J. Physiol. (1981), **319**, *pp.* 143–152
With 5 text-figures
Printed in Great Britain

INFLUENCE OF ELECTRIC FIELDS ON THE EXCITABILITY OF GRANULE CELLS IN GUINEA-PIG HIPPOCAMPAL SLICES

BY J. G. R. JEFFERYS

*From the Department of Physiology, University College London, Gower Street, London WC1E 6BT and the Sobell Department of Neurophysiology, Institute of Neurology, Queen Square, London WC1N 3BG**

(*Received 19 January 1981*)

SUMMARY

1. Monosynaptic evoked potentials were recorded from the granule cell layer of slices of guinea-pig hippocampus maintained *in vitro*. Current pulses of 25–250 msec duration were passed across the slices, between gross electrodes in the bathing liquid.

2. Polarizing current modified the excitability of the granule cells as judged by changes in their population discharge during postsynaptic responses. All durations of polarization had at least qualitatively similar effects. Conventional current from dendrites to cell bodies increased excitability (and vice versa). This is consistent with altered membrane potential of a spike trigger zone, at or close to the granule cell bodies, imposed by the fraction of polarizing current which flows intracellularly.

3. In some experiments polarization also affected the presynaptic volley and (hence?) the synaptic potential. When this occurred it was in the wrong sense to explain the concomitant changes in population spike.

4. Focal polarization, where currents were applied across the cell body layer between a small electrode on the mid or outer dendritic regions and a remote gross electrode, altered granule cell excitability in the same direction as in (2). Thus conventional current injected at the dendritic electrode increased excitability.

5. The smallest effective polarizing currents caused extracellular voltage gradients of 5–10 mV/mm, which is less than occurs in this tissue during synchronous activation of the neurones or during seizure activity. Therefore such field potentials could increase the synchrony of discharge of the granule cells.

INTRODUCTION

Extracellular voltage gradients of up to tens of millivolts per millimetre can be generated by synchronous activity of hippocampal neurones. The present study examines whether granule cell evoked responses can be modified by measured electric fields imposed on hippocampal slices.

Fields of up to 50 mV/mm have been recorded during granule cell evoked potentials (Lømo, 1971; Gardner-Medwin, 1976), 20 mV/mm during seizure activity

* Present address.

(Green & Petsche, 1961*b*) and of the order of 0·5–4 mV/mm during normal slow wave activity (Green & Petsche, 1961*a*; Winson, 1974). Steady potential gradients of 4 and 10 mV/mm have been recorded in hippocampus under physiological conditions and during seizures respectively (Gloor, Vera, Sperti & Ray, 1961; Gloor, Vera & Sperti, 1963), while 10 mV/mm has been found in normal rat neocortex (Bures, 1957). Previous work on extracellular polarization has used currents lasting many seconds or even minutes, passed between a remote electrode and one placed in or on the tissue or the overlying cerebrospinal fluid. Currents that modified the spontaneous firing rate of neocortical neurones produced voltage gradients of 50 mV/mm in the cat (Creutzfeld, Fromm & Kapp, 1962) and 2·5 mV/mm in the rat (Bindman, Lippold & Redfearn, 1964). In the latter example similar current strengths also altered evoked potentials. Polarization of the hippocampus with fields of 20–30 mV/mm altered the ease with which action potentials could be initiated in pyramidal cell dendrites (Purpura & Malliani, 1966; Purpura, 1969; see also Purpura, 1974).

Some of the extracellular current passing a neurone will cross its membrane to flow intracellularly. The precise pattern of the resulting membrane current depends on the extent of that neurone, in relation to its space constant, in the direction of the current. However, it will enter the cell through regions closer to the positive end of the extracellular potential gradient, and leave at those closer to the negative, resulting in hyperpolarization and depolarization respectively. Increased firing rate of neocortical pyramidal cells was obtained with current expected to depolarize their somata (Creutzfeld *et al.* 1962; Bindman *et al.* 1964), in contrast with the lobster stretch receptor where firing was increased by dendritic depolarization under fields as low as 1 mV/mm (Terzuolo & Bullock, 1956).

