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EFFECT OF TUBOCURARINE ON THE ELECTRICAL ACTIVITY OF THE CAT'S BRAIN UNDER CHLORALOSE

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Recently some of the effects of tubocurarine on the electrical activity of the brain have been studied in cats anaesthetized with pentobarbitone (Feldberg, Malcolm & Sherwood, 1956). The tubocurarine was either applied topically to the cerebral cortex or injected into the lateral cerebral ventricle. These experiments have been continued, mainly on cats anaesthetized with chloralose because of the different pattern of central excitability which this anaesthetic produces (Adrian, 1941; Marshall, Woolsey & Bard, 1941). Under pentobarbitone evoked responses to afferent nerve stimulation are obtainable in limited areas of the brain only, whereas under chloralose many more regions are accessible to afferent stimuli and the 'spontaneous' activity is greater (Amassian, 1954; Albe-Fessard & Rougeul, 1955).

In the previous experiments the main emphasis was on the effect of tubocurarine on responses evoked by afferent nerve stimulation, although the appearance of increased activity of the brain occurring independently of such stimulation was also described. In the present experiments more details are given about this 'spontaneous' increased activity. Further, it will be seen that the effect of tubocurarine, at least when applied topically, on evoked cortical responses differs under pentobarbitone and chloralose anaesthesia.

METHODS

The experiments were carried out in cats anaesthetized either with 40 mg/kg pentobarbitone sodium intraperitoneally, or with chloralose (70–80 mg/kg) intravenously. The technique of exposure of the cortex, insertion of the Collison cannula into the right lateral ventricle, stimulation of the left peroneal nerve and recording from the cortex and thalamic regions of the left cerebrum was essentially the same as that described previously, except that a fine wick electrode instead of a platinum one was used for recording cortical potentials and that the diameter of the tip of the focal electrode ranged from 3 to 8 μ. This electrode was placed in the desired position with the stereotaxic instrument, relying for localization on the drawings of the thalamus by Jiminez-Castellanus (1949). To apply tubocurarine topically the liquid paraffin covering the

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exposed cerebral cortex was displaced by warm Locke's solution containing the tubocurarine. The tubocurarine used was the tubocurarine chloride B.P. of Burroughs Wellcome and Co, London.

Histology. At the end of each experiment the left carotid artery was cannulated, the chest opened, the ascending aorta clamped close to the heart with a large Spencer Wells forceps and the head, still in the stereotaxic instrument, perfused, first for a few minutes with 0·9 % NaCl solution, and then with a 20 % formol-30 % alcohol-saline solution. After fixation with this solution, the whole brain was exposed and cut in the vertical Horsley-Clarke plane so that an appropriate transverse segment could be removed. Frozen sections were cut from this segment 24 hr later and stained by Klüver's method (Klüver & Barrera, 1953). In some experiments the position of the tip of the focal electrode was marked in the living animal by passing a few μA through it.

RESULTS

In the previous experiments under pentobarbitone sodium anaesthesia (Feldberg *et al.* 1956) stimulation of the peroneal nerve evoked a response in a limited region of the primary sensory area of the posterior sigmoid gyrus. The areas mapped did not include the secondary sensory area located in the anterior portions of the ecto-sylvian gyrus (Adrian, 1941). In the present experiments the mapping was extended to cover this gyrus and evoked responses were obtained both in the primary and secondary sensory areas, which are synonymous with the somatic areas 1 and 2 of Woolsey (1947). A typical experiment is illustrated in Fig. 1. Usually no evoked responses were recorded in any other regions.

Under chloralose anaesthesia the result was different. Evoked responses on stimulation of the peroneal nerve were not limited to the primary and secondary sensory areas but were obtained from a much wider area of the cortex. This is shown in Fig. 2. The pattern of the responses varied. In the primary sensory area it consisted usually of a surface-positive, followed by a surface-negative wave and, subsequently, by small fluctuations. In the secondary sensory area the response was usually smaller and sometimes either a positive or a negative wave only was observed. The voltage of the evoked responses in these sensory areas was usually greater than under pentobarbitone sodium. In the motor cortex the response consisted of a large positive followed by a slow negative wave, which was sometimes as large as the positive wave. Further evoked large positive waves occurred in regions mainly posterior to the primary sensory area after a small early negative wave. There were variations in the patterns of the responses in different experiments and in one, in addition to the early response, a large negative wave was consistently evoked about 60 msec after the stimulus in a number of regions posterior to the sigmoid gyrus. The positive waves decreased in amplitude laterally and posteriorly from the primary sensory area.

When pentobarbitone sodium was injected intraperitoneally into a cat under chloralose anaesthesia it was not possible to decrease the area from which evoked responses could be obtained but only to reduce their size and duration. The evoked responses became very small and only their initial part

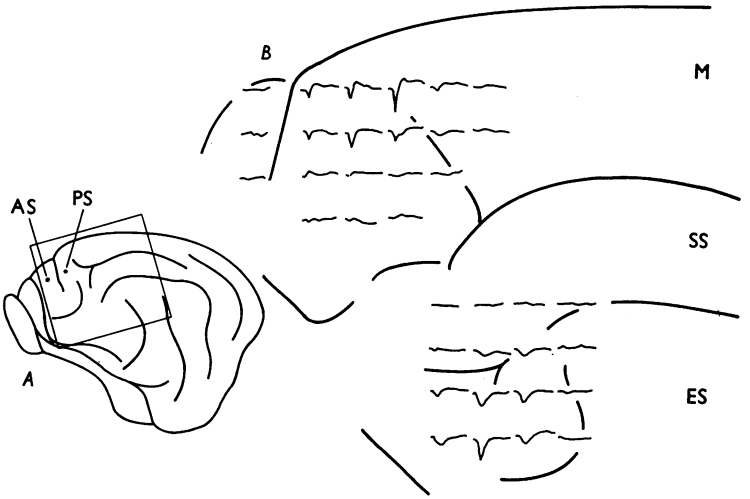

Fig. 1. Evoked cortical responses in a cat under pentobarbitone sodium on stimulation of right peroneal nerve. At *A*, diagram of cat's left cerebral cortex. AS and PS, anterior and posterior sigmoid gyrus. At *B*, enlargement of area in rectangle of *A*: M, SS, and ES, marginal, suprasylvian and ectosylvian gyrus. The responses shown were from the points at which each record begins. Upward deflexion recording electrode negative.

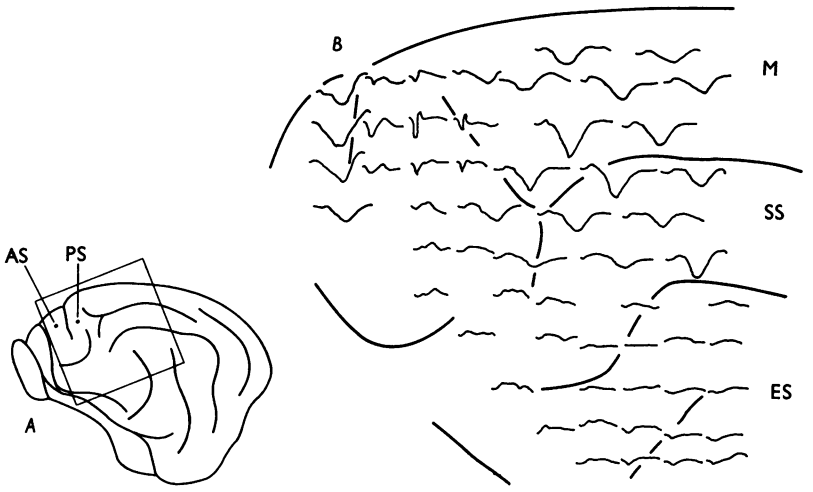

Fig. 2. Evoked cortical responses in a cat under chloralose anaesthesia on stimulation of right peroneal nerve. At *A*, diagram of cat's left cerebral cortex; AS and PS, anterior and posterior sigmoid gyrus. At *B* enlargement of area in rectangle of *A*; M, SS and ES, marginal, suprasylvian and ectosylvian gyrus. The responses shown were from the points at which each record begins. Upward deflexion recording electrode negative.

remained. This is shown in the experiment of Fig. 3. On the other hand it was possible to enlarge the area from which evoked responses could be obtained in a pentobarbitone cat by giving chloralose, as shown from a comparison of Fig. 4 *A* and *B*. In the area in which evoked responses had been obtained under pentobarbitone alone, the responses were somewhat reduced in size, but now small responses were evoked in previously unresponsive areas.

