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Entorhinal Activation of Dentate Granule Cells

By

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Abstract

Andersen, P., B. Holmqvist and P. E. Voorhoeve. *Entorhinal activation of dentate granule cells.* Acta physiol. scand. 1966. *66*. 448—460. — In rabbits, anesthetized with urethane-chloralose, the activation of the granule cells of the dentate area by perforant path fibres from the entorhinal area was studied by intracellular and extracellular recording. Intracellular recording showed that the perforant path produced a large EPSP in the granule cells, followed after 2—4 msec by a large IPSP, lasting some 100 msec. Field potential studies indicated that the synapses responsible for the EPSP were located on the middle third of the dendritic tree, whereas the IPSP was generated by synapses at, or very close to, the cell bodies. The most likely explanation is that the inhibition is mediated by a recurrent inhibitory pathway, in which the collaterals of the granule cell axons excite the basket cells These are inhibitory in nature and send their axons to terminate upon the somata of many granule cells. The inhibition of the granule cells produced by the perforant path is resistant to strychnine in doses up to 0.6 mg/kg. The efficiency of the perforant path excitatory synapses was greatly increased by raising the rate of stimulation from 1 to 10 a second.

The entorhinal area provides the most important afferent inflow to the hippocampus and the dentate area (Cajal 1911, Lorente de Nó 1934, Blackstad 1958). These afferent fibres form two distinct pathways. One is the alvear path of Lorente de Nó (1934) that distributes itself to the subiculum, the other is the perforant path which terminates in the stratum moleculare of the fields CA1, CA2, CA3 in the hippocampus and in the corresponding layer of the dentate area (Fig. 1). In the dentate fascia, the entorhinal afferents, the perforant path fibres, make synaptic contact with the dendritic branches at a distance of about 100 μ or more from the soma (Blackstad 1958) (Fig. 1B), and have an excitatory effect (Andersen and Løyning 1962, Fujita 1962, Gloor, Sperti and Vera 1962). The commissural fibres, on the other hand, terminate on the proximal part of the

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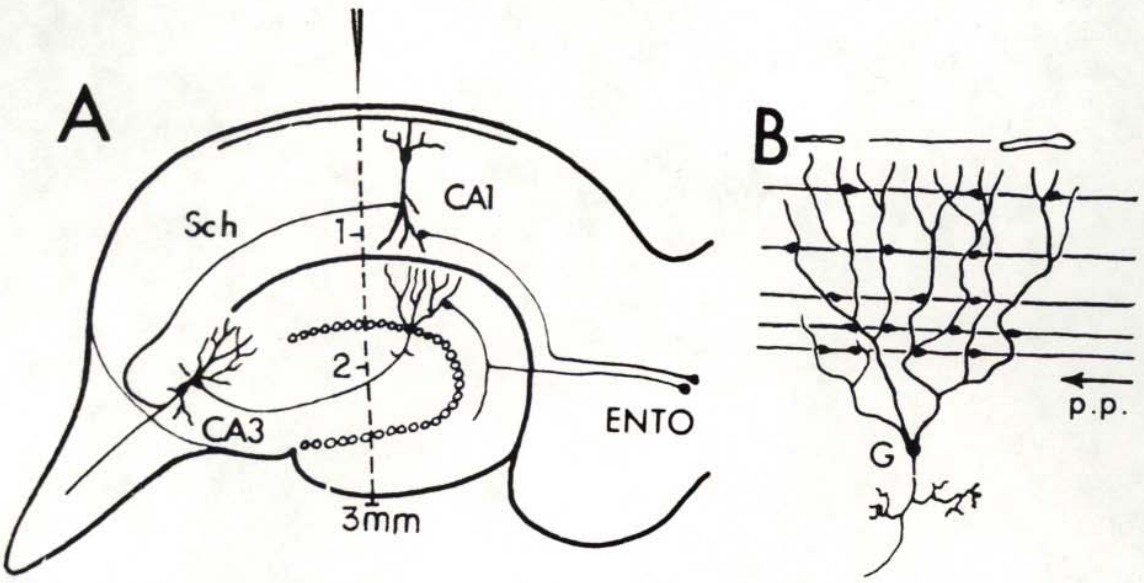

Fig. 1. *Diagram over the afferent pathways from the entorhinal area (ENTO) to the hippocampal formation. A.* The normal track of the microelectrode is indicated by the stippled line, which is marked with bars at 1, 2 and 3 mm. Sch — Schaffer collateral. *B* shows the perforant path (p.p.) synapsing with the dendrites of the granule cell (G).

dendrites. The granule cell bodies themselves receive the terminals of the basket cells. The latter have their somata just beneath the granular layer in the hilus of the dentate fascia, and have been named because of the basket-like structure which their terminal ramifications form around the cell bodies of the granule cells. Basket cells in the hippocampal fields CA1 and CA3, and in the cerebellum have been shown to produce large IPSPs in the somata of pyramidal and Purkinje cells, respectively (Andersen, Eccles and Løyning 1964, Andersen Eccles and Voorhoeve 1964).

The aim of the present investigation has been first to utilize the peculiar synaptic arrangement in the dentate fascia to study with intracellular electrodes the excitatory potency of synapses located at a distance from the cell body. Second, as a test of the generality of a pattern of somatic location of inhibitory synapses (Andersen, Eccles and Løyning 1964, Andersen, Eccles and Voorhoeve 1964, Andersen and Eccles 1965), it was of interest to see whether the basket cell terminals on the granule cell somata could produce IPSPs.

