J. Physiol. (1962), **162**, *pp.* 105–120
With 11 *text-figures*
Printed in Great Britain

THE NON-SELECTIVE BLOCKING ACTION OF γ-AMINOBUTYRIC ACID ON THE SENSORY CEREBRAL CORTEX OF THE RAT

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(*Received* 1 *December* 1961)

γ-Aminobutyric acid can be easily extracted from various parts of the nervous system and since it has been shown to have inhibitory effects on the activity of neurones, interest has centred upon its possible role as a transmitter substance. When it is applied to the surface of the cerebral cortex there is an apparent reversal in sign of any negative electrical activity that may be present. For example, the typical configuration of the normal evoked potential in the rat consists of an initial small positive wave (of latency 7 msec) followed by a second positive wave (latency 8 msec) and lastly a larger negative wave (latency 10–13 msec). This is changed by the application of γ-aminobutyric acid to either a single small positive wave or a positive wave followed by a second larger positive wave.

This effect has been interpreted by other authors (e.g. Purpura, Girado & Grundfest, 1957, 1958; Grundfest, 1958, 1960) as a selective blocking of the excitatory depolarizing component of the evoked potential complex. They suppose that the positive potentials are associated with inhibitory hyperpolarization and can be recorded from the surface unobscured under these conditions.

However, in the experiments quoted, the pharmacological activity of γ-aminobutyric acid was confined to a thin zone at the surface of the brain. If measures are taken to ensure that the whole thickness of the grey matter is exposed to the substance, we have found that almost all electrical activity in the cortex is abolished. This suggests that γ-aminobutyric acid has a non-specific blocking action on cortical neurones. If this is the case, topically applied γ-aminobutyric acid simply produces an inactive surface zone in the cortex. This zone would act as a source for electronegativity taking place underneath it and become positive, thus accounting for the observed reversal at the surface. This hypothesis assumes a fairly sharp boundary between the zone affected by γ-aminobutyric acid and the

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underlying zone not affected by the drug. In the experiments described below we have found that γ-aminobutyric acid does in fact produce a demarcation of this nature as it diffuses slowly into the cortex.

METHODS

Preparation. Twenty albino rats were lightly anaesthetized with intraperitoneal urethane (36%; 1·0 ml./200 g body wt.). The animals were immobilized with a head holder having a spike in each external auditory meatus and a clamp attached to the incisor teeth. The primary receiving area of the sensory cortex was exposed by a trephine hole 4 mm diameter; a polythene collar 3 mm in depth was cemented to the surrounding bone with Horsley's wax. This collar was filled to a constant level with 0·9% saline solution at constant temperature (Fig. 1). The body temperature of the whole animal was maintained at 36° C. The dura was removed in order to admit the micro-electrode.

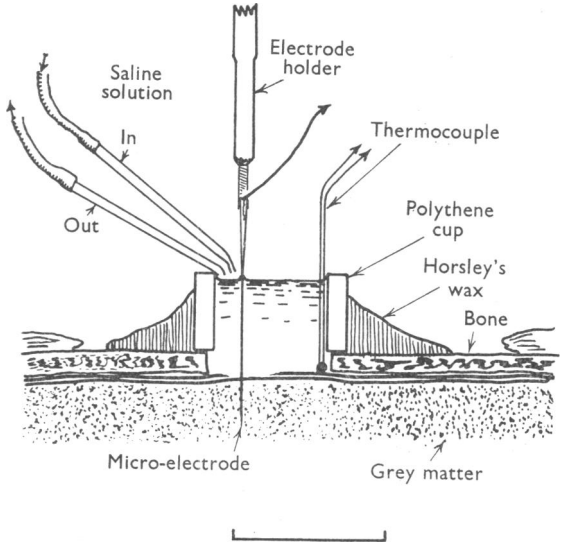

Fig. 1. The recording apparatus. A constant flow of saline at body temperature was maintained except during the application of drugs. The temperature was monitored with the thermocouple. Bar is 0·5 cm long.

Recording. Blood pressure was recorded in some experiments by means of a mercury manometer connected to a cannula in the carotid artery. Potentials were recorded from the surface of the brain by means of a low resistance (about 1 MΩ) saline-filled glass micro-electrode. A second micro-electrode, usually of smaller tip diameter and filled with 3 M-KCl was attached to a micromanipulator and could be introduced to known depths below the cortical surface. The 'reference' electrode was inserted into the musculature of the neck. The two recording electrodes were connected by chlorided silver wire and cathode followers to separate channels of d.c. amplification (having a flat characteristic up to 50 kc/s. Palmer & Read, 1962) and a twin-beam oscilloscope. When it was necessary to record unitary spike discharges of neurones the appropriate electrode channel had a time constant of 200 μsec interposed in the last interstage coupling. In all the figures published in this paper a positive potential at the micro-electrode tip is shown as an upward deflexion of the photographic trace.

