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ABSTRACT

Accelerated alpha particles from the Berkeley heavy-ion linear accelerator were used in a series of experiments designed to elucidate the conditions by which radiation can stimulate or modify nerve action in mammals. Single millisecond pulses in excess of 40,000 rads or pulse trains of less than 1 sec duration elicited the corneal blinking reflex when delivered to the cornea of unanesthetized rabbits. The lowest threshold dose was observed when the Bragg ionization peak was placed at 140 μ depth.

STIMULATION OF THE CORNEAL BLINKING REFLEX BY IONIZING RADIATION*

Cornelius Tobias, Jean Luce, Nicholas Yanni, Tor Brustad,†
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INTRODUCTION

Persons or animals exposed to ordinarily available sources of penetrating radiations usually do not indicate sensory perception of radiation. Nevertheless, it has been known for many years that the retina is very sensitive to x or γ rays;[1,2] an exposure to 1 roentgen or less has been reported to cause an alteration in the absolute threshold to light sensation.[1,3] Animal experiments have shown that moderate radiation exposures sometimes result in alteration in behavior patterns,[4] possibly because x rays cause light sensation, a circumstance already noticed by Axenfeld in 1896.[5] Conditional reflexes are reported to be quite sensitive to radiation.[6] Conard observed the stimulation of motility by radiation on exteriorized intestines of rats, rabbits, and guinea pigs.[7,8] Hug has observed the reactions of snails, sea urchins, and various sea animals to moderate doses of x rays.[9,10] He recorded peristaltic contractions of the body of the leech, <u>Hirudo medicinalis</u>, at low dose rates of 0.75 r/sec following 50 seconds of radiation stimulation. On the other hand, appreciable dose rates of 1000 rad/min of high-energy α particles in our laboratory failed to stimulate frog sciatic nerves (C. Gaffey, unpublished), and lasting alterations of ionic balance in

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†Norsk Hydros Institutt for Kreftforsknign, Norway; ICA Fellow at the University of California, 1958 to 1960.

‡Ophthalmology Dept., University of California Medical School, San Francisco.

peripheral mammalian nerves follows only after irradiation by many thousand rads.[11, 12] Evidently there are very widely varying limits of excitability of nerve or of muscle action. It is possible that high sensitivity to excitation by penetrating radiations occurs mainly in structures sensitive, or sensitized, to visible light, though there is not sufficient information on hand to prove this point. During the past two years workers at Berkeley have initiated a series of experiments designed to elucidate the conditions under which radiation can stimulate or modify nerve action in mammals. It appeared to us that a relationship must exist between radiation intensity, time sequence of exposure, and the physiological state or active response of various neural structures. In this preliminary report we deal with the blinking reflex that usually follows corneal stimulation in rabbits.

EXPERIMENTAL METHOD

The Berkeley heavy-ion linear accelerator (Hilac) was used as a radiation source. This instrument can deliver controlled single or multiple pulses of 40-Mev α-particle radiation ranging from 0.2 msec to 5.0 msec in duration and up to a 10^7-rad dose in a single pulse. Young, adult, unanesthetized rabbits (New Zealand white males) were used. Figure 1 shows the schematic arrangement.

These rabbits normally have a blinking rate of approximately once every 4 minutes. The α-particle beam emerging from the Hilac was focused into an approximately parallel bundle of particles, a few mm in diameter; individual beam pulses were monitored by means of a vacuum "foil chamber." The surface dose was measured with a parallel-plate ionization chamber.[13] Multiple trains of beam pulses could be produced at the rate of 15 pulses per second.