Methods

Adult rabbits were used, weighing from 2.3 to 3.6 kg. They were anesthetized by 0.75 g urethane and 40 mg chloralose per kg b.w., given i.v. Rabbits were preferred because in this species entorhinal stimulation can be performed in a simple and reliable manner (Cragg and Hamlyn 1957, Andersen and Løyning 1962). Some confirmatory experiments were performed in cats. Apart from slightly different depth measurements, the characteristics of the potential pattern were the same as in rabbits. The hippocampal formation was exposed on both sides by removal of the overlying neocortex and the corpus callosum, and the electrodes placed under visual control. From the diagram in Fig. 1A, it is seen that a penetrating electrode will traverse the hippocampal field CA1 for about 1.2 mm before entering the dentate area. After an additional 0.4 mm through the dentate molecular layer, the electrode will reach the first layer of granule cell bodies, called the granular layer. After penetrating the hilus of the dentate fascia, the electrode will traverse the granular layer for the second time, and then the molecular layer, because of the curved structure of the dentate fascia.

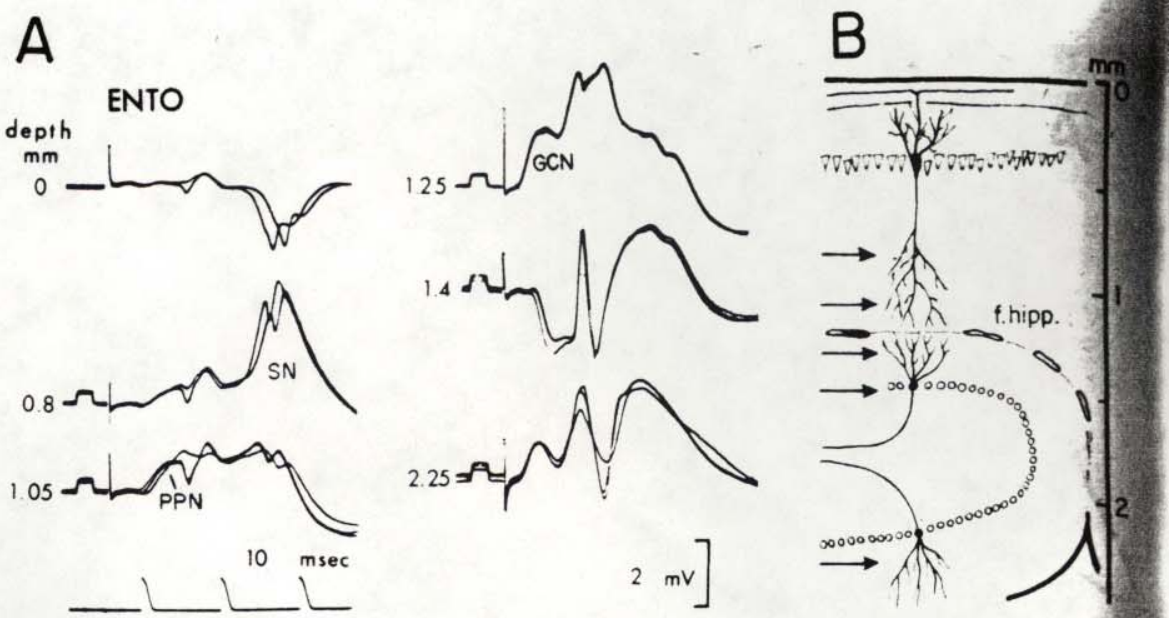

Fig. 2. *Typical potentials recorded from the CA1 and dentate area in response to entorhinal stimulation. A.* The records were obtained at the depths indicated in mm to the left of each record and also by the horizontal arrows in B. f. hipp. — fissura hippocampi, GCN — granule cell negativity, PPN — perforant path negativity, SN — Schaffer collateral negativity.

The recordings were made both with surface electrodes and glass micropipettes. The surface records were made with 0.5 mm thick platinum wire insulated with polyethylene and cut off squarely, leaving a flat face to rest on the hippocampal surface. The insulation prevented shunting by the Ringer-Locke solution or the agar that covered the surface. The micropipettes were filled with solutions of either 4M NaCl (1—4MΩ), 3M KCl (7—20MΩ) or 2M K-citrate (7—40MΩ), and were connected to a cathode follower. The output from the cathode follower was recorded both with a DC coupled amplifier to give the membrane potential of impaled cells and with an AC coupled amplifier. The time constant of the AC amplification system was usually 1 sec. The signals obtained from the micro-electrode were also fed to a display oscilloscope with a large picture tube having a phosphor with a long afterglow, facilitating the study of responses from very short-lasting cell impalements.

Histological checks were made by making small electrolytic lesions with a tungsten microelectrode after potentials typical of a certain depth had been recorded. The hippocampal formation was then removed and fixed in 10 per cent neutralized formalin. Paraffin sections of 15 μ thickness were cut and stained with thionine or by silver impregnation method.

Results

Hippocampal surface potentials following entorhinal stimulation. With the recording electrode placed on the surface of the field CA1 of the hippocampus, there was a quite typical potential in response to a stimulus delivered to the entrohinal area or to its efferent projection, the perforant path (Fig. 2). A small positive spike of 8 msec latency was followed by a slow wave of about 17 msec latency. In order to find out what part of this potential could be generated in the dentate area itself, an electrode was inserted vertically to the alveus of the CA1 and records were taken at various distances from the alveus. It was regularly found that only minor changes of the potentials could be detected in the superficial millimeter of the hippocampal cortex. At a depth of about 1.0 mm, however, there appeared a negative wave of 4—5 msec latency-PPN, possibly