Pharmacological preparation. The various drugs used were introduced into the polythene cup, made up in 0·9 % saline solution at body temperature. During the course of such experiments the flow of saline in the polythene collar was temporarily stopped until it was necessary to wash out the drug. A thermocouple was used to monitor the temperature of the saline in the cup; a thermometer inserted into the abdominal cavity measured deep body temperature.

Stimulation. Current pulses of variable strength and duration (usually 0·5–5 V and 100 μsec) were applied via a 1:1 transformer, whose secondary winding was floating with respect to earth, to the stimulating electrodes, which were two fine stainless-steel needles inserted subcutaneously into a selected area of skin distal to the earth electrode. A non-polarizable earth electrode was inserted beneath the skin of the forearm. A chlorided silver wire connected to a saline-filled micro-pipette was used when it was necessary to stimulate the cortex directly.

RESULTS

The normal configuration of the evoked potential from the surface of the cortex, recorded immediately over the primary receiving area of the forelimb, is as follows. There is an initial small positive potential (1), up to 100 μV in amplitude, 1–2 msec in duration, which begins 6·5 msec after the shock is given to the peripheral nerve. This is followed by a second larger positive potential (2), up to 2 mV in amplitude and 3 or more msec in duration, with a latency of 7–9 msec. There is lastly a negative wave (3), usually slightly larger than wave (2), in extreme instances 5 mV in amplitude, and of more variable latency (10–15 msec) and duration (up to 20 msec). The positive wave (1) represents the incoming thalamocortical volley (Chang & Kaada, 1950; Perl & Whitlock, 1955; A. Angel & G. D. Dawson, personal communication). The remaining two waves are generally agreed to be largely post-synaptic in origin, the positive phase at least in part being due to the surface processes of neurones (e.g. apical dendrites) acting as current sources for the depolarization of their deeper parts. The subsequent negative wave occurs when this excitation has spread to involve the surface elements also (Eccles, 1951; Cragg, 1954; Cragg & Hamlyn, 1955; Chang, 1959). The amount of this surface negativity, and the velocity and precise direction of its spread, are variable, particularly in lightly anaesthetized preparations in good condition (Lippold, Redfearn & Winton, 1961).

Surface application of γ-aminobutyric acid

When any drug is applied to the cortical surface it is essential to ensure that the exposed cortex and the solutions used are at body temperature. Small alterations in temperature can produce large changes in the size and form of evoked electrical activity (Lippold & Redfearn, 1960). Failure to observe these precautions may well have been responsible for certain anomalous results reported by other authors.

The effects of application of γ-aminobutyric acid were consistent. In most experiments concentrations of 0·5–10 % gave rise to a rapid change in the surface negative wave (3) which was replaced within 1–10 sec by a

positive deflexion of variable amplitude and duration (Fig. 2). The threshold concentration for producing the surface positive effect varied; it was higher in preparations already showing large stable negative waves on surface recording (e.g. those due to cooling). The actual values ranged from a minimum of 0·1 % to a maximum of 1·0 %.

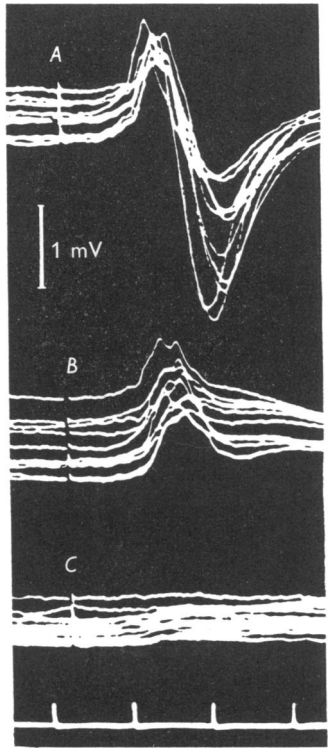

Fig. 2. The effect of topical application of 2 % γ-aminobutyric acid to the sensory cortex of rat anaesthetized with intraperitoneal urethane. Traces recorded from micro-electrode in contact with pial membrane. *A*. Control. 10 successive potentials at 2 sec intervals, superimposed. Stimulus artifact indicates shock given to contralateral forepaw. *B*. 5 sec (the first of the 10 traces) after application of the drug. *C*. 3 hr 40 min after application. Positivity of electrode tip is shown as an upward deflexion. Time marker, 10 msec.