The rabbits were bundled in a specially built straight jacket, preventing gross movements of their bodies, and then were placed in an electrically shielded cage. Usually four fine (5-mil) stainless steel wires, insulated with formvar and shielded, were inserted into the upper eyelid some days prior to the experiment, piercing the orbicularis oculi muscle responsible for closing movements of the eyelid. Electrodes were connected to a socket mounted near the rabbit's head. Electrical impulses received from two of the wirew were amplified and recorded by means of a Sanborn dual-channel

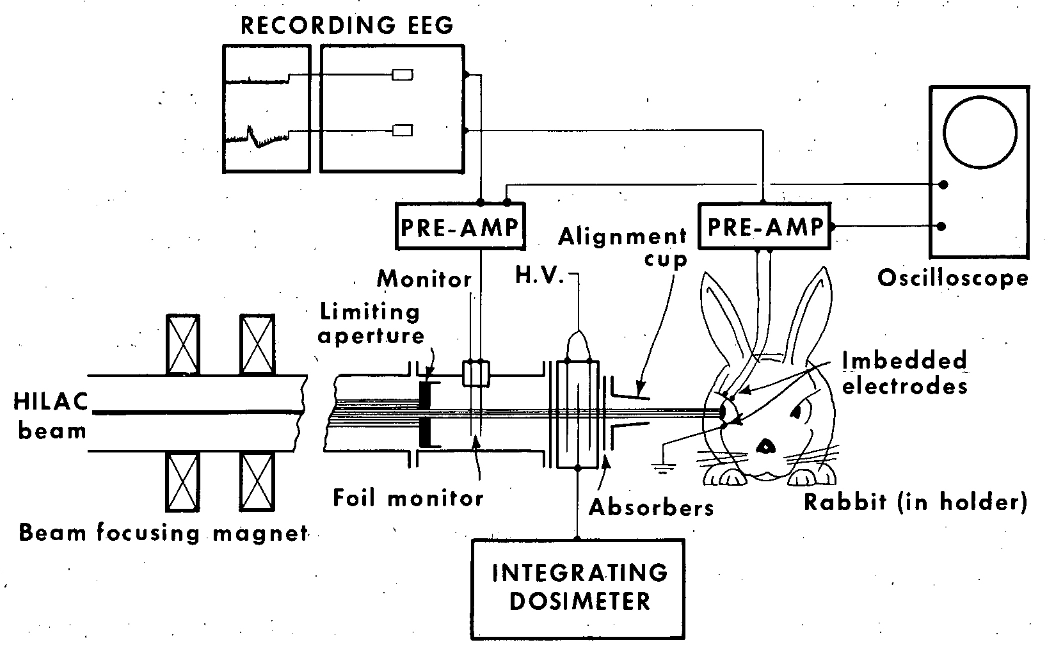

Fig. 1. Schematic of the experimental setup.

electroencephalographic recorder. A record of the beam pulse was recorded simultaneously. At the same time, the information was displayed on an oscilloscope. The animals were under constant observation by means of closed-circuit television; blinking could thus be directly observed.

Suitable aluminum absorbers were placed in the beam to control the depth of penetration into the cornea; the full-energy beam penetrated to about 1300 μ in tissue.

In some experiments, local corneal anesthesia was induced by 4 or 5 drops of 0.5% tetracaine hydrochloride (ophthalmic preparation, Alcon Laboratories); in others, blind rabbits were used whose optic tracts had previously been severed either bilaterally at the optic chiasma or unilaterally anterior to the optic foramen. (We are indebted to Professor E. Marg and Dr. H. Rose for furnishing the blind rabbits.) About forty rabbits were used in this study.

RESULTS

When the dose of α particles corresponded to less than 1000 rad per pulse, even prolonged radiation "stimulation" up to a total dose of 150,000 rads in the cornea in 100 sec or longer failed to elicit blinking or pain response. On the other hand, when the substantia propria received a dose of about 40,000 to 50,000 rads, either in a 2-msec pulse or in several evenly spaced pulses, all delivered within 1 sec, the blinking reflex resulted, observable directly or by means of electrical recording from the orbicular oculi muscle. Typical records of such stimulations are shown in Fig. 2.