In the present preparation some control over the direction of the extracellular current in relation to the cell layers is afforded by the careful positioning of pairs of polarizing electrodes in the solution bathing the slices. The mechanism of changes induced by polarization can be clarified by an analysis of the laminar evoked potentials.

METHODS

The preparation and maintenance of the 400–600 μm thick hippocampal slices were generally as described previously (Jefferys, 1979). Longitudinal slices were cut from the ventral/medial surface of the dentate area (Yamamoto & Kawai, 1967; Bliss & Richards, 1971). Transverse slices were cut perpendicular to the long axis of the hippocampus (Skrede & Westgaard, 1971). In more recent experiments transverse slices were cut using a Vibroslice tissue slicer (Campden Instruments Ltd., London W8 7TH, U.K.) in preference to the hand-held razor and recessed cutting-guide used previously.

Slices were immersed in oxygenated artificial c.s.f. which flowed at 1·5–2·5 ml./min through a water-jacketed bath. They were supported between a nylon net and a set of parallel fibres, each mounted on nylon rings, which were clamped in the shallow cylindrical recording chamber by a slightly oversized split ring.

Silver–silver chloride polarizing electrodes were mounted in the chamber in various ways. To pass current perpendicular to longitudinal slices (i.e. vertically) chlorided silver disks, with the same diameter as the recording chamber (24 mm), were clamped between nylon spacing rings approximately 2·5 mm above and below the slice. Stimulating and recording electrodes were admitted through a hole in the upper disk. Horizontal currents (across transverse slices) were passed either between chlorided silver foil plates (50 mm^2 in area) mounted in the lid of the chamber, or between

sintered silver–silver chloride cylinders (2 mm diam., 4 mm length) mounted on external clamps. In some experiments, silicone rubber blocks were used to restrict the spread of current to a rectangular area (5 × 20 mm) which contained the slice in the middle and pellet polarizing electrodes at both ends. For experiments on focal polarization, one chlorided silver wire electrode (75 μm diam. 1 mm length) was positioned on the mid-molecular (dendritic) layer of transverse slices, and the other larger electrode was placed in the solution away from the slice. Any risk of silver toxicity was minimized by discarding the artificial c.s.f. after a single passage through the chamber.

Conventional electrophysiological methods were used for extracellular field recordings, which were photographed from an oscilloscope or analysed and plotted on a minicomputer. Glass recording micropipettes had tips 0·5–1·5 μm in diameter and contained 3 M KCl or NaCl. Recording of evoked potentials during the current pulses was helped by positioning the reference electrode, using a micromanipulator, in order to balance out the imposed voltage changes at the recording site, though sometimes filtering was also necessary (typically 8 Hz high pass, 2·5 kHz low pass). Voltage gradients during polarization were measured from d.c. recordings from tracks in the slice which included the recording site. Current pulses lasting 25–250 msec were applied through the polarizing electrodes by isolated stimulator units (Digitimer, Welwyn, U.K.: types 2533 and DS2), usually with a series resistor of 1–50 kΩ. The polarizing electrodes deteriorated more slowly when the protocol was arranged so that equal currents were passed in opposite directions alternately.

