.

From these studies it was concluded that increased amounts of "protease" and "vasodilator polypeptides" in the cerebrospinal fluid are associated with: (i) active inflammatory or degenerative disease of the central nervous system, (ii) vascular headache of the migraine type (sustained vasodilation), (iii) prolonged noxious stimulation and pain (sustained excitation within the central nervous system), and (iv) chronic schizophrenic reactions.

Table 1. Results of bioassay.

Condition of patient	Type I	Type II	Negative
Active disease of CNS	8	36	2
Inactive or no disease of CNS	0	2	39
During migraine attack	3	14	1
Seven days after migraine attack	1	0	7
Pelvic or leg surgery (no pain)	0	2	11
Pelvic or leg surgery (sustained pain)	2	9	1
Chronic schizophrenia	4	35	2

A provisional formulation of these findings has been made: During periods of increased or faulty metabolism in the central nervous system, the augmented catabolism results in the accumulation of protease and possibly protein and protein breakdown products in the extracellular and cerebrospinal fluid. Through proteolysis either within or outside minute vessels, this protease forms vasodilator polypeptides that act with other mediators to enhance local blood flow. When appropriate in amount, this response to increased catabolic products serves to meet increased tissue needs by enhancing nutrient supply. When for whatever reason the accumulation of extracellular protease is excessive or there is excessive formation of vasodilator polypeptides, the enhanced capillary permeability and damage to tissue may interfere with the functional capacity of the central nervous system.

It has been shown previously in this laboratory (*5*) that subjects with disease classified as chronic schizophrenia exhibit a fall-off in highest integrative functions in many ways resembling that observed in subjects with serious brain damage. Impairment of highest level brain functions was also observed in subjects with prolonged adaptive difficulties and severe anxiety. The latter subjects, as well as many of those with schizophrenia, were referred to as being in the "unadapted state," a term employed to refer to the totality of reactions (physiological, behavioral, and attitudinal) present in subjects who for long periods have perceived themselves to be severely or overwhelmingly threatened and have been unable to achieve adequate overall adaptation. Prolonged disturbances in the modulation of excitation and inhibition within the central nervous system is inferred to be an aspect of the unadapted state.

It therefore is of special interest that the cerebrospinal fluid of patients with schizophrenia contains an abnormal amount of protease, as also was found in those with active inflammatory and degenerative diseases of the central nervous system and in those in whom a prolonged state of excitation within the central nervous system may be inferred. This observation suggests that a significant (yet perhaps reversible) alteration in metabolism occurs in the brain of patients with schizophrenia. The available evidence does not allow inferences as to whether the accumulation of protease and polypeptides in the cerebrospinal fluid is a manifestation of deranged metabolic function of the brain or is a causative factor in such derangement. However, whether primary or secondary, this accumulation could contribute to impairment of the functions of the brain.

It is suggested that the protease-polypeptide system is implicated in local vasomotor control within the central nervous system and when in excess the components of the system may be relevant to disease.

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29 August 1958

Electric Response of Glia Cells in Cat Brain

Abstract. An experiment is described suggesting that the glia cells in the mammalian cerebral cortex are capable of developing electric responses to direct stimuli. When hyperfine microelectrodes were pushed into the cortex of a cat, slow, reversible potential variations were recorded which resembled the "electric responses" from the glia cells in tissue culture.

In recent years, the technique of recording electric responses of single cells with intracellular microelectrodes has been employed by many physiologists working on the mammalian central nervous system. There has been, however, some ambiguity in this type of investigation with respect to the exact position of the recording microelectrode relative to the single unit recorded, or, in many cases, even with respect to the type of cell impaled by the microelectrode. This ambiguity can be completely eliminated if one uses the material in tissue culture and carries out electrophysiological observations under direct visual control.

In November 1957, we were given an opportunity to travel to the Tissue Culture Laboratory of the University of Texas, Galveston, with necessary electric equipment, and to work in collaboration with W. Hild on in vitro nervous elements taken from the cat cerebrum and cerebellum. It was found in this investigation (*1*), in the first place, that both nerve cells and astrocytic glias give sizable resting potentials on penetration with a microelectrode. In response to direct electric stimulation (with an extracellular electrode), nerve cells produced action potentials of duration of