If the γ-aminobutyric acid remained in the cup, the evoked potentials recorded from the surface retained the predominantly positive deflexion but the amplitude progressively declined until after 4–5 hr evoked electrical activity was often entirely absent when high concentrations were employed.

It is known that substances locally applied to the fronto-parietal cortex may produce blood pressure changes (e.g. adrenaline; Walaszek, 1960). Also γ-aminobutyric acid lowers the blood pressure when injected intravenously (Elliott & Hobbiger, 1959). It was therefore necessary to show that a similar effect was not responsible for part or all of the observed

changes in the evoked potential. This was done (*a*) by recording blood pressure during the application of γ-aminobutyric acid to the cortex; 10 % solutions produced little change in blood pressure over 1½ hr. (*b*) Normal evoked potentials could usually be recorded from the cortical hemisphere opposite to that treated with the drug. (*c*) When the blood pressure was lowered to about 60 mm Hg by venesection, normal evoked potentials were present.

When the solution of γ-aminobutyric acid was washed out, normal evoked potentials could once more be recorded after an interval which was dependent on the strength of the solution and the time during which it had remained in the cortical cup. In the case of a 1 % solution recovery occurred in about 30 min following an application of the drug for 30 min.

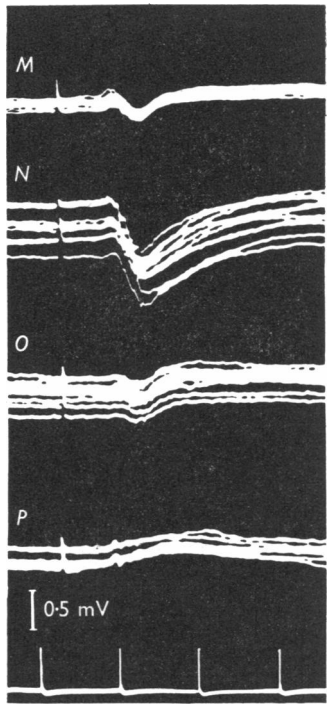

Fig. 3. Recordings of the evoked potential at a depth in the cortex after application to the surface of 2 % γ-aminobutyric acid. *M*. 600 μ below the cortical surface. 31 min after application. The negative wave is considerably reduced in size. *N*. 700 μ below the surface. 45 sec after record *M*. At this depth the electrode was recording from a zone not greatly affected by the drug. *O*. The same depth as *N* but 16 min later. The zone is now affected by the drug. *P*. As *N* and *O* but 3 hr 20 min following initial application. Time marker, 10 msec.

Electrical activity at a depth

The size and form of the normal evoked potential is altered when recorded from a micro-electrode below the cortical surface (Li, Cullen & Jasper, 1956*a*, *b*). In the rat, at depths below 300 μ the positive wave (2)

is reversed and the whole potential consists of a large negative wave. We found that at first the deep potential was unaffected by γ-aminobutyric acid. However, after an interval which depended on the concentration of the substance and the depth of recording there was a reversal in polarity of the deep negative potential (Fig. 3).

By moving the micro-electrode up and down whilst recording, it was possible to show the existence of a boundary within the grey matter. Above it the evoked potentials were entirely positive, while below it they had the

