2). Guanidine-dependent viruses may prove useful in producing immunization against poliomyelitis.

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Synchronous Sensory Bombardment of Young Rats: Effects on the Electroencephalogram

Abstract. *Rats were exposed to intense five-per-second synchronous clicks and flashes from birth to adulthood. Electroencephalographic recordings showed an abnormally high incidence of high-voltage burst activity in the visual cortex and thalamus of the animals; wave frequency within the bursts was five per second instead of the normal seven per second.*

It is nearly a century since the electrical activity of the brain was first studied, and, although considerable progress has been made in the last 35 years, some of the basic phenomena are not yet well understood. For instance, we still do not know the exact basis of the oscillations in recorded potential, nor do we know why the oscillations occur at the rate they do. This report is concerned with the second of these problems.

It is usually assumed that the frequency of brain rhythms depends almost entirely on the intrinsic organization of the nervous system. We have tried to find out what happens when rats are subjected to strong repetitive synchronous bombardment of two sensory systems during their early life. Such treatment might be expected to control the activity of large groups of neurones in the sensory areas and in other parts of the brain, and, by keeping these cells busy, prevent them from being integrated into the normal temporo-spatial organization. We were particularly curious to know whether we could establish a persistent rhythmic activity which would be determined by the rate of our synchronous stimulation.

Twelve hooded rats were reared from birth in a white box measuring 60 by 45 by 45 cm where they were constantly exposed to 10-msec flashes of light and synchronous clicks. The flashes were produced by a Grass model PS-2 photic stimulator, and the synchronous clicks were made by amplifying the pulses from the monitored output of the stimulator and passing them to a speaker attached to the box. The stimulation rate was five per second, the intensity of the clicks was of the order of 90 db (ref. 0.0002 dyne/cm^2), and the intensity of the photostimulator was rated by the manufacturer at 1.5 million candle power. Twenty-four control animals were reared in boxes of the same size. Half were kept in complete darkness; the other half were exposed for 1 hour to diffuse light and white noise each day (roughly the sum total time of the durations of the individual clicks or flashes to which the experimental animals were subjected). A "split litter" technique was used in assigning rats to these three groups so as to control genetic factors (each litter was divided at random and a third of each litter placed in each group), and the mothers were rotated between groups every 3 days.

Unfortunately, a failure in the air-conditioning system killed all the control animals after we had recorded from only two animals in each control group. Accordingly, a third group of eight rats reared in normal laboratory cages was used. Since no difference in brain activity was apparent between these eight animals and the four remaining from the original control groups, the results from all 12 subjects were pooled.

When the rats were 3 to 6 months old, bipolar electrodes made of 0.25 mm enameled Nichrome wire were implanted in the visual cortex and various subcortical structures; the rats were ultimately killed, and the placements were verified histologically. The animals were then allowed to recover for a week in the boxes in which they had been reared. Each rat in turn was then placed in a restraining sling of the type described by Kimura (*1*), and a light-diffusing dome was placed over its head. After an adaptation period of 1 hour, recordings were made for 52 minutes.

The test procedure was as follows. Control records were taken for 4 minutes. The rat was then photically stimulated at the rate of one flash per second for 2 minutes. This was followed by a 2-minute dark, control period. After this came stimulation by two flashes per second, followed by another 2-minute dark period, and so on until the rat had been exposed to stimulation at frequencies from one to nine flashes per second. Then came a 6-minute dark, control period. During the final 8 minutes of the session, 15 light flashes were presented, one every 30 seconds; only ten of the experimental rats were subjected to this procedure. This somewhat elaborate routine was followed to determine among other things whether the experimental treatment had any effect on photic driving. However, the data we are concerned with at present are based on the 26 minutes without stimulation, and on the last 8 minutes when 15 light flashes were presented.

It is known that light flashes cause a late response in the visual cortex of the unanesthetized rat (*1*). This response consists of spindle-shaped bursts of high-amplitude waves which generally have a wave and spike form; this was seen in all of the experimental and control animals. However, the late response of the experimental animals differed from that of the controls in two ways. First, it could be more easily triggered. For the 15 test flashes given each animal, the mean number of late responses was 11.6 for the experimental rats (range 10 to 13) and 3.2 for the controls (range 1 to 6). Second, measurement of the frequency of each individual wave in each response showed that the modal frequency for eight of the ten experimental rats tested was five waves per second, while for all the controls (and the remaining two experimental rats) it was seven per second. Typical responses are shown in Fig 1*A*.

The most striking difference between the groups, however, was in the spontaneous spindle-shaped bursts of waves, resembling the late response, which were seen in the tracings from the visual cortex of most animals. These bursts occurred, on the average, eight times as often in the experimental group (mean number of occurrences for the 26-minute observation period, 57.4; range, 24 to 28) as they did in

the controls (mean 7.1, range 0 to 22). The frequency of the waves in these high amplitude bursts was also different in the two groups. In all but two experimental animals the modal frequency was five waves per second, while for the controls it was seven waves per second. An example of typical spontaneous bursts is shown in Fig. 1*B*.

