0.046% fat grew normally for 6-8 weeks, at which time weight gains began to decrease slightly. No marked symptoms of deficiency developed, but depigmentation of feathers and scaliness of skin were observed. Fat-deficient male chicks had smaller testes and some females had immature oviducts, when compared with normal chicks. Water consumption by the deficient birds did not increase markedly until the 11th week on the fat-free diet.

1. Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, v82, 345.

2. Hansen, A. E., and Wiese, H. F., *Texas Rept. Biol. Med.*, 1951, v9, 491.

3. Reid, M. E., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 708.

4. White, E. A., Foy, J. R., and Cerecedo, L. R., *ibid.*, 1943, v54, 301.

5. Reiser, R., *J. Nutrition*, 1950, v42, 319.

6. Briggs, G. M., Spivey, M. R., Keresztesy, J. C., and Silverman, M., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 113.

7. Briggs, G. M., and Fox, M. R. S., *Poultry Sci*, 1955, v34, 1231.

8. Holman, R. T., *The Vitamins*, vol. II, W. H. Sebrell and R. S. Harris, ed., Academic Press, New York, 1954, 296.

9. Deuel, H. J., Jr., and Reiser, R., *Vitamins and Hormones*, vol. XIII, R. S. Harris, G. F. Marrian and K. V. Thimann, ed., Academic Press, New York, 1955, 43.

10. O'Dell, B. L., Boston, M. D., Savage, J. E., and Hogan, A. G., *Poultry Sci.*, 1952, v31, 559.

11. Waibel, P. E., Bird, H. R., and Baumann, C. A., *J. Nutrition*, 1954, v52, 273.

12. Assn. Off. Ag. Chem., *Methods of Analysis*, 1955, 210.

13. Wiese, H. F., and Hansen, A. E., *J. Biol. Chem.*, 1953, v202, 417.

14. Russell, W. C., Taylor, M. W., and Palskin, L. J., *J. Nutrition*, 1940, v19, 555.

Received August 27, 1956. P.S.E.B.M., 1956, v93.

Variations in After-Discharge from the Hippocampus. (22719)

R. E. CORRELL AND W. R. INGRAM

Departments of Psychiatry and Anatomy, University of Iowa

A number of investigators have noted the relative sensitivity of the hippocampal formation to the production of after-discharge(2,5, 8,9). It has also been noted that the after-discharge shows little or no spread to the rostral neocortex(3), although there is some disagreement on the extent of spread. Akert and Andy(1) describe the local after-discharge in the guinea pig as characterized by 18-25 c/sec. regular waves with occasional activity at slower or faster frequencies. Liberson and Akert(6) describe a tonic-clonic sequence. The tonic phase consists of 10-30 c/sec. activity associated with brief spikes of 100-250 c sec. frequency. The relation of the DC potentials, described earlier by Liberson and Cadilhac(7), to the clonic phase was indicated. Crutzfeldt and Meyer-Mickelett(3), using cats, describe 3 characteristic phases preceded by a period of 50-100 c/sec. activity. The first phase consists of 8-12 c/sec. waves and is followed by 18-28 c/sec. activity. The final phase consists of short pauses and single spikes.

The purposes of the present study were to determine the components of hippocampal after-discharge in the cat, and more specifically the different patterns which these components may form. A study was also made of variations in after-discharge threshold within the hippocampal formation, and an attempt was made to relate variations in pattern of after-discharge to a number of antecedent conditions.

Material and methods. Bilateral bipolar electrode placements were made in a series of 20 cats with the aid of a Horsley-Clark stereotaxic instrument. All operations were performed under aseptic conditions using sodium pentobarbital (Nembutal) anesthesia. In all cases placements were as nearly symmetric as possible, and at the level of the posterior portion of the lateral geniculate body. Some variation was introduced in the vertical plane

to cover different structures within the hippocampal formation. The bipolar electrodes were nichrome steel, insulated to within one millimeter of the tips, and placed with the tips one-half to one millimeter apart. Small stainless steel screws were placed in the skull over the middle suprasylvian gyrus to serve as cortical recording electrodes. All recording was done with a Grass Model III-D Electroencephalograph. Stimulation was by means of a Grass Model 3C Stimulator and isolation unit. Stimulation was with diphasic pulses of one millisecond duration at a rate of 80 pulses per second (pps.). To study the effects of stimulus frequency rates of 20 pps. and 300 pps. were also used. No records were taken for several days following operation to allow complete recovery of the animal. After-discharge was produced by stimulating the hippocampal formation with the stimulus parameters described for 10 seconds at intensities of 1 to 5 volts. In studies on effects of voltage the duration of stimulus was reduced to 5 seconds to minimize tissue destruction at high voltages. The duration of the after-discharge periods ranged from 2 or 3 seconds following termination of stimulus to just under 2 minutes. Most of them lasted between 30 and 40 seconds. For analysis of the patterns of after-discharge, only those which persisted long enough for development of a clear pattern were used.