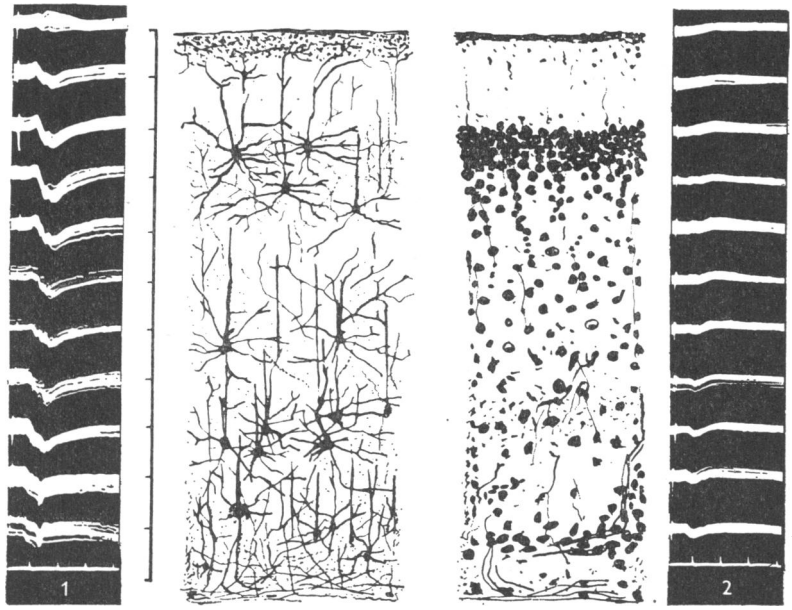

Fig. 4. Drawing from Golgi–Cox preparation (left-hand column) and Nissl preparation (right-hand column) of sensory cortex of a normal rat. Column (1) shows a series of superimposed records taken from another rat of comparable weight at the depths shown on the scale. Column (2) was taken about 30 min after the application to the cortical surface of approximately $2\frac{1}{2}\%$ γ-aminobutyric acid solution (in the same animal). Vertical scale divisions = 100 μ.

usual negative form (Fig. 4). At the demarcation there was a region about 50 μ in thickness in which the evoked potentials were variable in form and polarity, being sometimes positive and sometimes negative.

This boundary slowly travelled deeper into the thickness of the grey matter; it was possible to determine approximately the rate at which this took place by following the zone in which reversal occurred. Figure 5 shows the depth of the boundary plotted against the time after topical administration of γ-aminobutyric acid of various concentrations. The

electrical effects produced by 0·5 % solutions reached 200 μ in 60 min; those produced by 2·5 % solutions reached 800 μ in the same interval.

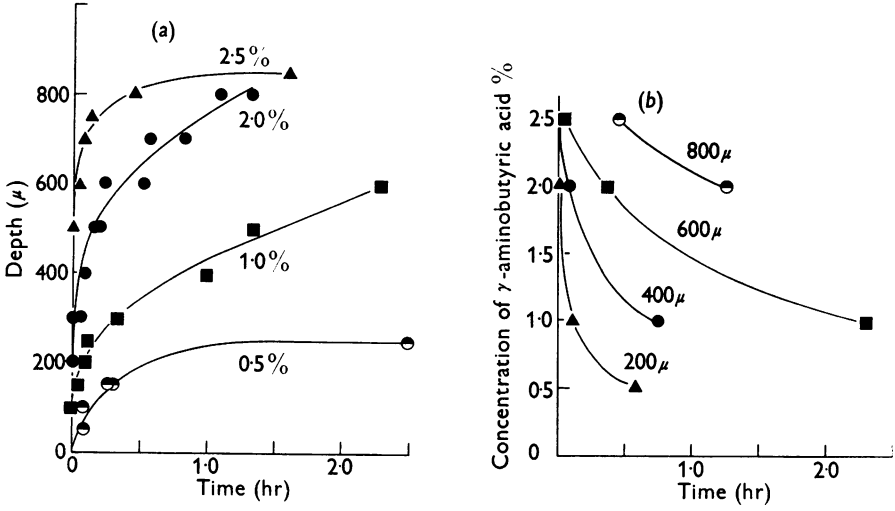

Fig. 5 (*a*). Rates of diffusion into the cerebral cortex of various concentrations of γ-aminobutyric acid. Each curve was obtained from two rats. The criterion for determination of each point was that 9 out of 10 successive evoked waves were completely positive-going at that depth. It should be emphasized that the measurement is of an electrical effect; it does not necessarily imply that the drug itself diffuses in the same way. (*b*) Graph showing the time–concentration relationship for four different depths below the cortical surface; points from 5 (*a*).

Complete abolition of electrical activity

When the cortical cup remained filled with 5 % γ-aminobutyric acid (or a stronger concentration) after 3–6 hr all the grey matter became affected by the drug. If γ-aminobutyric acid merely unmasked inhibitory activity, then as the drug diffused downwards, involving more and more of the cortex, the positive wave would become larger and it would still be possible to record it after all signs of depolarization had disappeared. As the boundary sank into the cortex, in fact, both the positive potentials above it and the negative potentials below it gradually declined in amplitude until eventually no electrical activity could be recorded (Figs. 2*C* and 3*P*).

It was noticeable that if the micro-electrode was moved up and down several times this process was hastened, and it also became difficult to define a sharp boundary. This was probably because the needle tracks served to convey the γ-aminobutyric acid from the surface.