When the dose in a single pulse was above the "threshold" for the animal, the blinking reflex followed the corneal radiation pulse by 100 to 300 msec. When the dose was near threshold, delayed blinking sometimes resulted, following the beam pulse by 300 to 10,000 msec (Fig. 3). When the stimulus was more than twice the threshold, or following 10 to 20 stimulations, frequently repeated blinks, shutting of the eyelid for several seconds, and release of secretions from the lachrymal glands followed (Fig. 4).

The origin of blinking reflexes is complex in nature and depends on the nature of the stimulus used. Blinking may result from strong light sensation within the retina because of fluorescent light produced by the beam. Heat absorbed by the iris can have a similar result.[14] Mechanical,

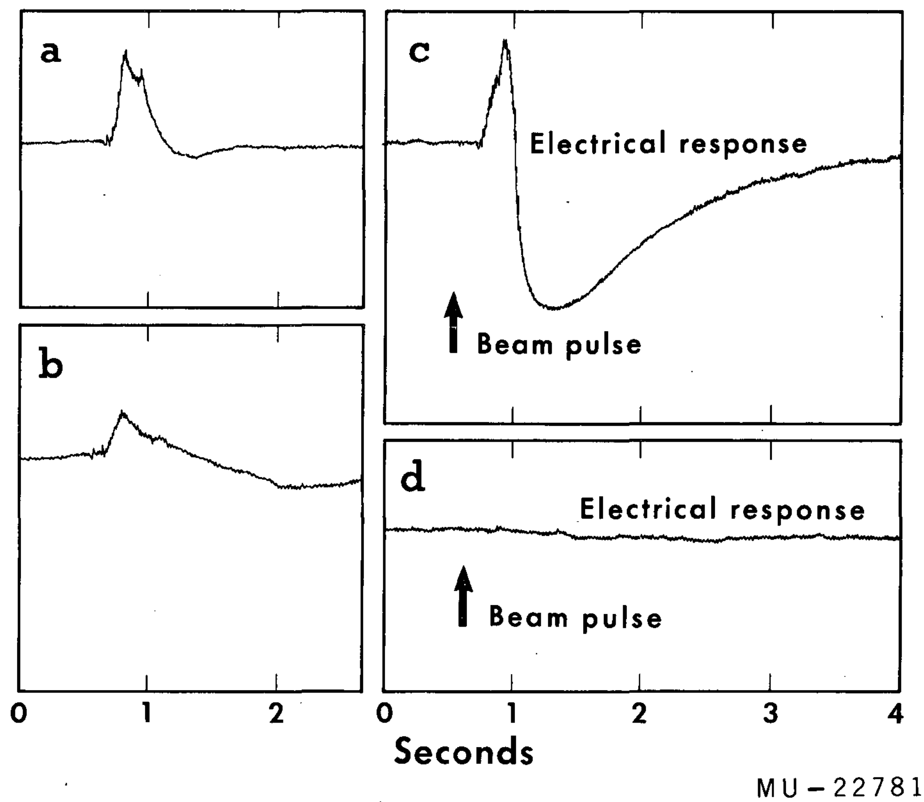

Fig. 2. (a) Response to mechanical stimulation by fine brush. (b) Response to radiant-heat stimulation. (c) Response following 50,000 rads in 2 msec—note delay of about 0.3 sec. (d) No response from subthreshold stimulation of 10,000 rads in 2 msec. (Figures redrawn from original recorder tracings.)