Analysis of the after-discharge activity is based on 91 seizure records of 16 cats. Studies of the effects of voltage, pulse rate, time between seizures and mode of stimulation (ipsilateral, bilateral or contralateral) were carried out on 4 additional cats. The right hippocampal tracing was the one analyzed in all cases. For bilateral stimulation the right and left hippocampal bipolar electrodes were connected in parallel. Voltage was monitored at all times by means of a cathode ray oscilloscope. Histological control was available for all animals.

Results. The most frequently seen components were spikes of varying duration. They tended to fall in bands of 15-20 milliseconds, 30-40 milliseconds and 60-80 milliseconds. Occasionally faster spikes were visible, but their characteristics could not be adequately determined with an inkwriter. The slower spikes were seen more frequently than the faster and ranged in amplitude from 1 to 2 millivolts. The faster spikes were usually at lower voltage. These faster spikes typically were diphasic or triphasic in form and characteristically ranged from 100 to 600 microvolts in amplitude. Slower waves, more sinusoidal in form, were seen at a frequency of 5-11 c/sec., and occasionally activity at ½ to 4 c/sec. was present. Rhythmic activity between 20 and 30 c/sec. was at times seen for brief periods. It was most often present at 26 c/sec., but also frequently at 22 c/sec.

These components tend to become organized into several relatively discrete patterns. Five such patterns were empirically defined on the basis of wave form, frequency and voltage of the predominant activity. Examples of these groups are shown in Fig. 1. Group 1 was defined on the basis of a low to moderate voltage (100 to 400 microvolts peak to peak) 1½ to 9 c/sec. rhythmic activity. The slower activity was usually at the lower voltages. This activity may occur alone, but often the shift across the baseline is accompanied by a burst of fast low voltage spikes. Occasionally this activity may occur with a near normal tracing superimposed on it. In some records the spikes bear a constant relationship to the slower wave, but often they do not. Rarely a classical "wave and spike" pattern was seen.

Group 2 was the largest group, and 12 of the 16 animals had records falling in this group. It includes those cases where the predominant activity is spikes. The slower spikes were more highly organized and occurred at a repetition rate of 7½ to 12 per second, but most frequently at 9 per second. These spikes are monophasic and occur on the crest of a low voltage (50 to 100 microvolts) slow wave. The faster spikes are more complex, as previously indicated, and also occur with slower waves of low voltage and rhythmic 20 to 30 c/sec. activity. These records tend to show more components with more variable relationships between them than do the records with slower spikes.