Correlation between positive and negative potentials

The fact that γ-aminobutyric acid greatly diminishes all electrical activity when it has affected the whole thickness of the cortex strongly suggests that its action is non-specific in the sense that it in some way prevents the propagation of all impulses. If this action is assumed to be the case, the positive waves recorded from the surface when only a narrow zone of grey matter has been affected by the drug can be explained by the supposition that this inactive surface layer is behaving as a source for the

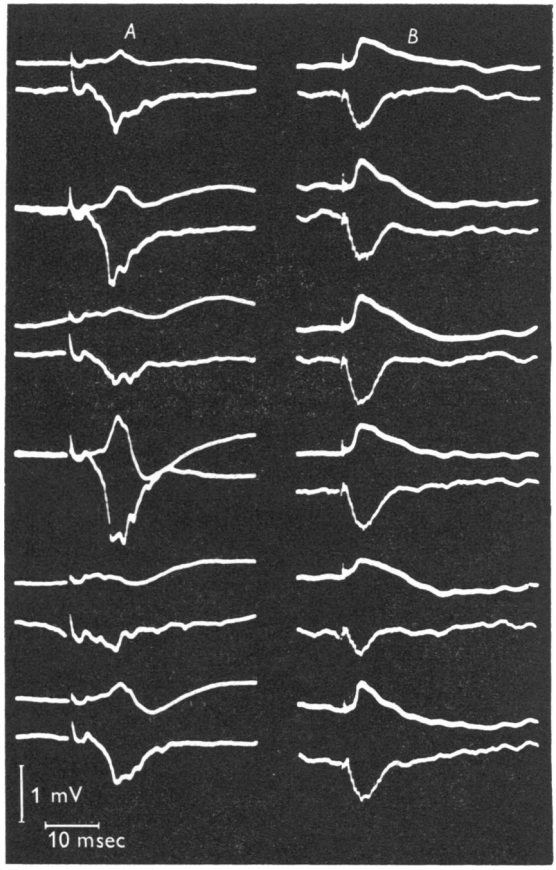

Fig. 6. Records of pairs of recordings taken at 2 sec intervals, the top trace of each pair on the surface and the lower taken simultaneously 500 μ below it. *A*. Normal evoked potentials, showing variability associated with low stimulus strengths. *B*. 1×10^{-3} D-tubocurarine was applied to the surface (in order to produce large negative waves). 2·5% γ-aminobutyric acid was applied 22 min later. At the surface, corresponding positive waves were then observed. 25 min later the ladder shown in *B* was taken.

electronegativity beneath. On this basis it might be expected that simultaneous recording just above and just below the boundary would show some measure of correlation between the amplitude and duration of the respective positive and negative evoked potentials. According to the hyperpolarization theory an inverse correlation would be more likely. Within limits, the former state of affairs was found to be the case (Fig. 6).

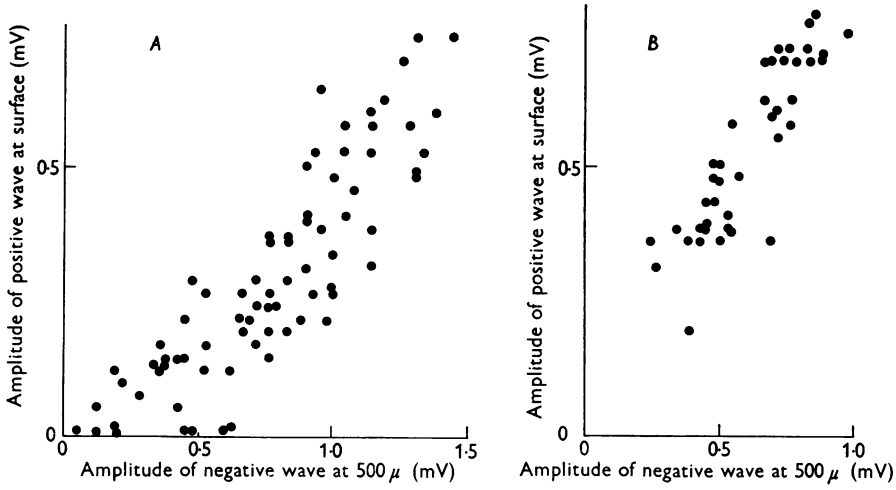

Fig. 7. The relation between positive wave (from surface) and negative wave (from a depth of 500 μ). Measurements taken from the experiment shown in Fig. 6. *A*. Normal cortex. *B*. 30 min after topical application of $2\frac{1}{2}$ % γ-aminobutyric acid.

