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THE HIPPOCAMPUS AS THE SITE OF ORIGIN OF THE SEIZURE DISCHARGE PRODUCED BY TUBOCURARINE ACTING FROM THE CEREBRAL VENTRICLES

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Tubocurarine perfused through the cerebral ventricles of an anaesthetized cat produces an abnormal discharge in the electroencephalogram (e.e.g.), resembling the seizure discharge of epilepsy. Recently it was shown that this discharge results from an action of tubocurarine on structures reached from the posterior half of the lateral ventricle; the discharge did not occur when this part of the ventricular system was excluded from the perfusion. It was stated that to produce the cortical discharge the tubocurarine must act on the hippocampus or on the amygdala, or on both (Feldberg & Fleischhauer, 1962).

To distinguish between these structures their electrical activity was recorded in the present experiments simultaneously with that of the occipital cortex, and it was found that the abnormal discharge appeared first in the hippocampus, next in the cerebral cortex, and last of all in the amygdala. The cortical discharge therefore results from an action of tubocurarine on the hippocampus.

METHODS

The experiments were carried out in anaesthetized cats weighing between 2½ and 3 kg. Anaesthesia was effected either with intravenous chloralose (60 mg/kg) or with intraperitoneal pentobarbitone sodium (36 mg/kg). The left femoral vein was cannulated. For the chloralose injections, this was done under anaesthesia with ethyl chloride and ether. The trachea was cannulated. With the cat lying on its belly the head was fixed to the ear bars and mouth-piece of a head holder similar to that of the Horsley–Clarke stereotaxic instrument.

The method of perfusion of the cerebral ventricles was the same as described previously (Feldberg & Fleischhauer, 1962). Both lateral ventricles were cannulated and perfusion was carried out by means of separate injectors, one containing artificial cerebrospinal fluid (c.s.f.) the other the tubocurarine solution. It has been shown that under these conditions the tubocurarine enters only the one lateral ventricle. The rate of infusion was 0·1 ml./min from each injector, and the outflow was collected from the cannulated aqueduct.

The electrical activity of the occipital cortex, hippocampus and amygdala was recorded simultaneously from monopolar electrodes; an earthed metal screw on the forehead served as indifferent electrode. The time constant of the Ediswan pen recorder was set at 0·3 sec

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and the high-frequency limit at 500 c/s. The epidural cortical electrodes, inserted through burr holes over the right and left middle suprasylvian gyri, consisted of platinum wire held in a nylon screw. The electrical activity of the hippocampus and amygdala was recorded from stereotactically inserted insulated steel needle electrodes with a free tip of about 0·5 mm. The location of the electrodes was determined histologically after the position of their tips had been marked at the end of the experiment by means of electrolytic lesions.

RESULTS

Figure 1 shows the sequence in the development of the rhythmic discharge of single spikes as they appear first in the hippocampal, next in the cortical and last of all in the amygdaloid lead of the left hemisphere, the lateral ventricle of which is perfused with tubocurarine. The position of the tip of the electrode in the hippocampus and in the amygdala is shown in Fig. 2. The experiment is on a cat anaesthetized with chloralose. Record *A* Fig. 1 is a control taken whilst both lateral ventricles were perfused with artificial c.s.f. The electrical activity of the amygdala consists of small surface-negative spikes which follow each other in quick succession. Record *B* is taken 16 min after the beginning of perfusion with tubocurarine 1/20,000 through the left lateral ventricle. The abnormal discharge has just begun, with the appearance of large positive spikes in the hippocampus, and the record begins with the fourth spike. No abnormal activity is as yet recorded from any other lead. Several minutes elapse before the abnormal discharge appears on the occipital lead, and within a few seconds each positive spike in the hippocampus becomes associated with a negative one in the occipital cortex. Record *C* is taken a few minutes later, when the discharge is just beginning to appear in the amygdala, i.e. when a hippocampal discharge is occasionally associated with a negative spike in the amygdala. In record *C* this is seen for two out of the nine hippocampal discharges. Meanwhile the voltage of the spikes in the hippocampus has increased so much that the gain of the amplifier had to be reduced. As the perfusion continues, more and more of the large positive spikes in the hippocampus become associated with negative spikes in the amygdala. At first they are of varying and relatively low voltage, but within a few minutes they increase in voltage and from then on there is a discharge synchronous in all three leads from the side perfused with tubocurarine; each positive spike in the hippocampus is associated with a negative spike in the amygdala as well as with one in the occipital cortex. There is, however, scarcely any spread of the discharge to the contralateral occipital cortex. This is shown in record *E*.

