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CORTICAL RESPONSES TO PAIRED STIMULI APPLIED PERIPHERALLY AND AT SITES ALONG THE SOMATO-SENSORY PATHWAY

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SUMMARY

1. Experiments have been performed on animals anaesthetized with various anaesthetics to determine the responsiveness of the cortex to the second of a pair of identical stimuli applied at three sites along the sensory pathway, i.e. to the periphery, the medial lemniscus and to thalamocortical fibres.

2. It has been found that in deeply anaesthetized animals the mass response recorded from the cerebral cortex to the second of a pair of peripheral or lemniscal stimuli became reduced in size if the interval between the stimuli was 30–500 msec. If the interval was less than 30 msec for peripheral stimuli or between 10 and 30 msec for lemniscal stimuli responses were not obtained to the second stimulus. This was found to be a basic pattern which could be modified in animals less deeply anaesthetized. In these animals, periods of relatively increased responsiveness were seen after peripheral stimulation.

3. The post-synaptic responses recorded from the ventrobasal thalamus showed the same behaviour to the second of a pair of peripheral stimuli as did the cortex both as regards size and latency of the responses.

4. The post-synaptic responses recorded from the cuneate nucleus rarely showed any reduction in size unless the separation between the stimuli was 10 msec or less; even at intervals as low as 3 msec there was no increase in the latency of the response.

5. When a pair of stimuli were applied to thalamocortical fibres, a different pattern of cortical responsiveness was found. At the time the cortical response to stimulation at pre-thalamic sites was reduced or abolished, the response to stimulation at post-thalamic sites was unaltered or increased in size.

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6. Finally an attempt was made to correlate the mass response recorded from the cortical surface and the activity of single cortical cells. Two types of cell could be distinguished in the rat. Those lying from 0·35 to 1·2 mm deep in the cortex showed a response pattern, to paired stimuli, closely resembling that of the cortical mass response. Others situated deeper in the cortex were found which had a very long absolute unresponsive time, from 50 to 80 msec and a very long relative unresponsive time of 1 sec.

INTRODUCTION

It has been shown by many authors (e.g. Marshall, 1941; Jarcho, 1949; Mountcastle, Covian & Harrison, 1952; Amassian, 1952; Towe & Amassian, 1958) that, in the cat deeply anaesthetized with barbiturates, response could not be obtained from the primary somato-sensory cortex to the second of a pair of electrical stimuli applied to the periphery when the interval between the stimuli was between 30 and 40 msec. This time was called the absolute unresponsive time (Marshall, 1941). When the interval between stimuli was 40–150 msec the cortical response to the second stimulus was reduced in size. This was called the relative unresponsive time. Much of this decrease in the cortical responses was shown to be due to a failure of transmission through the ventrobasal thalamus (Marshall, 1941).

In the present publication an account is given of the electrical response at various sites in the central nervous system to pairs of stimuli applied to the periphery, to the medial lemniscus or to the thalamocortical radiation fibres.

Stimulation of sites in the central nervous system was performed to discover whether the cortical unresponsiveness was due solely to the decreased thalamic transmission or if the cortex itself showed any change in responsiveness. Part of this work has been reported in brief (Angel, 1963*a*).

METHODS

For this study forty-eight rats and six coypus were used. They were anaesthetized with either urethane (1·5–1·8 g/kg), pentobarbitone sodium (Cyclonal, May and Baker 30–60 mg/kg) given intraperitoneally, trichloroethylene (Trilene, I.C.I.) as a mixture of its vapour in air, or a mixture of nitrous oxide and fluothane in oxygen via a tracheal cannula.

Access to the cerebral cortex and thalamus was allowed by an extensive craniotomy on the left-hand side, after reflexion of the temporalis muscle. The dura mater was opened and reflected. To expose the medulla, the neck muscles were reflected back from the base of the skull and the arch of the atlas and some of the skull overlying the posterior part of the cerebellum were removed. The cerebellum was always left intact. The animal was held firmly in a specially constructed frame (A. Angel & G. D. Dawson, in preparation). The skin of the head was clamped between an inner Perspex ring and an outer metal clip. The pool so formed over the cerebral cortex was filled with liquid paraffin B.P. (which had been saturated with physiological saline) at a temperature of 37° C.

The electrodes used to record potentials from the surface of the cortex were of silver wire insulated except at the tips, which were fused into small balls. One of these was placed over the primary somatic receiving area the other further back on the occipital cortex. Potentials were recorded from the cuneate nucleus and ventrobasal thalamus with glass micropipettes having a tip diameter of 2–5 μ, filled with an 18% sodium chloride solution and with a resistance of 2–4 MΩ.

The micro-electrode was connected to the grid of one of a pair of cathode-followers (grid current $< 1{\cdot}5 \times 10^{-11}$ A) via a short piece of chlorided silver wire. The grid of the other cathode-follower was earthed.

The potentials were amplified by resistance-capacity coupled amplifiers. The high-frequency response of the amplifiers was such that the deflexion produced by a rectangular voltage pulse at the input reached 90% of its full amplitude in 0·1 msec. The low-frequency response was usually set so that the deflexion produced by a rectangular input fell to 90% of its full amplitude in 5 msec, equivalent to a time constant of 0·032 sec.

Electrical stimuli were applied to the periphery by means of lint pads soaked in 3M-NaCl. One pad was wrapped around the wrist or ankle and the other around a digit. The polarity of stimulation was such that the pad around the wrist became negative with respect to the more distal pad. The pressure under the electrode was kept as low as possible.

The stimuli were rectangular pulses of 100 μsec duration, continuously variable in amplitude from 0 to 120 V and isolated from earth by a 1:1 low capacity transformer. The timing of the stimuli was controlled from a digital timing unit (Pitman, 1958). Stimuli were also delivered via the glass micropipette, in which case the pipette was made the negative electrode. The positive electrode was clipped either on to the neck muscles or to a lint pad soaked in 3M-NaCl wrapped around the left forearm.

Summed records of the responses were obtained with a Mnemotron Computer of average transients Type 400 B.

The site of the micro-electrode used either for stimulating or recording was determined by histological examination of the tissues after the nervous system had been fixed with the microelectrode *in situ*.

RESULTS

The results can be conveniently divided into three parts. The first is connected with locating the region of the cortex giving the shortest latency responses to stimulation at the periphery. This was found to be necessary because of the difference in the recovery times of short latency and long latency responses (see below). The second is concerned with an investigation of the responses of the cuneate nucleus, the ventrobasal thalamus and the primary somatic cortical receiving area to the second of a pair of peripheral stimuli applied at various intervals. The third is concerned with the recovery time of the cortical responses to pairs of electrical stimuli applied to the fibres of the medial lemniscus or the thalamo-cortical radiations.

