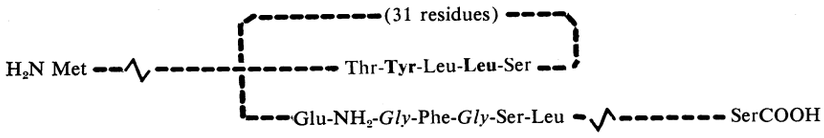

Fig. 2. Possible relationship between different regions of the folded wild-type A-protein molecule suggested by second-site reversion analysis. The amino acids changed in primary mutational events are shown in italics, while those changed by second-site reversion are shown in boldface type. (Abbreviations as in Fig. 1.)

er interest is the finding that, in mutant A-187, second-site reversion also occurs and results in a change in an amino acid situated 36 residues from the position of the original change (Fig. 1). The residue concerned, leucine, is two residues away from the tyrosine residue replaced in the second-site revertant of mutant A-46, revertant A-46 PR8. This leucine residue is replaced by arginine in the A-187 revertant, strain A-187 SPR1. Thus, mutational changes affecting amino acids situated two residues apart in the protein are reversed by second-site changes which are also two residues apart. This finding is diagrammatically represented in Fig. 2 in which a possible relationship between the respective regions of the folded wild-type A-protein is depicted. Whether or not the two regions are actually close together in the folded protein, it is clear that there is some functionally important relationship between the two regions of the A-protein. If structural relationships of this type continue to appear, the mutational approach may be of considerable help in the elucidation of those tertiary structure relationships which are of significance in determining enzyme activity *in vivo*.

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Reaction Time to Cortical Stimulation

Abstract. *A monkey was trained to release a lever when a stimulus was applied to the striate cortex. Reaction times to stimulation of the striate cortex were consistently 30 milliseconds shorter than reaction times to visual stimulation. The technique appears to be fruitful for analysis of neural mechanisms in a simple behavioral task.*

In a typical reaction-time experiment subjects are instructed to depress a key when a preliminary stimulus appears and to release the key at the onset of a second stimulus. The time between the preliminary stimulus and the second stimulus is termed the foreperiod. The interval between the appearance of the second stimulus and the response is termed the reaction time. Such experiments have yielded data which purportedly bear on problems extending from peripheral nerve conduction (*1*) to the underlying physiology and biophysics of perception (*2*). In most of these investigations, however, humans have been employed as subjects; thus neural processes could not be directly observed. Clearly, many of the conclusions from such studies might be established more firmly if they could be carried out with animal subjects.

In 1961 Stebbins and Lanson (*3*) described a technique for measuring reaction time in rats. We have used a modification of this technique for primates (*4*), to study reaction time to cortical stimulation.

In an adult monkey (*Macaca nemestrina*), bipolar platinium-iridium electrodes were implanted under direct observation in the occipital lobe at a point roughly 6 mm posterior to the lunate sulcus. This region lies near the central visual fields as determined by studies of photically evoked potentials (*5, 6*). The electrode assembly was a modification of that described by Doty *et al.* (*7*). The uninsulated tips (about 1 mm^2 in surface area) of each pair of electrodes were separated by approximately 1.5 mm. Electrodes terminated in a miniature Winchester plug. The intracranial stimulus was generated by a well-isolated constant current stimulator, with a continuously variable 60-cy/sec output of from 1 to 800 μamp (root mean square, rms).

Although we were concerned about the possibility of dural rather than cortical excitation, several factors tended to minimize this problem. (i) The electrodes were inserted 1 mm below the pial surface, which made excitation of dural receptors unlikely. (ii) Threshold current was quite small, approximately 50 μamp (rms) for the right hemisphere and 85 μamp for the left. (iii) High-amplitude differential recordings of evoked potentials could be made by using peripheral flashes to evoke bilateral responses and unilateral electrical stimulation to evoke interhemispheric responses. (iv) With current intensities over 15 times threshold and durations of up to 5 seconds, no behavior suggesting discomfort was observed.