On perfusion with stronger concentrations of tubocurarine the sequence in the appearance of the abnormal discharge in the different leads does not change, but the intervals shorten. In no experiment did the onset of the

discharge in the occipital cortex precede that in the hippocampus, yet the interval between the first discharge in the hippocampus and that in the cortex was only a few seconds, and in a few experiments the first hippocampal spike was already associated with a spike in the cortex. Even in these experiments it took a few minutes before the discharge appeared in the amygdaloid lead.

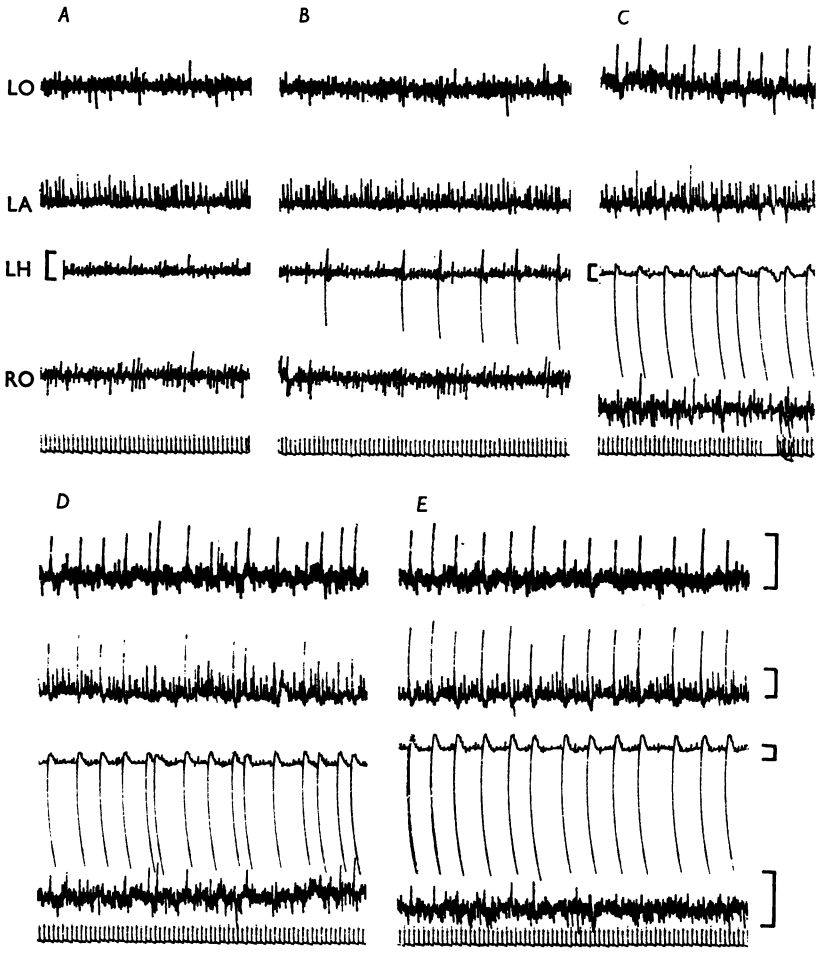

Fig. 1. Monopolar records of electrical activity of the cortex, amygdala and hippocampus in a cat anaesthetized with chloralose. Record *A* taken during perfusion of artificial c.s.f. from both cannulated lateral ventricles to aqueduct. Records *B*, *C*, *D* and *E*, taken 16, 25, 28 and 35 min after beginning of perfusion with tubocurarine 1/20,000 from the left ventricular cannula. LO and RO, left and right occipital leads, LA and LH, left amygdaloid and hippocampal leads. Calibration 1 mV. Time marker in seconds.