Localization of cortical responses

The region of cortex from which records were to be made was found by mapping the left cortex, while stimulating the right forepaw electrically. Records were made of twenty superimposed responses from each of sixty positions on the cortical surface. The mean latency to the start of the cortical responses was then measured for each site. For these experiments

seven rats were used; three were anaesthetized with trichloroethylene and three with urethane. The other animal was first anaesthetized with trichloroethylene and then, after the cortical surface had been mapped, the anaesthetic was changed to urethane and the experiment repeated to see if there was any difference in the cortical pattern of localization. The region of the cortex giving the shortest latency responses was found to be the same for both anaesthetics. It was found to have a sufficiently constant relation to the Y-shaped vein which joins the superior sagittal sinus at the level of the coronal suture for these to be satisfactory as guides in subsequent experiments. The area giving the shortest latency responses was always found to be in or near the fork of the Y. This region showed all the properties of the primary somatic receiving area described by Woolsey (1958) in the deeply anaesthetized rat and other mammals. In particular a response with a short latency could only be obtained in one part of the cortex by stimulation of a particular small area of the contralateral body surface.

Some differences were seen, however, in the distribution of the cortical potentials obtained with the two anaesthetics used. The over-all area of cortex from which definite potentials could be obtained was larger with urethane than with trichloroethylene anaesthesia. Another difference appears when the latency of the response was considered. The area of the cortex from which short latency responses could be recorded was found to be less extensive in the rostro-caudal direction in animals anaesthetized with urethane than in those anaesthetized with trichloroethylene. The mediolateral extent of the short latency response area appeared to be the same with both anaesthetic agents. The area which gave short latency responses in animals anaesthetized with trichloroethylene was roughly a square of 2 mm side, and in animals anaesthetized with urethane the comparable area was a rectangle approximately 1·5 mm in the rostro-caudal axis and 2·5 mm in the medio-lateral axis. Figure 1 shows plots along orthogonal axes, of the mean latency of the responses versus distance, in two rats, one deeply anaesthetized with trichloroethylene (Fig. 1*a*) the other with urethane (Fig. 1*b*). In each case, the intersection of the axes was arranged to coincide with the site of minimum latency. The surface blood vessels have been drawn in the small diagram (Fig. 1*c*); the site of minimum latency is near the fork of the Y-shaped vein. In these experiments the mediolateral axis fell just behind the coronal suture.

Responses to paired stimuli

(*a*) *Stimulation of the periphery*

Cortical responses. For convenience and descriptive purposes, the complex cerebral response to peripheral stimulation can be divided into three

major components. These are a first positive deflexion, which is followed by a first negative deflexion and lastly a second positive deflexion. Figure 2 shows the cortical responses, with the main components labelled, to twenty consecutive stimuli applied to the right forepaw in a rat (Fig. 2*a*) and a coypu (Fig. 2*b*) both deeply anaesthetized with urethane. When the size of the first positive deflexion (P_1, Fig. 2) or the first negative deflexion (N_1, Fig. 2) in response to the second of a pair of stimuli applied to the forepaw

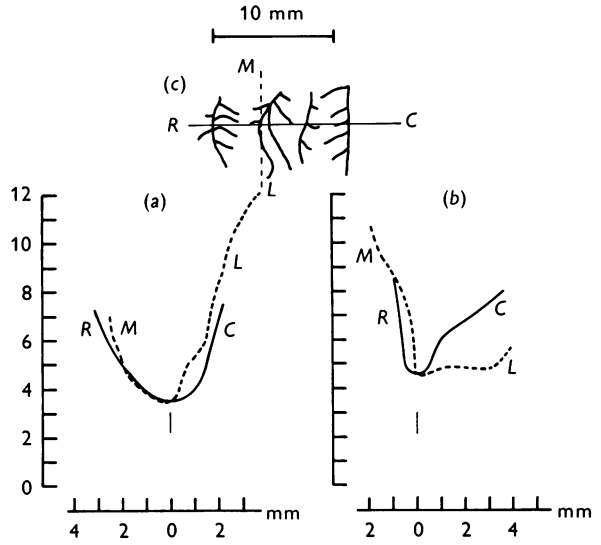

Fig. 1. Graphs showing a plot across the surface of the cortex of the average latency of the cortical response in rostro-caudal and mediolateral axes, in two rats deeply anaesthetized with (*a*) trichloroethylene, and (*b*) urethane. A tracing of the surface veins is shown (*c*) with the axes superimposed on it for the animal anaesthetized with urethane. A very similar vascular pattern was found in the animal anaesthetized with trichloroethylene. The position at which the axes cross was the site where the evoked potential had a minimum latency; the abscissae show the distance from this position in mm, the ordinates the latency in msec.

was plotted versus the interval of separation of the stimuli, two patterns of the recovery of the cortical responses were obtained. The difference was due to the depth of anaesthesia. The animals were either deeply anaesthetized, i.e. they showed no reflex withdrawal to a pinch, or relatively lightly anaesthetized, i.e. they showed a very slight withdrawal reflex.

Deep anaesthesia. In deeply anaesthetized animals the first negative wave of the cortical response was often too small to permit its accurate measurement. In those experiments where it was measurable no differences were seen in the pattern of recovery of either the first positive or first negative waves to paired stimuli. Usually a linear relation was found between the

percentage size of these two components of the cortical response. Figure 3*a* shows a plot of the size of the first positive and negative waves (expressed as a percentage of their respective maxima) of the cortical response versus the interval of separation of two peripheral stimuli from a coypu deeply anaesthetized with urethane. The relation between the percentage size of these two components is shown in Fig. 3*b* from another coypu deeply anaesthetized with urethane. In the deeply anaesthetized rat, the size of the response to the second stimulus was constant and equal to that to the first stimulus of the pair when the intervals between the stimuli was 500 msec or more. With intervals of less than 500 msec, the size decreased proportionally with the interval until, with intervals of 30–40 msec or less,

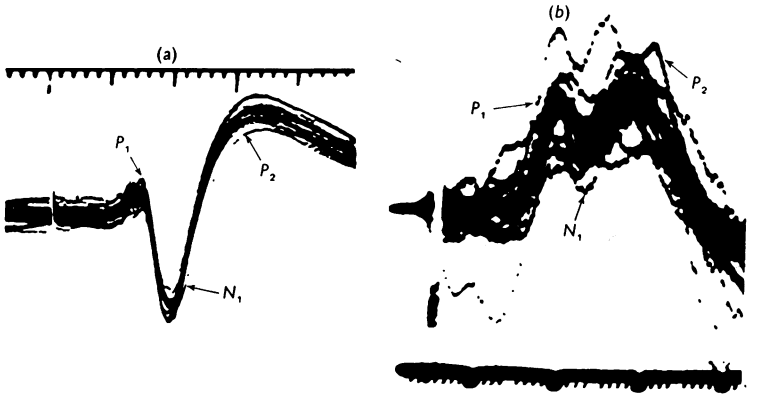

Fig. 2. This shows twenty superimposed responses from the primary cortical receiving area on the left hand side to electrical stimuli delivered to the right forepaw, (*a*) in a rat and (*b*) in a coypu both deeply anaesthetized with urethane. The main components of the cortical response have been labelled P_1 the first positive wave, N_1 the first negative wave and P_2 the second positive wave. The time scale in (*a*) shows 1 and 5 msec marks, that in (*b*) 10 msec marks. The pulse at the beginning of record (*b*) represents 500 μV for record (*a*) and 50 μV for record (*b*). In this and subsequent figures positivity at the active electrode is recorded as an upward deflexion.

