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THE ROLE OF INHIBITION IN THE PHASING OF SPONTANEOUS THALAMO-CORTICAL DISCHARGE

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In the production of the spontaneous cortical waves at a frequency of about 10/sec, thalamo-cortical discharges of this repetition rate undoubtedly play an important role (Bremer, 1958; Purpura & Cohen, 1962). However, the mechanism underlying the production of the thalamic rhythm is still poorly understood. Recently it has been possible, with the aid of intracellular recording technique, to identify the cellular mechanism underlying the 10/sec rhythmic response of the somatosensory relay nucleus in the thalamus evoked by a single cutaneous volley (Andersen & Eccles, 1962; Andersen, Brooks, Eccles & Sears, 1964). The dominating factor in the timing of this rhythmic activity is the large inhibitory postsynaptic potentials (IPSPs) recorded from thalamic cells under these conditions. Similarly, Purpura and collaborators (Purpura & Cohen, 1962; Purpura & Shofer, 1963) have shown the importance of a regular sequence of EPSPs and IPSPs in the intrathalamic recruitment of active cells following mid-line stimulation at 7/sec.

The present investigation represents, first, a description of an attempt to analyse the cellular reactions leading to, and maintaining, the spontaneous 10/sec rhythmic activity in the thalamus; and, secondly, to provide a theory for the development and decline of this spontaneous spindle activity. In order to achieve this, the analysis has been performed predominantly on cells within the ventrobasal complex (VBC) (Mountcastle & Henneman, 1949) where the histological arrangement and the connexions are such that control can be exercised over the experimental situation. In order to increase the generality of the observations presented, experiments have also been performed on cells in other thalamic loci, including the mid-line nuclei.

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METHODS

Adult cats were anaesthetized by intraperitoneal injection of 40 mg/sodium pentobarbitone per kg body weight. The cat was maintained in a lightly anaesthetized state characterized by a definite 'light reflex' and with the withdrawal of the front paw to a hard pressure just abolished; supplemental anaesthetic was given as required. The superficial radial (SR), median (M) and ulnar (U) nerves were isolated and mounted for stimulation on bipolar platinum electrodes. In many experiments, the cuneate nuclei were exposed so that a surface recording electrode could monitor the afferent volley from the peripheral nerves; in addition, by the application of a stimulating electrode, it was possible to provide monosynaptic activation of the thalamic sensory relay nucleus. Sufficient skull was removed to expose the convexity of the cerebral cortex. The middle portions of the lateral, suprasylvian and ectosylvian gyri were sucked away to open the lateral ventricle. With a thin glass seeker, the fimbria and hippocampus were deflected to expose the dorsal surface of the thalamus. The ventrobasal complex of the thalamus is situated 4–7 mm from the mid line and 6–9 mm deep to the dorsal surface of the thalamus according to the antero-posterior and lateral locations. The anterior-posterior landmark taken was the oblique groove between the thalamus and the anteriorly situated striatum. When the electrode was inserted vertical to the Horsley Clark reference plane and 6 mm from the mid line, the point of entry had to be between a point 2 mm in front of this groove and another point 2 mm behind it in order to enter the ventrobasal complex. Additional guidance was provided by using ordinary stereotaxic atlases. There was no great discrepancy between the two methods. The exposure of the dorsal thalamus gave shorter penetration distances and as a consequence there was greater accuracy in placing the electrodes, less possibility of damage to them, and reduction of the capacitive effect of the enclosing tissues on the electrodes. The VBC cells were identified by antidromic invasion following a stimulus to the post-cruciate gyrus. Most experiments were performed on n. ventralis postero-lateralis (VPL).

Recording from the thalamus was made with glass micro-electrodes filled with either 4 M-NaCl (1–4 MΩ), 2 M-KCl (5–15 MΩ) or K-citrate (5–15 MΩ). Recording from the cortical surface was made by a platinum wire ending in a small ball. When the surface electrode was shunted by seeping blood or cerebrospinal fluid, as in the recordings from the white matter, a straight wire electrode insulated to the tip was employed. Both a.c.- and d.c.-coupled amplifiers were used.

In addition to the acute experiments, thirteen experiments were performed on cats which had various parts of the cerebral cortex removed from 5 to 22 days previously.

Histological control was made by leaving the micro-electrode in the brain, and fixing it *in situ*. Sections parallel to the electrode track were made and stained by cresyl violet. Alternatively, the electrode was moved several times up and down, so producing an identifiable track in the histological section.

RESULTS

Thalamic spindles. The spontaneous rhythmic activity extracellularly recorded in the thalamus had a quite typical appearance, irrespective of the location of the electrode (Figs. 1*A*, *B*; 2*F*, *G*). The main elements were a burst of spike discharges followed by a positive wave (P-wave). The spike discharges occurred within periods of 20–40 msec whereas the P-wave lasted for 80–150 msec, largely depending upon the depth of the anaesthesia. Figure 1*A* and *B* show the start of two spontaneously occurring periods of rhythmic activity in the VPL nucleus. The activity appears

as groups of spikes separated by large P-waves. The P-wave has a steep onset followed by a slower return leading up to the next discharge. The development of rhythmic activity is characterized by augmenting P-waves, often with only a few spikes terminating the first few P-waves. A group of large spikes occurs first after the second P-wave in Fig. 1 *A* and similarly in Fig. 1 *B*, where only a single spike is seen after the first P-wave. The whole assembly of a series of burst discharges separated by P-waves will be called a spindle—in analogy with the cortical barbiturate spindle.

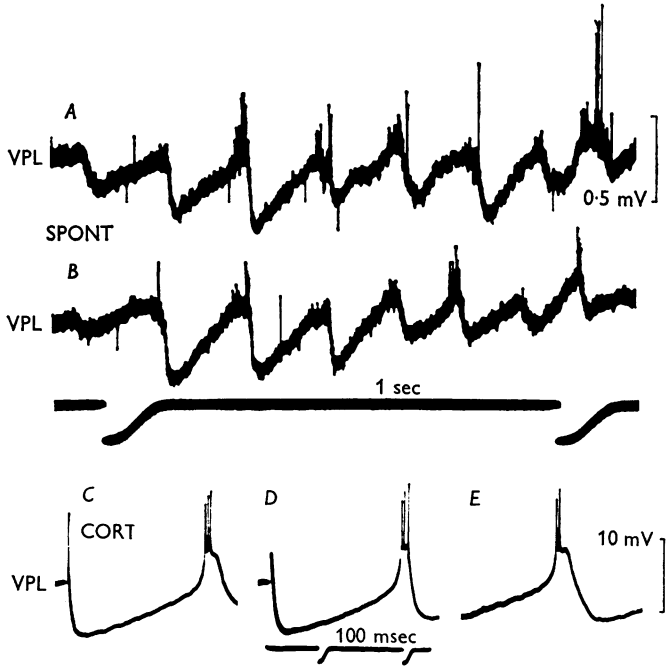

Fig. 1. Appearance of spontaneous rhythmic activity in the thalamus (spindles). *A* and *B*. Extracellular records of the start of two thalamic spindles. Negativity upwards. Spontaneous rhythmic activity. *C* and *D*. Intracellular records from a cell in the postero-lateral ventral nucleus (VPL) in response to a single shock applied to the post-cruciate subcortical white matter. *E*. As *C* and *D*, but the potential sequence occurred spontaneously during a spindle period. In *C–E*, positivity upwards. VPL, n. ventralis postero-lateralis. CORT, stimulation of subcortical white matter. SPONT, spontaneously occurring potentials.