During training and testing the subject was restrained in a primate chair in a darkened, sound-insulated room. The subject was trained initially to press a telegraph key following the onset of a 1000-cy/sec tone (ready signal) and to release the key at the onset of a light of approximately 215 lumen/m^2 which appeared after a variable foreperiod (0 to 5 seconds). Later the light stimulus was replaced by cortical stimulation (700 μamp). Releases of the key in the presence of both the tone and light (or tone and cortical stimulation) were rewarded with a 1-g banana pellet (*8*). Inappropriate key responses—that is, releases prior to the onset of the visual or cortical stimulation or presses prior to the tone resulted in a 30-second "time out" period (the duration of the intertrial interval). The change from light to cortical stimulation was effected by introducing the cortical stimulation and then slowly fading out the peripheral stimulus. This procedure caused little disruption of performance. Latencies of key releases were recorded for each day's run in the form of a frequency distribution on a CAT computer (*9*) and individually on a Cramer chronoscope.

To obtain responses of minimal latency a "limited hold" was introduced (that is, only key releases occurring during a specified interval following on-

set of the light were reinforced). This procedure was used as the counterpart of the verbal instructions given in the conventional human reaction time experiment to "respond rapidly." The "limited-hold" interval was reduced during training to a minimum of approximately 300 msec (until further decreases disrupted performance).

At the conclusion of limited-hold training, median reaction times to cortical stimulation were observed to be 30 msec shorter than to peripheral stimulation (Fig. 1). This 30 msec difference was consistently observed during a series of sessions in which the mode of stimulation was repeatedly varied, not only from day to day, but within daily training sessions as well. The median latency to peripheral stimulation was 260 msec with a semi-interquartile range of 15 msec; reaction time to unilateral cortical stimulation had a median latency of 230 msec and semi-interquartile ranges of 15 and 12.5 msec (for the left and right hemispheres respectively). This difference between peripheral and cortical reaction times is approximately equivalent to the latency of the primary photically evoked potential (*10*).

The reaction times to photic and central stimulation are based on the intensity of a single stimulus. Since reaction time is dependent on intensity (*11, 12*) we studied the changes of reaction time as a function of changes in stimulus intensity. Thus we examined the relative effectiveness of varying stimulus intensities in order to allow a meaningful comparison between peripheral and cortical stimulation. Figure 2 illustrates the effect of variation of intensity of cortical stimulation on reaction time. For this test series all intensities were randomly presented during each daily session and the limited-hold interval was extended to 1 second. (Comparison of Figs. 1 and 2 shows that this procedure tended to lengthen reaction time.) The points on the curves represent the median of a sample of the last 80 trials at each intensity following stabilization of behavior. Both curves are approximately exponential in form. A similar function was reported previously for this subject when photic stimulation was used (*11*). While the reaction times to photic stimulation in the present experiment were shortened by the limited-hold procedure, the intensities yielding the observed 30-msec difference between peripheral and cortical behavioral responses both lie on the brief latency asymptote of the curve. Thus, since 215 lumen/m^2 and 700 μamp each produced near minimal reaction times, these intensities may be meaningfully compared on the basis of their relative effectiveness in evoking the behavioral response.

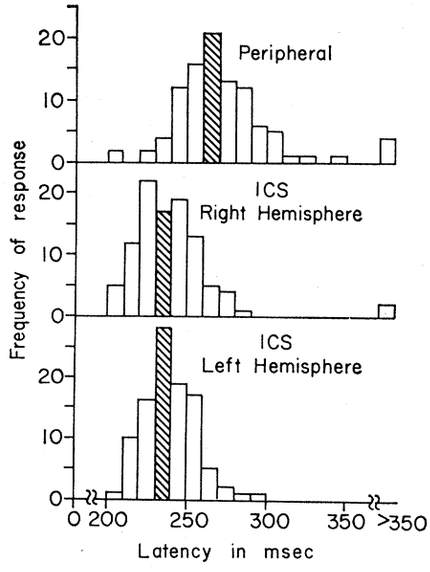

Fig. 1. Distribution of reaction times observed on final day of training with peripheral and unilateral cortical stimulation (*ICS*).

