Relationship of Form and Frequency of Experimental Epileptic Discharges to Age. (21007)

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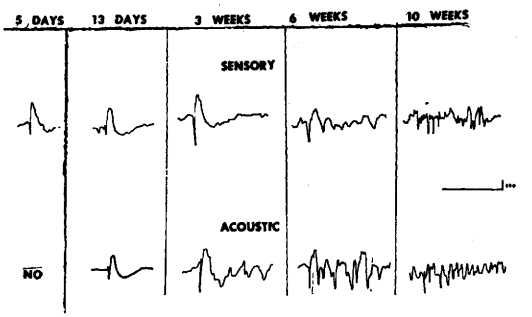
It was shown previously(1) that the development of the cortical activity in the cat proceeds postnatally according to a temporal and spatial order. The "primary" projection areas are the first to be electrically active and influenced by external stimuli, to be followed by other cortical areas. Ontogenetically, the "primordial" specific response of the cortex gradually assumes the form of a high voltage spike (followed by a single slow component under conditions of barbiturate narcosis).

The tendency of the immature cortex of the cat under barbiturate narcosis to respond to physiological sensory stimuli with a form of discharge seen in the EEG of epileptic children is of considerable experimental and clinical interest. Because the mechanism of the production of seizure discharges in general and the "spike and wave" pattern in particular is obscure, it seemed pertinent to investigate the rôle of the maturation factor in such a mechanism. Findings reported below seem to indicate convincingly that there is a definite relation between the various forms of epileptic seizure discharges and the ontogenesis of the electrical activity of the cortex.

Methods. 37 kittens distributed chronologically over the 3 postnatal months and 8 adult cats were anesthetized with nembutal or dial (2 to 5 mg per 100 g of weight, intraperitoneally). The electrocorticogram was secured through wick electrodes led into a 8-channel Grass electroencephalograph supplemented with a dual beam oscilloscope. The acoustic stimulus was a click produced by a 1 m second square wave led into an earphone with a repetition rate of .5/sec. Metrazol (Pentylenetrazol) (10%) was applied topically or was given intraperitoneally in small doses (as required to reach the desired state of excitability). "Monopolar" recording was obtained with a reference lead on the cut scalp. Surface stimulating electrodes on the cortex were bipolar with a separation of 3 mm. A Grass stimulator model S4A with all parameters variable was used for electrocortical stimulation.



EVOKED CORTICAL ACTIVITY AT VARIOUS STAGES OF POSTNATAL DEVELOPMENT

FIG. 1. Evoked responses recorded from specific somesthetic and acoustic cortex at various postnatal age. Note lack of acoustic response at 5 days.

Results. As seen in Fig. 1, at 5 days postnatally, the somatosensory cortex shows well established responses to afferent stimuli. At 13 days responses are elicited in the acoustic cortex and under moderate barbiturate narcosis both the sensory and the acoustic areas respond with a well developed "spike and wave" discharge. With increasing age the response becomes more and more complex. The "primary" spike response decreases in voltage and is followed by an "after-discharge".

It was found that the results of application of a convulsive agent like metrazol depend on the age of the preparation. In the first weeks of postnatal life of the kitten, electrical seizure activity induced in the specific areas of the cortex show characteristically repetitive "spike and wave" complexes without admixture of "fast" spike activity. At 3 weeks (Fig. 2A) the focal "spike and wave" after-discharge, induced in the left sensory cortical area by tapping the right forepaw (after topical or intraperitoneal application of metrazol), spreads slowly to the adjacent areas (acoustic and suprasylvian) and is confined to the homolateral hemisphere. At 6 weeks, (Fig.