Figure 7 shows the amplitude of the surface-positive wave plotted against that of the negative wave 500 μ below the pial membrane. The points were obtained from records taken simultaneously at both depths while the evoked response varied in amplitude. Another instance of such correlation was found in the action of D-tubocurarine in conjunction with γ-aminobutyric acid. Usually, surface application of tubocurarine produces abnormally large negative waves (Cairnie & Malcolm, 1960). If γ-aminobutyric acid is then applied to the surface after tubocurarine, very large surface-positive waves can be recorded simultaneously with the very large negative waves underneath (Figs. 6*B* and 8).

Effect of damage and of magnesium

If this action of γ-aminobutyric acid is non-specific it should be possible to mimic it in several ways. Damage to the surface layers might result in the loss of propagated activity and yet leave the cell processes in a condition in which they are still able to function as electrical sources. This state

of affairs was occasionally noted when the cortex was damaged in dissection or the surface allowed to become dry. In such cases the evoked potential had only a positive-going wave.

When saline containing 0·1 M-$MgSO_4$ was introduced into the cup, surface recording showed positive evoked potentials while the deep electrode recorded negative waves at the same time. The general appearance of the records (Fig. 9) resembled those obtained by using 1 % γ-aminobutyric acid, although the time course of the effect was more rapid.

The finding that magnesium sulphate also gave rise to positive evoked potentials at the cortical surface suggested the possibility that part or all of the effects produced by γ-aminobutyric acid were due to the osmotic action of the solutions. However, 25 % sucrose solution, topically applied, had little effect on the form of the evoked potential; iso-osmotic γ-aminobutyric acid on the other hand, was still effective.

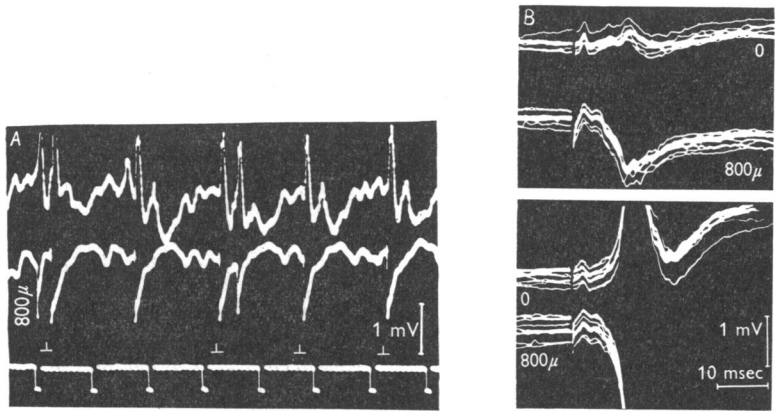

Fig. 8. *A*. Records from the cortical surface (top trace), and 800 μ beneath the surface (lower trace). The large waves, positive at the surface and negative below it, were produced by the initial topical application of 1×10^{-3} D-tubocurarine chloride. This was followed after 45 min by topical γ-aminobutyric acid (2 %). Stimulation at points marked with a $\perp$. The remaining waves were of spontaneous origin.

B. Records of ten superimposed evoked potentials taken on faster time base than *A*. Top block, before drugs applied to surface of brain; bottom block, a few minutes before record shown in *A* was taken. Note that −ve and +ve waves are smaller in *A* than in *B*, due to the further penetration of γ-aminobutyric acid into the grey matter.

Recording of unitary spike discharges

The normal evoked potential was accompanied by two, and in some cases three, distinct bursts of spikes. There was an initial abrupt, high-frequency and relatively synchronous discharge which corresponded in time with the surface-positive wave (2). The amplitude of these spikes was maximal deep in the cortex. The second burst, corresponding with the beginning of the

negative wave at the surface, consisted of smaller and more scattered discharges.

In the presence of γ-aminobutyric acid, the magnitude of these bursts decreased, as did the evoked potentials. Above the boundary level none could be recorded. When the evoked potentials were abolished, there were no spikes (Fig. 10). In addition, the normal random discharge of action potentials associated with general cortical activity was abolished by γ-aminobutyric acid (Fig. 11).

