maximum benefit in fruit size. These characteristics of NPA would appear to justify further work with this chemical as a thinning agent for peaches and perhaps other fruits.

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Brief, Noninjurious Electric Waveform for Stimulation of the Brain

John C. Lilly, John R. Hughes, Ellsworth C. Alvord, Jr., Thelma W. Galkin

National Institute of Mental Health and *National Institute of Neurological Diseases and Blindness, National Institutes of Health, U.S. Public Health Service, Bethesda, Maryland*

Long-term electric stimulation of the unanesthetized brain in animals and in man is becoming increasingly important in the fields of neurophysiology (*1*), psychology (*2*), and psychiatry (*3*). Despite indications that unidirectional pulses are injurious (*4*) their use in the afore-mentioned fields is quite extensive. In order to obtain reproducible results, injury to nerve cells by the passage of current must be avoided (*5*). We have found that one type of stimulating waveform does not injure the brain.

The processes that produce lesions by the passage of unidirectional pulses are apparently similar to those that produce lesions with small direct currents (*6*). The probable mechanisms of production of such lesions are those involving the displacement by the ionic current of charged particles (ions, enzymes, proteins, and so forth) from their key positions within and around the neurons. Presumably these displacements and the subsequent cellular destruction can be avoided by using sufficiently brief currents which pass an amount of electricity first in one direction and then an equal amount in the other within a short time. Conversely, any alternating waveform that has an additional small net flow in one direction will presumably cause net displacements and hence injury. If such a "zero net flow" waveform is brief enough, simple metallic nonreversible electrodes pass the current through the tissue without distortion of the waveform, and in addition the stimulus artifact is minimized.

With these points in mind, one of us in 1950 devised a waveform whose shape does not change with repetition frequency; it also has a net zero current and an invariant interval between the positive and negative peaks with change of frequency (Fig. 1). Despite the zero net flow and the brief time course of the pulse-pairs, it was found that such currents stimulate sensorimotor cortex and the responses to this stimulus are similar to those seen with rectangular pulses (*4*). These pulse-pairs probably are within the "constant coulomb threshold" region for nerve processes and cell bodies (*7*), and hence their absolute shape should not be critical.

The pulse-pairs (Fig. 1) are generated by quasi-differentiating (*8*) a rectangular pulse and amplifying the resultant with a Williamson type a-c amplifier that has a 100 kcy pass-band. The output of this amplifier is matched to the animal-electrode circuit by means of a high-frequency transformer (UTC-LS 63). The voltage drop across a 100-ohm resistor in series with the animal circuit is amplified and measured on a cathode-ray oscilloscope that has a pass-band of 0 to 10 Mcy (Tektronix type 535). This latter measurement is converted into current by calculation. By graphical integration on an enlarged photograph of these pulses, it has been shown that the quantity of electricity in the positive (upward) pulse is equal to that in the negative (downward) pulse within the experimental error of less than 0.4 percent. For a 10 ma peak current value, the quantity of electricity is about 0.2 μcoul per pulse.

In order to conduct these pulse-pairs through the cortex, an array of 25 or 36 electrodes is implanted over the sensorimotor cortex in a monkey with or without removal of the dura (*9*). This array is surrounded by a stainless steel ring screwed into the bone. The stimulating current is passed into the cortex from a given single electrode to the ring.

In our experiments, we have investigated the motor responses to these stimuli near the threshold (*4*). Pulse-pairs are used to stimulate the cortex on a schedule of a 2-sec train at 60 pulse-pairs per second every 30 sec for several hours a day.

A few results from observations on two unanesthetized monkeys (*Macaca mulatta*) are presented: the

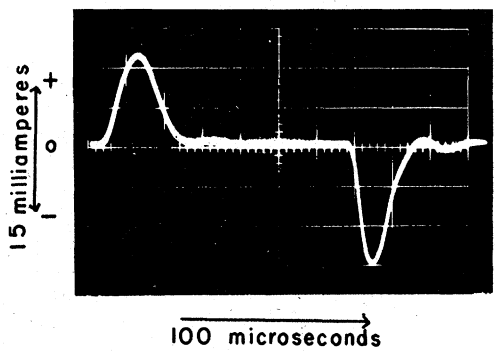

Fig. 1. Waveform of stimulating current: pulse-pairs of current resulting from quasi-differentiation of a rectangular pulse. Measured at 2 percent of the peak, the duration of the positive pulse (upward) is 34 μsec, and the duration of the negative (downward) is 28 μsec. The areas under the two pulses are equal; therefore, the net coulomb flow is zero for the pulse-pair algebraically summed during a time interval of 200 μsec from the beginning of the positive pulse.