Within a spindle the frequency of the repeating cycles, each consisting of a burst of spike discharges and a P-wave, varied between 5 and 12/sec, being usually around 8/sec.

During progressive circulatory failure, the spontaneous spindles and the evoked burst discharges disappeared before the thalamic field potential

evoked by a cutaneous volley. An increase of body temperature gave an increased repeating frequency of the groups of spikes within a spindle, as first described by Bremer (1958) for the cortical spindles and attributed by him to an effect on the thalamus.

The depth of the anaesthesia was the factor of most striking influence on the thalamic spindle pattern. Our observations on this topic are in full support of the excellent description given by Verzeano, Naquet & King (1955) on the effect of barbiturates on mid-line thalamic spindle activity. Injection of more barbiturate lengthened the P-waves and correspondingly there was a reduction of the frequency of burst discharges within the spindle. Comparable effects of barbiturate were observed with the rhythmic burst discharges to a single cutaneous volley (Andersen *et al.* 1964).

The time interval between spindles, and the duration of the latter, also varied with the depth of the anaesthesia, the spindles appearing more frequently and being of a longer duration as the anaesthesia lightened. A recurrence of the spindles at about 10/min was often recorded and each spindle usually lasted for 1–3 sec. Irrespective of variations in the spindle frequency, spindle duration and repeating frequency, the components were always the same, a burst of spikes followed by the long P-wave.

Often there was a definite correspondence between the spindles of the VBC and the rhythmic activity of the corticogram recorded simultaneously from the lateral part of the post-cruciate gyrus. Each burst of thalamic spikes was correlated with a cortical slow wave (Fig. 2*F*, *G*). However, in other cases, the thalamic spindle had a shorter duration than the cortical spindle and also occurred somewhat out of phase with the latter. Thus, the connexion in time between the two spindle types is not a rigid one.

Relation of spontaneous spindles to the rhythmic burst response. Spontaneous thalamic spindles can be recorded from all major nuclei, including the somato-sensory relay nucleus, the ventralis postero-lateralis (VPL) as shown in Figs. 1 and 2. In Fig. 1*C–E* are intracellular records from a VPL cell. Figure 1*C* and *D* show the initial part of the rhythmic response to a single antidromic volley delivered by an electrode in the white matter underlying the post-cruciate gyrus. All sensorimotor cortex, including area SII, had been removed 8 days earlier. Whether the antidromic spike was large (*C*) or small (*D*), there was a large and long-lasting IPSP. This was terminated by a depolarizing wave which gave rise to three spikes followed by another IPSP. For comparison, *E* is an excerpt from a spontaneous spindle recorded from the same cell some seconds later. The pattern of an IPSP followed by a depolarizing wave with spikes and another IPSP is nearly similar to the responses in *C* and *D*, except for a slower rise-time of the IPSP. The duration of the hyperpolarizing potential

was the same in the two cases, as also was the interval between two successive bursts. Finally, the repeating nature of the response with successive IPSPs was common to both types of responses.

The upper traces in Fig. 2*A* and *B* show the focal potential in VPL in response to a single volley in SR or U nerves, respectively. The lower traces are the records from the foreleg area of the post-cruciate gyrus. Figure 2*C* and *D* illustrate how a single cutaneous volley produces a rhythmic burst response, that is, a series of spike discharges with intervals

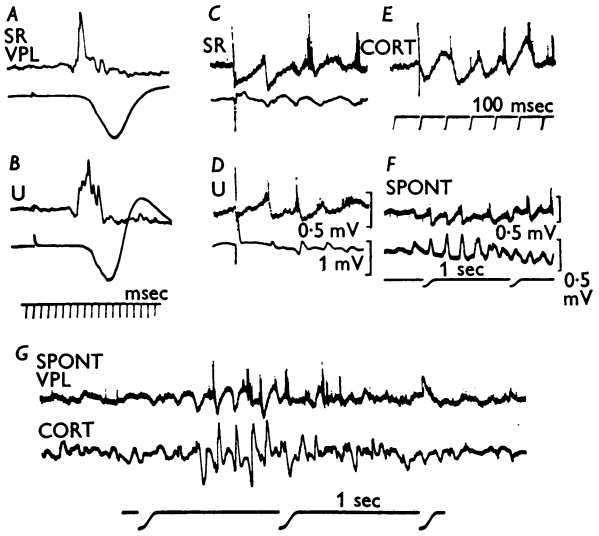

Fig. 2. Spindle activity in VPL. In each pair of records, the upper trace is recorded from the same site within VPL, and the lower trace from the foreleg area of the post-cruciate gyrus. In *A* and *B*, there are the field potentials in response to stimulation of the superficial radial (SR) and ulnar nerve (U), respectively. With slower sweep, single shock stimulation of the same nerves (*C*, *D*) is seen to evoke a rhythmic activity in VPL as well as in cortex. The deflexions in the cortical records occur simultaneously with the bursts of discharges in the VPL. *E*. Similar rhythmic thalamic activity as in *C* and *D*, but evoked by a single shock applied to the post-cruciate cortex. *F*. Spontaneous spindle activity with correspondence between the thalamic burst discharge and the slow cortical waves. *G*. Spontaneous spindle recorded with slow sweep speed. Extracellular recording, negatively upwards. Voltage calibration in *D* applies for all records except *F*. Time scale under *E* applies also for *C* and *D*.

of about 150 msec (Adrian, 1941; Andersen *et al.* 1964). Each group of spikes is followed by a positive wave (P-wave). Corresponding to each discharge, there appears a wave in the cortical record (lower traces), indicating that this rhythmic discharge takes place in thalamic cells projecting to the cortex. In Fig. 2*E*, a single shock was applied to the post-

cruciate cortex, close to the cortical recording electrode. In VPL, there appears after the antidromic invasion of a small number of cells, a typical series of P-waves, each terminated by a group of cell discharges, just like that produced by a cutaneous volley in Fig. 2*C* and *D*. Since the same rhythmic thalamic activity was recorded from VPL in cats in which all sensorimotor cortex had been removed 5–13 days earlier, so that the cortico-thalamic fibres presumably had degenerated, the reported result must presumably be due to the effect of antidromic conduction in thalamo-cortical fibres only.