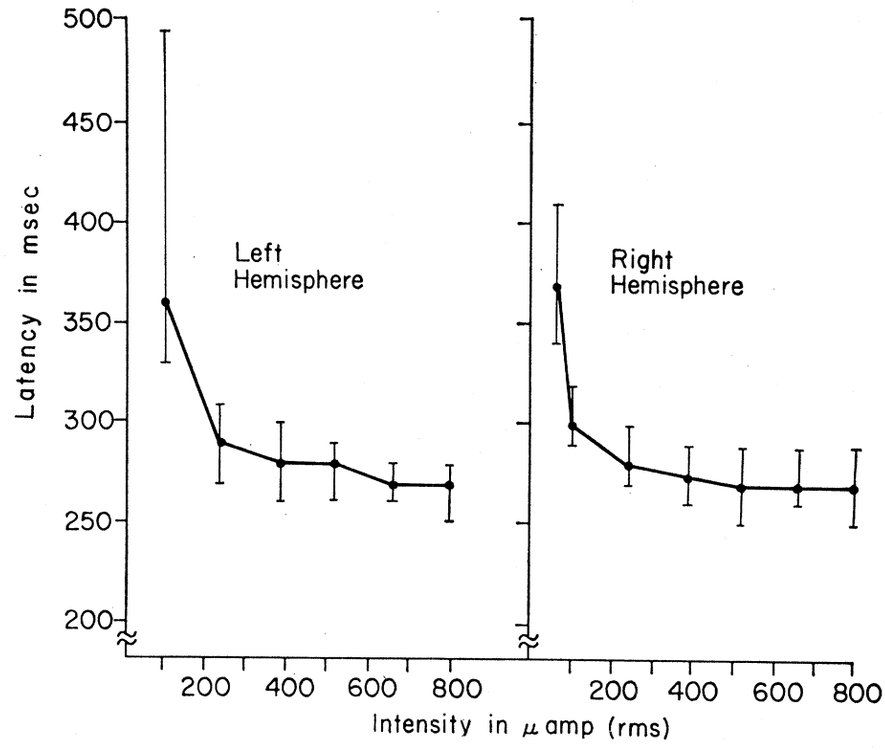

Fig. 2. Reaction times to unilateral cortical stimulation of differing intensities. Medians and interquartile ranges for a sample of 80 trials observed after stabilization of behavior. Thresholds are approximately 50 and 85 μamp (rms) for right and left hemispheres, respectively.

The observed difference in latency to visual and cortical stimulation suggests that cortical stimulation short-circuits the delays attributable to photochemical processes in the retina as well as synaptic and conduction delays in the visual pathways. We believe the technique may prove fruitful for the analysis of neural mechanisms in a simple behavioral task.

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activity beginning as early as 20 to 25 msec was observed (C. Stevens and J. Miller, unpublished observations). This decrease in latency in the unanesthetized preparation has previously been noted; M. A. B. Brazier, in *Brain Mechanisms and Consciousness*, J. F. Delafresnaye, Ed. (Blackwell, Oxford, 1954); R. W. Doty, D. S. Kimura, G. J. Mogenson, *Exptl. Neurol.* **10**, 19 (1964).
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16 October 1964

Telemetry of Homing Behavior by the Deermouse, Peromyscus

Abstract. *Miniature transmitters (weighing 2.5 to 2.7 grams, including encapsulation) implanted subcutaneously in deermice* (Peromyscus) *radiate a pulsed signal at 27 megacycles per second which can be detected by a simple antenna at a distance of 45 meters. The radio signal indicates movements of the deermice, periods of activity, and the location of occupied nests. One mouse was traced as it returned to its nest 300 meters in 1 hour. This rate of homing is many times more rapid than the rate usually determined by conventional methods for tracking small terrestrial mammals.*

The homing behavior of mice has been studied by repeated capture of the animals in live traps on their return to home ranges from release points in areas unfamiliar to them (*1*). But nocturnal mice usually enter the traps only at night, and may spend one or more days in the home area without entering available traps. In general, the maximum speed of homing, determined by the time between release and recapture, is only about 30 meters per hour from distances up to 1000 m or more (*2*). Since mice can travel at a much faster rate, the low measured homing speed could reflect extensive exploratory searching in the homing behavior. A few examples of homing at rates of 30 m per minute (*3*) suggest that mice may be able to navigate homeward in a manner analogous to that of homing pigeons (*4*). However, the paths taken by mice of the genus *Peromyscus* within 60 m of the release point, determined by trapping or visual observation, show no homeward direction (*1*). We have developed a radio transmitter small enough to be carried by a mouse to determine both the maximum speed of homing and all or most of the route taken.