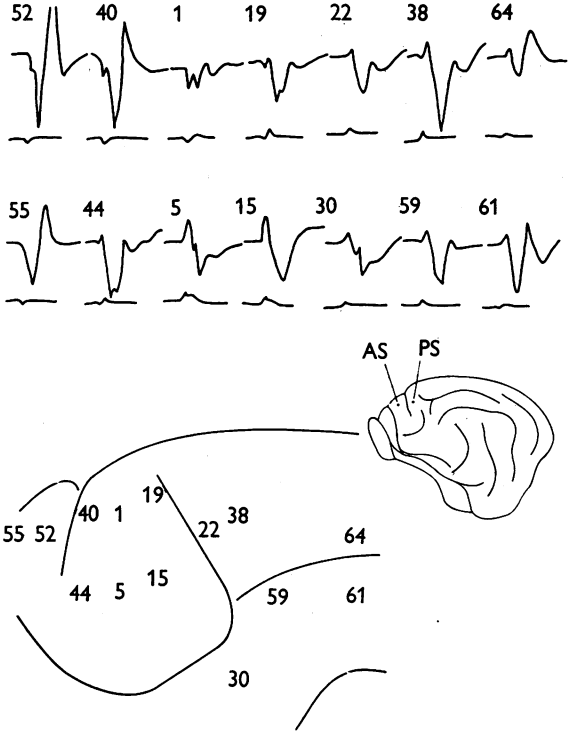

Fig. 3. Effect of intraperitoneal pentobarbitone sodium on the evoked cortical responses obtained under chloralose anaesthesia. Response evoked by stimulation of the contralateral peroneal nerve. In each case the upper record shows the response under chloralose before, the lower after, the pentobarbitone injection. The numbers beside the responses designate, in the accompanying diagram, the points from which the records were obtained. AS and PS anterior and posterior sigmoid gyrus. Upward deflexion recording electrode negative.

In the previous experiments on cats under pentobarbitone sodium anaesthesia, stimulation of the peroneal nerve evoked scarcely any response in the focal record with the electrode tip in the nucleus ventralis lateralis of the thalamus. It showed only a few spikes which sometimes also occurred without nerve stimulation; scarcely any slow waves were evoked. In contrast, in cats under chloralose, stimulation of the peroneal nerve elicited regularly large slow waves on the focal record. The pattern of this response consisted of a positive wave of a duration of up to 50 or 60 msec, often preceded and

followed by negative waves of varying size. Similar slow waves occurred from time to time also without stimulation. Fig. 5 illustrates the variation in the evoked responses obtained in five different experiments. Often an evoked group of sharp spikes of 2–3 msec duration was superimposed on the early negative wave. This group of spikes occurred either intermittently or was consistently present for periods of several minutes. In addition, similar sharp spikes, either in groups or singly, occurred unrelated to the stimulus (record *a*, Fig. 5, and records *j*, *k*, *l*, Fig. 6, column *A*) or without the nerve being stimulated (record *f*, Fig. 5, and record *m*, Fig. 6, column *A*). When they occurred in groups their frequency was up to 100/sec. The shortest latency for the evoked response in the thalamus was of the order of 15–20 msec, and invariably this was related to the commencement of a slow wave; the latency for an evoked spike varied from 20 to 60 msec.

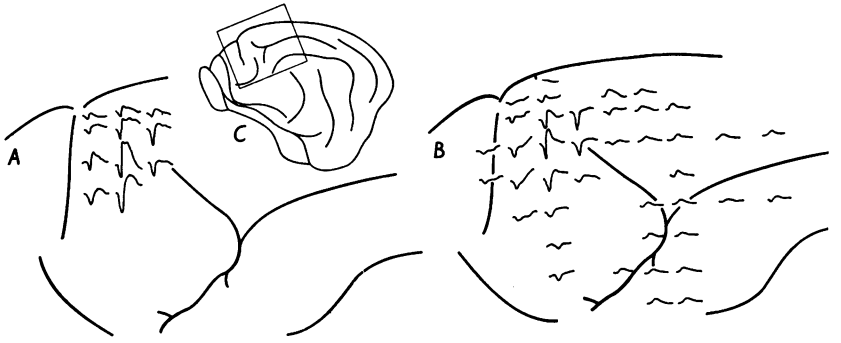

Fig. 4. Effect of intravenous chloralose on evoked cortical responses obtained under pentobarbitone sodium anaesthesia. Responses evoked by stimulation of the contralateral peroneal nerve. In *A* the limited distribution of the evoked cortical responses under pentobarbitone alone; in *B* the spread of the responsive area after subsequent injection of chloralose. In *C*, diagram of the left cerebral cortex, the rectangle indicates the area shown in *A* and *B*. Upward deflexion recording electrode negative.

An evoked response was obtained not only when the tip of the electrode reached the nucleus ventralis lateralis of the thalamus, but at all levels between the cortex and this region. The response consisted of a similar pattern of slow waves, though the relative size of the waves often varied at different levels. Similar slow waves occurred spontaneously at all levels and there was no difference in the frequency of this recurrence at different levels. At certain levels spike activity, evoked and spontaneous, was recorded. It was most prominent in a region immediately dorsal to the postero-lateral nuclei. A typical experiment is illustrated in Fig. 6, column *A*. The electrode was inserted stepwise and records were taken at about every millimetre. An evoked group of large spikes appeared in this experiment over a distance of 2 mm (at *f*, *g* and *h*). Subsequent histological examination showed that the electrode tip at this level was near the dorsal margin of the postero-lateral nuclei. When

traversing deeper this evoked group of large spikes disappeared but reappeared when the electrode was withdrawn to the former level. At 11 mm above the Horsley-Clarke zero plane groups of spontaneous spikes appeared in this experiment (at *k*, *l* and *m*).

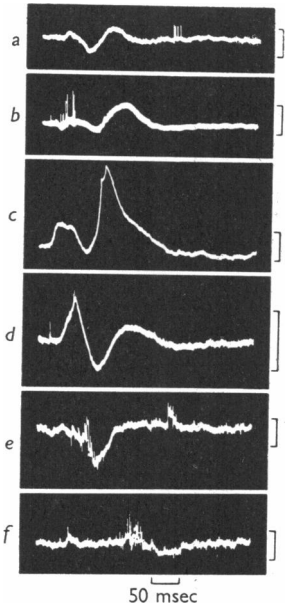

Fig. 5

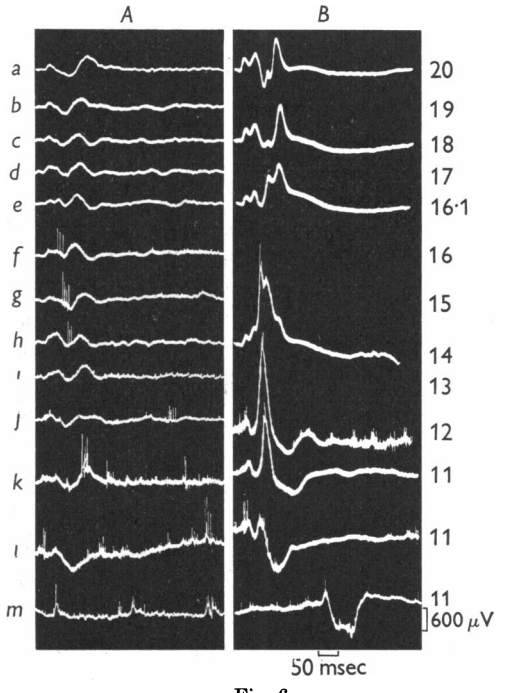

Fig. 6

Fig. 5. Records from the nucleus ventralis lateralis of five cats under chloralose anaesthesia. At *a–e* responses evoked by stimulation of the contralateral peroneal nerve; at *f* spontaneous activity from the same cat as record *e*. Upward deflexion recording electrode negative. The vertical bars represent 300 μV.