The records in Fig. 2*G* are taken from the same electrode location as Fig. 2*A–E*. Typically, the spindle consists of a series of growing P-waves and bursts of cell discharges. In the first half of the spindle, each assemblage of a group of spikes and a following P-wave has a definite slow cortical wave as its counterpart. Similar synchrony between the thalamic discharges and the cortical slow waves is seen in Fig. 2*F*, taken from VPL and the post-cruciate cortex of another cat. In the last half of the spindle in Fig. 2*G*, the thalamic cell discharges occur more asynchronously and correspondingly there are no synchronous large cortical waves.

In conclusion, there is a remarkable similarity in pattern between the spontaneously occurring thalamic spindles and the rhythmic burst discharges to a single cutaneous or an antidromic volley. The main element in both is a group of cell discharges followed by a positive wave. The main difference in appearance between the spontaneous thalamic spindle and the induced burst discharges is the slow building up of the sizes of the P-waves in the spindle, whereas in the induced burst discharges, the first P-wave is usually the largest. In the case of the burst discharges, intracellular recording has shown that the extracellularly recorded positive wave is due to the near-synchronous production of large and long-lasting IPSPs in a great number of cells in the somatosensory relay nucleus of the thalamus (Andersen & Eccles, 1962). It would now appear that the same mechanism is responsible for the P-waves of the spontaneous thalamic spindles.

Intracellular recording of spontaneous thalamic spindle activity. Figure 3 shows intracellular records obtained with a d.c.-coupled amplifier from two neurones within VPL (*A*, *B*) and from a neurone in the centre median, CM (*C*). In *A*, the two records *a* and *b* are continuous. The time scale serves as a reference line and indicates a potential of -100 mV. Two fully developed spindle periods are shown under the broken lines. The characteristic pattern of a spindle is a series of hyperpolarizing waves, each having a duration of about 150 msec. For the following reasons these hyperpolarizing waves can be classified as inhibitory post-synaptic potentials (IPSP): they may occur without an immediately preceding dis-

charge of the cell, they may be enhanced by the passage of a depolarizing current and reversed in sign by passing a hyperpolarizing current or by the passage of Cl^- ions out of a KCl electrode, and, finally, they are associated with inhibition of spontaneous discharges as well as inhibition of synaptically or antidromically evoked discharges of the cell.

The start of a spindle is particularly well seen in the first part of Fig. 3*Aa*. The successive IPSPs grow in amplitude and are summed to cause

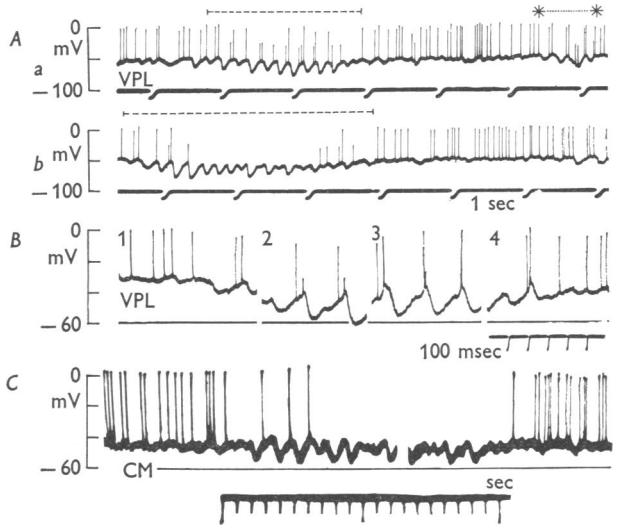

Fig. 3. Intracellular records of spontaneous thalamic spindles. All records are taken with a d.c.-coupled amplifier. *A*. *b* is the continuation of *a*. The time scale tracing also indicates the −100 mV level. Two spindles are indicated by broken lines, and an abortive spindle by two asterisks connected by an interrupted line. *B*. Excerpts of records taken with a d.c.-coupled amplifier at various stages during different spindles. 1 and 2 are two stages of the start of the spindle, 3 is from the middle, and 4, from the end of the spindle. *C*. Excerpts of records taken from a cell in the centre median (CM), the first showing the onset of a spindle, and the last, the end of another. Time scale, $\frac{1}{10}$ and 1 sec.

an average hyperpolarization of the cell. On top of each crest between two successive IPSPs, one or more spike discharges occur. Many of the spikes are not fully developed SD spikes, but appear as IS spikes only. The most probable reason for this partial failure was the hyperpolarization of the cell. Where this hyperpolarization became more pronounced, as in Fig. 3 *Ab*, the cell was prevented from firing altogether. The last spike before the pause during this spindle was an IS spike, and so were the first three which inaugurated the firing after the spindle. Between the spindles in *A*, the cell was in a state of continuous, but irregular, discharge at about 12/sec.

Not all spindles reach the full development just described. An abortive spindle is indicated by two asterisks to the right of Fig. 3*Aa*. The IPSPs are discernible and so is the phasing of the spike discharge between them. Two spikes occurring in a period of hyperpolarization were IS spikes. However, the spindle did not develop further than three consecutive IPSPs and the membrane potential then resumed its interspindle value.

Figure 3*B* is taken from another VPL cell. Excerpts from d.c. records taken during different spindles are mounted together to illustrate various stages of a spindle. Figure 3*B*1 shows the slightly irregular firing during the interspindle period and the increasing amplitudes of the IPSPs at the onset of the spindle. In Fig. 3*B*2, these IPSPs were very much larger and the membrane potential was increased from about -32 to -60 mV. On the declining phases of the IPSPs, there appeared spike discharges, the first being a SD spike, the following only an IS spike. Excerpt *B*3 illustrates the regular sequence of the fully developed IPSPs and how the spike discharges, consisting of both IS and SD spikes, were confined to a very limited span of time between two successive IPSPs. Thus, the IPSPs determine the time of occurrence of the cell discharges and, therefore, act as the main phasing device for the spontaneously occurring thalamocortical volleys. In *B*4, the IPSPs were decreasing, the membrane potential of the cell was falling and the cell resumed its irregular and continuous firing.

Figure 3*C* shows the start of one spontaneous spindle, and the end of another, recorded from a cell in the centre median (CM). As in the VPL, during the spindle period there was a series of large IPSPs which hyperpolarize the cell and first gave rhythmic discharges and later total inhibition of the cell discharges.