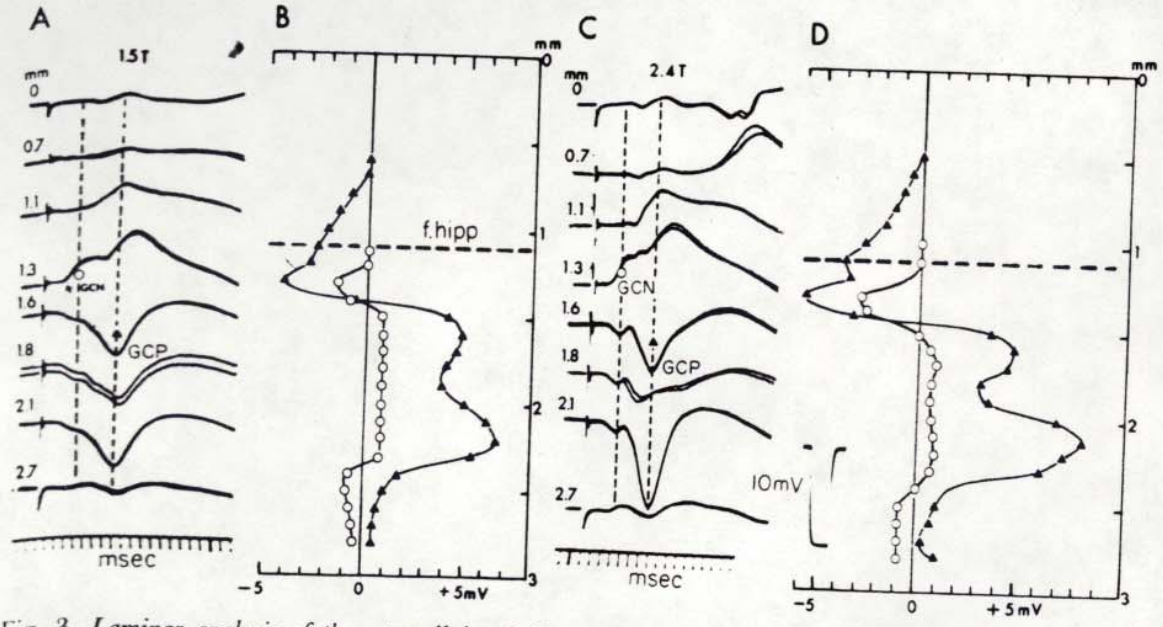

Fig. 3. *Laminar analysis of the extracellular field responses of the dentate area in response to entorhinal stimulation.* In *A* and *C*, extracellular records taken at 1.5 × threshold and 2.4 × threshold, respectively, as recorded from the depths indicated by the numbers to the left of each record. In *B* and *D*, the amplitude of the records, measured at times indicated by the two vertical dotted lines in *A* and *C*, has been plotted against the recording depth. Open circles signify the granular cell negativity (GCN) which is predominant at 1.3 mm depth. Filled triangles indicate granule cell positivity (GCP), that is predominant at 2.1 mm. The stippled lines in *B* and *D*, marked f. hipp., gives the depth of the hippocampal fissure.

related to the depolarization of the terminal dendritic branches of CA1 pyramids by perforant path fibres. On further penetration, a new negative wave appeared at about 1.3 mm deep to the hippocampal fissure, having a latency of only about 2 msec (GCN). This wave probably represents the EPSPs that are produced in dentate granule cells by the perforant path volley. This early negativity was succeeded by a series of irregular deflections. At 1.4 mm, the GCN wave had reversed its polarity, and was followed by a negative spike that probably signalled the discharge of granule cells. At 2.25 mm depth, the potential appeared similar to that observed at 1.25 mm, indicating that the electrode recorded from the second layer of granule cells. Fig. 3 shows the size and polarity of the two components of the potential that can be ascribed to granule cell activity. The components are the early negative wave at about 1.3 mm (GCN — open circles), and the positive wave observed at deeper levels (GCP — filled triangles). In the diagram, the level of the hippocampal fissure, determined from measurements on histological sections, is indicated by the broken line. In Fig. 3A, the entorhinal stimulus was 1.5 times threshold (T), and in Fig. 3C, it was 2.4T. The plottings are in Fig. 3B and D, respectively. The GCN wave (open circles) was negative at depths from 1.3 to 1.5 mm, corresponding to the outer two thirds of the molecular layer of the dentate fascia, and to the area of termination of entorhinal fibres (Blackstad 1958). In most cases, an equivalent negative wave was found at a deeper level. In Fig. 3B and D, it was at about 2.5 mm depth, corresponding to the lower blade of the molecular layer of the dentate fascia.