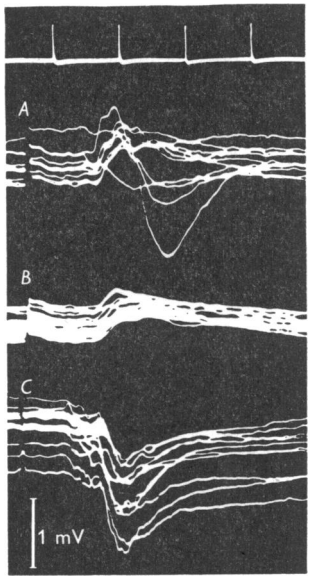

Fig. 9

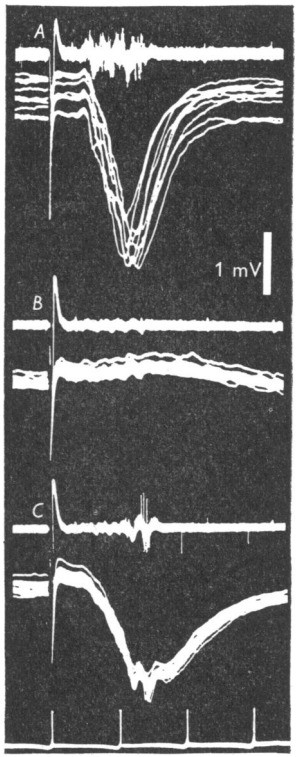

Fig. 10

Fig. 9. The effect of 0·1 M-$MgSO_4$ solution on the normal sensory cortex of the rat. The evoked potentials undergo a similar series of changes to those produced by γ-aminobutyric acid. *A* shows normal evoked potential from the surface. *B*, the same 5 min after application of $MgSO_4$. *C*, 1 min later, recording at 1000 μ below the surface. Time marker, 10 msec.

Fig. 10. The effect of γ-aminobutyric acid on unitary spike activity in the sensory cortex. *A*. Bottom trace, normal evoked potential recorded at 500 μ. Upper trace from same electrode tip at higher gain and with 200 μsec time constant. *B*. The same recorded 45 min after topical application of $2\frac{1}{2}$% γ-aminobutyric acid. *C*. After 46 min 30 sec, recording at 1000 μ.

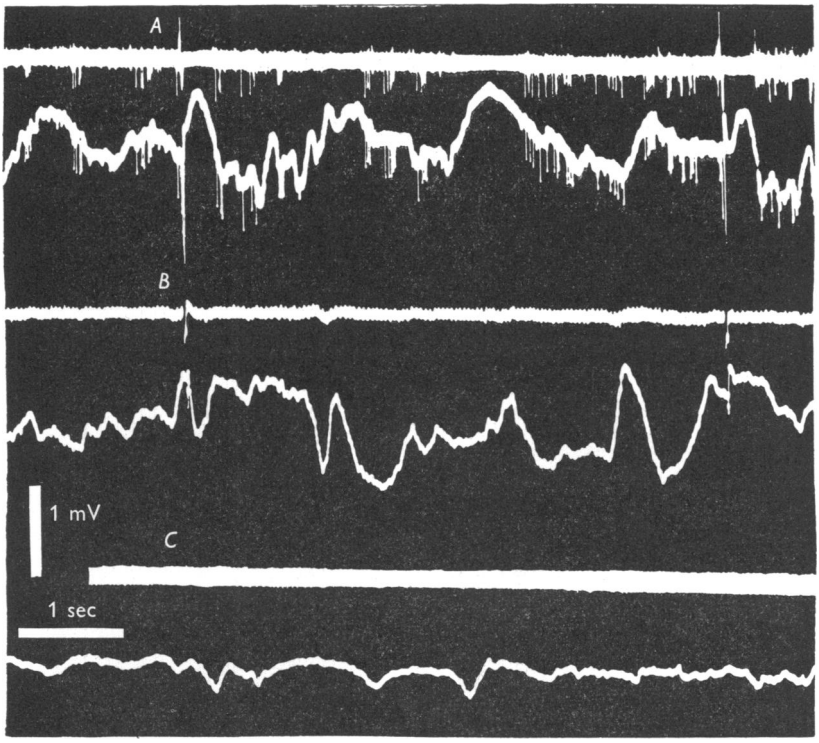

Fig. 11. *A*. General cortical activity recorded at 950 μ in the normal cortex. *B*. The same 45 min after topical 5 % γ-aminobutyric acid. *C*. The same 2 hr after topical 5 % γ-aminobutyric acid. Upper beam recorded of each pair with time constant of 200 μsec.

DISCUSSION

γ-Aminobutyric acid has been shown to cause inhibition by activating inhibitory synapses in crustacea (McLennan, 1957*a*; Kuffler & Edwards, 1958; Boistel & Fatt, 1958; Grundfest, Reuben & Pickles, 1959; Furshpan & Potter, 1959). It also bears close resemblance in its actions to an inhibitory substance that can be isolated from the mammalian central nervous system (Florey, 1954; Florey & McLennan, 1955*a*, *b*; McLennan, 1957*a*). It has therefore been tempting to assign to it the role of an inhibitory transmitter; moreover, various authors have shown that when it is locally applied to the cerebral cortex positive potentials appear (Purpura *et al.* 1957, 1958; Grundfest, 1958; Iwama & Jasper, 1957; Mahnke & Ward, 1960).