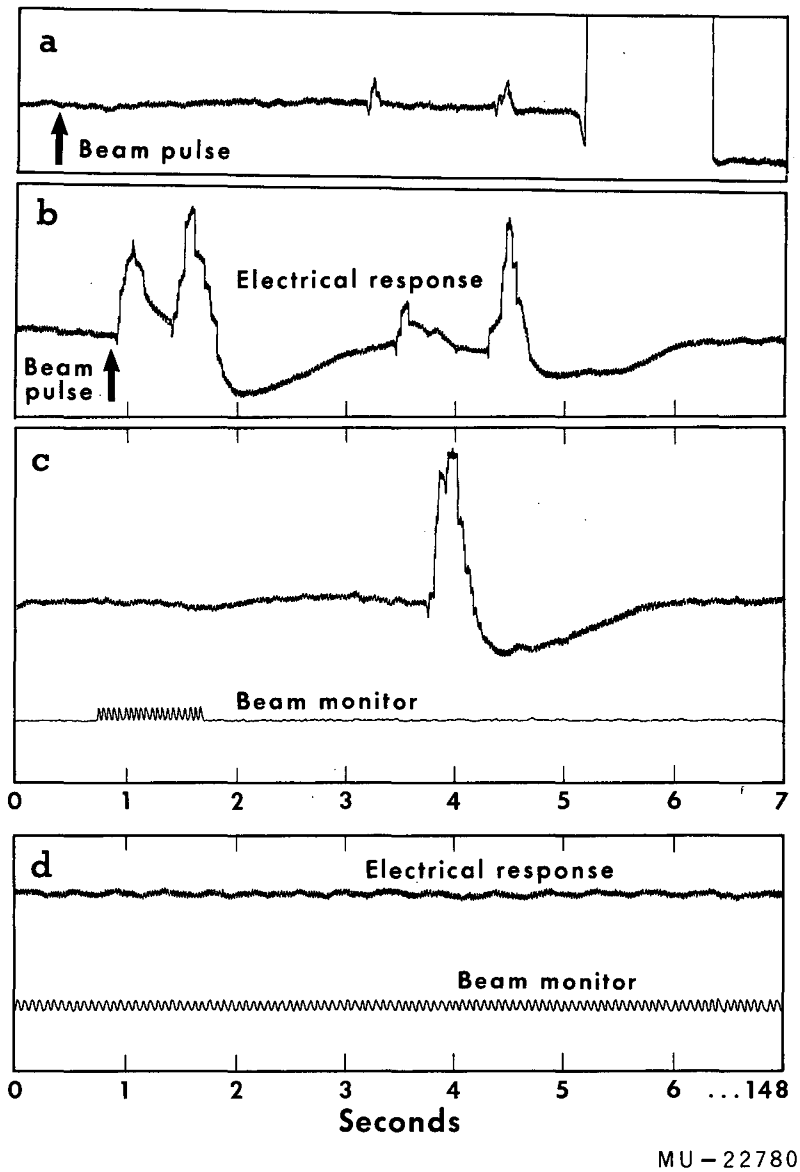

Fig. 3. (a) Delayed blink response following near-threshold dose. (b) Single stimuli sometimes result in multiple blinking. (c) Delayed blinking from 20 stimuli delivered in 1.25 sec–27,000-rads dose. (d) No response following prolonged subthreshold stimulation for 114,000 rads in 148 sec. (Figures redrawn from original recorder tracings.)