Radio transmitters have been used to track wildlife and telemeter physiological data from unrestrained animals (*5*), but with transmitters too large for mice. Our transmitter (Fig. 1*a*) provides a pulsed radio frequency signal at about 27 Mc/sec. Its Hartley oscillator circuit is sensitive to temperature, and the components for each circuit must be matched with great care to the individual transistor and tested at the subcutaneous temperature of mice 34°C. Our transmitters oscillate at all subcutaneous temperatures but not at or below 25°C; thus they work only in a living mouse. Rise in subcutaneous temperature caused by increased activity of the mouse or by its change in position in an insulating nest increases the frequency of the signal but does not notably alter the repetition rate of the pulses. The repetition rate, which can be adjusted by critical choice of values for R_1, R_2, and C_3 (Fig. 1*a*), serves to identify each transmitter (*6*). The radiated power is delivered from the circuit as a whole, principally from the coil L. The radiation pattern is directional, so that small movements of the animal carrying the transmitter effect changes of signal strength at the receiver. The weight of the transmitter is 2.2 g when constructed with the smallest commercial components (Fig. 2*a*); addition of a silicone rubber covering increases the weight

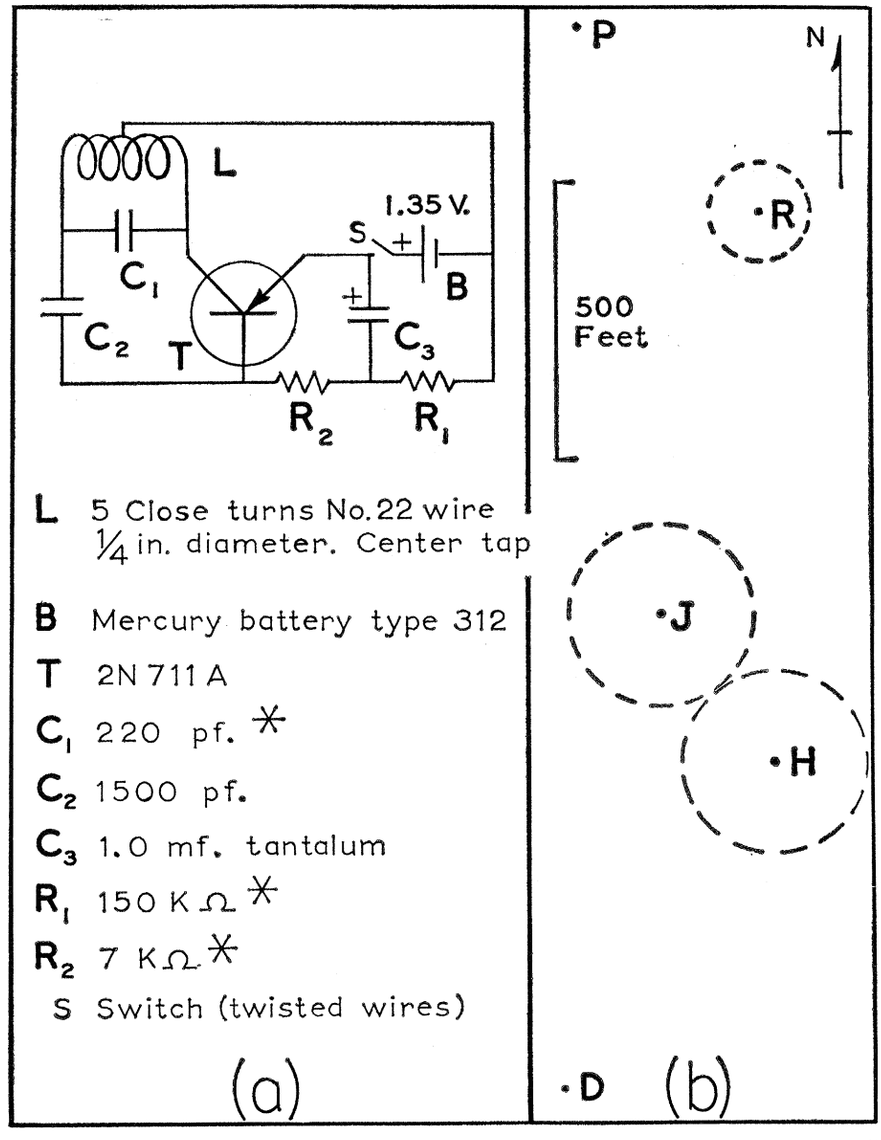

Fig. 1. (*a*) Schematic circuit of the transmitter. Ferrite chips are placed within the coil L for final radio-frequency tuning. Values marked * are typical and must be adjusted for each circuit (pf, picofarad; mf, microfarad; kΩ, 10^3 ohms). (*b*) Map relating release points to home (H) for the homing experiments described. Dashed circles show the approximate ranges of the receiver antennas (500 feet is equivalent to 152 meters).