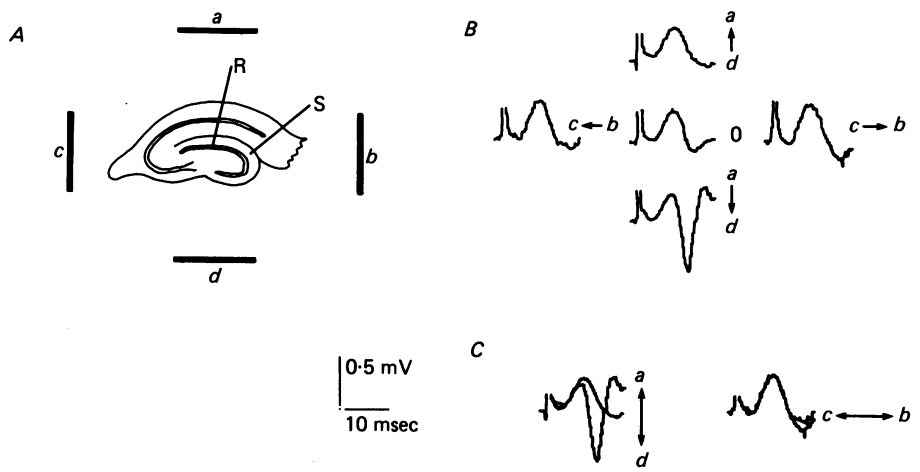

Fig. 1. Effect of direction of polarizing current. *A*, polarization currents were passed across a transverse slice, between gross electrodes positioned in the artificial c.s.f. at sites *a* and *d* or *b* and *c* (S, stimulus, site; R, recording site). The polarization potential gradient was 17 mV/mm, measured over a 250 μm track in the slice. Responses to afferent volleys were recorded from the cell body layer and are labelled with the direction of conventional current (*B*). To aid comparison, responses under both directions of current have been superimposed for each electrode pair (*C*). Temperature was 27 °C.

RESULTS

Responses to the stimulation of afferent fibres were recorded from the granule cell body layer of a transverse slice, while current was passed in the plane of the slice between a pair of chlorided-silver electrodes placed about 8 mm either side of the recording site (Fig. 1). Current perpendicular to the cell layer, making the molecular layer (dendrites) more positive than the cell body layer, greatly increased the amplitude of the population spike and reduced the time to its peak by 1 msec (Fig.

1*B*, *C*, $a \rightarrow d$). This current tends to depolarize the granule cell bodies and will be called soma-depolarizing for convenience. Current in the opposite direction ($d \rightarrow a$) reduced or abolished the spike. Currents of equal strength parallel to the cell layer ($b \leftrightarrow c$) had no consistent effect on the responses.

In most experiments polarization perpendicular to the cell layer which affected the population spike did not alter the synaptic wave. However there were a few exceptions, where soma-hyperpolarization enhanced the synaptic wave while still