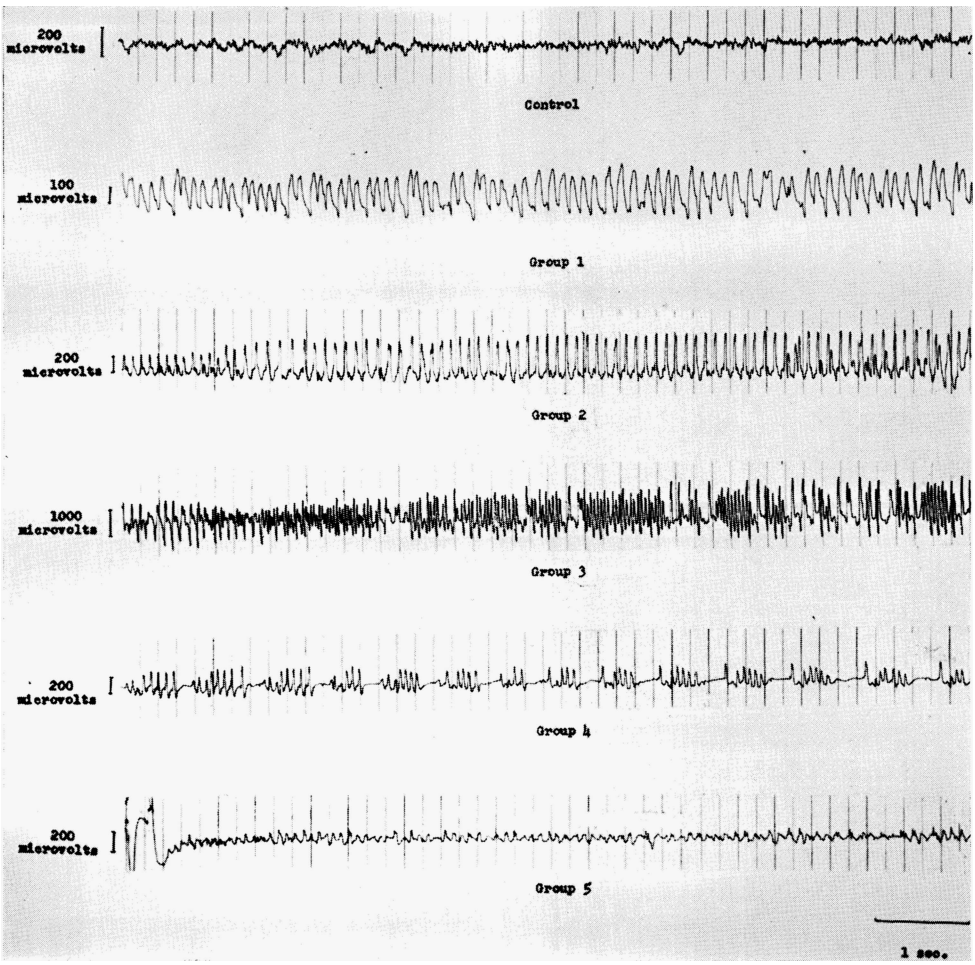

FIG. 1. Patterns of after-discharge obtained by stimulation of the hippocampal formation, and a representative section of normal hippocampal activity. These records were taken from different cats.

Group 3 is relatively rare, and was seen in only 3 of the 16 animals. It was defined on the basis of a predominant rhythmic fast activity ranging from 20 to 30 c/sec., but most often seen at 26 c/sec. Sharp spikes and slow waves are the most usual accompanying activity. Group 4 was defined on the basis of an alternating burst and depression. Most typically the bursts are made up of 4 or 5 15-30 c/sec. sharp waves which are separated by a silent period. This pattern was seen in 7 of the 16 cats. Group 5, seen in 5 of the 16 cats, was defined on the basis of a low voltage continuous rhythmic activity at frequencies of 5-15 c/sec. This may be mixed with low voltage fast spikes. More than one of these patterns may appear during a single seizure, though one is nearly always predominant. If the records are grouped on the basis of the predominant pattern, records from the same cat fall into several groups. The range was from 1 to 4 with a median of 2.5.

Fig. 2 illustrates a number of the characteristics of hippocampal after-discharge when the entire period of after-discharge is considered. When the after-discharge is compared with the control record, the absence of any great disturbance in the dorsolateral cortex is apparent. The pattern of after-discharge on the right and the left shifts markedly (more than is typical) throughout the course of the seizure, and the activity on