With the stronger concentrations of tubocurarine multiple spikes and episodes are seen as they appear in the different leads. Figure 3 is from an experiment under pentobarbitone sodium anaesthesia and with perfusion of tubocurarine 1/5000 through the left lateral ventricle. The figure illustrates single and multiple spikes recorded at a faster speed in the leads from the left hemisphere. The single spike at *A* has occurred during the early part of the tubocurarine perfusion, but at a time when the dis-

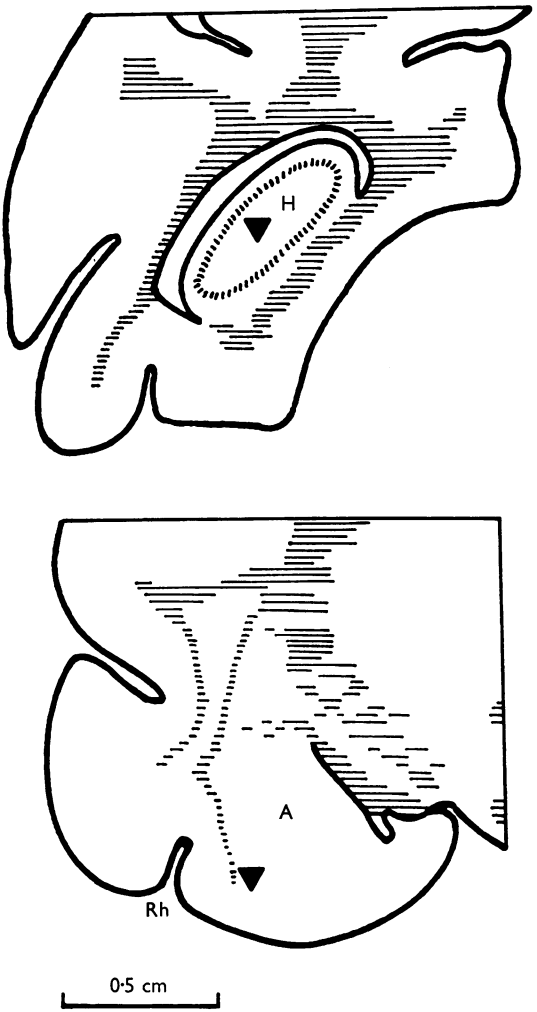

Fig. 2. Position of electrode tips in hippocampus and amygdala shown by triangles in diagrams of coronal sections through the brain of the cat from the experiment Fig. 1. Position of electrode tips in experiment Figs. 3 to 5 were nearly the same (A, amygdala; H, hippocampus; Rh, rhinal fissure.)

charge had already appeared in the amygdala; the record shows the difference in polarity between the spike discharge in the hippocampus and in the other regions. At *C* the multiple spike with an after-discharge of eleven small spikes is obtained later in the tubocurarine perfusion. The after-discharge occurs at the end of the negative phase of the large hippocampal spike and each small spike begins with a positive deflexion, whereas in the amygdala the after-discharge occurs at the end of the positive phase of this spike and all small spikes begin with a negative deflexion. In the record from the occipital lead the first few small spikes are not clearly distinguishable, but the later ones are seen to be of opposite