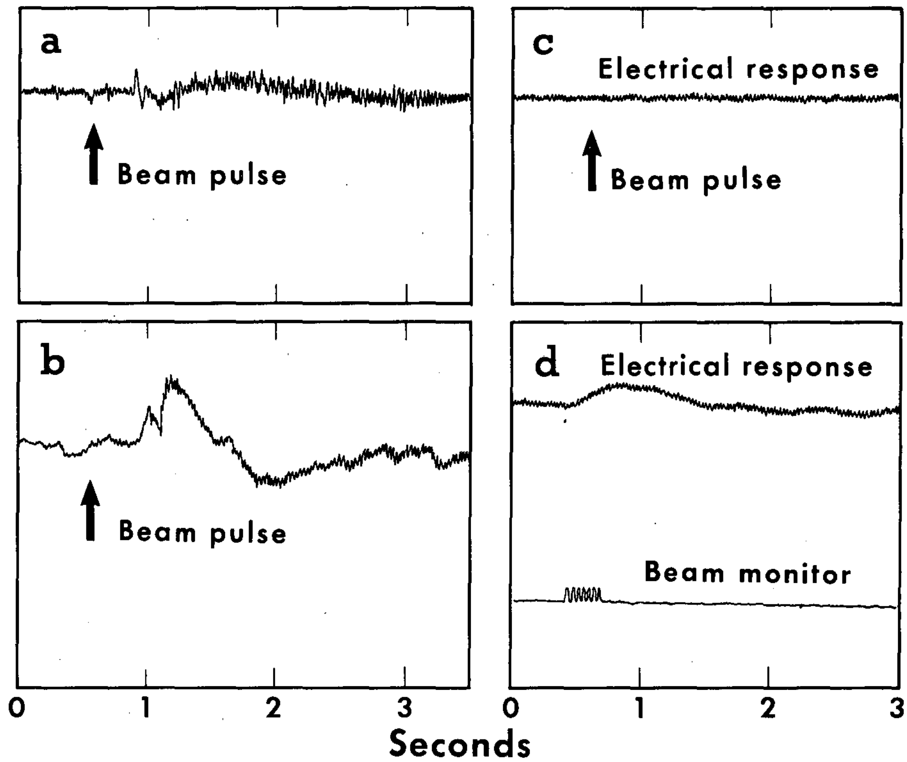

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Fig. 4. (a) Tetanus following single impulse of 120,000 rad (b) Stimulation of corneal blinking reflex in rabbits with severed optic nerve. (c) No response to corneal alpha-particle stimulation 15 minutes following surface anesthesia by tetracaine. (d) No response to the Bragg peak of alpha particles delivered in the top 50 μ of the corneal surface-dose greater than 150,000 rads in less than 0.5 sec. (Figures redrawn from original recorder tracings.)

electrical, heat, and cold stimuli in the cornea usually caused the same effect (Fig. 2 a, b). It seemed worth while to establish the site of origin of the radiation-induced nerve impulses that led to the blinking responses which we observed. Alpha-particle stimulation was then carried out under the following conditions: on normal rabbits; on rabbits under varying light illumination; on rabbits with their optic nerve cut; on rabbits with local corneal anesthesia; and finally, by placing the "Bragg ionization peak" of the beam pulse at various depths under the corneal surface. When the optic nerve was cut so that the animals did not exhibit blinking or contraction of the iris in response to visible light stimulation, the response to corneal α-radiation pulses remained unaffected (Fig. 4b). On the other hand, corneal anesthesia by tetracaine abolished blinking reflex at all radiation doses tested up to 100,000 rads (Fig. 4c); blinking response to visible light, mediated by the retina, remained unaffected.

Figure 5 shows accumulated results of a number of stimulations with α particles. Each stimulation was carried out with the beam penetrating to a different depth. Because of the Bragg ionization peak, the particles deliver up to 4.5 times as much ionization about 30μ before the end of the range as they do at their full energy (10 Mev per nucleon). A "threshold" dose-stimulation curve was constructed, which indicates that the threshold for blinking reflex is lowest if the peak of the Bragg curve is 140μ under the surface of the cornea. This is a strong indication that that part of a fiber which is most sensitive to radiation (possibly the nerve ending) lies at that depth.

The significance of these findings is better illustrated by comparing the rates of blinking of unstimulated normal rabbits with animals stimulated by radiation. The normal blinking rate under environmental conditions is about once in every four minutes, measured in tests on several rabbits. Assuming that blinking in the absence of external stimuli happens at random time intervals, the probability of finding a spontaneous blink in any given one-second interval is 0.0042. On the other hand, in 50 radiation stimulations above "threshold," as in Fig. 5, we found 47 definite responses, a probability of 0.94. The statistical significance of this finding is high.