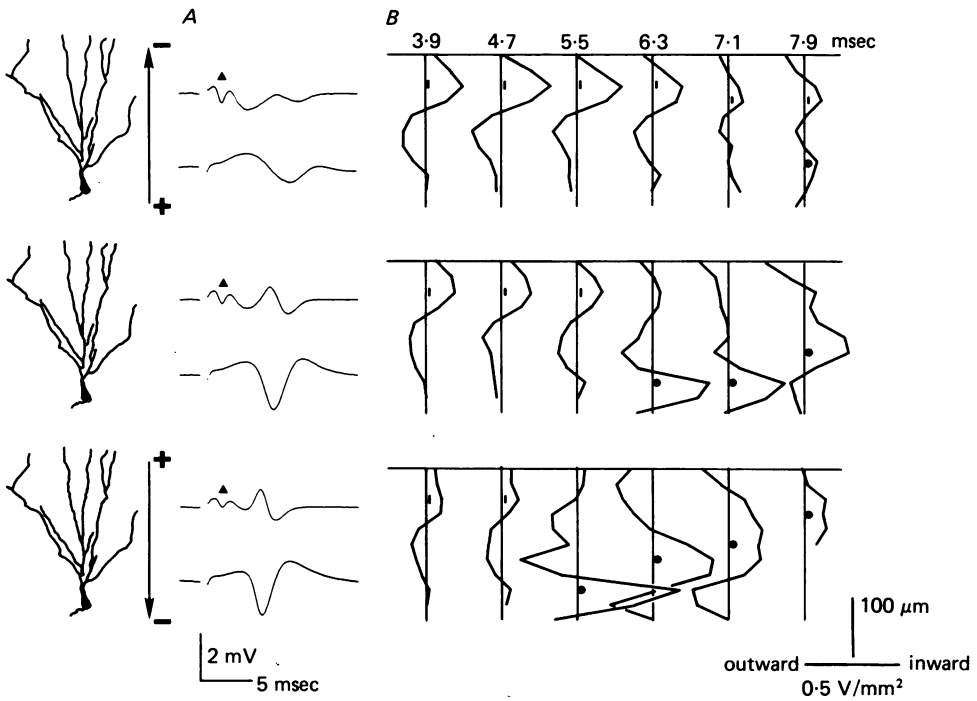

Fig. 2. Evoked potentials and 'membrane current' profiles under polarization. Responses were recorded from a longitudinal slice while current was passed perpendicularly from cell body layer to dendrites (upper row), in the opposite direction (bottom row) and in the absence of current (middle row). To the left of each row the tracing of the Golgi-stained granule cell indicates the approximate recording sites for parts *A* and *B*. *A*: evoked potentials recorded from cell body and afferent layers; afferent volley has been marked (▲). *B*: profiles of second spatial difference of potential provide estimates of membrane current at a sequence of times after the stimulus; maximum inward synaptic and population spike currents have been marked (—) and (●) respectively.

depressing and delaying the population spike (as described above), and soma-depolarization attenuated the synaptic wave, while potentiating the population spike (Fig. 2). Close inspection of responses from the mid-dendritic region (Fig. 2*A*) revealed a small biphasic potential preceding the synaptic wave, which can be attributed to the presynaptic volley (Lømo, 1971; Jefferys, 1979). Polarizing current which decreased the synaptic current also depressed this early potential by a similar amount, while the reverse polarization increased both. In the experiment illustrated each of these changes was by about one third of the control values.

Extracellular polarizing currents should alter the membrane potential of at least the distal portions of the dendrites in the opposite sense to the cell bodies. A depth profile analysis was performed to discover whether polarization qualitatively altered the pattern of events during the granule cell response. Recordings were taken at 25 μm steps on a track across the cell layer, and depth profiles were constructed from these for each of a series of times after the stimulus. The profiles were analysed using a numerical approximation of the second spatial derivative of voltage as an indication

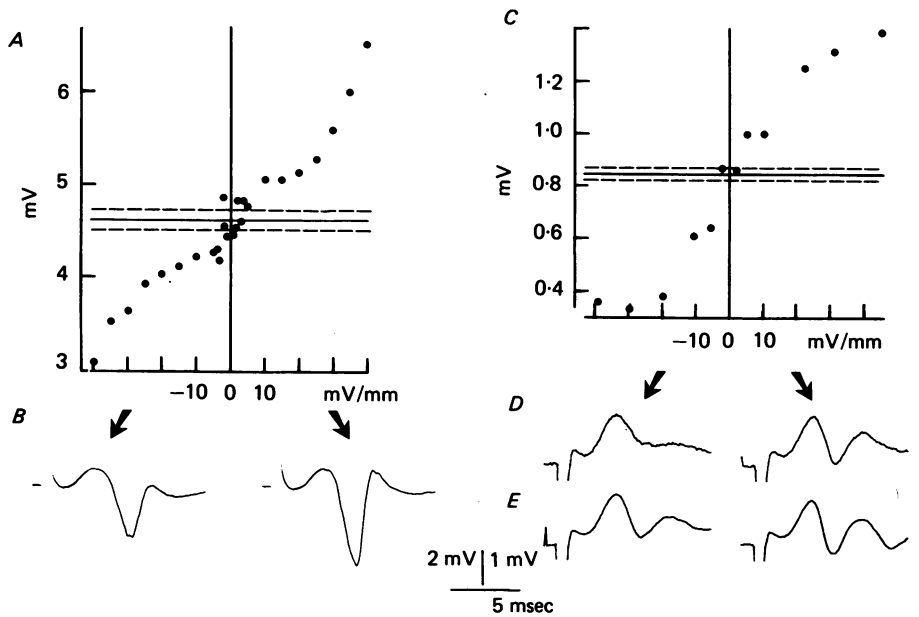

Fig. 3. Effect of varying strength of polarization on population spike. Population spike amplitude was measured from recordings from the cell body layers of a longitudinal slice (*A*, *B*) and a transverse slice (*C*, *D*, *E*). Polarization gradients were measured along tracks in the slices, 400 and 700 μm long respectively. Under positive gradients the granule cell dendrites are positive with respect to the cell bodies. Spike amplitude has been plotted against polarization gradient (*A*, *C*), and the mean $\pm$2 S.E. population spike amplitude without current is indicated by the horizontal continuous and dashed lines. Sample responses from these experiments are reproduced (*B*, *D*). The responses, *E*, were triggered after 10 msec polarization, compared with 100 msec for *C* and *D*. Calibrations: *B*, 2 mV, 5 msec; *D*, *E*, 1 mV, 5 msec. Temperature 35 °C.