The patterns of increasing IPSP that sums and phases the discharge of the cell, have been seen in cells in the following thalamic nuclei: nn. ventralis postero-lateralis, ventralis postero-medialis, ventralis medialis, ventralis lateralis, centre median, centralis lateralis, and the PO group of Mountcastle & Powell (1959). In all cells, the IPSPs produced either a phased discharge of the thalamic cells, or even brought the cell to such a hyperpolarized level that total inhibition of the cell discharges ensued.

Extracellular spindle activity was recorded from all the nuclei listed above, and also from nn. ventralis anterior, lateralis posterior, lateralis dorsalis, geniculatum laterale, geniculatum mediale, centralis medialis and dorsalis medialis.

Various patterns of spindle response. Although the basic characteristic pattern of summing IPSPs has been seen in all thalamic cells recorded intracellularly, certain variations have been encountered and are illustrated in Fig. 4. In Fig. 4*A–C* are sections of d.c. records of three different

spindles from a CM cell in a chronically decorticate cat. The average hyperpolarization produced by summed successive IPSPs is clearly seen in *A* and *B*. These two excerpts also illustrate the occurrence of the cell discharge on the crest between two IPSPs. *C* shows the return of the membrane potential to the lower interspindle level. It could be argued that the cessation of the spike discharges during the spindles is a sign of cell damage owing to the impalement. However, the described effect may also

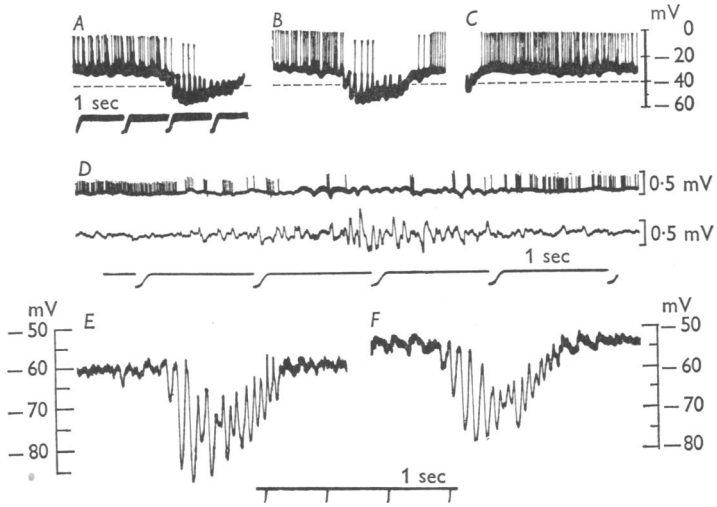

Fig. 4. Various forms of thalamic spindle activity. *A* and *B* are two spindles and *C* is the end of another spindle from the same VPL cell in a chronically decorticate cat. Intracellular records taken with a d.c.-coupled amplifier. *D*. Upper trace is a recording of a single VPL unit; lower trace is the corticogram of the post-cruciate gyrus. Extracellular records. The record shows cessation of the spontaneous firing of the VPL unit during the spontaneous spindle activity. *E* and *F* are intracellular records, taken with a d.c.-coupled amplifier, of two spontaneously occurring spindles in a cell in n. centralis lateralis.

be seen in extracellular records (Fig. 4*D*, upper trace). The lower trace is the corticogram recorded from the post-cruciate gyrus. Figure 4*E* and *F* show two successive spindles recorded from a cell in the nucleus centralis lateralis (CL). The time between the two records was 11 sec. This cell was not excited either in the interspindle period or during the spindles. In spite of this, it showed the typical spindle pattern of augmenting and summing IPSPs. Two remarkable features of the spindles can be seen. First, the hyperpolarization is very powerful, and brings the membrane potential up to -80 to -85 mV, which presumbly is very close to the E_{IPSP}. Secondly, the end of the spindle is strikingly different from the onset. It takes a longer time, and the IPSPs in the tail of the spindle are shorter

and smaller than those in the developing phase. The shorter duration and smaller size of the IPSP suggest that the cells responsible for their production have lost the synchrony they had in the early part of the spindle.

Some information on the process involved in the disruption of the rhythm could be gained from experiments like that illustrated in Fig. 5. In *A*, a single cortical shock causes an antidromic invasion of the cell, followed by a rhythmic activity where the first burst discharge is marked with a triangle. This indication mark is repeated under the other records. In *B*, *C*, and *H* a second cortical shock is delivered some time before the anticipated occurrence of the first burst discharge. This latter activity is markedly delayed in *B*, and abolished altogether in *C*. However, when

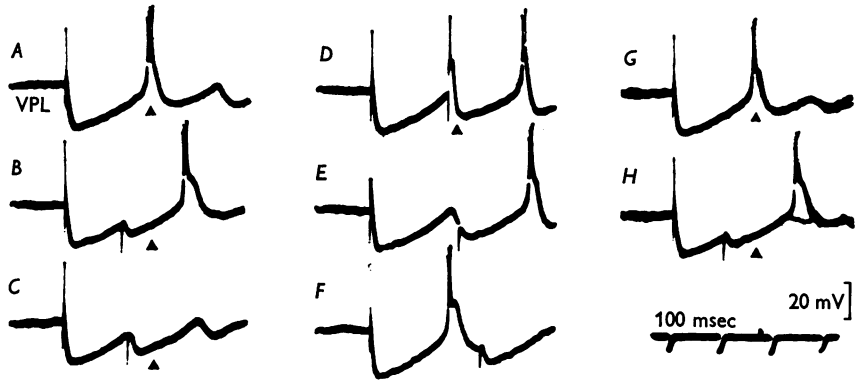

Fig. 5. Resetting of thalamic rhythm by an antidromic volley. All traces are intracellular records taken with a d.c.-coupled amplifier from a VPL cell. All sensorimotor cortex was removed 8 days earlier, presumably resulting in the degeneration of any cortico-thalamic fibres. *A*. Rhythmic activity evoked by a single volley conducted antidromically in the thalamo-cortical fibres. The antidromic spike is followed by an IPSP, then a depolarizing wave with spike discharge—the first burst discharge—then a second IPSP, terminated by a smaller depolarization, and, finally, a third IPSP. A second antidromic volley arriving before the anticipated time of occurrence of the burst discharge (▲) delays the latter (*B*), or even abolishes it (*C*). Arrival of the second volley at the time of the first discharge (*D*) re-inforces the rhythmic activity, but when the second volley is delivered later, the following burst discharge is again delayed (*E*) or abolished (*F*). The resetting of the rhythm can be seen clearly by comparing *G* and *H*. Three superimposed traces.

the second shock coincides with, or occurs just before the anticipated appearance of the first discharge (*D*), the rhythmic activity is re-inforced. On the other hand, if the second shock is delivered somewhat later, it either delays the occurrence of the second burst discharge (*E*) or this discharge is abolished altogether (*F*), depending upon how late the second shock is given. The re-setting of the rhythm is particularly well seen in *G*

and *H*, both records being composed of three superimposed traces. In *H*, the burst discharge is delayed twice and abolished once.