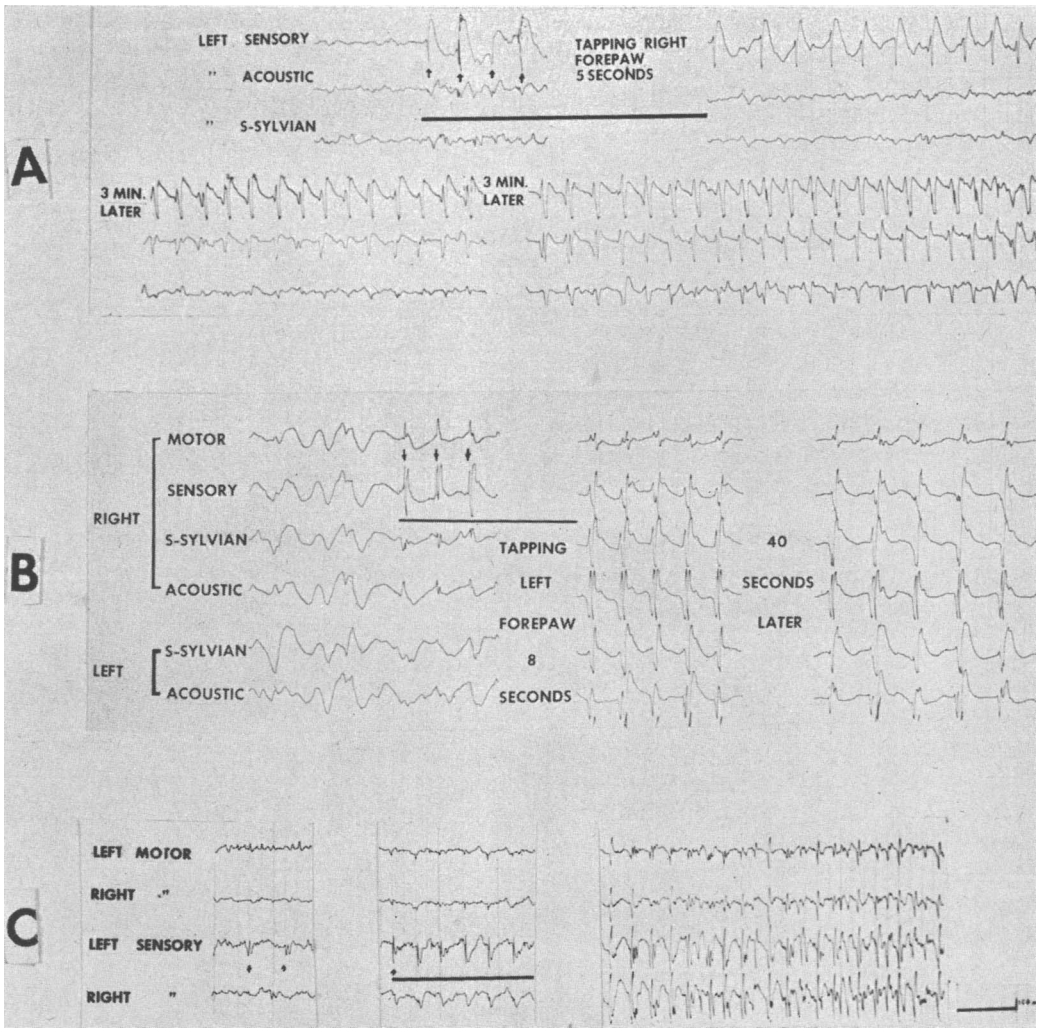


FIG. 2A. Self-sustained after-discharge in a 3-wk-old kitten evoked by stimulation of the right forepaw after topical application of 10% metrazol on left sensory cortex.

FIG. 2B. Bilateral spread of after-discharge evoked in a 6-wk-old kitten after intraperitoneal injection of metrazol and stimulation of left forepaw.

FIG. 2C. Responses to tapping of right forepaw before and after gradual increase in dose of injected metrazol. Kitten 12 wk old.

2B) after intraperitoneal application of metrazol and tapping of the left forepaw, seizure discharges are induced in the right sensory area. The seizure discharges show a rapid spread and are displayed bilaterally and synchronously in both hemispheres. At this age the "fast" spike activity is still not brought out and the seizure activity consists of "spike and wave" complexes. Only with further ontogenetical development the "fast" frequencies begin to appear more prominently during an induced generalized seizure (Fig. 2C). Such a fast activity is an almost invariable component of any generalized epileptic after-discharge produced in an adult animal.

The same tendency of the cortex to respond with a "spike and wave" discharge is revealed at earlier stages of development, by direct electrical stimulation of the cortex, without the use of a convulsive agent. In Fig. 3A typical "after-discharge" that follows the

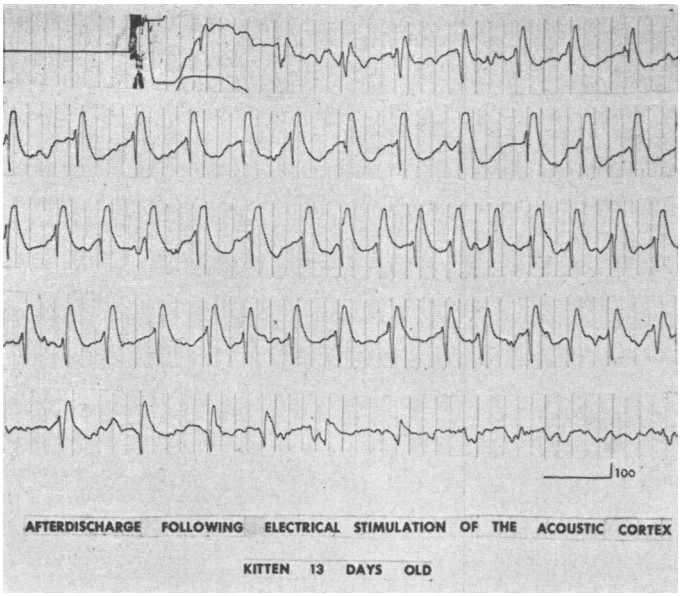


FIG. 3.

electrical stimulation of the acoustic cortex in a 13-day-old kitten is shown. The initial fast component or the "tonic" component that precedes the "clonic" component in the adult animal (Fig. 4) is here completely missing.