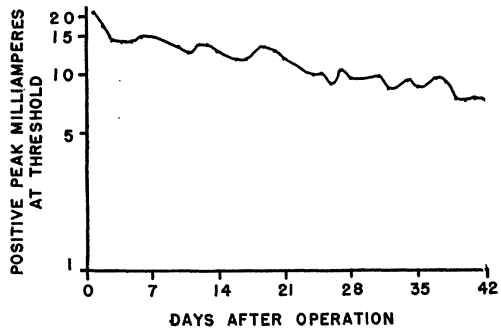

Fig. 2. Variation of threshold with time. The stimulus frequency is 60 pulse-pairs per second; the train duration is 2 sec; and the threshold is the positive peak current necessary to elicit a visible movement. The total quantity of the electricity for the positive pulse summed for the whole train is 18 μcoul. Time is given as days after operation. For the subsequent 9 wk, the threshold remained constant at the value shown at 42 days.

first monkey was observed for 7 wk; at the time of writing, the second monkey had been observed for 17 wk.

Figure 2 shows the slow fall of the threshold current (about 50 percent in the first month) for the pulse-pairs at 60 per second for a typical electrode during a period of 6 wk. Some of this decrease in threshold is probably due to recovery from anesthesia and operative trauma, and some to a slow decrease in distance between the electrode array and the cortex. In the earlier work with rectangular pulses, it was found that a relatively rapid increase in threshold takes place in a few hours (*4*).

Electrocorticograms taken from the electrodes showed no signs of injury. Neurological and behavioral examinations postoperatively showed only some minimal transient signs of injury. Slight flexor weakness in the contralateral hand was seen, but this weakness disappeared gradually during a period of 3 wk. We attribute this to mechanical operative trauma.

Some of the neurohistological studies (frozen sections) have been completed on the first monkey. Under the area of the trephined opening in the skull, there is a moderate proliferation of astrocytes and a slight meningitis. Minute amounts of lipid are present in a few macrophages in the leptomeningeal exudate but not elsewhere. The nerve cells are intact and have approximately the same appearance and population density as on the opposite, nonoperated hemisphere. Whether this degree of change is greater than that due to operative trauma alone remains to be determined.

From these results, it is concluded that this form of electric current does not detectably injure cellular function or structure when it is passed through the cortex near threshold values 4 to 5 hr per day for 5 to 15 wk. Other similarly balanced brief waveforms would probably give similar results. Further details of these observations and their interpretation are in preparation.

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Use of Haploid and Diploid Embryos of *Habrobracon* in the Study of Cell Poisons

A. M. Clark and W. C. Beiser, Jr.

Department of Biological Sciences, University of Delaware, Newark and *Brookhaven National Laboratory, Upton, New York*

Various genetic and cytological tests have been used in the evaluation of the injurious effects of x-rays on cell nuclei. These have included the study of radiation-induced mutations and chromosome breaks. Additional means of evaluating substances that affect cell nuclei are needed. One such test for chemical agents is reported here and is based upon the fact that the radiosensitivity of cells and organisms may be modified by their degree of ploidy (*1, 2*).

Studies on the parasitic wasp, *Habrobracon juglandis* (Ashmead), have shown that haploid embryos are more resistant to x-rays than diploid embryos when the embryos are treated during the cleavage stages of development (*2*). Similar results have been obtained with the nitrogen mustard, methyl bis(beta-chloroethyl)amine hydrochloride (*3*). Since both x-rays and this nitrogen mustard compound are known to affect cell nuclei and also to be more injurious to diploid than to haploid embryos of *Habrobracon,* various other chemicals were tested (*4*). The fact that some chemicals were found to be more toxic to diploid embryos than to haploid embryos, whereas other chemicals were not, is of interest and points to the possibility that the criterion of greater toxicity to diploid than to haploid embryos might be of some use in the evaluation of cell poisons.

In *Habrobracon,* haploids develop from unfertilized eggs and diploids from fertilized eggs. Haploid cultures are obtained from unmated females, whereas cultures containing both haploid and diploid eggs ("mixed cultures") are obtained from mated females. Usually about 65 percent of the eggs from mated females are fertilized and, accordingly, are diploids. In material used in the present report, all haploids developed into males and all diploids into females. Eggs stored in the uterine sacs of the females remain