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7. The present results were reported at the annual meeting of the Association for Psychophysiological Study of Sleep in Washington, D.C., March 1965. At the same session Rechtschaffen, Cornwell, and Zimmerman of the University of Chicago reported quite similar elevations in temperature in the cat brain during paradoxical sleep.
8. Supported by grants from NIH (NB 01162) and the Ford Foundation. We thank J. N. Hayward for advice in making thermocouples and David Whitmoyer for technical assistance.

16 August 1965

Prolonged Excitation in the Visual Cortex of the Cat

Abstract. *Intense light flashes produce maintained increased in cortical activity not dependent on continuous excitation from the retina.*

Cells in the visual cortex of the cat respond to precise stimulation in specific ways, but these responses are transient (*1*). Other workers have shown that the tonic output of the retina is modified by changes in illumination (*2*). In man long-term changes in absolute threshold may be produced by exposure to bright sunlight (*3*) and, following a brief, intense stimulus, a characteristic persistence of vision commonly referred to as an after-image occurs.

We have produced persisting changes in the firing rate of cells in the primary visual cortex of the cat following an intense, diffuse light flash to the contralateral retina (*4*). We now present results which confirm our original findings and in addition show that repeated flashes of equal intensity may produce step-like increases in firing rate. The persistence of these effects is a cortical phenomenon and is not due to a maintained increase in retinal output.

Twenty cats weighing between 3 and 5 kg were anesthetized with ethyl chloride followed by ether. After insertion of tracheal and venous cannulae, a mid-collicular section was made through the brainstem (*5*) and the animals were then maintained by artificial respiration. The skull and dura were removed to expose a large area of the visual cortex of one hemisphere. A well, filled with warm mineral oil, was made by suturing the scalp to a ring above the head (*6*). The eye to be used in the experiment was protected from desiccation with a contact lens. The cat's temperature was maintained within 0.5° of 37°C by a heating pad controlled by a thermistor probe inserted in the rectum. Anesthesia was not maintained, but the preparations were paralyzed with continuous intravenous infusion of 10 mg of gallamine triethiodide (Flaxedil, M&B) per hour. Continuous infusion was employed because it had been found in a previous study that a single injection of 40 mg, as normally used, sometimes stopped spontaneous unit activity and reduced the amplitude and high-frequency components of the electrocorticogram. In some cases the preparation was kept for 2 or 3 days without any apparent deterioration or change in properties of the cortex. To do this it was necessary to supply glucose intravenously and penicillin by intramuscular injection.

Glass micropipette electrodes with internal tip diameters of 1 to 5 μ and resistances between 0.2 and 1 megohm, filled with 90-percent saturated NaCl solution, were used to make extracellular recordings of unit activity; data were stored on magnetic tape for subsequent analysis. The electrodes were suspended from a steel wire tensioned in such a way that they were effectively weightless and hence free to move with the cortex (*7*). Single units could therefore be "held" for several hours without dislodgement of the electrode tip from the recording site by movements caused by heartbeat and respiration.

An intense, diffuse flash was provided by a 1000-joule flash bulb fired 15 cm from one of the cat's eyes, the pupil of which had been dilated with atropine. No attempt was made to correct for refractive errors. The other eye was covered with an opaque shield. In some experiments pressure of 100 g was applied to the contact glass to produce retinal ischemia (*8*). In pilot studies the effectiveness of the ischemia in preventing transmission from the retina was tested by stimulation with a flashing neon bulb (Fig. 1).

Recordings were made from 75 cells. Figure 2 shows the effect of a single flash on the mean firing rate of a single cell in the primary visual cortex. Characteristically, the unit exhibits an immediate sharp discharge