the second stimulus elicited no response (Fig. 4*a*). This pattern was seen in rats deeply anaesthetized with urethane, trichloroethylene, hexobarbitone, or pentobarbitone-sodium and in coypus deeply anaesthetized with urethane or a mixture of oxygen, nitrous oxide and fluothane. In the deeply anaesthetized coypu, however, the response to the second stimulus was depressed with intervals as long as 1 sec (Fig. 3*a*).

Relatively light anaesthesia. In these experiments, the recovery cycles showed, at some intervals of separation of the stimuli, an increased responsiveness relative to those obtained from deeply anaesthetized animals. When the stimuli were separated by between 50 and 200 msec,

the response to the second stimulus was enhanced, its voltage reaching in some preparations 5–6 times the voltage of the response to a single stimulus. In addition to this, some preparations showed responses to the second stimulus at separations of 2–20 msec. In all the animals responses to the second stimulus with intervals of separation of 20–40 msec were not obtained. Examples of each of these patterns are shown in Fig. 4. Each point on the graphs is the mean response height of the first positive wave (Fig. 4*a*) or first negative wave (Fig. 4*b*, *c*) of the cortical responses to twenty stimuli applied at each interval. The response size is expressed as a percentage of the response to the first stimulus of the pair. In Fig. 4*c* the behaviour of the responses to both foreleg and hind leg stimulation are

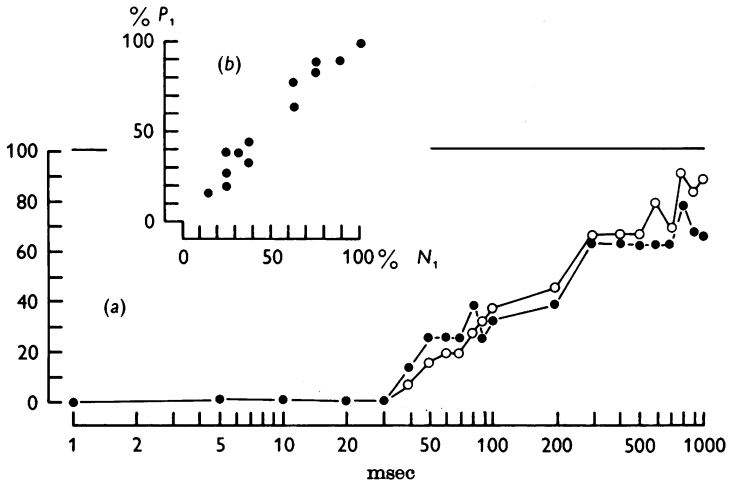

Fig. 3. (*a*) A graph of the size of the first positive wave ○ and first negative wave ● of the cortical potential to the second stimulus of a pair, expressed as a percentage of the potential to the first stimulus (ordinate) for various intervals of separation of the stimuli (abscissae, plotted on a log scale). Each point is the average of twenty consecutive responses obtained from a coypu deeply anaesthetized with urethane. (*b*) This shows graphically an identical experiment to that shown in (*a*) but the percentage size of the first positive wave (ordinate) has been plotted against the percentage size of the first negative wave (abscissae) recorded at the same time to the second of a pair of stimuli. Each point is the average of twenty consecutive responses.

plotted for the same animal (in this animal the area giving the shortest latency responses to hind leg stimulation was determined). The later cortical waves (P_2, Fig. 2) showed a much more prolonged recovery time, taking in some experiments, as long as 5 sec to recover fully.

As well as the alteration in size of the cortical response to the second stimulus of a pair, the latencies of the responses were also changed. The

pattern of this latency change was always the same. In the rat, when the stimuli were 100–300 msec apart, the latencies of the response to the second stimulus became slightly increased; with shorter intervals between stimuli, the latencies became greatly increased. For example in one animal, showing the response pattern of deep anaesthesia, the mean latency of the responses to the first stimulus was 4·2 msec. After a pair of stimuli the latency to the second stimulus at an interval of 100 msec was 4·5 msec, and at an interval of 50 msec, 11·5 msec. Further examples of this latency change can be seen in Figs. 6*b* and 7*b*. When the response pattern of Fig. 3*c*

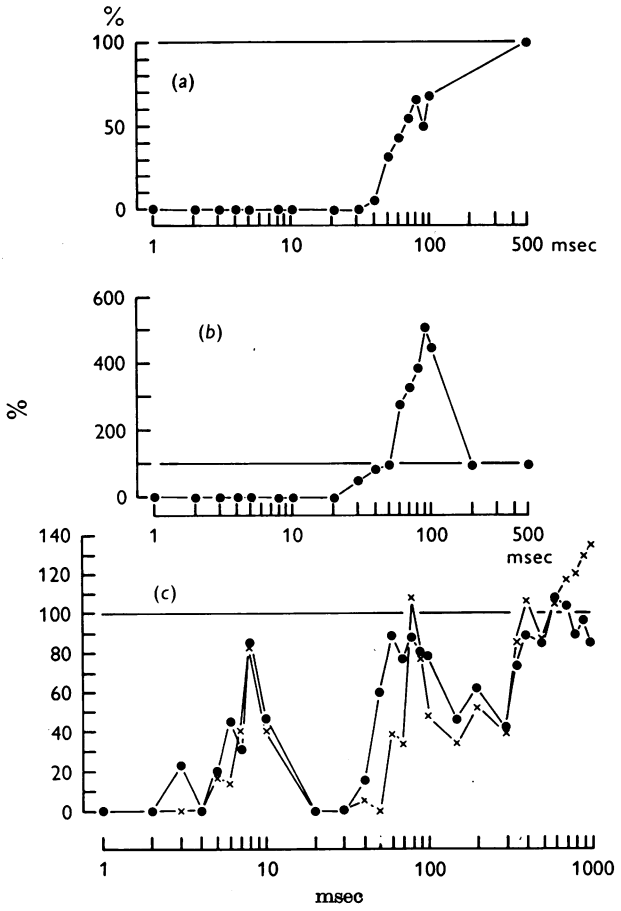

Fig. 4. Each graph shows the size of the first positive wave (*a*) and first negative wave (*b*, *c*) of the cortical potential to the second stimulus of a pair, expressed as a percentage of the potential to the first stimulus (ordinate), for various intervals of separation of the stimuli (abscissae). Note that the interval of separation of the stimuli is plotted as a log scale. Each point in the graph is the average of twenty observations.

occurs, with intervals between stimuli of 10 msec or less, the latency of the response to the second stimulus is the same as, or only slightly greater than, that to the first stimulus, e.g. in this animal at an interval between stimuli of 10 msec, the latency to the first response was 8·2 msec and to the second 8·4 msec.