Phasing of the thalamic discharges during a spindle. In Fig. 6 are shown a.c. records from a relay neurone in VPL to illustrate in more detail the manner in which the spikes are initiated and timed during the spindle. Columns 1, 2 and 3 show excerpts from the start, middle and the end of various spindles, and *A* and *B* are taken at two different speeds. When a spike discharge occurs, it does so between two consecutive IPSPs. How-

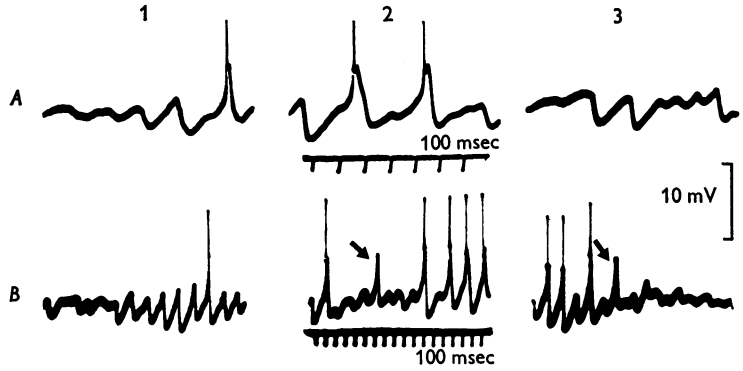

Fig. 6. Triggering of discharges within a spontaneous thalamic spindle. Intracellular records taken from a VPL cell with an a.c.-coupled amplifier. *A* and *B* are recorded at two different sweep speeds. Column 1 is from the start, 2 from the middle, and 3 from the end of different spindles. The spike discharges arise from slow depolarizing waves, usually occurring after large IPSPs. Some depolarizing waves do not result in the discharge of the cell (arrows).

ever, a spike is not generated merely by the return of the membrane potential to an average level, but requires a distinct depolarizing potential to trigger it off (*A* 1, 2; *B* 1, 2, 3). Sometimes these depolarizing potentials were not large enough to reach the critical firing level for the spike (arrows). The depolarizing waves occur at the end of an IPSP (Fig. 1*C–E*; Fig. 6*A* 1, 2; *B* 1, 2), but not after all IPSPs (Fig. 6*A* 1, 3; *B* 1). It is a possibility that they represent excitatory post-synaptic potentials (EPSPs), but their rise-time and duration are very slow, 5–20 and 20–80 msec, respectively. The EPSP generated in the same cell type by a synchronous cutaneous volley had a rise-time of only 1–1·5 msec and a total duration of about 10 msec (Andersen, Brooks & Eccles, 1963; Andersen *et al.* 1964). Since the nature of these waves is not known, the term depolarizing waves will be adhered to in this presentation.

Thalamic cells were not brought to discharge following each IPSP. Even if they gave a rhythmical discharge in the early part of a spindle, as in Fig. 5*A*, *B*, they usually failed to discharge later in the spindle.

Relation between repetitive driving of thalamic cell discharges and spontaneous spindle activity. Orthodromic and antidromic repetitive stimulation at a frequency near that of the spontaneous spindle burst discharges will recruit new cell and subsequently faithfully drive many neighbouring cells (Andersen *et al.* 1964). Since the average membrane potential in VPL cells increases during a spontaneous spindle (Fig. 3, 4), it can be asked what effect this increase has on the response of the cell to repetitive antidromic invasion. In Fig. 7, each horizontal line shows d.c. records

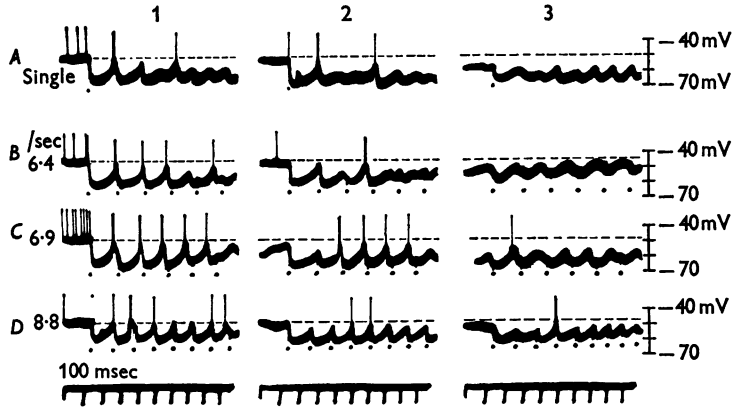

Fig. 7. Interference between repetitive antidromic volleys and spontaneous spindle activity. All traces are intracellular records taken with a d.c.-coupled amplifier. In column 1, the records are taken in a period between spindles, in column 2, during the start of a spindle, and in column 3, during a fully developed spindle. *A*. Single antidromic volley. *B–D*. Stimulation of the post-cruciate white matter at the indicated repetition frequencies, eliciting antidromic volleys in the thalamo-cortical axons. The repetitive antidromic volleys produce less rhythmic activity when they arrive during a spontaneous spindle (column 3) than in interspindle periods (column 1).

from a VPL cell taken at various stages of spontaneous spindles. Column 1 is from the interspindle period, column 2 from the initial, and column 3 from the fully developed stage of a spindle. The animal had its somatosensory area I removed 13 days earlier. Figure 7 *A* shows the responses to single shock stimulation of thalamo-cortical axons in the white matter of the post-cruciate gyrus: *B* to *D* show the effect of repetitive stimulation of the white matter at the frequencies indicated to the left of each line. The interrupted lines are drawn to indicate the membrane potential of the cell at the onset of the stimulation. In the interspindle period, a single shock (*A* 1) caused a series of repeating IPSPs, each leading to the cessation of the spontaneous firing and the start of rhythmic firing on the termination of two IPSPs. At a slightly higher membrane potential (*A* 2) the pattern

was similar, but at a still higher membrane potential (*A* 3), caused by a spontaneous spindle, the effect of the repetitive stimulation of the white matter was markedly less. Only small repeating IPSPs were seen, and no discharge of the cell occurred.