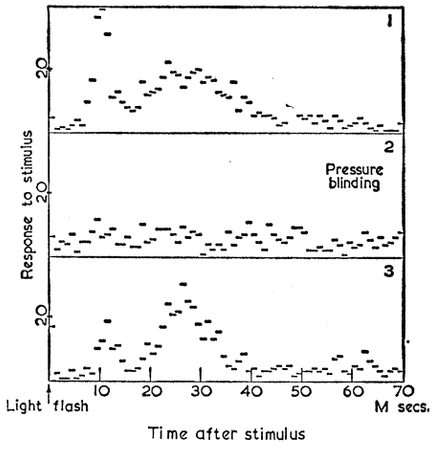

Fig. 1. Post-stimulus histograms showing the effect of "pressure-blinding" on the response of a cortical cell to a flashing light at the retina. Each histogram is the total response of the unit to a flash repeated once a second for 3 minutes: part 1 is before pressure-blinding, part 2 during, and part 3 after. Ordinates are number of impulses recorded from the unit at time after flash on the abscissa.

followed by a "silent" period of up to 30 seconds. From this point, activity increases steadily. Peak activity is normally attained within 10 minutes of the flash. Firing rate may then subside to the control level, the whole sequence taking approximately half an hour. About 70 percent of the cells tested with a single flash responded in this way.

Figure 3 shows the effects of flashes repeated at regular intervals; their effects summate, confirming the finding reported in our previous note (*4*).

While individual flashes invariably provoke an immediate burst of firing, long-term changes are not always present. Flash 2 in Fig. 3, for example, seems to have had no tonic effects on maintained, or tonic activity, though the transient post-stimulus burst was observed. Flashes 3 and 4, however, show marked summation effects, and the remaining flashes hold the mean firing level at a generally constant value. (The time scale of Fig. 3 is too coarse to show transient responses; in any case these responses have little effect on mean firing rate, for they include a brief burst of firing followed by a period of inactivity.) Flash 3 clearly provoked a surge of firing that reached a peak approximately 5 minutes after the flash, the rate subsequently falling to the basic level. This response was consolidated by the flash 4, which produced a large increase in firing rate, reached a peak 9 minutes

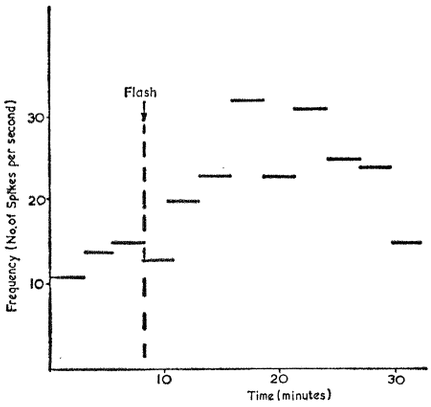

Fig. 2. Change in the rate of firing following a single intense flash of light to the retina.

after the stimulus, and fell to a mean level significantly higher than the previous basic level. The tonic activity doubled, but it was not affected by subsequent flashes. Twenty cells showed effects of summation between flashes.

Figure 4 gives another example of summation following a series of flashes, all but one of which set the cell to a new, higher level of tonic activity. It should be noted that in the undisturbed brain the mean frequency of discharge of cortical neurons appears to remain constant indefinitely (*9*).

This graph also shows the results of a study of the effect of "pressure-building," undertaken in an attempt to isolate peripheral and central components of these responses. At point A a flash produces the expected phasic response, which is abruptly terminated by pressure-blinding, though the tonic increase is not affected. At point B a flash, unaccompanied by pressure-blinding, produces the usual effects, while at point C pressure-blinding can be seen to have no effect on the tonic increase. That the system is still capable of a marked response to new stimuli, however, is demonstrated by the increase in firing at point D, where a further flash was given.

We previously reported changes in both short- and long-interval components of the histogram of the interspike interval, in contrast to the findings of Smith and Smith, who showed (*7*) that any changes in firing rate following less powerful stimulation of cortical cells could be accounted for by changes in the slope of the distribution of long intervals—their "slow process." Figure 5 demonstrates that with our intense flashes a permanent change in the distribution of short intervals does take place, almost entirely accounting for the increased firing rate. We think this result is important because it has been shown that the interval distribution is a characteristic property of each cortical cell and that, in the spontaneously active cortex, it is very stable (*7*).