Finally, with intervals between stimuli of 200 msec or less, the shape of the responses was changed. Considering first the deeply anaesthetized animal, at intervals between stimuli of less than 200 msec, the response to the second stimulus consisted only of a positive wave, which gradually

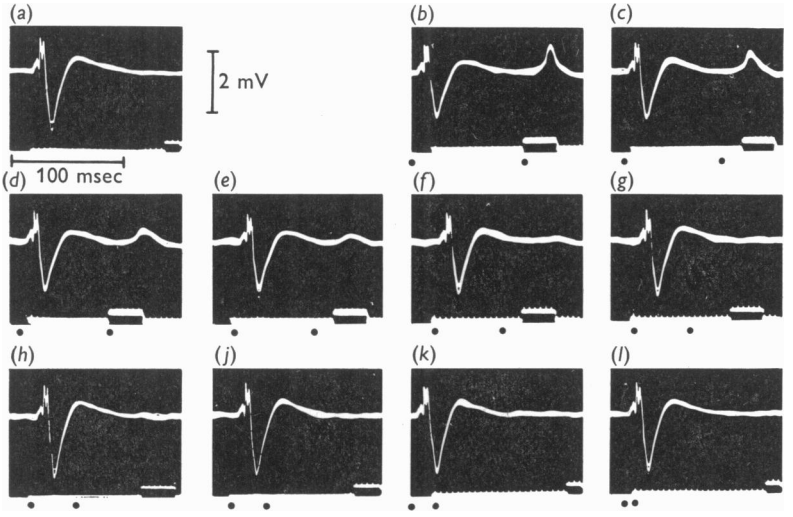

Fig. 5. Each record is of ten superimposed responses from a rat deeply anaesthetized with urethane, (*a*) shows the response to the first stimulus alone, (*b*) to two stimuli applied with a separation of 100 msec, (*c*) 90 msec, (*d*) 80 msec, (*e*) 70 msec, (*f*) 60 msec, (*g*) 50 msec, (*h*) 40 msec, (*j*) 30 msec, (*k*) 20 msec and (*l*) 10 msec. The black dots below the records show the positions of the stimuli. Positivity at the active electrode is recorded as an upward deflexion. The vertical bar by record (*a*) represents 2 mV, the horizontal one 100 msec.

became smaller as the intervals between the stimuli were reduced, until no response was obtained. In the case of animals which were lightly anaesthetized but which showed, as with deep anaesthesia, little increase above the 100% level at long intervals, e.g. Fig. 4*c*, the same changes were seen. However, in those animals in which a large increase in the response to the second of a pair of stimuli was seen (as for example in Fig. 4*b*) the responses to a second stimulus still showed a first negative deflexion at intervals as small as 60 msec. Figure 5*b* shows that the cortical response to the second stimulus was a positive wave only, the latency to its peak being 23·1 msec.

As the interval between stimuli was reduced this wave diminished rapidly in size and in Fig. 5*e* had a latency to its peak of 35 msec after which it could no longer be measured with any accuracy. The records in Fig. 5 are from a rat deeply anaesthetized with urethane. Ten superimposed cortical responses are shown either to single stimuli to the right forepaw (Fig. 5*a*), or to pairs of stimuli applied at various intervals of separation (Fig. 5*b*–1).

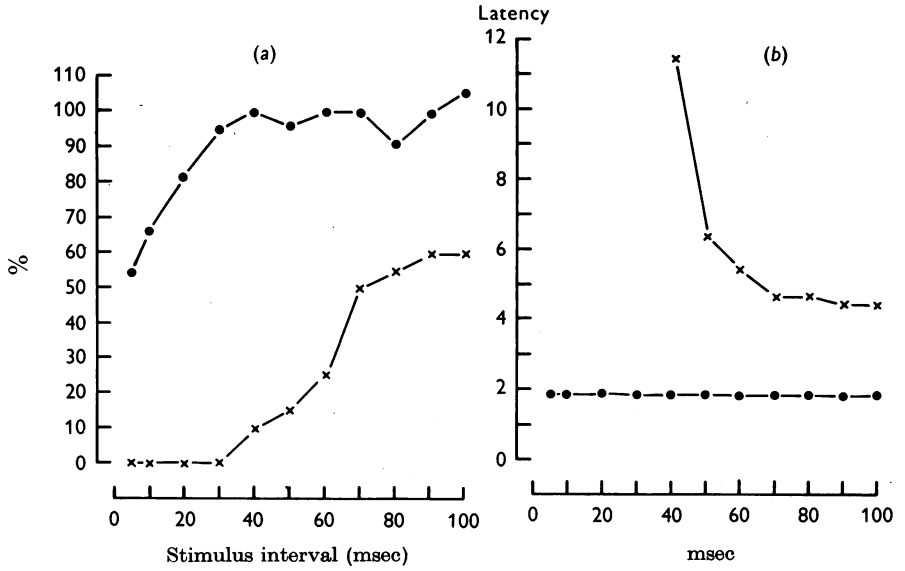

Fig. 6. (*a*) The size of the response to the second of a pair of stimuli, expressed as a percentage of that to the first stimulus of the pair, of the first positive wave of the cortical response (× --- ×) and the post-synaptic discharge recorded from the cuneate nucleus (●--●) versus the interval between the stimuli. (*b*) The latency of the cortical response (× -- ×) and beginning of the post-synaptic response from the cuneate nucleus (●--●) to the second stimulus of a pair versus the interval of separation. These results were obtained from a rat deeply anaesthetized with urethane. Each point on the graphs is the average of twenty observations.

Cuneate responses. An examination of the responsiveness of the cuneate nucleus showed that generally the size of the post-synaptic response to the second of two peripheral stimuli was not reduced unless the interval between stimuli was 10 msec or less. In a few cases the responses from the cuneate nucleus showed a reduction of 20% with stimulus intervals of 20 msec. The latency of the post-synaptic mass response of the nucleus remains unchanged with separation of the stimuli as little as 3 msec. Figure 6 shows the sizes and latencies of cuneate post-synaptic mass response and cortical surface positive wave, from a rat deeply anaesthe-

tized with urethane, as a function of the intervals of separation of the peripheral stimuli. Each point of the graph is the mean of twenty responses.