A related pattern was seen in response to repetitive stimulation (*B–D*). In the interspindle periods (column 1), the most regular discharge occurred at stimulation of 6·9/sec. At stimulus rates slightly above or below this value, there was also some following of the stimulus frequency. However,

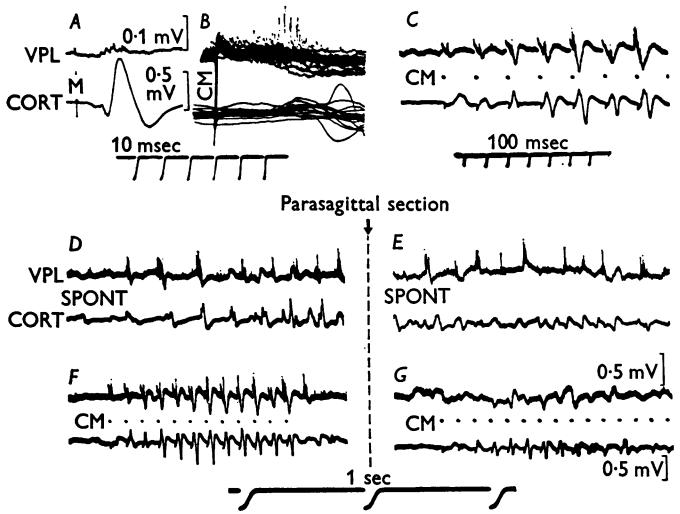

Fig. 8. Independence of VPL rhythm of the connexions from the thalamic mid line. All records are extracellular. Upper trace is the activity of VPL, lower trace shows the post-cruciate corticogram. *A*. Thalamic field potential (VPL) and cortical surface record (CORT) in response to a single shock to the median nerve (M). *B*. Superimposed traces of VPL and pericruciate responses to 7·5/sec stimulation of the centre median (CM). *C*. As *B*, but recorded on moving film. *D*, *E*, spontaneous spindle activity before and after a parasagittal section 4 mm from the mid line, severing all connexions between the mid-line structures and the VPL, respectively. *F*, *G*, VPL and cortical responses to 7·5/sec CM stimulation before and after the same section as described above, respectively. The VPL recruiting responses disappear after the section, whereas the corresponding cortical responses are greatly reduced.

during the developing phase of a spindle (column 2), only the critical stimulation frequency was capable of producing a regular cell discharge (*C* 2). During the fully developed spindle (column 3) the cell was so hyperpolarized that not even the optimal repetition frequency could produce driving of the cell and there were only a few random discharges.

In conclusion, the hyperpolarization during the spindles markedly impairs the ability of the cell to respond with a series of regular discharges

to repetitive antidromic invasion. This interference clearly indicates the inhibitory nature of the summing IPSPs that produce the typical spindle pattern. Basically, there are similar results with orthodromic stimulation, but the pattern is slightly more complex because of the synaptically evoked discharges with short latency.

Local nature of the thalamic spindle activity. The spindle activity does not require the presence of an intact sensorimotor cortex. This is the case both for acute (Figs. 1*A*, *B*; 6; 8*E*) and chronic decortication (Figs. 1*C–E*; 3*B*; 4*A–C*). Spontaneous thalamic spindle activity is also known to persist after section of the brain stem at a mesencephalic level (Bremer, 1937; Dempsey & Morison, 1943). In order to test whether the spindle activity recorded in VPL is initiated by impulses generated in or near the mid-line nuclei, the following section experiment was performed (Fig. 8). The electrode was first placed in the VPL by employing as a criterion the short latency focal potential in response to stimulation of three foreleg nerves, the response to M being shown in Fig. 8*A*. A stimulating electrode was placed in the centre median and stimuli given at 7·5/sec (Fig. 8*B*, *C*). In *B*, the superimposed records show that the spikes so produced in the VPL have a considerably longer latency than the field potential due to a cutaneous volley. The cortical record (lower trace) indicates that there was a growth of cortical potential which coincided with the appearance of the spikes and a following positive wave (P-wave) in VPL. This relation is better seen in *C* where the responses are recorded on moving film. The build-up of burst discharges separated by augmenting P-waves is similar to the alternating sequences of EPSPs and IPSPs in cells in the ventral thalamic nuclei following mid-line stimulation at 7/sec (Purpura & Cohen, 1962).

There was spontaneous spindle activity (Fig. 8*D*) from the same location as for the records in Fig. 8*A–C*. The thalamic P-waves appear shorter than usual because a shorter time constant of the amplifier was used. A parasagittal section, 4 mm from the mid line was then made, severing all connexions from the mid-line nuclei and the centre median to the VPL. After the sectioning, spontaneous spindle activity was recorded from VPL as before (Fig. 8*E*), and from other nuclei lying more dorsally, whose connexions from the mid-line nuclei were also severed. The definite recruitment of cell discharges in VPL in response to 7·5/sec stimulation in CM (Fig. 8*F*) disappeared after the parasagittal section (Fig. 8*G*) and the corresponding cortical recruitment was also considerably reduced. This was not due to damage to the VPL, since the field and spike responses evoked by nerve stimulation were attained as readily as before. Thus, although stimulation of mid-line thalamic structures can produce powerful recruitment in VPL, connexions from the mid-line nuclei are not necessary

for producing or maintaining the spontaneous spindle activity recorded in that nucleus. We conclude, therefore, that the spontaneous thalamic spindle activity is neither dependent on the integrity of the connexions with the cortex, nor with the lower parts of the brain stem, nor with mid-line structures in the thalamus itself. This spontaneous activity seems to be purely an intrathalamic event. The minimum amount of thalamus necessary to provide conditions for thalamic spindle activity remains to be determined, as also the importance of the striatum in this context.

DISCUSSION

Appearance of thalamic spindle activity. The typical pattern of the thalamic spontaneous spindles, as seen in extracellular records, is identical with that recorded by Galambos, Rose, Bromiley & Hughes (1952) from the medial geniculate nucleus and by Verzeano & Calma (1954) and Verzeano *et al.* (1955) from the mid-line nuclei of the thalamus. Furthermore, this pattern is virtually the same as that comprising the rhythmic burst discharges evoked by single shocks either to a peripheral nerve or to the sensorimotor cortex (Andersen *et al.* 1964).

The increasing number of spikes in the first few groups of cell discharges indicate that a growing number of neighbouring thalamic neurones are brought to fire in near synchrony. However, it is not a prerequisite for the development of a spindle that each neurone fires in each of the successive groups of spikes. A given cell may fire in one burst, then pause during several of the following groups of spikes, and resume firing in later bursts of the same spindle (Figs. 1*F*; 2*G*, *H*). Such a pattern shows that the link between the participating neurones is not rigid, but that the individual thalamic neurone needs to be conditioned to a certain stage of excitability in order to be brought into the spindle firing.