Fig. 6. Records obtained in a cat under chloralose anaesthesia from different levels of the thalamus before (column *A*) and after (column *B*) flooding the cortex with tubocurarine solution 1:1000. At *a–l* responses evoked by stimulation of the contralateral peroneal nerve; at *m* spontaneous activity. The figures on the right side refer to the height in mm above the Horsley-Clarke zero plane. Upward deflexion recording electrode negative.

When pentobarbitone sodium (35 mg/kg) was injected intraperitoneally into a cat already anaesthetized with chloralose, the slow waves evoked in the nucleus ventralis lateralis of the thalamus disappeared and spontaneous spike activity was greatly reduced.

Topical application of tubocurarine

In cats under pentobarbitone the flooding of the cortex with tubocurarine 1:1000 did not produce flushing of the cortex, which is a typical effect of the

intraventricular injections (see page 189) nor did it produce any muscular movements except in one experiment in which some weak contractions of the neck muscle occurred about half an hour after the flooding. These, however, did not necessitate intravenous tubocurarine. In cats anaesthetized with chloralose there occurred always at some time after the flooding small contractions either in the neck, jaw or limb muscles, confined to the contralateral side; again they were too weak to require intravenous tubocurarine. In two of these cats some flushing of the cortex was observed.

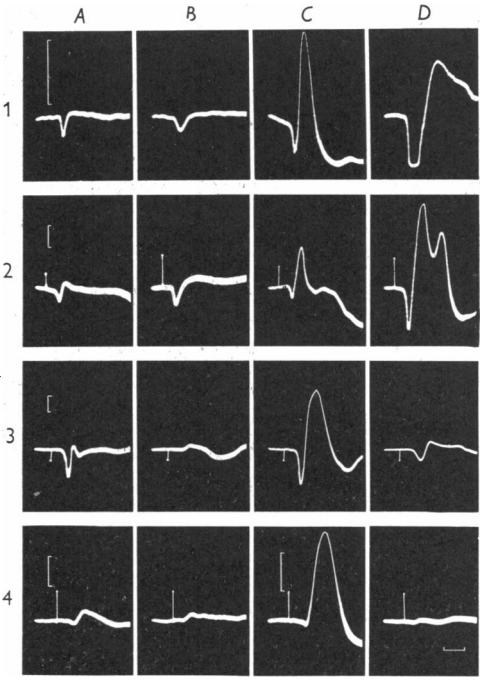

Fig. 7. Responses evoked by stimulation of the contralateral peroneal nerve in the primary (columns *A* and *C*) and secondary (columns *B* and *D*) sensory areas from two cats under pentobarbitone sodium (1 and 2) and from two cats under chloralose (3 and 4) anaesthesia. Columns *A* and *B* before, columns *C* and *D* after, flooding the cortex with tubocurarine solution 1:1000. All vertical lines represent 300 μV. The horizontal bar represents 50 msec. Upward deflexion recording electrode negative.

Effect on cortex. In previous experiments on cats under pentobarbitone sodium it was found that topical application of tubocurarine on small filter-paper disks increased the early evoked response in the primary sensory area (Feldberg *et al.* 1956). This finding was confirmed in the present experiments, but the concentration of tubocurarine required to produce a pronounced effect of this kind was found to be higher, i.e. 300μg/ml. For these experiments the usual method employed for topical application of tubocurarine was to

flood the whole exposed cortex for about 10 min with tubocurarine at a concentration of 1:1000 in Locke's solution. The main difference in the action of tubocurarine applied in this way between cats under pentobarbitone and under chloralose anaesthesia is illustrated in Fig. 7. Under pentobarbitone (Fig. 7, 1 and 2) there was a great increase particularly in the surface-negative component of the early evoked response in both the primary (Fig. 7, *C*, 1 and 2) and secondary (Figs. 7, *D*, 1 and 2) sensory areas. Under chloralose (Fig. 7, 3 and 4) this augmentation occurred in the primary sensory area only (Fig. 7, *C*, 3 and 4); the evoked response in the secondary sensory area being either unchanged or only insignificantly increased (Fig. 7, *D*, 3 and 4).

As in the previous experiments under pentobarbitone sodium, the portion of the primary sensory area from which an evoked response could be obtained after topical application of tubocurarine did not significantly increase even though much higher concentrations of tubocurarine were used in the present experiments for flooding the cortex. Similarly, the portion of the secondary area from which an evoked response was obtained did not significantly increase after flooding the cortex with tubocurarine. In cats under chloralose anaesthesia, in which evoked responses could be elicited from a wide area, the enhancement of the early response in the primary sensory area was limited to the small area which corresponded to the area of enhancement under pentobarbitone anaesthesia.

The flooding of the cortex with tubocurarine produced, however, additional changes both in the cats anaesthetized with pentobarbitone and with chloralose. It produced in many regions increased 'spontaneous' activity consisting of large irregular waves, and the appearance or enhancement of slow waves following the early evoked responses. The regions where these slow waves were particularly conspicuous were not identical in each experiment and their pattern varied, though the following generalizations can be made:

(1) In the primary sensory area the enhanced evoked early response was often followed by irregular fluctuations. This is illustrated in Fig. 8 for an experiment under pentobarbitone sodium (at *a*) and under chloralose (at *d*).

(2) In the secondary sensory area the enhanced evoked response obtained under pentobarbitone sodium was also followed by irregular fluctuations (Fig. 8, *b*). In cats under chloralose in which tubocurarine did not enhance the evoked response, late sharp waves were sometimes recorded (Fig. 8, *e*). They were also seen in other regions and occurred without stimulation but less frequently.

(3) In the motor area the flooding with tubocurarine caused the appearance of irregular waves in the experiments under pentobarbitone. They were dependent on the stimulus in so far as they occurred less frequently with-

out stimulation. In the experiments under chloralose in which the evoked response consisted of a large positive wave followed by a variable negative wave before tubocurarine, the pattern was interrupted by a number of irregular fluctuations.

(4) Both in cats under pentobarbitone and under chloralose, random fluctuations and large waves occurring as late as 100 msec after the stimulus were recorded in adjacent areas, lateral and posterior to the primary sensory area (Fig. 8, *c*). Again, they were only dependent on the stimulus, inasmuch as they occurred less frequently without stimulation. In cats under chloralose, the large positive waves which were often recorded in some of these regions before the flooding, were interrupted by irregular fluctuations (Fig. 8, *f*). There was a definite decrease in this more or less irregular activity when the electrode was moved more and more posteriorly.