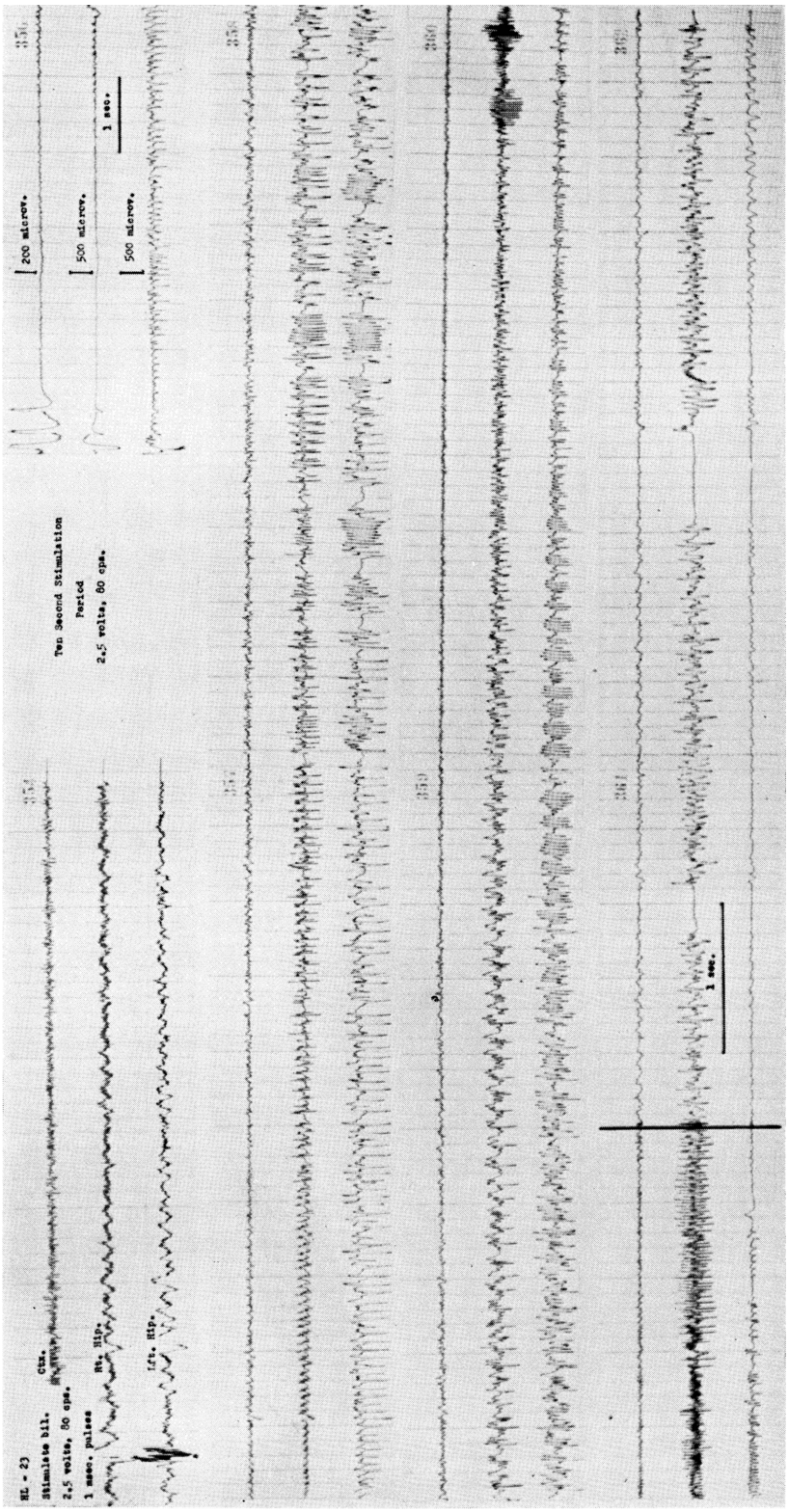

FIG. 2. A complete record of hippocampal after-discharge obtained by bilateral stimulation at 2.5 volts, 80 pps. See text for discussion.

the two sides is almost never identical. However, the brief silent periods, when they occur, usually occur simultaneously on the right and the left. Shifts in after-discharge form nearly always occur simultaneously in the 2 hippocampi. The long build-up which is present on the right is frequently seen, and may not be equal bilaterally. The gradual ending of the seizure activity on the left is atypical, as the seizures usually end quite abruptly and simultaneously on both sides. If there is a difference in the voltage of the after-discharge on the right and the left, the higher voltage is usually on the side stimulated. There is usually a brief period of depressed electrical activity following a seizure. In rare instances random spikes persisted for several minutes following a seizure, and appeared independently on the right and the left.

Of the locations investigated, the dentate fascia had the lowest thresholds for the production of after-discharge and the subiculum the highest. In some cases after-discharge could not be elicited from the subiculum. The alveus also has a high threshold in the only 2 placements in that location, as did the ventromedial edge of the hippocampus. In general, the fiber tracts running between the pyriform and retrosplenial areas and the hippocampus yielded an intermediate threshold.

Since the pattern of after-discharge was not dependent on the location of the electrodes, at least entirely, other factors were investigated. When the patterns were ranked by mean threshold and by mean time since operation there was a statistically significant relationship between the rankings ($r = .77$). Threshold means were computed by finding the overall mean for each cat and giving each record a value in terms of deviation from this mean. These deviation values were then averaged for each pattern. Group 4 showed the lowest threshold and Group 2 the highest. There was very little difference between the stimulus intensities required to produce after-discharge at 20, 80 and 300 pps., but 80 pps. seemed slightly more effective (not statistically significant). There was no evidence that any of the components of after-discharge, or the patterns they formed was related to the stimulus frequency, or to the voltage used provided it was above threshold. There was consistent evidence of reduced excitability two minutes following the end of a seizure. Occasionally longer intervals were tested, but the results were inconsistent.

Discussion. The results disclose considerably more variability in the form of the after-discharge from the hippocampal formation than has previously been indicated, although the components seen confirm those previously reported for the guinea pig(1,5) and the cat (4,8). The sequences of activity reported by Liberson and Akert(6), and by Crutzfeldt and Meyer-Mickelett(3) were both seen, but were no more frequent than other sequences noted. The complete tonic-clonic sequence was rather rare. The gradual build-up frequently seen would seem to indicate a recruitment process within the hippocampus. The fact that quite different patterns of after-discharge could be recorded from the same electrode implant with identical stimulus parameters would strongly suggest that the nature of the recorded activity is dependent upon the state of the organism at the time the stimulus is applied.