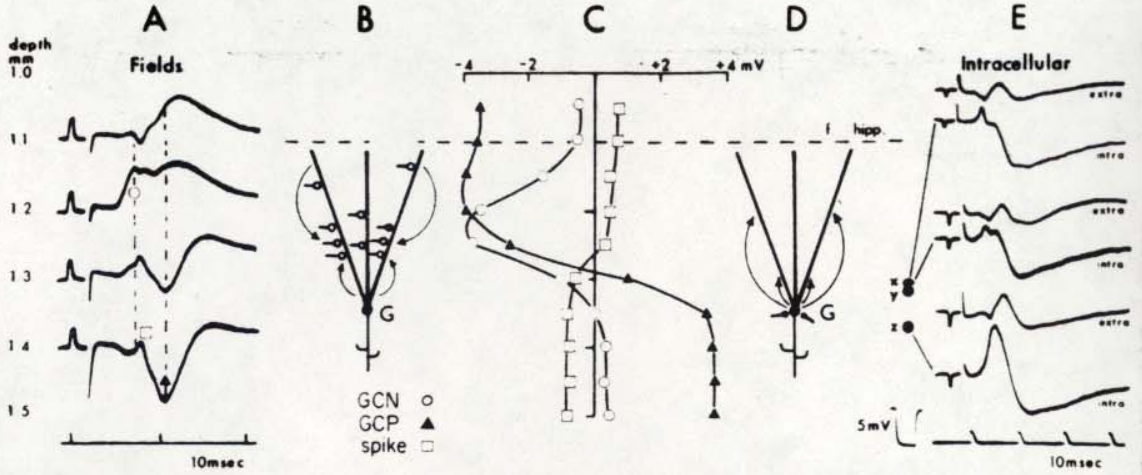

Fig. 4. *Extracellular field potentials in relation to the depths at which intracellular penetration took place.* The records in *A* were taken at the indicated depths below the ventricular surface in response to entorhinal stimulation. Open circles — GCN wave, solid triangles — GCP wave. *B.* Schematical diagram showing the flow of current at the time indicated by the left vertical dotted line in *A*. *C.* The sizes of the GCN, GCP wave and the spike are plotted against recording depth. The peak of the GCN wave corresponds to the location of the perforant path synapses. *D.* Flow of current at the time indicated by the right vertical stippled line in *A*, corresponding to the onset of the IPSP. *E.* The lower traces of each pair was obtained by impalement of three cells, x, y, z, whose depths are indicated by the filled circles. The upper traces in each pair is the record taken just extracellularly with the same gain and polarity as the intracellular record.

The GCP wave (filled triangles) shows two conspicuous amplitude peaks in Fig. 3B and D, at a depth of 1.6 mm and at 2.2 mm. These depths correspond very closely to the two blades of the granular layer (Fig. 1).

Because of the complex histological arrangement in this area, it is not possible to deduce from extracellular records only, to what extent the deflections recorded in the dentate area are the results of active synaptic depolarizations or hyperpolarizations of parts of the dentate granulè cells, and what is due to a passive flow of current. It was, therefore, necessary to correlate the extracellular pattern with intracellular records. However, it proved extremely difficult to obtain satisfactory impalements of the granule cells. This was not unexpected, since the diameter of the granule cell bodies are only 10 to 15 μ, and since they also have an extensive dendritic tree which is very likely to be damaged by the microelectrode approaching the soma. However, it was possible to penetrate 10 cells for a sufficiently long time to allow the determination of the perforant path synaptic activity on the cell.

Since the soma most likely forms the only target for the impaling microelectrode, the depth of the electrode when penetrating cells will give the level of the granule cell bodies as shown in Fig. 4E. All records are taken from the same experiment. In Fig. 4A, there are field potentials recorded at the indicated depths below the surface of the alveus, and in C, the amplitudes of the GCN wave (open circles), the GCP wave (filled triangles), and the spike (open squares) are plotted against the recording depth. The level of the hippocampal fissure is indicated by the broken line. The components are measured at the two broken lines in A. The different components demonstrate a typical sequence at increasing depths (Fig. 4C), comparable to that in Fig. 3. The spike was rather small since the stimulus stregnth intentionally was kept relatively weak.

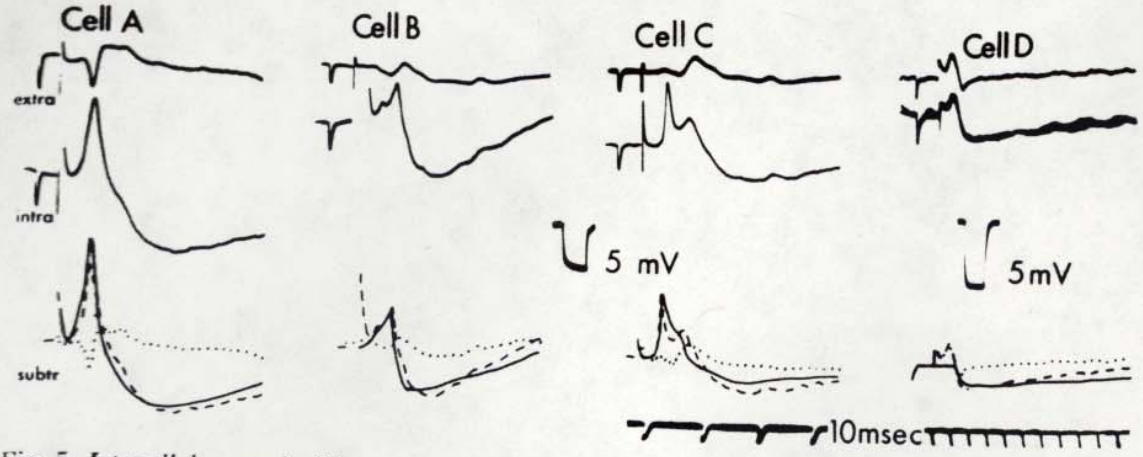

Fig. 5. *Intracellular records of dentate granule cells.* Upper row: Extra- and intracellular records from four granule cells. Lower row: dotted lines are tracings of the extracellular records, the broken lines of the intracellular records, and the fully drawn lines give the geometrical subtraction between the two sets of records, giving the actual trans-membrane potential changes. In cell A, B and C, an initial EPSP is followed by a large IPSP. In cell D, the IPSP is seen alone. All IPSPs are of long duration, whereas the EPSP duration is usually of the order of 5 msec.

Since the GCN wave had a short latency and a definite maximum at a depth corresponding to the termination of the afferent perforant path fibres, it was considered likely to be the extracellular sign of an EPSP in the granule cell dendrites produced by the entorhinal volley. The GCP wave that followed after the spike could either be an active hyperpolarization of the cell, or it could be the source for a current flowing into some distant sink. Since the depth recordings showed that the positive wave had two maxima, each corresponding to the two layers of the granular layer, it was anticipated that the positive wave was generated by the granule cells and not by the activity of neurones in the hilus of the dentate fascia.