The earlier descriptions of the thalamic spindles in the pioneering work by Morison and his associates (Morison, Finley & Lothrop, 1943*a*, *b*; Dempsey & Morison, 1942, 1943), mostly concerned the positive waves. The slow waves recorded by Morison and his group are identical with the positive waves of the micro-electrode recordings. It is now known that the P-waves are due to the IPSPs produced by a recurrent inhibitory pathway (Andersen & Eccles, 1962; Andersen *et al.* 1964). Morison *et al.* (1943*a*) concluded that spontaneous spindles were seen only in the medially situated nuclei of the thalamus, but not in the more laterally located sensory relay nuclei. A similar conclusion was reached by Verzeano & Calma (1954). However, typical spindles have been recorded from the medial geniculate body (Galambos *et al.* 1952) and, in the present investigation, from all major thalamic nuclei, including the relay nuclei, nn.

geniculatum laterale and ventralis postero-lateralis. Hence it can be concluded that spontaneous spindles may occur in all thalamic areas, providing the conditions are suitable, especially with regard to the level of anaesthesia.

Local origin of thalamic spindles. Spontaneous thalamic spindle activity may be recorded virtually unaltered after total removal of all neocortical areas with connexions to and from a given thalamic nucleus (Morison *et al.* 1943*b*; Galambos *et al.* 1952; Bremer, 1953; and Fig. 8 of the present communication). The same holds true for the related phenomenon of repetitive burst discharges to a single cutaneous volley (Adrian, 1941, 1951; Bremer & Bonnet, 1950; Bremer, 1953; Andersen *et al.* 1964) and to recruiting responses recorded in the thalamus or from the white matter (Morison & Dempsey, 1943; Morison *et al.* 1943*a*; Morison & Basset, 1945; Arduini & Terzuolo, 1951). Evidence at hand leads to the conclusion that the functioning cerebral cortex is not a prerequisite for spontaneous thalamic spindle activity, nor for the production of the repetitive burst responses due to a single cutaneous nerve volley. It is, however, possible that cortical activity may modify the rhythmic thalamo-cortical discharges during spindles and burst discharges.

Thalamic spindles are not abolished by severance of the connexions between the rest of the brain stem and the thalamus. In fact, this procedure increases the frequency as well as the amplitude of the component waves of the spindles (Bremer, 1937; Dempsey & Morison, 1943). The only neural structures between the precollicular section and the white matter underlying the cortex, that may be responsible for the spontaneous spindles are the thalamus itself, the basal ganglia and the hypothalamus. Removal of the latter does not alter the cortical activity (Morison *et al.* 1943*a*). It also appears likely that the substrate for the spontaneous thalamic spindles is to be found within the thalamus itself. It may be that the thalamic spindle pattern may be influenced by striatal activity.

Regarding the question of the origin of the rhythmic activity in the thalamus, Morison *et al.* (1943*a*) stated that 'several thalamic areas... have a tendency to produce spontaneous rhythmic bursts, but that they may be under the additional control of a master area associated especially with the internal medullary lamina'. The results of the present experiments give strong support to the concept that several thalamic areas have a tendency to produce spontaneous rhythmic activity. However, the persistence of spindles in the VPL of a decorticate cat, after severance of its connexions from all the mid-line nuclei and centre median, shows that a limited thalamic area contains a complete mechanism for spindle activity, and also that the control of the proposed master area can only be an additional one, as was in fact suggested by Morison *et al.* (1943*a*). Our

findings lead us to conclude that all thalamic areas possess the ability to produce spindle activity, provided the area is over a certain minimal size.

Inhibitory phasing as the mechanism for the production of thalamic spindles. We present here an hypothesis which provides a new explanation for the production of spontaneous thalamic spindles. It is based largely on a comparison between intracellular records of spindles and corresponding records of the burst discharges previously described (Andersen & Eccles, 1962; Andersen *et al.* 1964). Most of the experimental evidence supporting the hypothesis is derived from records of VPL cells, but a sufficient number of records has been made from other nuclei, including the mid-line structures, to ensure its general applicability.

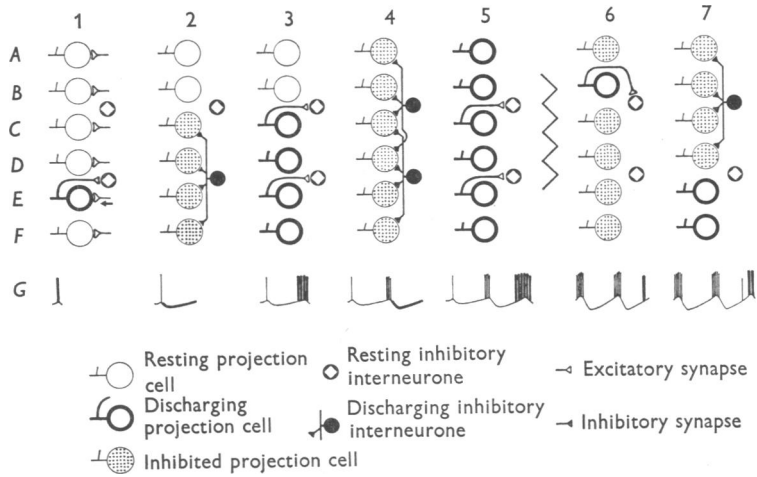

Fig. 9. Diagrammatic representation of the inhibitory phasing theory. Columns 1–5 illustrate various stages in the development of a thalamic spindle, and 6 and 7 show two stages of the end. Further explanation in the text.

In describing the inhibitory phasing theory for the thalamic spindle mechanism, reference will be made to the diagram in Fig. 9. The columns 1–7 represent various stages in the development of the spindle, which are also diagrammatically represented in the traces of row G below each column. In these traces, the heavy lines represent the neuronal event (spike or IPSP) illustrated in the column above. The open circles represent six thalamic neurones (*A–F*) with their axons projecting to structures outside the nucleus, either the cortex, the basal ganglia or other thalamic nuclei. The axons of these projection cells are postulated to have one or more collaterals that excite a particular type of interneurone drawn black with a white square. These interneurones are inhibitory to a large number of thalamic projection cells by way of a set of ramifying axonal branches. The afferent axons (e.g. lemniscal fibres) are drawn diagrammatically in