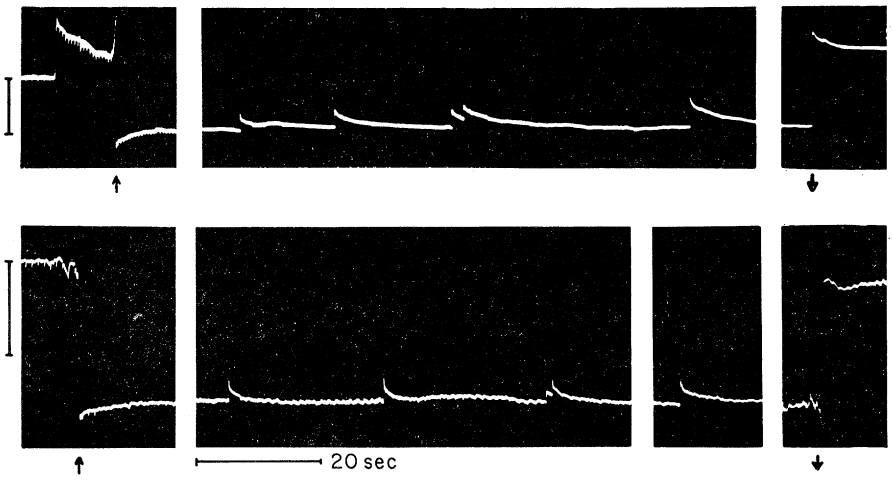

Fig. 1. (Top) Electric responses of an astrocytic glia from the midbrain of a kitten, cultivated in vitro for approximately 1 month. The recording microelectrode was introduced into the glia cell under direct visual observation by means of a phase-contrast microscope, at a magnification of 600. When the microelectrode was pushing the surface membrane of the culture, there was an upward deflection of the oscillograph beam. Penetration of the cell membrane is indicated by the arrow. The voltages applied to the extracellular stimulating electrode of 3.5-megohms resistance were −60 v, +60 v, two shocks at −60 v and +80 v, respectively. Room temperature was 28°C; voltage calibration, 25 mv; time calibration, 20 seconds. [This record was obtained by us in the laboratory of Dr. C. M. Pomerat at the University of Texas, in collaboration with Dr. W. Hild.] (Bottom) Electric responses of a "glial element" in the striate cortex of a cat, recorded with a hyperfine microelectrode. The beginning and the end of the resting potential are indicated by the arrows. The ripples of the base line are due to the pulsatory movement of the cortex. The intensities of stimulating current pulses (applied through a glass tubing of 1.5-mm diameter) were −10 ma, +10 ma, two shocks at −10 ma and +14 ma, respectively. Voltage calibration was 50 mv; time calibration, 20 seconds.

the order of 1 msec (and of amplitude of from 40 to 70 mv). It was found in this connection that the astrocytes in the same culture media produced, on direct stimulation, "electric responses" which had a duration more than 1000 times as long as that of the action potential of the nerve cells. This response of the astrocyte was characterized by a sudden depolarization (up to 40 mv) followed by a slow, roughly exponential return of the recorded potential to the resting level (see Fig. 1, top). The time constant of the exponential potential variation was around 4 seconds at 30°C and was nearly independent, within a certain limit, of the strength of the brief (10 msec or less) stimulating pulse. When two or more stimulating pulses, spaced over a short interval, were applied to the impaled astrocytes, the responses were found to summate. These electrophysiological properties of the glia cell resemble those of a slime mold, *Physarum polycephalum,* investigated previously by Tasaki and Kamiya (*2*). Later on, Chang and Hild (*3*) found that such electric stimulation of the glia evokes a slow mechanical contraction which lasts as long as 7 to 16 minutes.

In the investigation described in this report, an attempt was made to record slow electric responses from the glia cells in vivo. Cats were anesthetized with Nembutal. The skull was opened on both sides to expose the lateral, suprasylvian, and ectosylvian gyri. After removal of the dura, the 3- to 4-mm tip portion of a pair of sharp stainless-steel forceps was pushed diagonally into the cortex; these forceps served to reduce the movement of the cortex and were also used as one of the stimulating electrodes. The other stimulating electrode was a glass tube, approximately 1.5-mm in diameter, filled with Ringer agar gel; it was placed on the surface of the cortex in the region between the two legs of the forceps. The stimulating circuit was completely isolated from ground and was closed for a period of from 5 to 20 msec by means of a mechanical switch operated by an electromagnet. The intensity of stimulating pulses was between 10 and 30 ma. The recording microelectrode was pushed into the region of the cortex where the density of the stimulating current appeared to be maximal. The ground electrode was a large coil of silver wire imbedded in Ringer agar gel; it was placed on the surface of the contralateral cortex. The resistance of the microelectrode was measured, during the course of penetration of the electrode into the cortex, by means of short electric pulses repeated at a rate of 1 or 2 per second.

When a negative direct-current potential of from 40 to 70 mv was recorded with the microelectrode, a pulse of stimulating current was delivered to the cortex. This resulted on many occasions in a sudden small reduction in the observed "resting potential," followed by a gradual repolarization. The time course of this variation in potential was very similar to that observed in the glia cells in tissue culture (see Fig. 1, bottom). The amplitude of the variation in potential was found to vary with the stimulus intensity, within a limit. A reversal of the polarity of the stimulating current reversed the shock artefact (not shown in the figure), but the time course of the slow variation in potential which followed the artefact remained essentially unaffected (the only variation was in amplitude). Two stimulating pulses delivered a short time apart gave rise to a summation of potentials. No slow variation in potential was observed when the recording microelectrode was slowly withdrawn and the resting potential had disappeared.

The experimental findings described above suggest very strongly that the elements in the cortex of the cat which gave rise to slow "electric responses" are actually glia cells, and that these glia cells respond to electric stimuli as the glia cells in tissue culture do. It is probable that this slow electric response of the glia cell is followed by a slow mechanical contraction of the cell. The present experimental findings raise many interesting new problems in the field of brain physiology.

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2 June 1958

Extracranial Responses to Acoustic Clicks in Man

Abstract. Electronic averaging of potentials recorded from the human scalp reveals the presence of small average responses following the presentation of click stimuli. These responses are first detectable near the subject's psychophysical threshold and vary in amplitude with click intensity. It is suggested that the short-latency components of these responses are cortical in origin.

Averaging techniques have recently been used by several investigators to detect extracranial responses to sensory stimuli. These responses are imbedded in