Our results show that the presence of these positive waves does not in itself constitute evidence in favour of inhibitory hyperpolarization (as pointed out by Jasper, Gonzalez & Elliott, 1958) and although we have no

direct information regarding the physiological role of γ-aminobutyric acid, we have found that the administration of it has a generalized blocking effect on all neuronal activity. There were apparently no positive post-synaptic potentials (of long duration) nor was any spike activity present in the affected region when the whole thickness of the cortex was affected by the drug. Thus it probably acts in a non-specific way on the excitable membrane and alike abolishes the electrical signs of the incoming afferent volley, post-synaptic dendritic potentials and propagated spike activity. Such a hypothesis agrees with the view of Curtis & Watkins (1960), who have shown in spinal motoneurones that γ-aminobutyric acid increases their threshold both to excitation and inhibition, probably by giving rise to an increase in chloride permeability (Boistel & Fatt, 1958; Curtis, Phillis & Watkins, 1959).

Our results can be explained in terms of simple current flow between the excited depolarized regions and the inactive regions of neurones whose dendrites are orientated normal to the surface of the brain. Thus synaptic spread of underlying electronegativity (at below 300 μ in depth) produced by the incoming volley cannot extend to the surface zone inactivated by the γ-aminobutyric acid. This activity can, however, still draw current and give rise to a prolonged surface positivity. In this connexion it is interesting to note that Purpura (1958) claims that in the case of cerebellar evoked potentials no surface positivity is produced by the topical application of γ-aminobutyric acid. He construes this as evidence that there is a relative absence of inhibitory systems in the cerebellum. It must be remembered, however, that the incoming fibres enter the molecular layer and have their synapses close to the cerebellar surface. It is therefore possible that γ-aminobutyric acid acts by gradually depressing activity in these fibres first of all, thus giving rise to the observed effect of a diminution and eventual abolition of the evoked potential.

Further evidence against the inhibitory origin of the positive wave recorded at the surface, both normally and after the application of γ-aminobutyric acid, is the fact that the beginning of the positivity is simultaneous with spike activity recorded at a depth, and that there is a strong correlation both in size and form between the positive wave at the surface and the negative wave at 300 μ and below.

Purpura & Grundfest (1956) suggest that the greatly enlarged negative wave of the evoked potential which follows the topical application of curare is due to a blocking of hyperpolarizing activity. If this were so, the application of γ-aminobutyric acid, which they state selectively blocks depolarizing synapses, should be followed by the abolition of evoked electrical activity. Instead of this, large positive waves are observed at the surface corresponding with large negative ones at a depth.

γ-Aminobutyric acid will not pass the blood–brain barrier and thus it has little effect on the normal animal brain when injected intravenously. When in contact with the surface of the cortex it apparently penetrates the brain substance at a very slow rate. This is surprising, since a number of studies on diffusion in brain slices indicate that the diffusion coefficients, e.g. of ^{131}I, Cl, sucrose, are not greatly different from those measured in water (McLennan, 1957*b*; Davson & Spaziani, 1959). If the γ-aminobutyric acid were strongly adsorbed or metabolized by the tissue, the building up of the inhibitory concentration at any distance would be slowed down considerably and it may well be that this is the explanation.

SUMMARY

1. The electrical response of the primary sensory cortex of the lightly anaesthetized rat was recorded at various depths and after various intervals of time following the application of γ-aminobutyric acid to the cortical surface.

2. As the γ-aminobutyric acid diffused through the depth of the cortex, a boundary could be discerned above which evoked potentials were entirely positive in polarity and no unitary spikes could be recorded, and below which evoked potentials were negative, and in which there were spikes in association with the evoked response.

3. 0·5% solutions were effective at a depth of 200 μ in 1 hr; 2·5% solutions reached 800 μ in the same time.

4. When the whole thickness of the cortex was involved, electrical activity could not be recorded in the affected region.

5. There was a correlation between the amplitude and form of simultaneously recorded potentials above and below the boundary.

6. It is concluded that γ-aminobutyric acid acts by a non-specific blocking of all neuronal activity, both post-synaptic and propagated, and that the positive waves produced by the drug at the surface are not necessarily due to hyperpolarization.

We are indebted to Mr G. W. Cambridge of John Wyeth and Brother, Ltd., for a generous sample of γ-aminobutyric acid. We are grateful to the Nuffield Foundation for providing a grant.

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