Thalamic responses. When the responses from cells located in the ventrobasal thalamus were studied, it was found that they showed the same behaviour to paired stimuli as the cortical responses. This was seen both in the size of the mass response, or the probability of discharge of single thalamic cells; and also the latencies of the responses. Figure 7*a* shows the

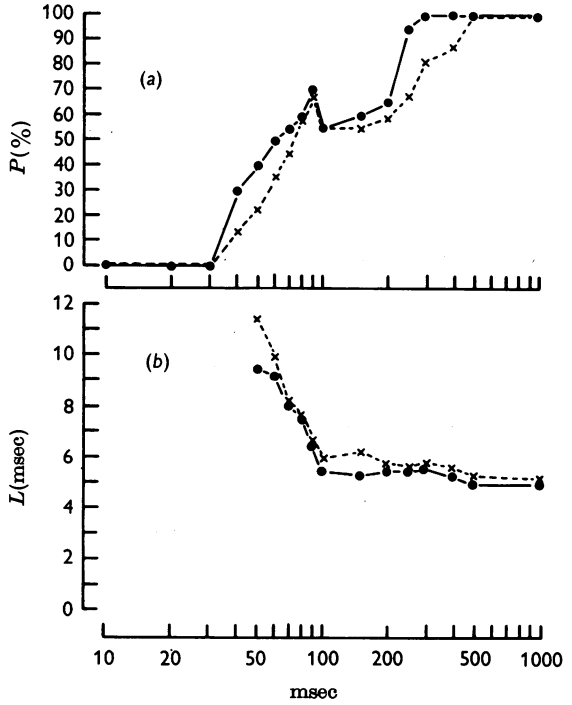

Fig. 7. (*a*) The size of the cortical responses (× ---- ×) to the second of a pair of stimuli at various intervals of separation expressed as percentages of the control size and the probability of response of a single thalamic cell (●--●). (If the cell responded 20 times in twenty trials, the probability of response was plotted as 100). (*b*) The latency of the cortical responses (× ---- ×) and thalamic cell discharge (●—●) to the second of a pair of stimuli versus the interval of separation of the stimuli. For this experiment, a rat anaesthetized with urethane showing a just perceptible withdrawal of its hind leg in response to a strong pinch was used.

size of the early part of the cortical response and the probability of discharge of a single thalamic cell; Fig. 7*b* shows the latency of the earliest discharge of the thalamic cell and the mean cortical latency versus the intervals of separation of the two stimuli.

The results so far show that the decline in responsiveness to two peripheral stimuli occurred first at the level of the second sensory synapse, in the ventrobasal thalamus, and that responses of cells in this location showed a behaviour very similar to the responses recorded from the primary cortical receiving area of the cerebral cortex. To see if the reduced cortical responses to the second of a pair of peripheral stimuli was solely a reflexion of the decreased thalamic transmission with the cortex playing a passive role, experiments were performed stimulating the somatosensory pathway at sites below and above the ventrobasal thalamus via a micropipette.

(*b*) *Stimulation of the sensory pathway, centrally*

In all the experiments in which the post-synaptic mass responses to the second of a pair of peripheral stimuli were recorded from the cuneate nucleus, no changes in their amplitude or synchrony were seen, with intervals between stimuli of 25 msec or more. Clearly, then, this nucleus played no part in the reduction of the cortical responses to the second of a pair of stimuli at intervals greater than 25 msec.

Stimulation of the medial lemniscus. Whether or not the micro-electrode was in the medial lemniscus was determined histologically at the end of the experiment. At the time of the experiment its location was judged by the stereotaxic position and by the latencies of the cortical responses to stimulating via the micropipette. The determination of a pre- or post-synaptic placement of the stimulating micropipette rests upon the fact that in the presynaptic position the latency of the response recorded from the cerebral cortex will consist of three parts: (i) presynaptic (lemniscal) conduction time, (ii) synaptic (thalamic) delay, and (iii) post-synaptic (thalamocortical) conduction time, whereas the latency to stimulation at a post-synaptic site will only include the last. The latencies of the presynaptic component of the thalamic mass and cortical responses to peripheral stimulation were measured early in the experiment with the micropipette recording from the cells of the ventrobasal thalamus. The micropipette was then moved to a new position 0·5 mm or less caudal to the recording site and the latency of the cortical response, to stimulating via the micropipette, was measured. If the difference in latency between the cortical and thalamic responses to peripheral stimulation was equal to the latency of the cortical response to stimulation via the micro-electrode, the tip of the electrode was assumed to be in the medial lemniscus near to the thalamus. This was confirmed by the fact that the thalamo-cortical conduction time subtracted from the cortical latency to stimulation via the micro-electrode, gave a difference equal to or in excess of 0·8 msec, roughly the delay in transmission through one synapse (Eccles, 1957).

For example, in one experiment the following values were obtained from the superimposed records:

Cortical latency to peripheral stimulation	5·6 msec
Thalamic latency to peripheral stimulation	4·3 msec
Difference	1·3 msec
Cortical latency to stimulation via microelectrode	1·3 msec
Thalamo-cortical conduction time	0·5 msec
Difference	0·8 msec

The scatter in the latencies was found to be not more than $\pm 0{\cdot}1$ msec.

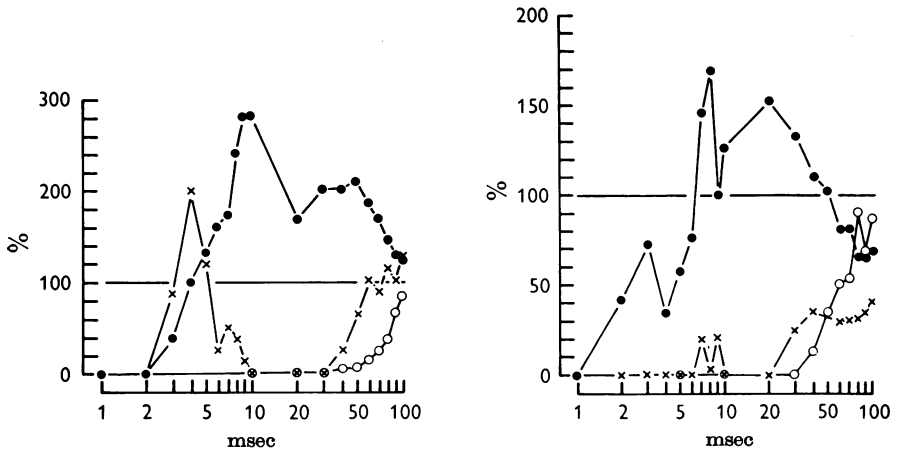

Fig. 8. Each point on the graphs represents the mean size of twenty cortical responses to the second of a pair of stimuli, expressed as a percentage of that to the first stimulus, to stimulation of the periphery (○--○), the medial lemniscus (×—×) and the white matter underneath the primary cortical receiving area (●--●), at various intervals of separation, in two rats deeply anaesthetized with urethane.