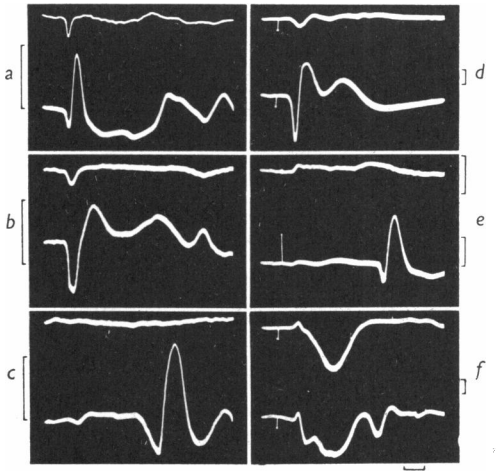

Fig. 8. Responses evoked by stimulation of the contralateral peroneal nerve in various cortical areas before and after flooding the cortex with tubocurarine solution 1:1000. In each pair the lower record is after the flooding. Records *a–c* from a cat under pentobarbitone; records *d–f* from two cats under chloralose anaesthesia (*d* and *f* from the same cat). Records *a* and *d* from primary, *b* and *e* from secondary sensory area, *c* from an area postero-lateral and *f* from an area posterior to primary sensory area. All vertical lines represent 300 μV. The horizontal bar represents 50 msec. Upward deflexion recording electrode negative.

Effect on thalamus. Flooding the cortex with tubocurarine 1:1000 had either no, or only a slight, effect on the focal record from the postero-lateral thalamic nucleus. In two out of four cats under pentobarbitone no effect was seen after the flooding. In the other two there was some increase in random slow wave activity and the appearance of an evoked late, very small but consistent slow wave, which in the one experiment was preceded by a few spikes. In the cats under chloralose the flooding of the cortex seemed always to increase the

random slow activity. In one experiment it also affected the evoked response. This experiment is illustrated in Fig. 6, column *B*. The lowest record shows a typical random slow positive wave and the two records above show the increase in the evoked response about 30 min (at *l*) and 70 min (at *k*) after the flooding. Whereas 30 min after the flooding it was mainly the late positive wave that increased, after 70 min it was the second negative wave which dominated the pattern. At this time the electrode was withdrawn stepwise. As shown in records *j* and *h* the negative wave became even more prominent when the tip of the electrode was raised first 1 and then 2 mm. Further, at all higher levels the focal electrode recorded a greatly enhanced evoked response after the flooding.

Intraventricular injections of tubocurarine

Small doses. In the previous experiments under pentobarbitone sodium (Feldberg *et al.* 1956) the intraventricular injection of 15 μg tubocurarine produced no consistent change in the cortical, and a doubtful increase in spike activity from the focal record from the posterior lateral nuclei. The present experiments were performed in cats anaesthetized with chloralose. In these the intraventricular injection of 20 μg tubocurarine produced no immediate effect on cortical activity, but within 10–20 min, sometimes even later, the pattern of the evoked cortical response changed. Records taken from various parts of the cortex showed that the most consistent changes which developed were an accentuation of the positive, and an attenuation or even disappearance of the negative waves. There was further a greater variability in the evoked responses and the appearance of 'spontaneous' activity. As in the previous experiments the intraventricular injections of 20 μg tubocurarine produced muscular contractions of sufficient intensity to require intravenous tubocurarine.

On the focal record the intraventricular injection of 20 μg sometimes produced spike activity, 'spontaneous' and evoked; however, an intraventricular injection of 0·3 ml. Locke's solution sometimes had the same effect. Apart from this spike activity, which could not be attributed for certain to the action of the tubocurarine, the injections produced no immediate change; but again an effect developed in the course of 10–20 min. The evoked response became more accentuated, particularly the positive wave, and was often followed by the appearance, or an increase in the number, of late slow waves. In addition, 'spontaneous' slow waves appeared. This is illustrated in Fig. 9.

The intraventricular injection of 40 μg tubocurarine accentuated the changes already seen after 20 μg, both on the cortical and thalamic focal records. On the focal record the afferent stimulus now provoked not only accentuation of the positive wave, but more prolonged activity, so that instead of the typical pattern of the waves previously seen there were many smaller fluctuations and

slow waves which sometimes obscured or even obliterated the original pattern. Spikes, if previously present, were sometimes no longer recorded.

In some experiments the focal electrode was inserted stepwise into the thalamus before, and raised again after, the injection of tubocurarine, and records were taken at different levels. A typical experiment is illustrated in Fig. 10, and the diagrams of the coronal section in the plane of the needle (Fig. 11) indicates the tip of the electrode at the various levels from which the records were taken. The upper two were taken with the tip of the electrode in the hemisphere, the third with the tip in the upper margin of the thalamus and the lower four with the tip well within the thalamus.

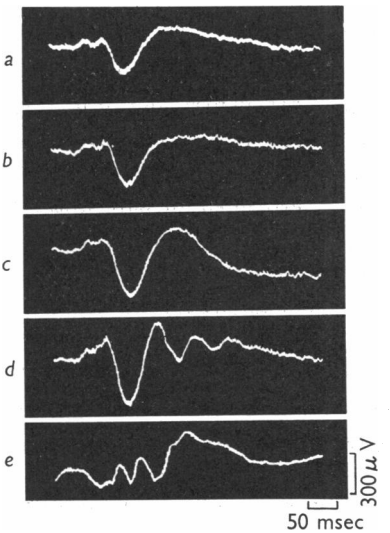

Fig. 9. Changes produced by intraventricular injection of 20 μg tubocurarine in the responses evoked by contralateral stimulation of the peroneal nerve in the postero-lateral nucleus of the thalamus. Evoked responses before (*a*) and 2, 11 and 20 min after the injection (at *b*, *c* and *d*); spontaneous activity 17 min after the injection (*e*). Upward deflexion recording electrode negative.

Before the tubocurarine injection the evoked responses consisted at all levels of a small positive wave only commencing approximately 120 msec after the stimulus. After the intraventricular injection of 40 μg tubocurarine these positive responses were slightly increased in size in the thalamus and followed at all levels by a variable negative wave which was small in the thalamic regions and large in the hemispheres. In the thalamus the negative wave was interrupted by a large positive wave about 250 msec after the stimulus and was followed by irregular negative deflexions. Similar patterns of large waves occurred 'spontaneously' and were positive in the thalamus and negative in the hemispheres.

Large doses. Most experiments were done on cats under chloralose anaesthesia but the results were essentially the same as in cats anaesthetized with pentobarbitone sodium. The intraventricular injection of 200 μg or more tubocurarine caused a large increase in the evoked responses in all cortical areas as well as in the thalamic region. In addition, there were periods of increased activity independent of stimulation of the peroneal nerve. The evoked responses could only be studied in the quiet between these episodes.

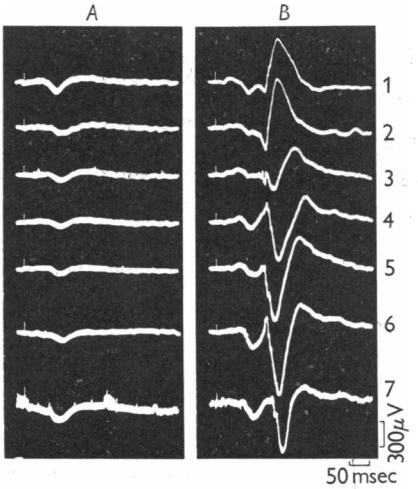

Fig. 10

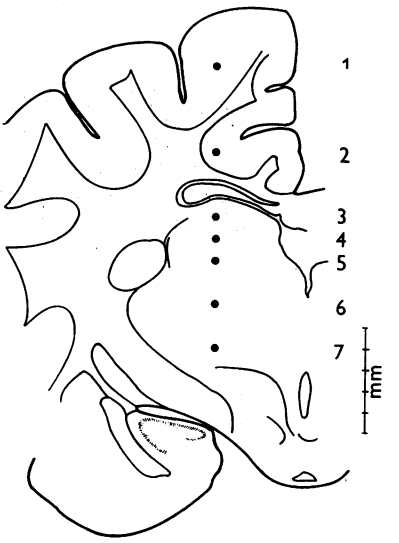

Fig. 11

Fig. 10. Responses evoked in a cat under chloralose anaesthesia by contralateral stimulation of the peroneal nerve at different heights of the thalamus before (column *A*) and after (column *B*) an intraventricular injection of 40 μg tubocurarine. The figures on the right side refer to the points shown in the diagram (Fig. 11) of the coronal section in the plane of the electrode track. At the lowest point the tip of the electrode was in the postero-lateral nuclei. Upward deflexion recording electrode negative.