of membrane current (Humphrey, 1968; Jefferys, 1979 for this preparation). These experiments showed that the normal sequence of events was not affected by polarization (Fig. 2*B*). Synaptic current entered the mid-dendritic region and could trigger a spike at, or near to, the soma by passive current spread. The spike then invaded the dendritic tree. Polarization affected only the size and timing of the membrane currents, and not their sequence or location.

The polarization was varied systematically to determine the minimum strength that affected the population spike. Potential gradients caused by the polarization were measured from d.c. recordings in the slice. The current flow in the slice, as judged from the voltage gradients, was mainly perpendicular to the cell layers. In typical

experiments on a longitudinal and a transverse slice, polarization profiles parallel to the axes between the polarizing electrodes gave gradients of 24·8 ± S.E. 0·6 mV/mm over 600 μm, and 21·0 ± S.E. 0·6 mV/mm over 750 μm, respectively. The corresponding gradients perpendicular to this axis were less than 1·0 and 1·3 mV/mm respectively.

The population spike was affected by polarization of 10 mV/mm or less in each of six experiments. In the two examples illustrated in Fig. 3 changes in population spike amplitude were detected with polarizing gradients of 5·4 and 5·0 mV/mm, while the variability of the responses made it difficult to discern any effects with gradients below 4·1 and 2·3 mV/mm respectively.

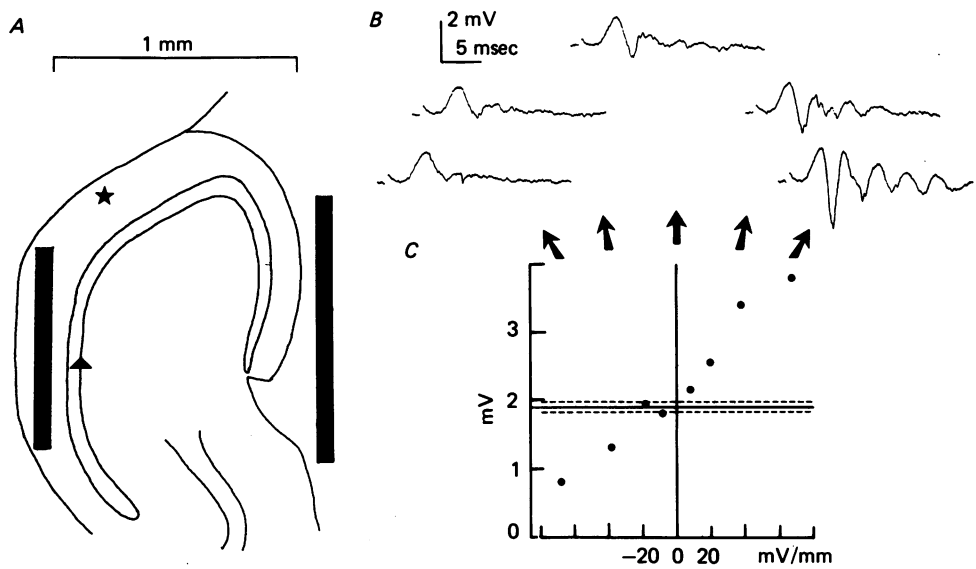

Fig. 4. Local polarization. Polarizing electrodes were positioned on the middle molecular layer of, and adjacent to, a transverse slice (filled bars on drawing of dentate area of this slice: *A*, stimulus site has been marked by a star and recording site, in the granule cell body layer, by a triangle). Polarizing current was varied and the population spike amplitude plotted against imposed potential gradient measured over 100 μm from the inner molecular layer to the hilar edge of the cell body layer (*C*). Evoked potentials from this experiment are reproduced above (*B*). Calibrations: *A*, 1 mm; *B*, 2 mV, 5 msec. Temperature 35 °C.