These conclusions were partly confirmed and partly amended by the intracellular records. In Fig. 4E, the three filled circles indicate the depths of three granule cells penetrated by the microelectrode, and the records obtained are displayed to the right. Each pair consists of the intracellular record below, with the extracellular record above. The large size of the extracellular field potentials in the hippocampal formation makes it imperative always to subtract the extracellular from the intracellular records in order to obtain the potential difference occurring across the impaled membrane. It is obvious that the GCN wave is associated with a depolarization of the cell membrane, an EPSP. This can be more clearly seen in Fig. 5. This figure shows the intra- and extracellular recordings of four different granule cells. In the construction in the lower row, the intracellular potentials are redrawn in broken lines and the extracellular field potentials in dotted lines. The fully drawn lines give the subtraction of the two and thus represent the potential changes across the membrane.

The intracellular recordings showed an initial depolarizing wave, corrresponding to the extracellular GCN wave (Fig. 5A, B, C). Since this depolarizing wave increased gradually with augmenting stimulus strengths, and was sometimes associated with granule cell discharges, it is an excitatory postsynaptic potential (EPSP). The failure to observe a regular association with impulse generation is no doubt due to the grave depolarization of the small cell by the impalement.

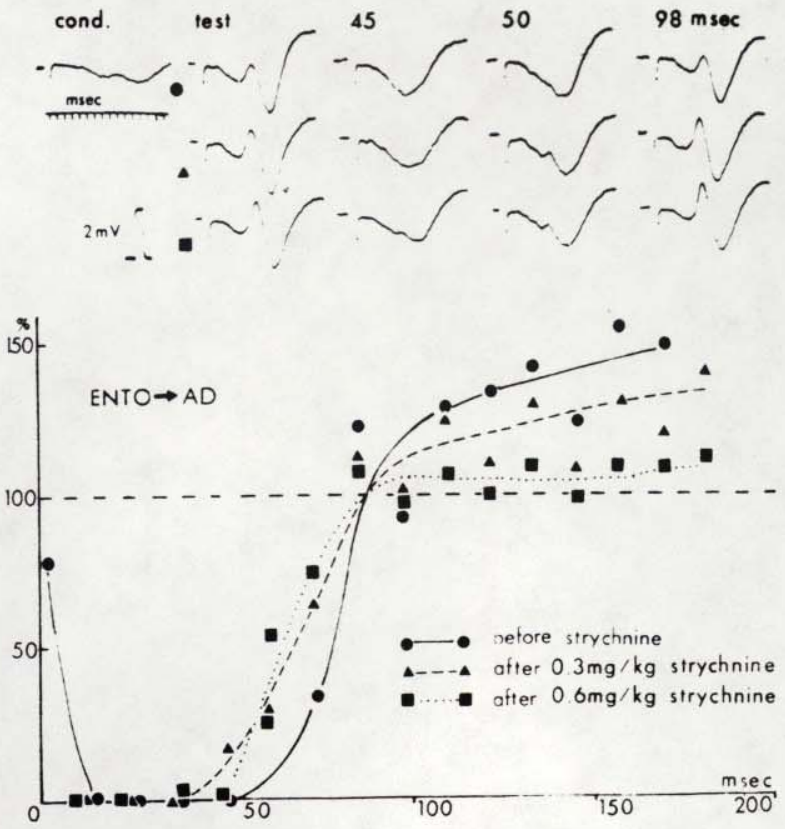

Fig. 6. *Inhibition of dentate granule cells.* Extracellular records obtained from the granular layer of the dentate area in response to paired entorhinal stimulation. The conditioning shock was kept weak (cond.), so as to exite only a small number of granule cells. The upper row shows the test shock unconditioned (test), and 45, 50 and 98 msec after the conditioning shock, respectively. In the lower half of the figure, the amplitude of the test response is plotted (filled circles) against the conditioning-test interval. The duration of the inhibition is about 80 msec. The middle and lower rows of records are comparable responses after 0.3 mg/kg strychnine and 0.6 mg/kg strychnine, respectively, given i.v. No significant change in the duration of the inhibition can be seen, only a reduction in the post-inhibitory facilitatory period.

Following the EPSP, there was a large and longlasting hyperpolarization of the neurone. This was regularly seen in cells without previous cell discharge (Fig. 4, 5), and was associated with cessation of injury or spontaneous discharges and reversed its polarity on chloride diffusion into the cell; it is, therefore, an inhibitory postsynaptic potential, IPSP. Correlation with an extracellular potential was possible when the stimulation was weak so that the EPSP was reduced and the onset of the IPSP detectable. Under these conditions, it was observed that the extracellular positivity was associated with the initial part of the IPSP (Fig. 5B). The duration of the IPSP was around 100 msec. The exact amplitude of the IPSP has not much meaning in view of the depolarization due to the damaged cell membrane. They were of the order of 5—15 mV.

With the information from the intracellular recordings, it is possible to utilize the size of the positive wave at the granular layer as a measure of the intensity of the outward current and thus to localize the focus of the inhibitory synapses. It is evident from the depth records in Fig. 3 and 4 that the positivity was related to the cell bodies or somata and not to the dendritic tree. In the initial phase of the IPSP, current is flowing as indicated in Fig. 4D.