These experiments were suggested by studies of after-images in humans, in whom an intense, brief stimulus gives rise to a persisting response in the visual system, the most characteristic feature of which is intermittent disappearance of the image. Subjectively, an after-image consists of a brief, extremely brilliant "positive" image, followed by a variable period when the image is not seen. Subsequently the image reappears in whole or in part and enters into a cyclic course involving changes in color and intensity. A diffuse after-image of the kind employed in these experiments may last in the human for as much as half an hour, though at the end of this period perception is so weak that the image may be observed only when background illumination varies.

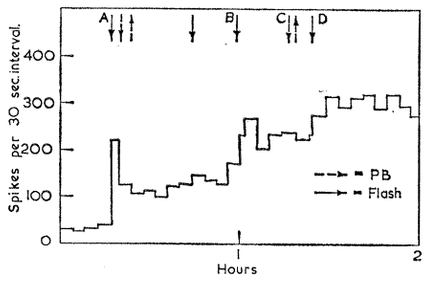

Fig. 4. Effect of pressure-blinding on firing after an intense flash. Broken arrows show start and finish of each period of pressure-blinding.

If it is possible to detect the effects of an after-image in a cortical cell, a reasonable prediction might be that the change would follow a cyclic course roughly comparable to the varying periods of appearance and disappearance reported by human subjects. In fact, during these experiments when populations of cells were being sampled, it was possible to detect a cycling in firing rate which appeared as increased noise on the gated loudspeaker used for monitoring spikes. The time course of these waves was of the order of seconds and thus might be roughly compared with the reported time course of the human after-image. However, one might not expect to detect from a single cell a clearly defined rhythmic activity emerging from a background of spontaneous activity. Apart from these features our results do not seem to be related to known psychophysical phenomena.

Two processes seem to be contributing to the general picture we have described. The first, which is sensitive to pressure-blinding (Fig. 4) must be dependent on continual output from the retina and may therefore represent a reasonable analogue to the "diffuse after-image" (*10*). The second, which was found in 50 percent of all cells tested whether with one or several flashes, consists of an increase in the level of firing maintained throughout periods of pressure-blinding and persisting for up to 2 hours. Figure 5 shows that this effect is almost entirely due to an increase in the slope of the short interval distribution of the cell's interval histogram as plotted by Smith and Smith (*7*). In other words, the frequency of the firing of the cell within bursts, as defined in their paper,

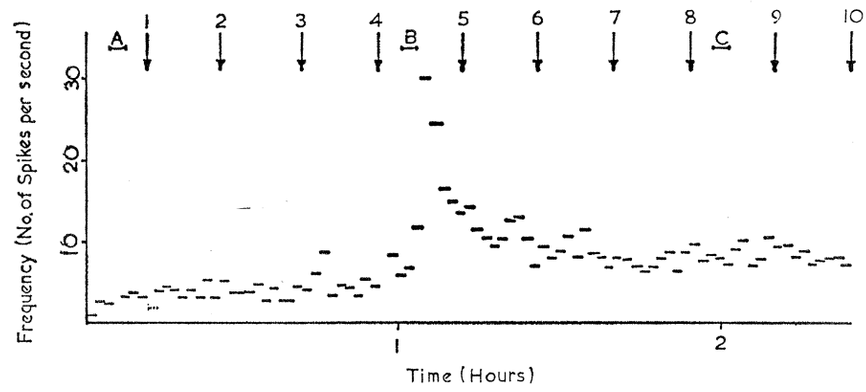

Fig. 3. Changes in the rate of firing following intense flashes repeated at regular intervals. Arrows indicate individual flashes and horizontal bars A, B, and C indicate the periods over which interval histograms shown in Fig. 5 were taken.

was increased, but the frequency and length of bursts were hardly affected.