With particularly careful positioning of the reference recording electrode it was possible to record evoked responses during brief polarizations. The responses illustrated were evoked 100 and 10 msec (Figs. 3*D* and *E* respectively) after the start of polarization pulses (potential gradients of 21·5 mV/mm). The effects of the longer and the short polarizations were at least qualitatively similar.

The polarizing fields used above differ from those generated by the granule cells themselves in being uniform across the cell layer. In order to mimic more closely endogenous granule cell fields, a small polarizing electrode (1 mm × 75 μm diameter chlorided silver wire) was placed on the surface of the middle molecular layer of a transverse slice, so that the closest edges of the wire and cell body layer were 70–80 μm

apart (Fig. 4*A*). Constant current, from the Digitimer stimulator in series with a 370 kΩ resistor, was passed between this electrode and another in the bathing solution. The effect of polarization was as described above. Thus current between the dendritic and remote electrodes (soma-depolarization) enhanced the post-synaptic population spike while the opposite current depressed it. The polarizing current path cannot have been perpendicular to the cell layers in this situation, especially as the

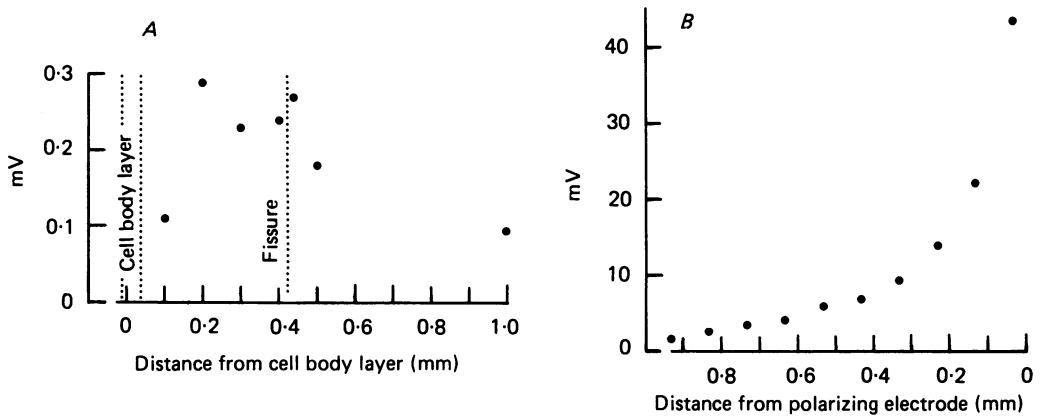

Fig. 5. Effect of site of local polarization. During an experiment similar to Fig. 4, the local polarizing electrode was moved between the granule cell body layer and the adjacent CA1 region (*A*). The increase in population spike amplitude under positive polarization has been plotted against distance between the cell body layer and the nearest edge of the polarizing electrode. The polarization field from this electrode is plotted in *B*.

upper surface of the slice had been exposed to moistened and warmed oxygen in order to reduce current leakage. However as the previous results (Fig. 1) showed that the most effective direction of the current was perpendicular to the laminae, the resulting potential gradient was measured in this direction, over 100 μm from the dendritic layers to the cell body layer, at the depth from which the responses were recorded. In the experiment illustrated soma-depolarizing fields as low as 7·7 mV/mm had a detectable effect on spike amplitude and depolarizing fields of greater than 38 mV/mm resulted in repetitive discharges in the response. Focal polarization was effective from the mid and outer dendritic regions, but not from the proximal dendritic laminae (Fig. 5*A*: immersed slice). The effect also decreased as the focal electrode was moved across the hippocampal fissure into the CA1 region, presumably because the current density decreased with distance from the local source (Fig. 5*B*).