Fig. 11. Diagram of the coronal section of the cat's brain at 9 mm in front of the Horsley-Clarke zero plane. The numbered parts indicate the positions of the tip of the electrode from which the corresponding records in Fig. 10 are taken.

One of the earliest effects of these intraventricular injections was a conspicuous vasodilatation, which produced a flushed appearance of the exposed surface of the cortex. The effect occurred within a minute after the injection and persisted as long as the episodes occurred.

In the primary sensory area the effect on the evoked response was similar to that described in cats under pentobarbitone sodium. The increase was particularly evident in the surface-negative wave which encroached on the initial surface-positive wave. This accentuation occurred sometimes only 20–30 min after the injection. There were, however, already changes in the

pattern of the evoked response within a few minutes after the injection which consisted of the appearance of a variable number of small waves after the initial complex. This development is shown in Fig. 12. In motor and association areas there was also a period, before the large increase in the evoked response occurred, in which late irregular waves followed the initial evoked patterns. The evoked response in the postero-lateral nuclei of the thalamus increased earlier than that in the cortex and the first change consisted often of additional late waves. Typical changes are illustrated in Fig. 12.

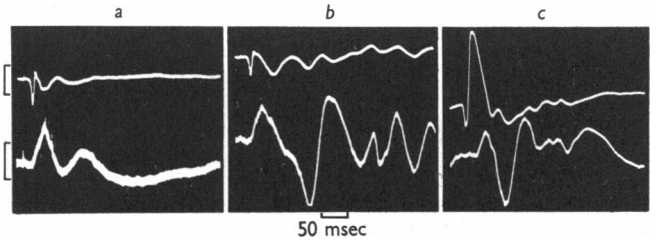

Fig. 12. Responses evoked in a cat under chloralose anaesthesia by contralateral stimulation of the peroneal nerve in the primary sensory area (upper records) and in the postero-lateral nuclei of the thalamus (lower records). At *a* before, at *b* 5 min and at *c* 25 min after an intraventricular injection of 200 μg tubocurarine. All vertical lines represent 150 μV. Upward deflexions recording electrodes negative.

The 'spontaneous' activity which occurred in periods of varying length between which the records were more or less quiescent was observed in all cortical areas from which recordings were made and in the various regions of the thalamus examined. The development of this periodic spontaneous activity followed a characteristic pattern and is illustrated in Figs. 14 and 15. The exact position of the tip of the focal electrodes as obtained from the histological examinations is indicated in the diagram Fig. 13. In the experiment of Fig. 14 it is in the nucleus ventralis pars lateralis; in the experiment of Fig. 15, at the margin of the zona incerta. In both experiments 200 μ tubocurarine was injected and the main changes observed were as follows:

(1) Periods of spontaneous activity occurred first, within 3–5 min, in the focal record without concomitant changes in the cortex and at a time when the evoked cortical responses were not yet increased. They consisted of regular waves occurring first at an increasing frequency and increasing amplitude and eventually stopping more or less abruptly. Fig. 14*a* shows the second episode of such activity occurring 5 min after the injection, and the record starts when the maximal frequency was reached. The first episode had appeared 3 min after the injection. Fig. 15*b–d* shows a similar episode, this time the first, and was obtained whilst the peroneal nerve was stimulated at a frequency of 16/min. In the cortical record this stimulation produced an evoked re-

sponse similar to that obtained before the injection (Fig. 15*a*) but in the focal record the response was occluded by the spontaneous activity.

(2) Between these initial episodes of spontaneous activity when both records were relatively quiescent there occurred from time to time isolated short bursts, again only in the focal record. On stimulation of the peroneal nerve similar large complexes were evoked without pronounced concomitant changes in the cortical response. This phase is illustrated in Fig. 15*e* and *f*.

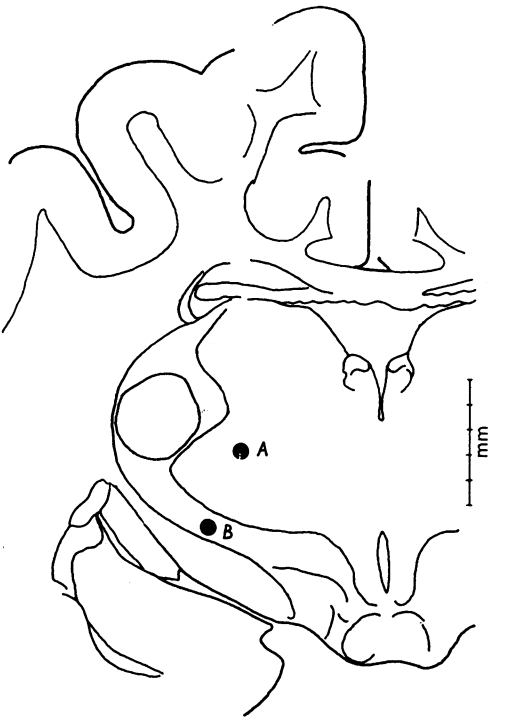

Fig. 13. Coronal section of cat's brain 8 mm in front of the Horsley-Clarke zero plane. The black point *A* gives the position of the tip of the electrode in experiments of Fig. 14; point *B*, position in Figs. 15 to 18.

(3) The beginning of the next phase (Fig. 16) was indicated by the fact that the evoked cortical responses increased in amplitude and showed additional late waves (at *a*). At this stage the occasional short burst of spontaneous activity in the focal record was accompanied by a large cortical wave (at *b*). Before these short bursts developed into episodes of intense activity in both records, they increased in frequency and, in the focal records, underwent characteristic changes. An initial large diphasic wave was followed by a kind of after-discharge consisting of a variable number of regular waves which were only slightly reflected in the cortical record. As these waves developed in the