Since individual rabbits exhibited considerable variation in their threshold sensitivity, and repeated stimulation appeared to lower the threshold, we

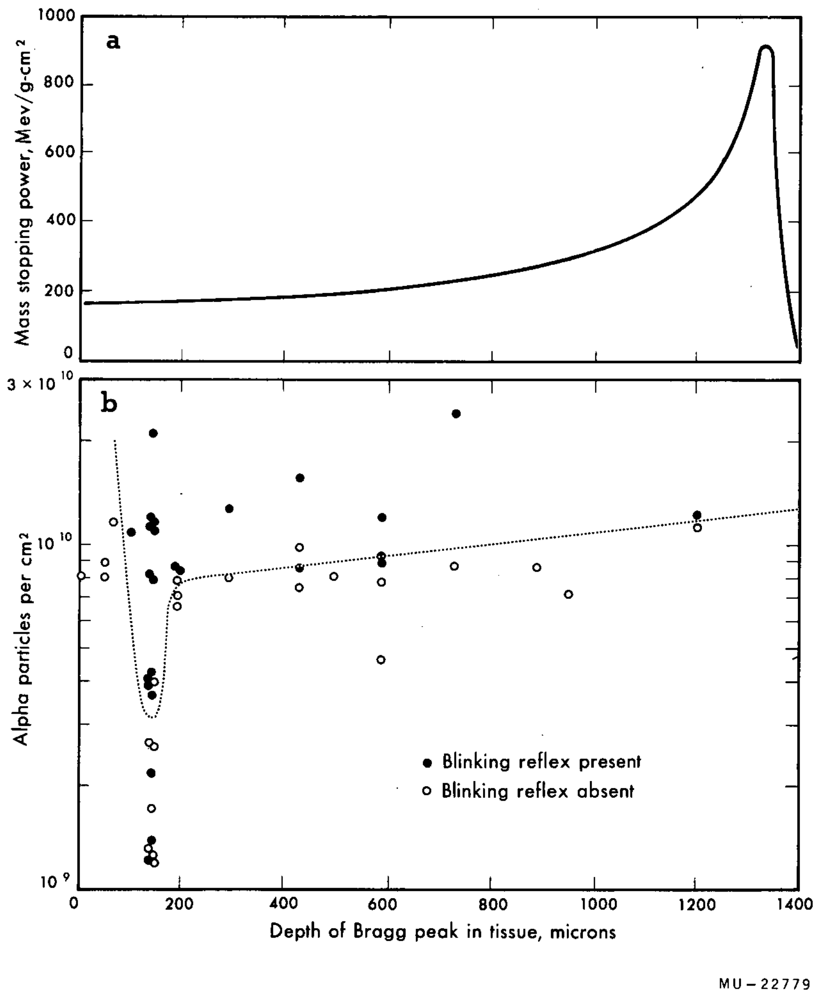

Fig. 5. (a) Bragg ionization curve in tissue-equivalent material of 10.4 ± 0.2 Mev/amu He^4 (calculated from unpublished data, Brustad and Lyman). (b) Presence of blinking reflex ●, and absence of reflex O, with the Bragg peak at different depths in tissue. The dose is given in terms of particles per cm^2 in the beam pulse.

Data from six normal animals were used in the construction of this graph; the dotted line represents a depth-threshold curve fitted to the data.

attempted to influence the corneal response by chemical means. Although this study is only beginning, application of the photodynamic substance Rose bengal, in the form of droplets of 10^{-6} g/cc concentration, appeared to change the character of the electrical response and to lower the threshold by a factor of two.

Each large pulse of radiation had some residual deleterious effect. Ten to twenty strong stimulations, depending on the depth, resulted in stimulation of lachrymal secretions and prolonged closure of the eye. Within a few hours following radiation, disciform keratitis was observed. This is a localized corneal involvement with thickening of the cornea; the lesion appears milky. Eventually, healing takes place. Histopathologically, the corneas appear quite normal (observation by Samuel Kimura).

DISCUSSION

We have demonstrated that intense millisecond pulses of high-energy α particles — 4×10^9 particles cm^2, or 40,000 rads or more — at the proper depth cause reflex closing of the eyelid, the "corneal radiation blinking reflex." It appears that nerve fibers in the upper layers of the substantia propria are responsible for the afferent impulses initiating the reflex.[15] The amount of energy deposited by the Bragg ionization peak over a layer of perhaps 30μ in depth is about 0.1 g cal/cm^3 — sufficient to raise the local temperature, during the pulse, by about 0.1°C.