The patterns of recovery of the cortical response to the second of a pair of stimuli delivered to the medial lemniscus were found to be essentially similar to those of peripheral stimulation with intervals of separation between stimuli greater than 10 msec. They tended, however, to show an increased responsiveness with inter-stimulus intervals in the range 6–10 msec. Figure 8 shows the recovery curves of the cortical responses in two rats both deeply anaesthetized with urethane. Each point on the graph is the average size of the first negative wave of the cortical response to the second of a pair of stimuli, expressed as a percentage of that to the first stimulus, applied to the periphery (Fig. 8, circles) and to the medial lemniscus (Fig. 8, crosses) at various intervals of separation of the stimuli. One major difference between stimulating the medial lemniscus and the

periphery was that, in some experiments, large amplitude responses were obtained to a second lemniscal stimulus, with intervals of 2–5 msec.

Stimulation of thalamo-cortical fibres. When the fibres leaving the ventrobasal thalamus, or the fibres underlying the primary somatic receiving area were stimulated, a completely different pattern of responsiveness to

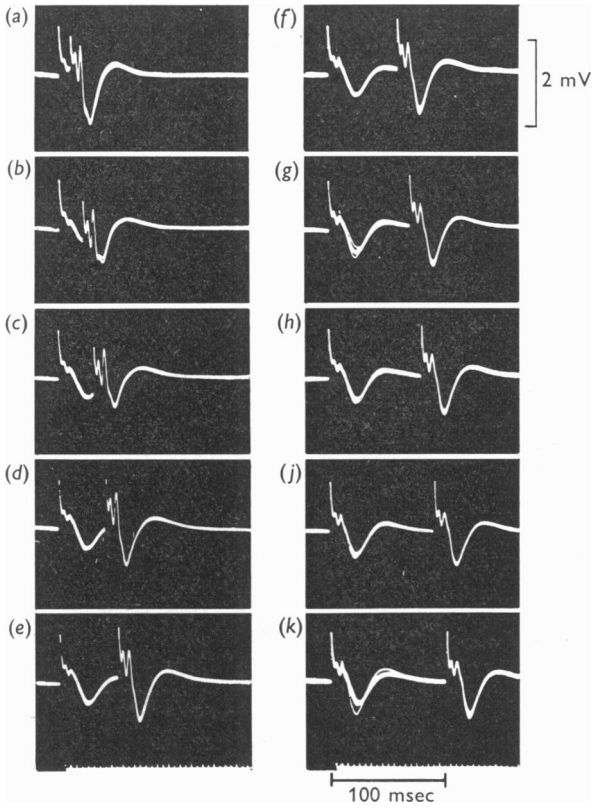

Fig. 9. Each record is of ten superimposed cortical responses to two stimuli applied to the white matter underlying the cortex at the following intervals: (*a*) 10 msec, (*b*) 20 msec, (*c*) 30 msec, (*d*) 40 msec, (*e*) 50 msec, (*f*) 60 msec, (g) 70 msec, (*h*) 80 msec, (*j*) 90 msec and (*k*) 100 msec. Positivity of the active electrode is recorded by an upward deflexion.

the second of two stimuli was obtained. In rats anaesthetized with urethane, at inter-stimulus separations of 8–100 msec, the cortical responses to the second of a pair of stimuli applied to thalamo-cortical fibres were found to be increased in size, in which circumstance the cortical responses to the second of a pair of stimuli delivered to the periphery or to the medial lemniscus were absent or reduced. Figure 8 shows this pattern of cortical responsiveness to post-thalamic stimulation (dots)

compared, in the same animals, to peripheral stimulation and stimulation of the fibres entering the thalamus. The records of Fig. 9 show ten superimposed cortical responses, to stimulation of the white matter underneath the primary somatic receiving area of the cortex at various intervals of separation of the stimuli. Comparison of this figure with Fig. 5 shows the differences in the sizes of the cortical responses at stimulus separations of

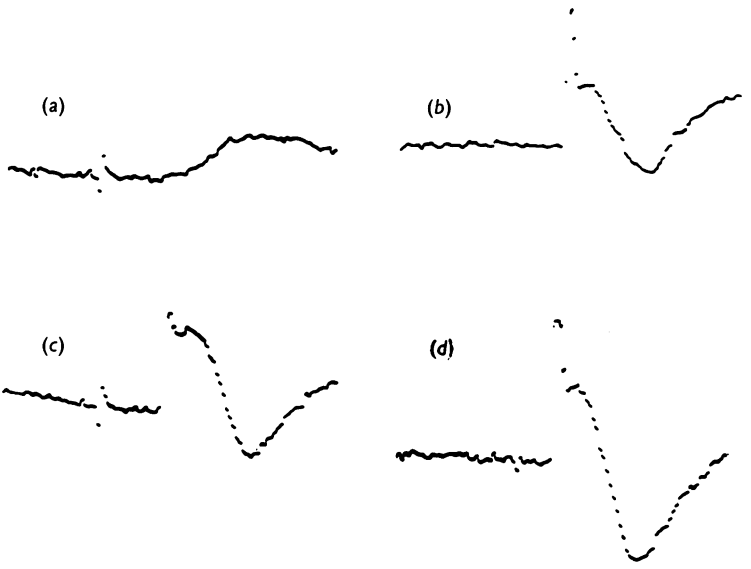

Fig. 10. All the traces are the sum of forty cortical responses obtained with a Mnemotron CAT. (*a*) The response to the second stimulus of a pair applied peripherally at an interval of 50 msec. (*b*) The response to stimulation of the white matter below the primary cortical receiving area. (*c*) Stimulation of the white matter during the cortical responses to the second peripheral stimulus. (*d*) The same as (*b*) but the response to the second peripheral stimulus alone has been subtracted. Each record lasts for 20 msec.

10–100 msec. In these experiments it was found that both the first positive and negative components of the cortical responses were facilitated. The same pattern of increased responses were seen if the thalamo-cortical stimulation was preceded by a peripheral stimulus. An increased cortical response to thalamo-cortical fibre stimulation could also be obtained if the ascending volley from such a stimulus was timed to arrive at the cortex during the first positive wave of the response to a peripheral stimulus. To illustrate this point the following type of experiment was performed.

(*c*) *Combined peripheral and central stimulation*

Three stimuli were used, two peripheral and one thalamo-cortical. The function of the first peripheral stimulus was to render the sensory pathway

relatively unresponsive so that the cortical response to the second peripheral stimulus consisted only of a positive wave (Fig. 10*a*). The thalamo-cortical stimulus alone gave the responses shown in Fig. 10*b*. When the thalamo-cortical response (Fig. 10*b*) was timed to start at the beginning of the response to the second peripheral stimulus (Fig. 10*a*) the resultant was the response shown in Fig. 10*c*. If the contribution of the response to the second peripheral stimulus (Fig. 10*a*) was subtracted from (Fig. 10*c*) the resultant was the response shown in Fig. 10*d*. The facilitation of the response to the thalamo-cortical stimulus can be seen if Fig. 10*b* and *d* are compared. Each trace (Fig. 10) is the sum of the responses to forty consecutive stimuli.