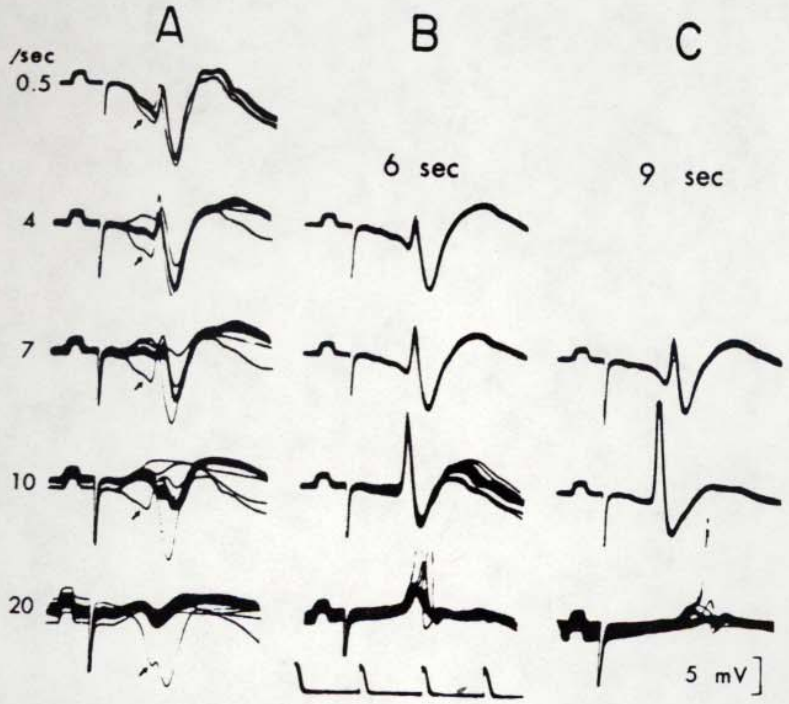

Fig. 7. *Frequency potentiation of perforant path synaptic activity on dentate granule cells. A.* Effect of increasing rates of stimulation, indicated to the left of each row. Extracellular records. The arrow indicates the first trace in each series. *B.* Responses to higher stimulus frequencies, 6 sec after the onset of the tetanus. *C.* Responses at the three highest frequencies, 9 sec after the onset of the tetanus. An initial depression of the high frequency stimulation is replaced by a facilitation of the discharge of the cells, as indicated by the larger extracellular spike.

Therefore, the combined use of intracellular and extracellular recordings at various depths leads to the conclusion that the entorhinal afferent volley induces an EPSP in the dentate granule cell dendrites having a maximum at about one third of the distance from the cell bodies to the dendritic terminal branches at the hippocampal fissure. This EPSP is followed by a longlasting IPSP, which probably is generated at the soma itself.

The inhibitory effect of the granule cell IPSPs can be demonstrated by the use of the double shock technique, varying the interval between the first conditioning shock and the following test stimulus (Fig. 6). The recording electrode was located at a depth of 1.8 mm, just deep to the upper blade of the granular layer, and the spike signalled the simultaneous discharge of a number of granule cells. A preceding conditioning shock (cond.), too weak to produce any appreciable occlusion, completely depressed all signs of spike discharges in the test response for about 50 msec (upper row and filled circles). Full resumption of the excitability was not attained until after about 90 msec, corresponding to the duration of the IPSPs recorded intracellularly. Stronger stimulation gave an inhibition lasting for 150 msec. Since the inhibition of the granule cell discharge was not dependent of a previous discharge of the same cells, the effect was due to a true inhibitory process.

Postsynaptic inhibition in the spinal cord is reduced by the administration of strychnine. A dose of about 0.2 mg / kg body weight virtually abolishes both the direct and the recurrent inhibition in motoneurones (Bradley, Easton and Eccles 1953, Eccles,

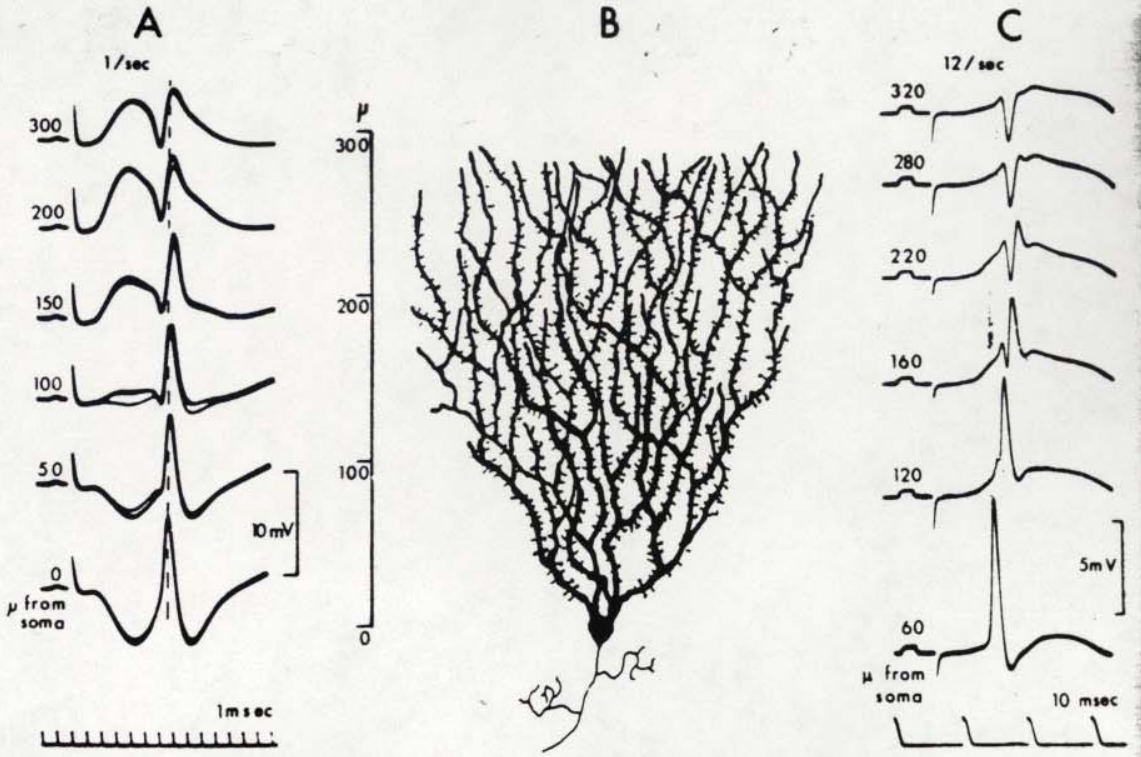

Fig. 8. *Propagation of spikes along the dendritic tree of dentate granule cells.* Row *A* shows extracellular records taken at the indicated distances from the soma by an electrode that was withdrawn along the dendritic tree. The stippled line is drawn through the peak of the spike, as seen at the soma layer. The increased latency of the peak negativity indicates somatofugal conduction. *B.* Diagrammatic dentate granule cell, drawn to scale to facilitate comparison with the records in *A* and *C*. C. Records take during frequency potentiation (10/sec) through the perforant path, by a microelectrode being withdrawn from the soma. The increasing diphasicity with increasing distances from the soma is indicative of a somatofugal conduction of the spike.