Lele, and Weddell[16] have shown that radiant or conducted heat is capable of eliciting sensation in the human cornea, and that sensations of heat, cold, and pain can be distinguished. The threshold temperature difference appeared to be +1.5 or -1.0°C when stimulus was applied to the corneal surface for some seconds. Later, they demonstrated afferent nerve impulses in <u>in vitro</u> preparations of the ciliary nerves of the cat eye, when the preparations were stimulated with similar heat intensity.[17] The thermal stimulus applied was usually a temperature difference of several °C, and the time lag for afferent impulses varied inversely with the strength of the stimulus. According to Dawson, when the cornea of the cat is stimulated <u>in vivo</u> by radiant or contact heat, a temperature rise ΔT must occur prior to the observation of afferent impulses in the ciliary nerves. This temperature rise varied with the threshold temperature difference, ranging between 0.058 °C and 7.47 °C.[14] The greatest sensitivity to heat is obtained when the time rate of warming, dT/dt, is slow. For example, when $dT/dt = 0.1\,^{o}C/sec$, $\Delta T = 0.4\,^{o}C$; when $dT/dt = 0.8\,^{o}C/sec$, $\Delta T = 3\,^{o}C$.

In the experiments reported herein, the energy was delivered in 2-msec pulses, producing dT/dt of about 20 °C/sec. In our experiments more total energy was needed to stimulate the corneal reflex when the dose rate was low than when it was high. The three sets of experiments are not strictly comparable; however, qualitatively the present findings are in closer agreement with those of Lele and Weddell[16, 17] than with those of Dawson.[14] If comparison is made for specific rates of temperature rise, then the effectiveness of ionizing radiation appears to be 4 to 20 times that of heat.

It seems desirable to plan specific studies, using the same animal and similar techniques of measurement, to clarify the relationship of particle stimulation of the cornea to heat and cold stimulation. Experiments using radiant or contact heat are complicated by the fact that several heat-sensitive structures actually warm simultaneously, whereas the depth and localization of stimulation can be varied at will when α-particle beams are used. This method of stimulation should be useful in studies of Frey's specific-receptor theory.

There is still considerable disagreement as to the nature of nerve endings in the cornea; however, there seem to be no encapsulated forms of nerve endings.[16] The majority of the nerves terminate in the basal layer of the epithelium in fine branches; nerves also penetrate the substantia propria. The finding that the depth most sensitive to radiation lies about 140 μ below the surface of the eye, near the uppermost layers of the substantia propria, signifies the need for further histological studies. These are necessary to establish the nature of the neural mechanism responding to α-particle stimulation.

Obviously, there is a very large range of threshold sensitivities among various types of cells. The availability of intense ion beams from accelerators opens up a new field of study of the central nervous system. It now appears possible to deliver messages to the brain by means of spatially and temporally coded radiation-pulse signals instead of via sensory organs or need stimulation.

In two preliminary experiments carried out by this group, the exposed surface of the brain of anesthetized rats was scanned by a beam of pulsed 40-Mev α particles. At locations in the vicinity of the motor centers, definite and reproducible stimulation of movement of front and hind limbs, the opposite side of the face, and of individual whiskers could be elicited for some rats, but we failed to do so in others. Work is in progress to enable us to control localization more accurately. As particles with deeper penetration become available, it should become possible to carry out such experiments with increasing refinement. One form of a possible device for this purpose is shown in Fig. 6, where a deflection system is used to "scan" a limited region of the brain surface. Time modulation of pulsing can be achieved, and spatial and depth modulation is achieved by a suitable "mask" absorber in the beam. Near the surface of the brain considerable resolution can be achieved, because the area of a single-pulse bundle of particles need not be greater than a few μ^2. At greater depth, because of scattering and straggling, the definition of the particle beams is largely lost. Accelerated heavy particles of proper kinetic energy can reach any part of the brain without the need for surgery.[18] Of course, much stimulation can be applied to nerves and muscles generally. Since the beam can be split into more than one branch, simultaneous stimulation of neighboring regions of the brain may be used as a tool to study facilitation and occlusion.