DISCUSSION

Extracellular currents applied perpendicular to the granule cell layer in hippocampal slices altered the excitability of these cells. The evidence presented suggests this was due to modification of the membrane potential at a spike trigger zone in the vicinity of the cell bodies by the fraction of current which flowed intracellularly. Polarizing currents in the direction soma to dendrites would be expected to hyperpolarize the

soma and was found to depress the population spike. The opposite 'soma-depolarizing' current potentiated the spike.

It can be concluded that the predominant effect was on the granule cells rather than on, for instance, the presynaptic axons, because (*a*) current parallel to the cell layer had little effect on the population spike and (*b*) currents perpendicular to the cell layer, which did affect the population spike, either did not influence the synaptic wave at all or altered it in the wrong sense to explain the changes in the spike. When changes in the synaptic wave were found they were associated with changes in the amplitude of the presynaptic tract potential, and probably resulted from stray currents affecting the membrane potential of the presynaptic axons either at the synapse or the stimulating site.

Analysis of the laminar evoked potentials showed that the pattern of events was not affected by polarizing currents. Only the sizes of the various components and the delays before them were altered. As was described elsewhere (Jefferys, 1979), there was no evidence of dendritic spikes triggered directly by synaptic potentials and preceding the population spike, but once a spike had been triggered at or near the cell body it invaded the dendrites. The lack of clear changes in events in the dendrites does not necessarily mean that altering their membrane potential has no effect, as this extracellular polarization is likely to have its maximum effect at either end of the soma-dendritic complex and may have little effect on most of the dendritic membrane in between.

The currents applied during this study resulted in potential gradients of up to 70 mV/mm, which were usually perpendicular to, and generally uniform across, the cell laminae. The smallest currents that had a detectable effect on the responses described here produced gradients of 5–10 mV/mm. Such gradients correspond to a potential difference across the granule cell layer ($<400\ \mu$m) of 2–4 mV, which provides an upper limit to the change in the soma membrane potential caused by these currents. The sensitivity to extracellular fields of spike initiation in granule cells, shown by the presented data on population responses, suggests it may be fruitful to look for ephaptic interactions between neighbouring granule cells in the layers of densely packed cell bodies (Laatsch & Cowan, 1966).

These results also suggest that fields generated during evoked potentials and seizures can alter the properties of granule cells. During evoked population spikes the soma-negative fields, of up to 50 mV/mm in intact preparations and 10–20 mV/mm in the present submerged slice (e.g. 12 mV/mm for the data of Fig. 2), could depolarize and reduce the threshold of cell bodies that had not discharged, thus tending to increase the synchrony of the granule cell discharge (Gardner-Medwin, 1976). These endogenous fields are not linear across the cell layers but have negative and positive potential peaks, which in the case of the population spike would be in the cell body layer and 100–150 μm away in the dendritic laminae respectively. However granule cell excitability was also increased by 'focal polarization' which mimicked the population spike field potential by injection of current to the mid-dendritic laminae. The focal electrode had to be at least 70–100 μm from the cell body layer to be effective (presumably limited by the length constant of the granule cells). Such polarization was effective when it set up potential gradients as low as 8 mV/mm in the vicinity of the cell body layer. During seizures the soma negative

steady potential described by Gloor *et al.* (1961) would tend to depolarize the cell bodies, while the oscillating seizure potential would help synchronize granule cell discharges during its soma-negative phases.

This work has not revealed any effects due to polarization of 4 mV/mm or less. It is not clear whether this represented a threshold field, or whether the variability of the response limited the resolution of the experiments so that averaging would have revealed weaker fields to be effective. The latter possibility receives some support from the observation that spike amplitude was proportional to polarization field over the range ± 20 mV/mm. Therefore this work neither supports nor excludes modification of granule cell excitability by spontaneous slow potentials or steady potentials under non-pathological conditions. Whether direct modification of granule cell excitability by any of these endogenous fields has any functional significance depends on how the relay operates normally. Relevant factors might include the variability in excitability through the cell population, the pattern of afferent activity, and the safety factor of transmission through the relay.

I am grateful to Dr A. R. Gardner-Medwin and Professor T. A. Sears for valuable discussion and encouragement, to Dr T. V. P. Bliss for critical reading of the manuscript, and to the Brain Research Trust and the Medical Research Council for support.