Records from subcortical structures also differed in the two groups of animals. Data are available for two rats in each group with electrodes situated in the medial thalamic nucleus, and for two with electrodes in the midline thalamic nuclei. All the experimental rats showed spontaneous bursts from the thalamus during the observation period, while, surprisingly enough, none of the controls did. On the whole, bursts from the thalamus tended to be associated with bursts from the cortex and to precede them, though there were many instances of independence of the two structures. Bursts from the thalamus were sometimes very prolonged, lasting for as long as 30 seconds, with periodic waxing and waning. Thalamic activity is shown in Fig. 1*C*.

When the experimental rats were taken out of the boxes in which they had been reared, and kept in a normal environment, the modal frequency of the waves changed within 1 week (in the case of one rat, within 24 hours) to the seven waves per second typical of the control rat (Fig. 1*D*). However, the incidence of the bursts was not reduced, even after the rats had been in a normal environment for 6 weeks. Of the four experimental rats tested, two showed no change, while two showed increases in incidence and duration of the bursts.

We also tried to find out what happens when a normally reared adult rat is kept in the flashing and clicking environment for long periods. When three 90-day-old animals were treated in this way for 10 weeks, they showed a most unusual prevalence of seven-per-second waves in cortical and subcortical structures which persisted for long periods in the recording session, although they could be blocked by an "arousing" stimulus. Figure 1*E* shows tracings from the visual cortex, midline thalamus, and tegmental periventricular gray; all of these structures show trains of these regular waves. However, these rats did not differ from the controls either in the incidence of spindling, or in the frequency of waves in the spindle-shaped bursts.

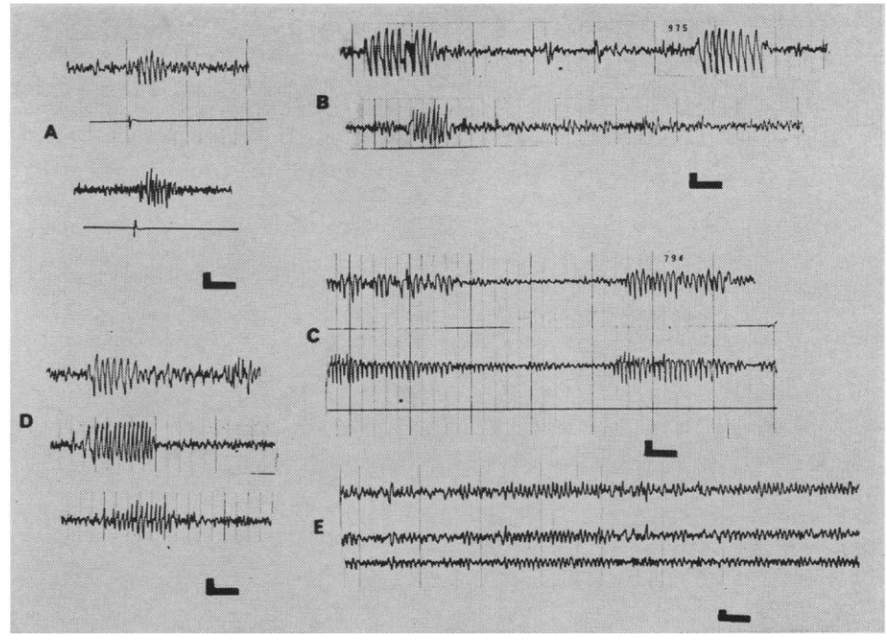

Fig. 1. (*A*) Examples of the delayed cortical response to a single flash for an experimental rat (top) and control rat (bottom). (*B*) Typical cortical tracings showing spontaneous bursts for an experimental rat (top) and control rat (bottom). (*C*) Tracings from an experimental rat showing spontaneous bursts in the cortex (top) and thalamus (bottom). (*D*) Cortical tracings from an experimental rat kept in a normal environment for 1 hour (top), 1 week (middle), and 1 month (bottom). (*E*) Tracings from cortex (top), thalamus (middle), and tegmental central gray (bottom) of a normally reared adult animal kept in the flashing and clicking environment for 10 weeks. Calibrations: 100 μv; 1 sec.

The results indicate that long-term exposure to strong repetitive sensory stimulation can have dramatic effects on the organization of brain activity in the rat, whether the animal is exposed during adulthood or infancy. Exposure during infancy seems to transitorily affect the mechanisms which determine the frequency of waves within bursts of activity, and to have lasting effects on the mechanisms which determine the frequency of occurrence of burst activity.

It is tempting to believe that the greater incidence of bursts in the experimental rats reflects an increased synchrony of activity of the cells concerned, possibly because of interconnections established by the prolonged sensory driving. Such an increase in synchrony might also account for the widespread seven-wave-per-second activity found in the adult rat subjected to the experimental procedure. Here the cells, again more closely connected and tending to wax and wane in excitability together, may be controlled by the seven-wave-per-second generator mechanisms in the animals' brain (such mechanisms seem to predominate in the rat). Because of the closer association of the cells, the effects of generators of other frequencies, which are prominent in normal animals, are greatly reduced.

It would be interesting to know whether the experimental rats are more susceptible to seizures than the controls. We suspect that they are more reactive to metrazol, and have observed that animals which show prolonged bursts, especially bursts involving the thalamus, tend to be unresponsive during the burst period.

Previously, it was shown that rats reared under conditions similar to those used in our experiments showed some abnormalities of behavior. They were much more active in an open-field situation, and were abnormally dull when tested on the Hebb-Williams maze (*2*).

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