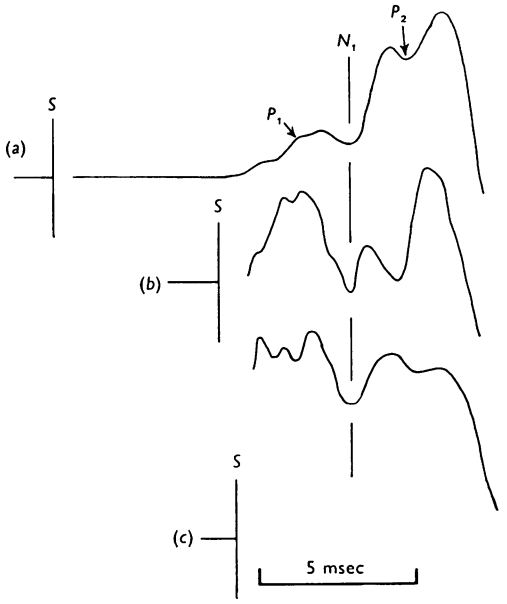

Fig. 11. Each trace represents the average of twenty cortical responses. (For the averaging, the deflexion of each response was measured at 330 μsec intervals and the average obtained by hand.) (*a*) to peripheral stimulation, (*b*) to stimulation of fibres of the medial lemniscus and (*c*) to stimulation of the white matter underneath the primary somatic cortical receiving area. The vertical lines marked *S* show the start of the stimulus artifacts. P_1 first positive wave, N_1 first negative wave and P_2 second positive wave.

The responses, resulting from peripheral, medial lemniscal and thalamo-cortical fibre stimulation are compared in Fig. 11, in which the averaged responses from the cortex to twenty stimulations at each site, in the same animal, are shown. To facilitate comparison of the response, the troughs of the first negative deflexions have been aligned. It can be seen that the responses are alike in shape and timing of the various inflexions. There is

the difference, however, that the components of the first positive wave (P_1) became compressed as the site of stimulation approached the cortical level. This was presumably because the temporal dispersion of the ascending volley became less.

Responses of single cortical cells

Finally, an attempt has been made to see if the early parts of the cortical response, i.e. the first positive and negative waves (Fig. 2, P_1, N_1), give

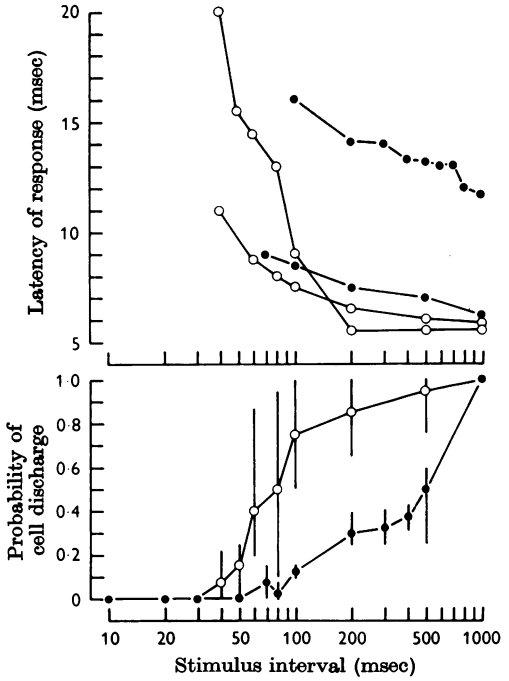

Fig. 12. (*a*) The latency of the response to two superficial cortical cells, (○--○), and two 'deep' cells, (●—●), to the second of a pair of stimuli applied at various intervals to the periphery, in a rat deeply anaesthetized with urethane. (*b*) The average probability of discharge of ten superficial cells (○—○), and three 'deep' cells (●—●) versus the interval of separation of a pair of stimuli. (If the cell responded 40 times out of 40 trials, the probability has been plotted as unity.) The vertical lines indicate the highest and lowest values found for the cells. Each point on the graphs is the average of forty observations at each interval for each cell.

any indication of the discharge of cells making up the response recorded from the cortical surface. Extracellular records have been made from single cortical units which responded to peripheral stimulation. These units were quiescent in the absence of stimulation. Two types of unit have

been found, both with receptive fields localized to one digit or a part of one digit. The first type of unit, between 0·35 and 1·2 mm below the cortical surface, responded to stimulation within its receptive field by discharging a single spike. This discharge was always during the first negative wave of the cortical surface response. When the two peripheral stimuli were delivered, these units discharged in 100 % of the trials after each stimulus so long as the inter-stimulus interval exceeded 500 msec. With intervals in the range 100–500 msec, the second stimulus evoked a spike in at least 70 % of the trials. With intervals less than 30 msec, this type of unit failed to respond to the second stimulus.

The second type of unit lay 1·2–1·8 mm below the cortical surface. This type also discharged a single spike potential after peripheral stimulation but the latency was greater; the spike occurred during the later parts of the surface cortical response. When two stimuli were delivered with an inter-stimulus interval of 500 msec, the unit responded to the second stimulus in about 50 % of trials. With 100 msec between the stimuli, the stimulus evoked a spike in only 10–15 % of trials and with intervals of 50 msec or less between the stimuli, the unit never responded to the second shock. Figure 12*b* (upper curve) shows the mean probability of discharge of ten cells of the first type found in two rats deeply anaesthetized with urethane. For each cell, the number of times it responded in forty trials to the second of a pair of peripheral stimuli at various intervals was determined. For each interval, the mean probability of discharge of all the cells was determined and plotted, together with the highest and lowest values obtained. The lower curve (Fig. 12*b*) was obtained from four cells of the second type. Again, the mean value has been plotted together with the upper and lower values. Figure 12*a* shows the changes in latency of two cells of the first type (circles) and two cells of the second type (dots). Of these, one was located in the forefoot area (lower curve) and the other in the hind foot area (upper curve).

These results show that, when the interval between a pair of peripheral stimuli is reduced, the probability of firing of the first type of cortical cell (lying between 0·35 and 1·2 mm deep) falls at a rate closely comparable with the diminution in size of the cortical mass response under the same conditions. The latency of the cortical cell response rises in the same way as does the latency of the cortical mass response.

DISCUSSION

In general, the results presented in this paper resemble those of other workers (Marshall, 1941; Jarcho, 1949; Mountcastle *et al.* 1952; Amassian, 1952; Towe & Amassian, 1958) in that the cerebral cortex showed no

response to the second of a pair of identical electrical stimuli applied to the periphery, in the deeply anaesthetized animal, when the interval between the stimuli was 30–40 msec or less.