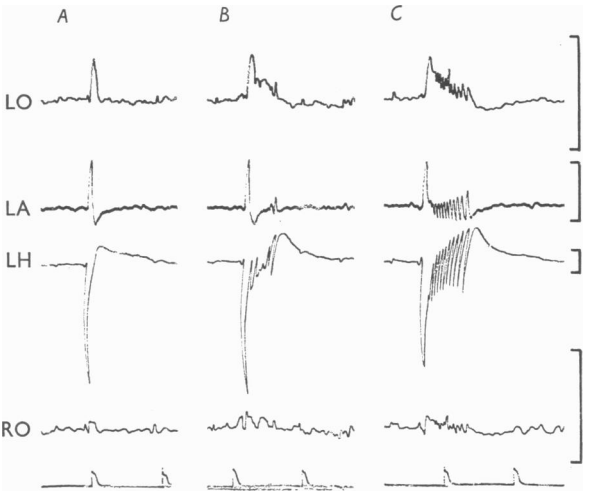

Fig. 3. Abnormal spikes recorded monopolarly from cortex, amygdala and hippocampus in a cat anaesthetized with pentobarbitone sodium during perfusion of tubocurarine 1/5000 from left, and artificial c.s.f. from right lateral ventricle to aqueduct. Record *A* taken 18 min afer beginning of tubocurarine perfusion, records *B* and *C* taken 20 sec and 7 min later. Calibration and lettering as in Fig. 1.

polarity to those of the hippocampus. During the period of transition from single to multiple spikes the small spikes are sometimes seen in the hippocampal and occipital leads but not in the amygdaloid lead. In the experiment of Fig. 3 this happened several times when the spike discharge reappeared after a period of electrical silence following an episode; one such spike is illustrated at *B*.

The development of episodes is shown in Figs. 4 and 5. In Fig. 4 record *A* illustrates the first episode, which occurred 1 min after the onset of the abnormal spike discharge at a time when not every spike in the hippocampus and occipital cortex was associated with one in the amygdala. Only one of the six hippocampal spikes preceding the episode was accom-

panied by one in the amygdala, and the activity of the episode itself was scarcely discernible in the amygdaloid lead, although the activity was clearly seen in the ipsilateral and also in the contralateral occipital leads. The later episode, shown in record *B*, Fig. 4, was taken when the spike discharge in the amygdala had become firmly established. Each of the spikes preceding the episode appears in the amygdala and the episode was now recorded in all three leads including that from the amygdala.

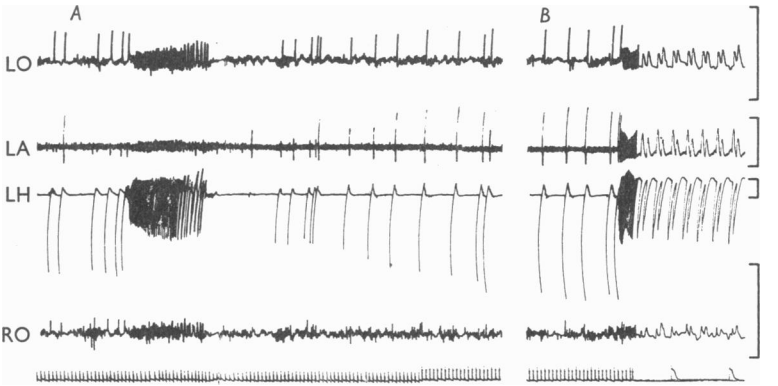

Fig. 4. Same experiment as Fig. 3. Record *A* taken 13 min and record *B* 17 min after beginning of tubocurarine perfusion. Lettering and calibration as in Fig. 1.

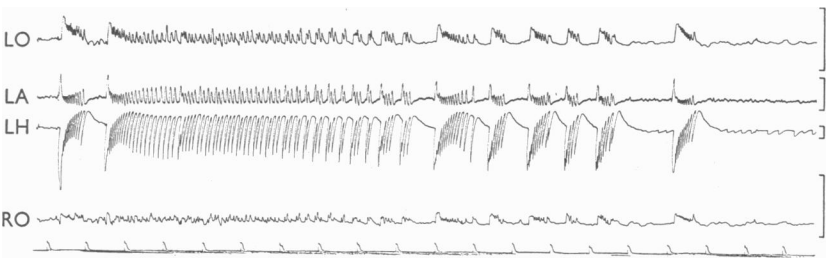

Fig. 5. Same experiment as Figs. 3 and 4. Episode taken on fast paper 19 min after beginning of tubocurarine perfusion. Lettering and calibration as in Fig. 1.