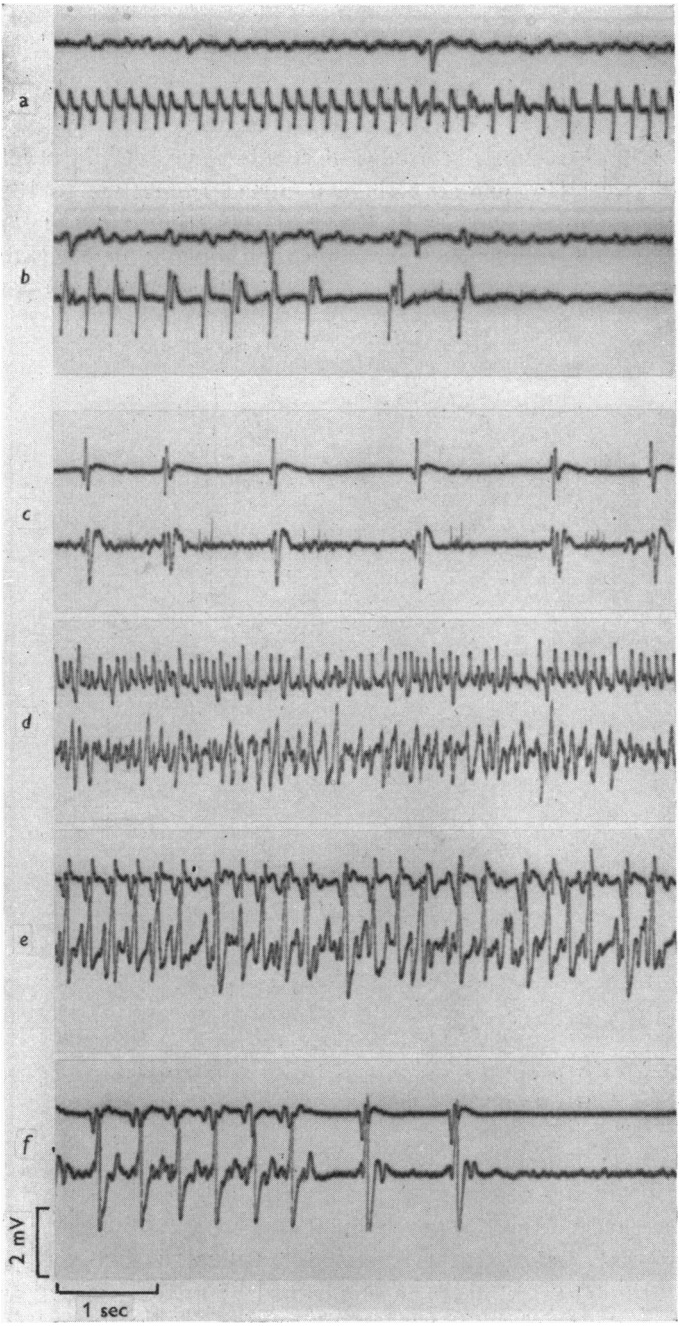

Fig. 14. In each record the upper tracing is from the primary sensory area of the cortex and the lower from the postero-lateral nucleus of the thalamus, in a cat under chloralose anaesthesia after intraventricular injection of 200 μg tubocurarine. Position of tip of thalamic electrode shown in Fig. 13*A*. Records *a* and *b* part of the second episode 5 min after the injection; records *e*, *f* part of an episode starting 70 min after the injection. Between *c* and *d*, 30 sec, between *d* and *e* 95 sec and between *e* and *f*, 40 sec interval. Upward deflexions recording electrodes negative. The voltage calibration refers to the focal electrode (for details see text).

focal record they increased in frequency and number, but decreased in amplitude and often filled nearly the whole interval between the bursts. This phase passed rather abruptly into maximal cortical and thalamic activity consisting of more or less regular waves. Synchronization between cortical and thalamic activity developed only gradually throughout the episode, being more apparent first between the large waves and becoming most complete at the end before the episode ended abruptly. These features are illustrated in Fig. 14, *c–f*, and Fig. 16, *b–e*. Fig. 17 illustrates in addition the asynchronisms during the early phase of an episode. For instance, the large cortical waves at *a*

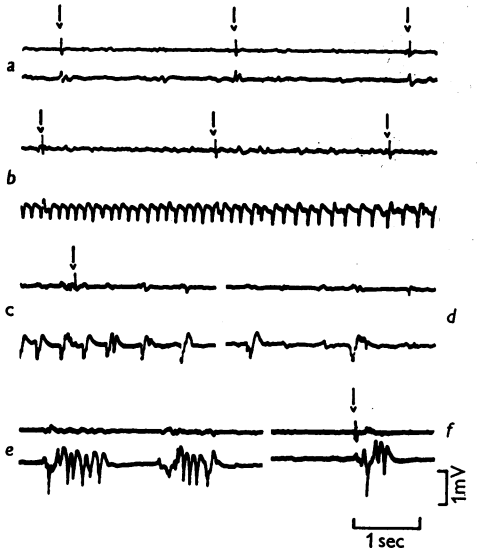

Fig. 15

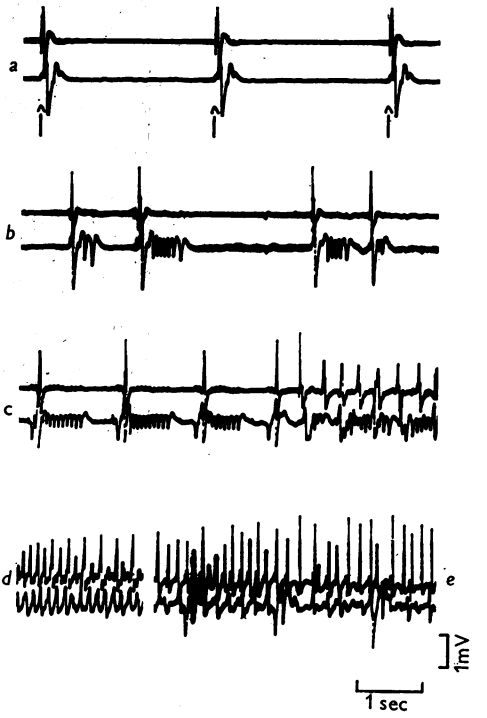

Fig. 16

Fig. 15. In each record the upper tracing is from the primary sensory area of the cortex and the lower from the margin of the zona incerta in a cat under chloralose anaesthesia. Position of focal electrode shown in *B* of Fig. 13. Stimulation of contralateral peroneal nerve at a frequency of 16/min; times of stimuli indicated by arrows. Record *a* control; record *b* first episode confined to focal electrode about 3 min after intraventricular injection of 200 μg tubocurarine. Between *b* and *c* 7 sec, between *c* and *d* 11 sec interval; *e* and *f* 22 min after the injection. Upwards deflexion recording electrode negative. The voltage calibration refers to the focal electrode (for details see text).

Fig. 16. Continuation of the experiment shown in Fig. 15. Record *a* obtained 43 min after the injection. Interval between *a* and *b* 10 min, *b* and *c* 12 sec, *c* and *d* 25 sec and *d* and *e* 32 sec. Stimuli applied to the peroneal nerve at the times marked by arrows.

occurred at relatively regular intervals and the pattern of the focal record seen in *b* also occurred regularly.

(4) The episodes of intense activity often occurred at such regular intervals that the approximate time of onset was predictable. They occurred first at intervals of a few minutes and lasted for 1–4 min. When larger doses than 200 μg were given, the intervals between the episodes became much shorter and at times there was no quiescent period between.

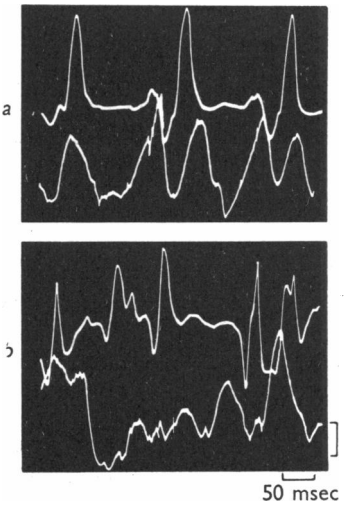

Fig. 17. Same experiment as Fig. 16 showing asynchronism (at *a*) at an early stage of a full episode obtained after a second intraventricular injection of 200 μg tubocurarine given 107 min after the first. Upper tracings from the primary sensory area and lower tracings from the margin of the zona incerta. (Point *B*, Fig. 13.) The horizontal bar represents 50 msec; the vertical bar 250 μV for the cortical tracings and 500 μV for the tracing from the zona incerta.