Attempts to determine the preceding conditions responsible for the variations in form of the after-discharge have not been very successful. The fact that there was a relationship between threshold and pattern suggests that the level of excitability at the time the stimulus is applied may be one significant variable. This is further supported by the significant relationship between threshold and time since operation for the different patterns, and would suggest some progressive modification of the hippocampus as one determining variable. If elements within the hippocampus are differentially affected by previous stimulation, then certain variations in pattern could represent the absence of participation of some elements. Additional work is necessary on this aspect.

Conclusions. The after-discharge of the hippocampus is formed by a number of components which can combine to form several fairly discrete well organized patterns. These

patterns seem to be related more to the state of the organism at the moment the after-discharge is produced than to the precise anatomic location of the electrodes or to the parameters of stimulation. Although the activity emanating from symmetrical electrode placements may show differences in pattern, there are systematic relationships between the right and the left which would indicate some bilaterally expressed control on the after-discharge. Extremely high voltage electrical after-discharge may be present in the hippocampal formation with only minimal disturbance in the dorsolateral cortex, at least by transverse recording, and without the precipitation of a major convulsion.

1. Akert, K., and Andy, O. J., *Trans. Am. Neurol. Assn.*, 1953, 194-195.
2. Andy, O., and Akert, M., *EEG Clin. Neurophysiol.*, 1953, v5, 320.
3. Crutzfeldt, O. D., and Meyer-Mickelett, R. W., *EEG Clin. Neurophysiol.*, 1953, Suppl. III, 43.
4. Green, J. D., and Shimamoto, T., *Arch. Neurol. Psychiat.*, 1953, v70, 687.
5. Liberson, W. T., and Akert, K., *EEG Clin. Neurophysiol.*, 1953, v5, 320.
6. ———, *ibid.*, 1955, v7, 211.
7. Liberson, W. T., and Cadilhac, J. G., *ibid.*, 1953, Suppl. III, 42.
8. MacLean, P. D., *J. Neurosurg.*, 1954, v11, 29.
9. Morin, F., and Green, J. D., *Anat. Rec.*, 1953, v115, 433.

Received August 27, 1956. P.S.E.B.M., 1956, v93.

The Effect of Molybdenum on Chick and Poult Growth. (22720)

B. L. REID, A. A. KURNICK,* R. L. SVACHA, AND J. R. COUCH
Departments of Poultry Husbandry and Biochemistry and Nutrition, Texas A. and M. College System, College Station, Texas

The ash of unidentified factor sources has been reported to stimulate the growth of both chicks and poults when supplemented to a purified type diet(1,2). The ash of dried whey, fish solubles or distillers dried solubles has also been found to stimulate the growth of chicks fed a practical type diet under field conditions(3).

Single additions of 40 elements to a purified diet for chicks were ineffective in duplicating the growth response obtained with the ash of a mixture of 6 unidentified factor sources(8). A reconstituted ash mixture, which was made up of chemically pure, reagent grade chemicals and supplied the elements found in the ash of distillers dried solubles upon spectrographic analysis, stimulated the growth rate of both chicks and poults (4). A similar reconstituted ash mixture produced a growth response under practical conditions (3).

Molybdenum has been found to be a co-factor of xanthine oxidase in the rat(5,6). The maximum molybdenum required by the rat for xanthine oxidase activity has been estimated as 0.2-0.3 μg per day(7). However, the growth rate of rats receiving the diets low in molybdenum was not affected. Molybdenum deficiency in the chick was associated with the feeding of 4.5-9.4 mg % tungsten in the ration(9). Such a deficiency could be overcome through addition of molybdate to the diet. The deficiency was characterized by growth depression, mortality, lowered tissue molybdenum concentrations, reduced xanthine dehydrogenase activity in the liver, intestine, kidney and pancreas, and decreased uric acid excretion.

Methods. Chicks were obtained from dams which had been reared from one day of age on an all-vegetable protein diet deficient in unidentified growth factors. The maternal diet used was the same as previously reported by Dannenburg *et al.*(1). Two types of chicks were used, New Hampshire and a New Hampshire X Barred Plymouth Rock Cross.

* Predoctoral Research Fellow of National Cancer Institute, N.I.H., P.H.S., Bethesda, Md.