Fatt and Koketsu 1954). However, several reports indicate that postsynaptic inhibition in various locations in higher levels of the central nervous system is resistant to strychnine (Green, Mancia and Baumgarten 1962, Andersen *et al.* 1963, Crawford *et al.* 1964). For this reason it was of interest to test whether the postsynaptic inhibition in the dentate granule cells was strychnine-resistant or not. In Fig. 6, the second row is similar to the records in the upper row, but taken 2 minutes after an intravenous injection of 0.3 mg strychnine hydrochloride per kg body weight. The records of the lower row were taken 4 minutes after an additional dose of 0.3 mg strychnine per kg. The graph below is a plotting of the size of the test spike against the conditioning-test interval. The strychnine administration does not alter the degree or duration of the inhibition, but abolished the late facilitation seen at intervals beyond 100 msec. Thus the postsynaptic granule cell inhibition is resistant to strychnine in doses up to 0.6 mg per kg.

The inhibitory curves and the intracellularly recorded IPSPs both indicate a powerful inhibitory process lasting for about 100—150 msec; hence it was anticipated that stimulation at a frequency of 7 / sec or more would reduce the probability of discharge of the dentate granule cells. This was found to be the case. In Fig. 7A, each assemblage consists of 5—20 superimposed records, the arrrows pointing at the response to the first stimulus of the tetanus. On increasing the stimulus frequency from 0.5 / sec to 4 / sec, a reduction of about 30 per cent appeared in the height of the population spike.

At a rate of 7 / sec, the reduction in spike amplitude was much more marked, and at a stimulus frequency of 20 / sec, the spike was abolished completely. This description relates to short-lasting tetani (0.5—1 sec). If, however, the tetanic stimulation was maintained for several seconds, the depression was slowly replaced by a facilitatory process, resulting in the reappearance and steady growth of the spike, usually to a larger amplitude than that observed at about 0.5 / sec stimulation (Fig. 7B, C, 10 / sec). Further, simultaneous with the increased amplitude of the spike, its latency showed a definite reduction (Fig. 7B). This facilitatory process by tetanic stimulation will be called frequency potentiation. At higher stimulus rates, the potentiation was soon replaced by an extinction of the cell discharges.

The process of frequency potentiation is slow, requiring several seconds of tetanic stimulation to make itself manifest. Conversely, after the cessation of the tetanus, a state of increased excitability of the granule cells is observed for several seconds, sometimes for as long as half a minute.

When the excitability was high, a large number of granule cells discharged virtually simultaneously, and it was then possible to test whether the spike is conducted along the dendritic tree. In Fig. 8A, the recording electrode was placed at various depths of the molecular layer. The response to stimulation at a rate of 1 / sec showed a spike, preceded and followed by a positive wave. The electrode was withdrawn in steps indicated by the figures to the left of each record. During the withdrawal, the excitability was stable as judged from the appearance of the surface record. Initially, the spike was purely negative, corresponding to the thick branches of the dendritic tree. Upon further withdrawal, the spike became increasingly diphasic with an initial positivity, until it appeared purely positive at a distance of 300 μ from the soma, corresponding to the distal portions of the dendritic tree. The increasing latency of the negative part of the spike during the withdrawal indicates the conduction of the spike along the branches of the dendritic tree until eventually the conduction was blocked about 300 μ from the soma, which is very close to the termination of the dendritic branches. A comparison of the records taken at 0 and 150 μ from the soma, gives a difference in the peak latencies of about 0.3 msec, indicating a mean spike conduction velocity of about 0.5 m/sec.

In Fig. 8C is shown a similar experiment that was performed in the state of frequency potentiation with a slowly changing excitability. The same qualitative changes of the spike configuration occurred at various depths. In this case, the latency shifts taking place during frequency potentiation prevents the estimation of a conduction velocity.

Discussion

Site of the perforant path activation of the dentate granule cells. The responses to entorhinal stimulation obtained from an electrode penetrating the dentate fascia showed an early negative wave with a maximum among the granule cell dendrites. This component corresponded closely in time to the intracellularly recorded EPSP and is most likely the extracellular sign of this potential. The amplitude of the early negative wave can, therefore, be taken as a sign of the intensity of the current flowing inwards through the activated excitatory synapses of the perforant path fibres (Fig. 4B). The plotting of the size of the negative wave against depth showed that it had its maximum among the dendrites, some 100 μ from the granule cell somata in the granular layer. This localization is in good accord with the histological findings (Blackstad 1958) of degeneration of