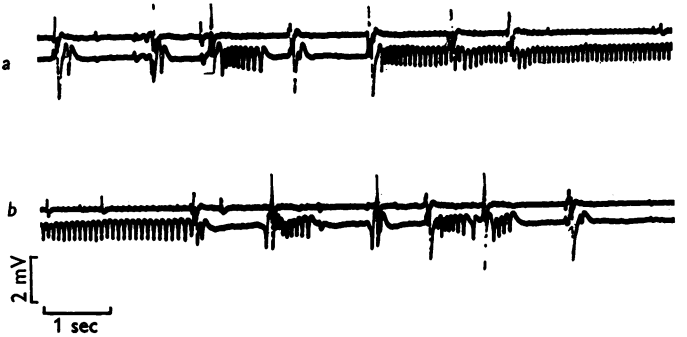

Fig. 18. Continuation of experiment shown in Fig. 16 showing part of an abortive episode about 70 min after the first injection of 200 μg tubocurarine; *b* taken a few seconds after *a*. The voltage calibration refers to the focal electrode.

(5) When the effect of tubocurarine declined, usually after 1·5 hr, the intervals between the episodes lengthened and the duration of the episodes shortened. Later only short abortive episodes were seen which did not develop beyond the usual prodromic symptoms characterized by periods of regular waves in the focal record. Such an abortive episode is illustrated in Fig. 18. When a new injection of 200 μg tubocurarine was given intraventricularly full episodes were again elicited.

(6) In several experiments it was possible to precipitate a full episode, or at the later stages abortive episodes, by a few regular stimuli to the peroneal nerve.

DISCUSSION

The difference in the responsiveness of the brain to afferent nerve impulses under the two conditions of anaesthesia examined requires comment. What is it that determines the differences in the action of pentobarbitone and of chloralose, so that under the influence of the former there is much less spontaneous activity, the response to afferent impulses in the peroneal nerve is restricted to the two discrete sensory areas in the cortex and none or only a small response is evoked in the posterior lateral nuclei of the thalamus; whereas under chloralose anaesthesia wide areas of the cortex respond to the afferent volley which in addition evokes large responses in the thalamic nuclei, and at all levels between them and the cortex, showing that under chloralose the responsiveness to afferent impulses is widespread in cortical and in subcortical regions?

We know from the experiments of French, Verzeano & Magoun (1953), as well as from Arduini & Arduini (1954), that the evoked cortical response in the sensory area, which ascends the classical somatic afferent pathway and is relayed through the ventral nucleus of the thalamus, is resistant to pentobarbitone sodium, whereas the afferent impulses, which pass via collaterals sent off from the classical afferent path to the ascending reticular system, are vulnerable to this anaesthetic so that evoked responses recorded in this region disappear quickly under pentobarbitone anaesthesia. In our experiments under pentobarbitone it was found that the sensory areas of the cortex remained responsive to the afferent impulses, even if the response was smaller than that evoked under chloralose anaesthesia. Infrequently was any evoked response recorded with the focal electrode inserted into the thalamus, and the cortical areas from which evoked responses could be elicited were restricted to the sensory areas. It is not surprising that in our experiments under pentobarbitone scarcely any evoked responses were recorded from the thalamus although it is the site of relay for the primary classical afferent pathway, because, as shown by Mountcastle & Rose (1952) and by Gaze & Gordon (1954), the sites of the relays are located in discrete areas and no attempt was made

to search for them. In addition, these authors reported on the appearance of evoked spikes only, whereas the present experiments were mainly concerned with the large slow waves evoked by the afferent impulses. The unresponsiveness of the thalamus under pentobarbitone could mean either that the activity in this region, as in the ascending reticular system and in other subcortical structures, is vulnerable to pentobarbitone, or that these regions depend upon facilitation from the ascending reticular system and therefore become silent when this system is blocked by pentobarbitone.

The difference observed in the evoked cortical responses between the two types of anaesthesia could also be explained on the grounds that pentobarbitone limits the spread from collaterals of subcortical structures to the cortex. However, this explanation cannot account for the finding that the cortical area from which an evoked response is obtained under pentobarbitone increases on subsequent administration of chloralose. To account for this finding one can suppose that chloralose has a direct excitatory effect which partly counteracts the pentobarbitone. Another possibility is that chloralose blocks mainly inhibitory, and pentobarbitone mainly excitatory, connexions. Neither of these possibilities is sufficient to explain the finding that with topical application of tubocurarine the evoked response in the primary sensory area is augmented under pentobarbitone and chloralose, whereas the evoked response in the secondary sensory area is augmented under pentobarbitone only, giving the impression that the tubocurarine-sensitive units in this area are blocked by chloralose.

The spontaneous activity as well as the evoked response encountered in the thalamic penetration under chloralose was of two types, slow waves and sharp spikes. The fact that the spontaneous slow waves were found with equal frequency at all levels made it impossible to designate any particular area as being their probable source. On the other hand, spontaneously occurring sharp spikes appeared most consistently in a region immediately dorsal to the postero-ventral nuclei. This level is close to the areas depicted by Gaze & Gordon (1954) as responding to tactile stimulation of the head and thus suggests a similar sensory origin. Since in the preparation used in the present experiments there must be considerable stimulation of sensory receptors by the ear, mouth and orbital clamps of the stereotaxic apparatus as well as by the cut margins of the skin of the cat's head, the spikes may well represent axonal action potentials from receptors in these regions arriving at the thalamic relay areas.

The range of latency for both types of evoked thalamic responses is within the range given by Gaze & Gordon (1954) for afferent volleys in the different components of the saphenous nerve, and it is conceivable that while the evoked spikes indicate the arrival of action potentials in axons or their cells at different times the slow waves may be interpreted either by temporarily

dispersed short duration activity of a large number of cells, or, more likely, by longer lasting activity in dendrites of relatively fewer cells following the arrival of volleys in the fastest axons. The relatively discrete localization of electrical signs as a result of activity in axons in comparison with the wide area involved when dendrites are active would account for the fact that the earliest slow or dendritic wave is recorded at all levels of the thalamus but that spikes are recorded at localized points only and with varying latencies.

In the previous paper it was pointed out that tubocurarine by topical application does not reproduce all the effects it produces on intraventricular injection. This conclusion is confirmed by the results of the present experiments in which the exposed cortex was flooded with a tubocurarine solution of a concentration of 1:1000. Even this high concentration did not elicit the characteristic features of intraventricular injections, i.e. the intense flushing of the cortex, the widespread muscular contractions and the generalized increased electrical activity over wide areas of the cortex and in the thalamus. Instead, the topical application, apart from its effect on the evoked responses in the sensory areas, caused only occasional flushing of the cortex, slight motor activity and some random fluctuations, and large waves in areas lateral and posterior to the primary area. Thus topical application of a strong concentration of tubocurarine can produce some cortical excitation. Some of it is corticofugal as evidenced by the slight motor activity. In contrast to the strong activation of the thalamus by the intraventricular injection, the topical application of tubocurarine had usually no, or only a slight, effect in this region, suggesting that no, or only a few, corticothalamic connexions are involved in this cortical excitation.