Figure 5 illustrates an episode, recorded at a fast speed, which lasted 17 sec. It followed a rhythmic discharge of multiple spikes which occurred at a frequency of 6–7/min. The initial part of the episode consisted of a quick succession of high-voltage spikes, and the latter part of a train of multiple spikes. All the events in the hippocampal lead were of opposite polarity to those in the amygdaloid and occipital leads. There was little spread to the contralateral occipital cortex. Following this episode there was a period of 27 sec during which no abnormal spike discharge was recorded, i.e. the period of electrical silenc

DISCUSSION

The problem has been solved as to whether intraventricular tubocurarine acts on the hippocampus or on the amygdala, or on both, when eliciting on the cortical e.e.g. the abnormal discharge resembling the seizure discharge of epilepsy. The tubocurarine must act on the hippocampus when producing this discharge because it appears first in the hippocampus, then in the occipital cortex, and last of all in the amygdala.

Since tubocurarine does not reach the cortex the discharge in this part of the brain cannot result from a direct cortical action of tubocurarine but must be explained by the spread of the hippocampal discharge. The amygdala on the other hand borders the lumen of the posterior part of the lateral ventricle and is reached by the tubocurarine when penetrating the brain from the ventricular surface. Therefore a direct action of tubocurarine on the amygdala is not excluded as the cause for the amygdaloid discharge. Yet this discharge, like that in the cortex, also appears to originate in the hippocampus and to be due to spread of the hippocampal discharge. Otherwise it would be difficult to explain why each spike and each episode in the amygdala coincide with a spike and an episode in the hippocampus, and why this coincidence occurs not only after the amygdaloid discharge has become fully established, but from its onset. The fact that several minutes elapse before the discharge spreads to the amygdala, even if the spread to the occipital cortex occurs within seconds, would then mean that for the invasion of the amygdala stronger or longer activation of the hippocampus is required than for the spread to the occipital cortex.

The difference in polarity between the abnormal discharge when recorded from the hippocampus and when recorded from the other leads appears to be a consequence of the peculiar anatomical arrangement of the cellular layers in the hippocampus. The positivity of the abnormal spikes in the hippocampal lead suggests that the relatively large tip of the needle electrode placed inside the concentric shell of the layer of orientated pyramidal cells is recording incoming current flow from a synchronous discharge of a large number of cells.

There is a close resemblance between the activity recorded from the hippocampus during an episode produced by intraventricular tubocurarine and the after-discharge obtained on electrical stimulation of the hippocampus. In Creutzfeldt's experiments (1956) on anaesthetized cats, for instance, the after-discharge had about the same duration as the episodes we observed, i.e. it lasted for less than $\frac{1}{2}$ min. Further, the after-discharge showed the same two kinds of activity as that observed in the present experiments during an episode, i.e. an initial phase of fast synchronous high-voltage waves followed by a train of multiple spikes. This

resemblance suggests that the same neuronal elements are activated and are responsible for the rhythm of firing in both conditions, i.e. during the after-discharge following electrical stimulation and during an episode produced by tubocurarine.

SUMMARY

1. The electrical activity of the occipital cortex, amygdala and hippocampus was recorded monopolarly in cats anaesthetized with chloralose or pentobarbitone sodium whilst tubocurarine was perfused from one, and artificial c.s.f. from the other lateral ventricle to the aqueduct.

2. The abnormal discharge recorded in the cortical e.e.g. under this condition originates in the hippocampus, where it appears first; it then spreads to the ipsilateral occipital cortex. There is little spread to the contralateral cortex.

3. The abnormal discharge in the amygdala, which appears last of all, is also explained by spread of the hippocampal discharge.

4. The abnormal discharge in the hippocampal lead is of opposite polarity to that in the cortical and amygdaloid leads.

REFERENCES

CREUTZFELDT, O. (1956). Die Krampfausbreitung im Temporallappen der Katze. *Schweiz. Arch. Neurol. Psychiat.* **77**, 164–194.

FELDBERG, W. & FLEISCHHAUER, K. (1962). The site of origin of the seizure discharge produced by tubocurarine acting from the cerebral ventricles. *J. Physiol.* **160**, 258–283.