Smith and Smith suggested that the process defining burst length and frequency was a gate, switching a cell on to standard activity; there is evidence for this in their paper. It is likely that the frequency of firing within bursts is either an intrinsic property of the cell itself or some index of activity in the network to which the cell belongs. Although the latter seems more likely it is not necessary to distinguish between the two possibilities here—the important fact is that a generally stable property of a cortical cell has been changed. Similar long-term changes have been reported by other workers (*11*). The general feature of these results is that maintained increases in firing rate of cortical cells throughout the cortex, in both rats and cats, have been produced by strong physiological or electrical stimulation. The stimulation must either be very intense or be continued for several minutes. For example, Bindman *et al.* found that, to produce a maintained increase in firing rate by passing a current of 0.5 μa/mm^2 of cortical surface through the cortex it was necessary to polarize for at least 5 minutes. One of their figures (*11*, fig. 4) shows striking similarities to our Figs. 3 and 4. Further, peak activity was not reached until about 5 minutes after the start of polarization. Gartside and Lippold (*12*), using electrical stimulation of the rat's forepaw to excite cells in the appropriate sensory receiving area of the cortex, have shown the same effect. Stimulation must last for at least 5 minutes from the start of an increase in cortical activity, and peak activity is not reached for at least 5 minutes. The similarities among the results produced by different forms of stimulation in different parts of the cortex in different animals suggest that prolonged excitation depends on a fundamental property of the cortex.

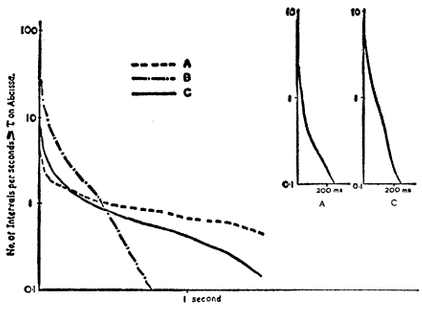

Fig. 5. Interval histograms taken over periods shown on Fig. 3. Inset shows the permanent increase in the number of short intervals; this increase accounts for the increase in firing rate maintained after an intense light flash.

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9 September 1965

Marmosets (Hapiladae): Breeding Seasons, Twinning, and Sex of Offspring

Abstract. *Our records on marmosets, primarily* Oedipomidas oedipus, *plus data from the literature, confirm that these animals customarily have twins. Demonstrated chimerism for several tissues is significant, for virtually all twins are of biovular origin. Furthermore, a single birth may often be a survivor of twins. Births occur during any month, but springtime appears to be the most common period. An average interval of 240 days between births predicts the production level of a captive colony. A gestation period of about 140 days appears to be a valid estimate.*

We are aware of several serious efforts to breed and use marmosets in the laboratory. The small size, low cost, and relative availability of these animals are only some of the reasons that make their use desirable. In comparison with higher primates, marmosets have a high production rate (three to four young per year) and short generation time (1 to 2 years). The fact that marmosets not only give birth to twins but that these twins are chimeric for blood, splenic tissue, lymph node (*1*), bone marrow (*1–3*), and testicular tissue (*4*) suggests their use as unique models for studies of tissue immunity. Also, an enzymatic function of the placenta (*5*) which appears to prevent free-martinism has been reported in these animals; their twins are characterized by extensive anastomotic connections via their placentae (*6*).

About three-fourths of all marmosets born in captivity are multiple births, presumably chimeric. Since the percentage of twins which are heterosexual is high, the sex chromosomes provide a useful and unique autosomal tag. The number of offspring born as singletons does not mean, however, that marmosets are unlikely to carry cellular contributions from undetected, resorbed twins. In fact, Wislocki (*6*) has reported a well-developed embryo beside a dead, macerated one, and in both our laboratory and that of Gengozian (*7*) similar observations have been made. It is sometimes unfortunate that each individual marmoset must be suspected of being phenotypically (and, perhaps, genotypically) contaminated, for studies of blood groups and other characters are made much more difficult.

Our data have come from a breeding colony of marmosets, all but two pairs of which were *Oedipomidas oedipus*. The others were one pair of *Tamarinus mystax* (two pregnancies) and one pair of *T. nigricollis* (five pregnancies). Although we have maintained a colony for nearly 5 years, breeding has taken place over the past 2½ years; 30 females have been pregnant 65 times. Ten of these females died for a variety of reasons, some intentional; thus the opportunity for multiple pregnancies did not come to all females. Twelve females have been pregnant once; 7, twice; 6, three times; 4, four times; and 1, five times.