column 1 as coming one to each of the thalamic neurones. The spindle activity is visualized as being triggered off by a weak afferent nerve volley ascending one of these axons (arrow in *E* 1). After the discharge of this cell (signified by thick lines in *E* and thick spike in the trace in *G* 1), the axon collateral conveys the volley to excite an inhibitory interneurone (Fig. 9, 2). Through its extensive axonal ramifications, this interneurone (black) produces relatively synchronous IPSPs of about 120 msec duration in four projection cells (column 2, *C–F*—stippled). When the IPSPs decline, these cells are then in a state of post-anodal exaltation (Andersen *et al.* 1964), and with the help of random or phased excitatory influences derived from various sources, including excitatory interneurones, these four neurones, *C–F*, are brought to discharge synchronously (column 3), some 120 msec after the initial firing of cell *E*. Because more cells are brought in action, their axon collaterals excite more inhibitory interneurones (two in column 4) which in turn produce IPSPs in a larger number of cells (six altogether in row 4) and as illustrated, larger P-waves in the extracellular records (*G* 4). Consequently, in that thalamic area, all cells which receive sufficient inhibitory synaptic influence from one inhibitory interneurone will eventually beat in unison. Many inhibitory interneurones are thus excited nearly simultaneously (see row 4) and this will make for an even greater degree of synchronous discharge of the projection cells. However, after some cycles of the rhythm, indicated by the zig-zag line, some cells drop out of the regular beating. This occurs because in some cells the excitatory drive is stronger, and, consequently, the inhibitory period is shorter than in other neurones. As a consequence, these cells will be discharged earlier than the neighbouring neurones (column 6*B*). By way of its axon collaterals and associated inhibitory interneurones such a precociously discharging cell inhibits some surrounding cells from firing during the next cycle (column 7). These cells thus discharge later, but then out of phase with the majority (cf. Fig. 5). However, through their own recurrent inhibitory pathway, they recruit other thalamic cells to their rhythm. This breakaway from the dominant spindle rhythm is followed by an increasing number of cells, until all neurones in the area are firing irregularly.

Since a precociously occurring excitatory influence will tend to destroy the rhythmic activity (Fig. 5), the prerequisite for starting a new spindle is that the starting cell is synaptically excited and then not disturbed for about 120 msec. If the cell at this time is subjected to a new excitation, it may initiate a spindle by recruiting the partly excited neighbouring cells into rhythmic activity.

Histological evidence for both axon collaterals of the projection cells and the presence of cells with short and extensively ramifying axons in the

somaesthetic nucleus was given by Cajal (1911). Descriptions of small and large cells in various thalamic nuclei are numerous (von Monakow, 1905; Turner, 1903; Cajal, 1911; Sheps, 1945; McLardy, 1950; Powell, 1952; Clark & Powell, 1953; Lemieux, 1954). Following cortical removal, the large cells in the relay and association nuclei degenerate to a varying degree, whereas the smaller cells, present in a considerable number, survive to a large extent (Sheps, 1945; McLardy, 1950; Powell, 1952; Clark & Powell, 1953). In the present series, we have confirmed these histological observations.

The proposed mechanism uses negative feed-back to produce the IPSPs, but the development of the spindles is an example of positive feed-back, as is evident from the records of both thalamic and cortical spindles.

The theory also explains how a spontaneous spindle interferes with the evoked burst discharges and with driving by repetitive stimulation. The degree of the interference will depend on the level of the membrane potential in individual cells when the stimulus is applied. If the stimulus employed produces a large EPSP in the cell, it may reset the rhythm of that neurone and its neighbours (Morison & Dempsey, 1943; Andersen *et al.* 1964).

The hypothesis avoids the postulate of a localized pace-maker area within the thalamus or other parts of the brain stem. All areas of thalamus may give rise to spindle activity provided there is activation of a sufficient number of projection cells and inhibitory and excitatory interneurones. Naturally, the thalamic areas which have the richest intrathalamic connexions, especially axons of inhibitory interneurones, will be favourably situated to impose their rhythm on other nuclei.

Relation to the electroencephalogram. Since the thalamic spindle activity in VPL occurs in cells sending their axons to the cerebral cortex, a spindle will produce a series of rhythmic thalamo-cortical volleys at about 8–10/sec. Like any other thalamo-cortical volley, they will evoke post-synaptic responses which in the electroencephalogram appear as slow waves. Consequently, the rhythmic thalamic activity will produce cortical slow waves of the same frequency (Fig. 1 *F*, *G*). Thus, to a certain extent, the thalamic spindle governs the frequency of the waves in the cortical spindles recorded from animals lightly anaesthetized with barbiturates, or from *encephale isolée* preparations. Although the phasing of the cortical slow wave activity may be sought partly in a thalamic mechanism, cortical slow waves must be generated in the cerebral cortex itself.

Clare & Bishop (1956) have suggested that the rhythmic nature of cortical waves may be produced by a purely intracortical substrate. The basis for this view is their finding of recruiting cortical potentials showing waxing and waning in response to repetitive stimulation at about 8/sec

of the white matter, and to the cyclic variations in cortical excitability which follow a single stimulus to the white matter. However, such a stimulation would not only excite the thalamo-cortical axons orthodromically but also antidromically. Since antidromic activation of thalamic neurones produces rhythmic 8/sec activity leading to phased thalamo-cortical discharges (Andersen & Eccles, 1962; Figs. 1*C*, 2*E*, 6, 7), the observed cortical recruitment and the cyclic excitability variations following single white matter stimulation may be mediated, at least in part, by a thalamic mechanism. Presumably, a mechanism similar to that found in the thalamus is present in the cerebral cortex. Thus, Andersson (1964) found rhythmically occurring IPSPs or EPSP/IPSP sequences from cells in the chronically isolated somato-sensory cortex in response to a single antidromic volley along their axons.

SUMMARY

1. In cats, lightly anaesthetized with pentobarbitone sodium, periods of rhythmic activity were recorded from all major nuclei of the thalamus, including the mid-line nuclei and the relay nuclei.

2. In extracellular records, the rhythmic activity appeared as groups of spike discharges separated by positive waves (P-waves). The spacing of the cell discharges of about 120 msec gave a repetition frequency of about 8/sec. The rhythmic activity occurred in periods, each lasting 1–2 sec and being 5–20 sec apart. On analogy with the simultaneously recorded cortical slow waves, a period of rhythmic activity in the thalamus will be called a spindle. The cell discharges occurred nearly synchronously in a large number of neighbouring cells sending their axons to the cortex.

3. Intracellularly, the onset of a spindle appeared as a series of augmenting and summing inhibitory post-synaptic potentials, each lasting about 120 msec. During each IPSP, the cell was prevented from discharging. Spike discharges occurred only on the crest between two successive IPSPs.

4. Comparison is made between the spontaneously occurring thalamic spindle activity and the rhythmic discharges produced by a single orthodromic or antidromic volley. On the basis of such a comparison, an inhibitory phasing hypothesis is presented to account for the development of spontaneous rhythmic thalamic activity of about 10/sec. The basis of the hypothesis is a recurrent inhibitory pathway producing the large and long-lasting IPSPs obtained from thalamic cells during the spindle activity.

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