REFERENCES

BINDMAN, L. J., LIPPOLD, O. J. C. & REDFEARN, J. W. T. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J. Physiol.* **172**, 369–382.

BLISS, T. V. P. & RICHARDS, C. D. (1971). Some experiments with *in vitro* hippocampal slices. *J. Physiol.* 214, 7–9*P*.

BURES, J. (1957). Ontogenetic development of steady potential differences in cerebral cortex in animals. *Electroen. Neurophysiol.* **9**, 121–130.

CREUTZFELD, O. D., FROMM, G. & KAPP, H. (1962). Influences of transcortical d.c. currents on cortical neuronal activity. *Expl. Neurol.* **5**, 436–452.

GARDNER-MEDWIN, A. R. (1976). The initiation of action potentials in hippocampal granule cells. *Exp. Brain Res. Suppl.* **1**, 218–222.

GLOOR, P., VERA, C. L. & SPERTI, L. (1963). Electrophysiological studies of hippocampal neurons. I. Configuration and laminar analysis of the 'resting' potential gradient, of the main transient response to perforant path, fimbrial and mossy fiber volleys and of 'spontaneous' activity. *Electroen. Neurophysiol.* **15**, 353–378.

GLOOR, P., VERA, C. L., SPERTI, L. & RAY, S. N. (1961). Investigations on the mechanism of epileptic discharge in the hippocampus. *Epilepsia* (Series *4*) **2**, 42–63.

GREEN, J. D. & PETSCHE, H. (1961*a*). Hippocampal electrical activity. II. Virtual generators. *Electroen. Neurophysiol.* **13**, 847–853.

GREEN, J. D. & PETSCHE, H. (1961*b*). Hippocampal electrical activity. IV. Abnormal electrical activity. *Electroen. Neurophysiol.* **13**, 868–879.

HUMPHREY, D. (1968). Re-analysis of the antidromic cortical response. I. Potentials evoked by stimulation of the isolated pyramidal tract. *Electroen. Neurophysiol.* **24**, 116–129.

JEFFERYS, J. G. R. (1979). Initiation and spread of action potentials in granule cells maintained *in vitro* in slices of guinea-pig hippocampus. *J. Physiol.* **289**, 375–388.

LAATSCH, R. H. & COWAN, W. M. (1966). Electron microscope studies of the dentate gyrus of the rat. I. Normal structure with special reference to synaptic organization. *J. comp. Neurol.* **128**, 359–396.

LØMO, T. (1971). Patterns of activation in a monosynaptic cortical pathway: the perforant path input to the dentate area of the hippocampal formation. *Exp. Brain Res.* **12**, 18–45.

PURPURA, D. P. (1969). Mechanisms of propagation: intracellular studies. In *Basic Mechanisms of the Epilepsies*, ed. JASPER, H. H., WARD, A. A. & POPE, A., pp. 441–451. London: Churchill.

PURPURA, D. P. (1974). Evidence contradicting neuron sensitivity to extracellular fields. *Neurosci. Res. Prog. Bull.* **12**, 143.

PURPURA, D. P. & MALLIANI, A. (1966). Spike generation and propagation initiated in the dendrites by transhippocampal polarization. *Brain Res.* **1**, 403–406.

SKREDE, K. K. & WESTGAARD, R. H. (1971). The transverse hippocampal slice: a well-defined cortical structure maintained *in vitro*. *Brain Res.* **35**, 589–593.

TERZUOLO, C. A. & BULLOCK, T. H. (1956). Measurement of imposed voltage gradient adequate to modulate neuronal firing. *Proc. natn. Acad. Sci. U.S.A.* **42**, 687–694.

WINSON, J. (1974). Patterns of hippocampal theta rhythm in the freely moving rat. *Electroen. Neurophysiol.* **36**, 291–301.

YAMAMOTO, C. & KAWAI, N. (1967). Presynaptic action of acetylcholine in thin sections from the guinea pig dentate gyrus *in vitro*. *Expl. Neurol.* **19**, 176–187.