A common feature of records obtained at all levels in penetrating the thalamus is a positive wave. It is sometimes the sole response, sometimes it is preceded and followed by negative waves of varying size and duration. When the positive wave is the sole response, or when it is followed by a negative wave, it is reasonable to assume that it is from units which are activated some distance away from the tip of the electrode and that the response spreads to the region of the tip in a varying number of these units to produce the negative wave. Since an intraventricular injection of a small dose of tubocurarine increased this positive wave and led to a following negative wave, even when it had not been present previously, the injection must have produced an increase in the excitability of the pathway involved in the early positive response. In addition the injection greatly increased the excitability of another slower or longer pathway to the thalamus as indicated by the large, late, positive wave which occurred at a time at which the evoked response, recorded before the tubocurarine injection, was over. Thus the tubocurarine excites previously quiescent pathways and this excitation is sufficient to start propagated responses which are conducted to the tip of the electrode. As with

the earlier response, the positivity indicates that the main site of excitation is some distance away from the electrode, but no evidence is supplied as to the actual site of this excitatory effect, except that it is clearly not from the lateral regions of the thalamus. Since the injection of 40 μg tubocurarine causes the appearance of a large evoked negative wave at about the same time as a large positive wave occurs in the records taken from the subcortical white matter, there is the possibility that the cortex is the source of the excitation for the late evoked positive wave in the thalamus. In that case the evoked negative wave recorded in the hemisphere would be the sign of a volley descending from the cortex to the thalamus. This would imply, however, that a highly active cortex is produced by the intraventricular injection of 40 μg tubocurarine. Yet this dose never produced such a condition. Moreover, the results obtained by topical application of tubocurarine are also against this explanation. Even in the one experiment in which flooding the cortex with tubocurarine 1:1000 affected the evoked response in the thalamus, the change consisted in an enhancement of the earlier negative wave, which implies that the activity reached right to the region of the tip of the electrode, and was therefore not comparable to that indicated by the late positive wave obtained after the intraventricular injection of a small dose of tubocurarine and resulting from activity at some distance from the tip of the electrode. It may well be that the evoked negative waves in the hemisphere are the result of the increased activity following the initial positive wave occurring about 120 msec after the stimulus, originating in subcortical structures and interrupted in the records obtained from the thalamus by the late positive wave. The abrupt onset of this wave from a small negative one is consistent with this interpretation. At present it is therefore only possible to say that the main centre of excitation produced by the intraventricular injection of a small dose of tubocurarine probably does not lie in the direct afferent pathway, but in a region on some indirect route.

Apart from the changes in evoked responses, the intraventricular injection of tubocurarine produced episodes of abnormal 'spontaneous' activity in cortical and subcortical regions. As pointed out by Samson Wright (1955) this activity showed all the features usually associated with convulsive activity and is therefore possibly responsible for it. However, at the present stage of our knowledge it is not possible to associate the episodes definitely with the convulsive activity produced by tubocurarine on intraventricular injection. The finding that in many instances it was possible apparently to induce an episode by afferent nerve stimulation suggests that the background activity may often act as the conditioning mechanism for these episodes. We do not know at which region of the brain the tubocurarine acts when it causes these episodes of abnormal activity; if, as we suppose, the site is close to the ventricular lining, the abnormal activity recorded from a focal electrode

in the thalamus, several millimetres away from the ventricular lining, must be from regions which are not directly affected by the tubocurarine itself but activated by connexions with those regions on which the tubocurarine acts. It is interesting that these thalamic regions are activated earlier than the cortex because it was found that the initial episodes occurred in the thalamic region but not in the cortex. At this early stage the waves were very regular, which suggests that the neurones of a small section of the total population in the region were discharging synchronously. When at later stages of the tubocurarine action the number of neurones excited became larger, the activity became less synchronous, and spread so as to involve the cortex. The fully developed episodes often began and terminated with bursts of activity that started simultaneously at both sites, but with only a certain amount of synchronism between them during the height of the episodes. At even later stages, when the tubocurarine action was wearing off, cortical episodes were sometimes recorded without or with little concomitant activity in the thalamic region. These findings give no information of either the site of origin, or of the actual spread of the abnormal activity which characterizes the episodes, but they do indicate that the nature of the episode must be determined to some extent by the local conditions in the various regions involved.

The flushing of the cortex which occurred regularly on intraventricular injection of large doses of tubocurarine could not be due to a direct action of tubocurarine on the vessels of the cerebral cortex since it did not occur, or only occasionally, when the cortex was flooded with strong tubocurarine solution. It must be the result of the increased neuronal activity. In fact it is known that during increased cortical activity vasodilatation occurs in those regions of the brain which are activated (Penfield, von Sántha & Cipriani, 1939). According to Penfield & Jasper (1954) the vasodilatation is brought about in the active brain tissue by the formation of a substance 'such as carbon dioxide or perhaps an unidentified substance'. The substance may well be a polypeptide such as substance P which is known to occur in the brain, or bradykinin which might be formed by a mechanism analogous to that responsible for the functional hyperaemia in the salivary glands described by Hilton & Lewis (1955).

SUMMARY

1. The evoked responses of the cat's brain to stimulation of the contralateral nerve were compared under pentobarbitone sodium and under chloralose anaesthesia.

2. Under pentobarbitone sodium evoked cortical responses were obtained in a limited region of the primary and secondary somato-sensory area only, whereas under chloralose a much wider area of the cortex was responsive to the afferent volley.

3. Pentobarbitone sodium injected into a cat anaesthetized with chlora-

lose reduced the size of the evoked cortical responses but not the area involved, whereas chloralose injected into a cat anaesthetized with pentobarbitone sodium enlarged the area responsive to the afferent volley.

4. Under pentobarbitone sodium scarcely any evoked responses were recorded in the nucleus ventralis of the thalamus whereas under chloralose large slow waves were evoked from all levels of the thalamus. In addition, spike activity, spontaneous and evoked, was recorded at various levels of the thalamus. When pentobarbitone was injected into a cat anaesthetized with chloralose the evoked slow waves disappeared and the spike activity was reduced.

5. The effects of topical application of tubocurarine were examined by flooding the exposed cerebral cortex with a solution 1:1000 and compared in cats under pentobarbitone sodium and under chloralose anaesthesia.

6. Under pentobarbitone sodium the topical application caused no flushing of the cortex, i.e. no visible vasodilatation, and only once weak muscular contractions, whereas under chloralose it produced sometimes flushing and always weak muscular contractions.

7. Under pentobarbitone sodium the topical application greatly increased the evoked responses to contralateral peroneal nerve stimulation in the primary as well as in the secondary somato-sensory area, whereas under chloralose it affected in this way the evoked responses in the primary area only. Under both anaesthetics it increased the spontaneous activity and led to the appearance or enhancement of slow waves following the early evoked responses in many cortical regions.

8. The topical application had little effect on the activity in the thalamus. An effect was more regularly obtained under chloralose than under pentobarbitone sodium. It consisted of an increase in random slow wave activity and the appearance or enhancement of an evoked slow wave response.

9. The effects of intraventricular injections of small (20–40 μ) and large (200 μg or more) doses of tubocurarine were examined in cats anaesthetized with chloralose. The results were essentially the same under pentobarbitone sodium anaesthesia.

10. The intraventricular injection of small doses of tubocurarine caused definite changes in the responses evoked by contralateral stimulation of the peroneal nerve. The evoked cortical responses showed an accentuation of the positive and an attenuation or disappearance of the negative waves. The evoked thalamic responses showed an accentuation of the positive wave followed by the appearance of a number of late slow waves. In addition, spontaneous slow waves appeared in the cortex as well as in the thalamus.

11. The intraventricular injection of large doses of tubocurarine caused flushing of the cortex and a great increase in the evoked responses and in the spontaneous activity in all cortical areas as well as in the thalamus. The first

change in the evoked responses consisted of the appearance of a variable number of small waves after the initial complex. The increased spontaneous activity occurred in periods of varying length (episodes) between which the records were more or less quiescent. The development of the episodes showed the following characteristic pattern:

(*a*) Episodes occurred first in the thalamus, without concomitant changes in the cortex, at a time when the evoked cortical responses were unaffected. These initial episodes consisted of regular waves.

(*b*) Later short bursts of activity in the thalamus and in the cortex developed into episodes of intense asynchronous activity at both sites.

(*c*) Synchronization between cortical and thalamic activity developed gradually and became more pronounced before an episode ended abruptly.

(*d*) When the effect of tubocurarine declined, there occurred abortive episodes in many ways resembling the initial ones.

(*e*) It was often possible to precipitate an episode by a few regular stimuli to the peroneal nerve.

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