Discussion. The findings described above make it more comprehensible why previous attempts by many investigators to reproduce the "spike and wave" after-discharge without admixture of "fast" activity were followed by rather controversial results. The main reason for this seems to be the fact that these investigators have been working exclusively on adult animals. And so it was reported(2)

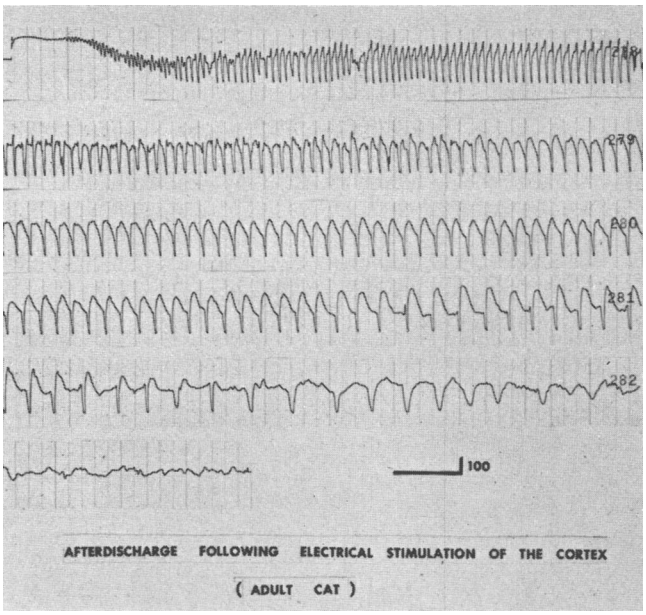


FIG. 4.

that stimulation of the medial thalamic structures may result in a "spike and wave" activity of large areas of the cortex. This activity was dependent on a stimulus frequency of about 3 per second. It was also found(3) that with metrazol sensitization and photic stimulation, repeated stimuli applied with a frequency of about 3 per second produce "spike and wave" formations in the cortex. However, the "spike and wave" discharges lasted only as long as the stimulation and have not been produced as a consistent self-maintained seizure activity after the withdrawal of the stimulus. If a self-sustained discharge occurred it consisted of "fast" spikes of the type seen during the "tonic" phase of a "grand mal" seizure.

The principles of development of the electrocorticogram of the cat indicate a tendency of the immature cortex to produce "slow" frequencies. This physiological electrical characteristic of immaturity is also manifested during the pathological state of an epileptic after-discharge. On this basis it is possible that the electrical picture of focal and non-focal "spike and wave" discharges so frequently found in the EEG of epileptic children may be explained by the readiness of the immature cortex to produce this type of discharge.

Summary. Metrazol and electrically induced seizure activity in the cortex of kittens at various stages of maturation reveals a relation of form and frequency of discharges to age. Focal and non-focal "spike and wave" discharges of frequencies seen in the EEG of the epileptic human infant and child may be reproduced in young animals. With increasing age spikes of faster frequencies replace this slower "spike and waves". The findings indicate the importance of electro-ontogenetic studies in investigations of the electrical patterns of epileptic seizures.

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1. Grossman, C. *Epilepsia,* 1953, v2, 145.
2. Jasper, H. H., and Droogleever-Fortuyn, *Proc. Assn. Res. Nerv. Ment. Dis.,* Baltimore 1947, p272.
3. Gastaut, H., and Hunter, F., *EEG Clin. Neurophysiol.,* 1950, v2, 287.

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Electrophoretic Mobility—Ionic Strength Studies of Proteins. I. Heterogeneity of Human Serum Albumin.* (21008)

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The moving-boundary method of electrophoresis as developed by Tiselius(1) offers a powerful tool for testing one aspect of the purity of proteins, *i.e.,* their homogeneity with respect to mobility(2). Such crystalline proteins as serum albumin(3) conalbumin, chymotrypsin, ribonuclease, etc.(4), B-lactoglobulin(5) and many other proteins were found to give a single moving boundary over a certain range of pH but *2 or more* boundaries under other conditions. By the technics of electrophoretic mobility, v.s. pH (3,6-10), prolonged electrophoresis(11,12), or electrophoresis in the presence of certain anions (13,14), human serum albumin showed *2* or more moving boundaries. Leutscher(3,6) carried out his original studies at pH 4.0 and with low ionic strength (.02) acetate buffer and reported separation of normal human serum albumin into 2 "components". Miller, *et al.*(9) also used acetate buffer at pH 3.9 but of high ionic strength (0.2, 90% NaCl) and reported separation of human serum albumin into *4* "components". It was our belief that this discrepancy in the number of boun-

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