The duration of the relative unresponsive time was found to be much longer than the 150–200 msec reported by the above authors. In rats the relative unresponsive time usually lasted for 400–500 msec, and in the coypu for 1 sec.

Furthermore, comparison of the cortical responses to paired stimuli applied at pre- or post-thalamic sites has shown that the cortex is capable of responding to closely spaced stimuli. The reduced cortical responses to the second of two peripherally applied stimuli is solely a reflexion of the responsiveness of the thalamus. The periods of increased cortical responsiveness seen in lightly anaesthetized animals, and in the response to stimulation of the medial lemniscus were probably due to three factors. The increased response to lemniscal stimulation with intervals of 2–5 msec was probably due to the second of the volleys occurring in the first positive wave of the cortical response, which has been shown to give a facilitated response (see Fig. 10). The excitatory influence of the cortex on the thalamus (Angel, 1963*b*) could explain the increased cortical response to peripheral and lemniscal stimulation at intervals of 6–20 msec. The increased cortical response to the second of two peripheral stimuli at intervals of separation of 50–200 msec appears to be due to at least two factors. In those experiments where the recovery curves showed a slight departure from those obtained in the deeply anaesthetized animal (e.g. Fig. 4*c*) the increase in responsiveness can be accounted for by the presence of thalamo-cortical after discharge (Andersen, Brooks, Eccles & Sears, 1964). However, thalamo-cortical after discharge is insufficient to account for the large increases in the cortical responses in some animals (up to 6 times the test response size, Fig. 4*b*). An alternative explanation would be the following. In all the experiments reported the peripheral stimuli used were supramaximal for the evoked cortical response. This intensity of stimulation will alter the discharge of cells located in the thalamic reticular nuclei, and such an alteration has been shown to be accompanied by a large increase in thalamic transmission (Angel, 1964).

The superficially located cortical neurones having the shorter latency of response to a peripheral stimulus showed the same pattern of recovery as did the cortical surface potentials, i.e. when a pair of peripheral stimuli are brought closer together, the probability of firing of the first type of cortical cell (lying between 0·35 and 1·2 mm deep) falls at a rate closely comparable with the diminution in size of the early components of the cortical evoked response under the same conditions. In the same way, the latency of the cortical cell response rises in the same way as does the

latency of the cortical mass response. Both had an absolute unresponsive time of 20–40 msec and a relative unresponsive period of up to 500 msec to peripheral stimulation. This relative unresponsive period is much longer than that found in cortical neurones in the cat by Mountcastle, Davies & Berman (1957). These authors found an absolute unresponsive time of 20–30 msec, in agreement with that found in this investigation, but a relative unresponsive period of only 160 msec. Three of the cortical cells studied here showed normal probability of response to the second of two stimuli when the interval between the stimuli was in the range 100–200 msec, but the latency of the discharge was increased and so the cells were still behaving in a subnormal fashion. Mountcastle *et al.* (1957) did not report observations on the behaviour of the cells with intervals of separation longer than 160 msec so that the possibility of a relatively supernormal phase associated with thalamo-cortical after-discharge cannot be excluded. The recent work of Andersen *et al.* (1964), has shown that, in animals with a moderate level of Nembutal anaesthesia, the rhythmic after-discharges of thalamic cells have a period of approximately 100–150 msec which are associated with periods of relatively increased thalamic transmission. Additional Nembutal administered intravenously was shown by these authors to prolong this period to 300 msec or more.

The other cortical cells found in the present investigation, with an absolute unresponsive time of 40–70 msec and a relative unresponsive period of 1 sec, were located deeper in the cortex than those with the shorter absolute unresponsive time, and showed a pattern of recovery comparable with the later components of the evoked cortical response.

A striking similarity has been shown for the behaviour of the thalamic and cortical responses to paired stimuli, either as mass responses or the responses of single cells. The behaviour of single thalamic cells has shown that they have an absolute unresponsive time of 20–40 msec, which is in agreement with the observations of Poggio & Mountcastle (1960) except that the relative unresponsive period of the cells was found to be as long as 500 msec against 160 msec found by these authors. This discrepancy may again be related to the presence of thalamo-cortical after-discharge.

It must be emphasized that, if one accepts the amplitude of the post-synaptic mass discharge recorded from a nucleus as an indication of the size or effectiveness of the volley transmitted by the nucleus, then these experiments indicate that the cuneate nucleus plays little or no part in the decrease in the responsiveness seen at the cortex. This observation appears to be at variance with that reported by Marshall (1941) and Andersen, Eccles, Oshima & Schmidt (1964). It is possible that this difference could by due to either the species, the type of anaesthesia or the depth of anaesthesia used.

To what extent co-stimulation of cortical efferent fibres affects the cortical recovery curves to stimulation of the white matter underlying the cortex (Fig. 8) is not clear. However, the similarity between the cortical responses to stimulation at this site and the periphery (Fig. 11) seems to indicate that both stimuli excite the same cortical elements. Also there is little evidence in the records obtained from the cortex, in response to stimulation of the white matter, of a positive wave with a short latency indicative of antidromic activation of cortical cells (Porter & Sanderson, 1964). Moreover, there appears to be no part of the cortical response which follows a high stimulation rate, as the antidromic response does (Porter & Sanderson, 1964). In other experiments, it has been found that the antidromic cortical response to stimulation at the level of the pyramidal decussation shows little or no reduction in size to the second of a pair of stimuli unless the interval of separation is 3 msec or less (A. Angel, unpublished observations).

As to the mechanism of the decreased thalamic transmission there are at least three possible factors to consider: (*a*) presynaptic inhibition of fibres entering the ventrobasal thalamus (Andersen *et al.* 1964), (*b*) an inhibitory feed-back system resembling Renshaw inhibition (Andersen, Eccles & Sears, 1964) and finally (*c*) an inhibitory feed-back from the sensory cortex to the thalamus. This last factor is known to be of importance since it has been shown (Angel, 1963*b*) that (i) the absolute unresponsive time of thalamic cells was decreased after cortical ablation, and (ii) the relative unresponsiveness of thalamic cells was accentuated after the sensory cortex had been made hyperexcitable with topically applied strychnine.

In conclusion, these results show that transmission through the thalamus is depressed for a long period of time after the arrival of a synchronous volley, confirming earlier work although the relative unresponsiveness, as judged by both size and latency of the responses, indicates a much longer recovery cycle than has been reported previously. The responses recorded from the primary sensory cortex, either as a mass response or from single cells, reflect this decreased thalamic transmission. In animals anaesthetized with urethane, the thalamo-cortical volley may actually be amplified by the cortex at a time when transmission through the thalamus is decreased. It is interesting to note that if two equal shocks are applied to a single locus in man, their temporal separation must be greater than 15–40 msec before they are felt as discrete events (Rosner, 1961).

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