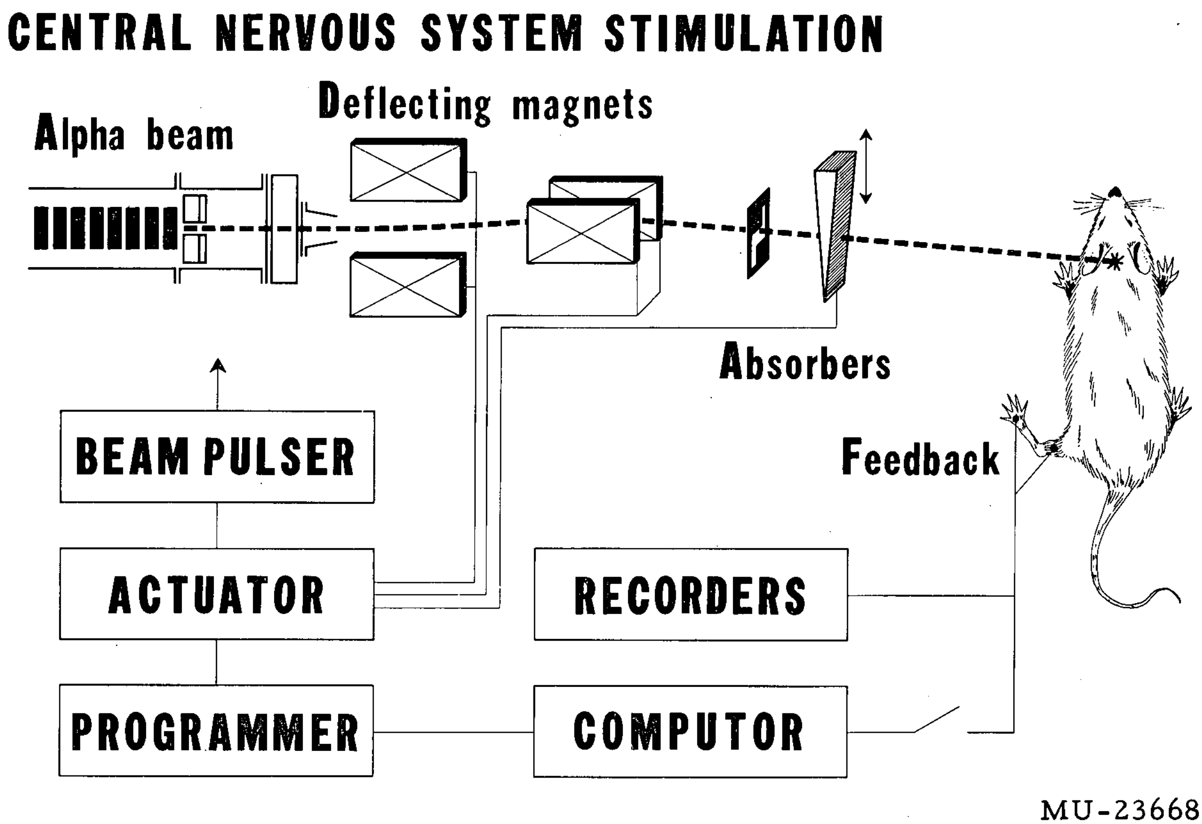

Fig. 6. Schematic concept of future uses of heavy-ion pulses for conveying "coded" messages to the brain. The coding may occur by (a) deflection of beam in predetermined sequence, e. g., scanning; (b) passage of beam through absorbers of predetermined profile to position the Bragg ionization peak where stimulation occurs; (c) coding time sequence and intensity of pulses of beam; (d) using "feedback" information from periphery to change coded messages.

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