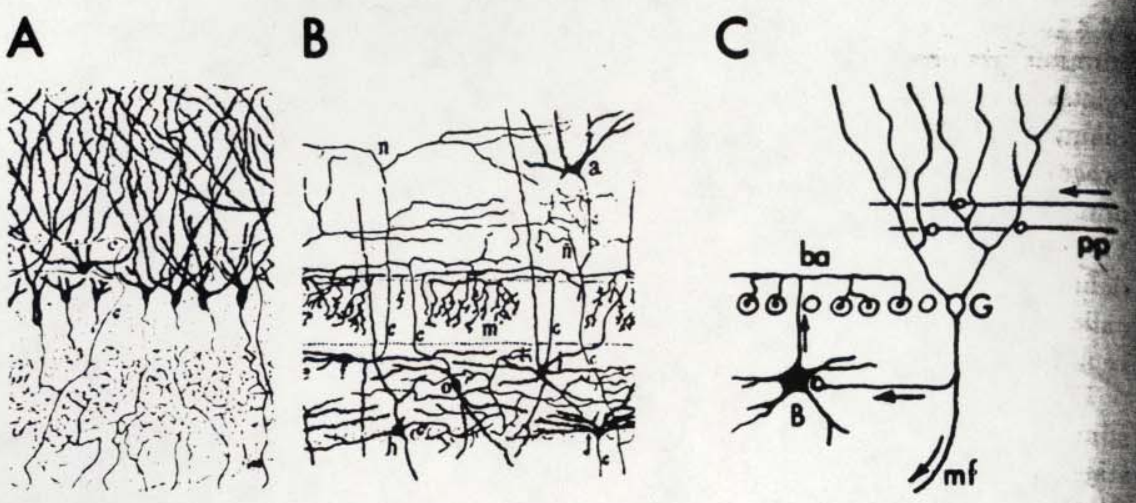

Fig. 9. *Possible pathway involved in the production of the large hyperpolarization of dentate granule cells.* *A* and *B* from Cajal (1911). *A*. Dentate granule cells with their axons, having profuse ramifications in the layer just beneath the granule cells. *B*. Some of the cells in this layer, marked o, i, are basket cells that send their axons, c, to ascend and branch profusely to provide basket-like terminations around the cell bodies of the dentate granule cells. *C*. Diagrammatic representation of the proposed pathway involved in producing the large hyperpolarizing responses of the dentate granule cells by perforant path stimulation. An afferent volley (pp) excites synapses on the dendritic tree of the granule cells (G) and will bring some of these cells to discharge. The nerve impulse will be carried along the mossy fibre (mf) and also along some axon collaterals (arrow) that will excite basket cells (B). These will in turn, through their axon (ba), produce postsynaptic inhibition of a large number of dentate granule cells.

perforant path fibres in the outer two thirds of the molecular layer, with the heaviest concentration of the degeneration in the part bordering the inner third. In rabbit, this would give the most massive synaptic excitation on the dendrites at about 100—150 μ from the granule cell somata.

Location of inhibitory synapses on the granule cells. The extracellular counterpart of the initial part of the granule cell IPSP was the positive wave that follows the spike discharges of the granule cells. Even if the positive wave was curtailed by a slow negative wave, it can still be used as an indicator of the intensity of the current flowing outward across the cell membrane due to the activity of the inhibitory synapses (Fig. 4D, E). The tracking shows that this current had a maximum at the level of the cell bodies, hence it is concluded that the inhibitory synapses are located at, or very close to, the somata of the granule cells.

In this respect the inhibitory synapses on the dentate granule cells are similar to those on CA3 and CA1 hippocampal pyramidal cells (Andersen, Eccles and Løyning 1964) and on cerebellar Purkinje cells (Andersen. Eccles and Voorhoeve 1964); and on neocortical pyramids (Kubota *et al.* 1965).

Undoubtedly, a part of the positivity at the granular layer is due to this layer acting as a source for the current flowing into the activated excitatory synapse on the dendritic branches about 100 μ from the soma. However, this can only apply for the initial part of the granular layer positivity since this wave always outlasted the intracellularly recorded EPSP. Furthermore, with the use of weak stimulation, the EPSP was small, but both the IPSP and the extracellular positive wave were still present.

Evidence for an inhibitory interneurone producing the dentate granule cell IPSPs. The wide distribution of the IPSPs, to all granule cells successfully impaled, even when the stimulus was so weak that only a limited number of granule cells were fired, makes it likely that the inhibitory potentials are produced by some mediating cell having wide axonal ramifications. This assumption is strengthened by the observation that the EPSP always had a shorter latency than the IPSP recorded from the same cell. The quality of the intracellular records do not allow exact measurements to be made of this latency difference, but a range of 2—4 msec was observed.

Possible histological substrate for the inhibition of granule cells. Having in mind the physiological findings of a wide IPSP distribution and a somatic location of the inhibition, there is hardly more than one histological candidate for the postulated inhibitory interneurone: the dentate basket cell. This neurone fulfils all the requirements put forward. The axon of each basket cell ramifies profusely and forms, with the axons of fellow cells, basket-like networks around, and makes synaptic contacts with the cell bodies of a large number of granule cells, hence their name (Cajal 1911, Lorente de Nó 1934). Fig. 9A and B is taken from Cajal (1911), showing the granule cells, their axons (Fig. 9A) with profusely branching axon collaterals, and the basket cells (Fig. 9B i, o) with their terminations (Fig. 9B, m).

The diagram in Fig. 9C explains the hypothesis of entorhinal activation of the dentate area arrived at in the present investigation, to be compared with the histological information in Fig. 9A and B. The perforant path fibres (pp) terminate in excitatory synapses on the granule cell dendrites, at some distance from the soma, where they evoke EPSPs. In some granule cells, the EPSP will be large enough to discharge the cell. The impulse travels along the axon, the mossy fibre (mf) and also along its numerous axon collaterals, ramifying beneath the graular layer. These collaterals are assumed to to excite the cells in the subgranular layer (B), where the majority of neurones are basket cells (Cajal 1911). By way of their terminals (ba) on the granule cell bodies, the basket cells will influence a large number of granule cells. The basket cell synapses on the granule somata are proposed to be inhibitory, and responsible for both the large IPSP observed in the granule cells, and for the positive wave recorded extracellularly from the granular layer.

It is pertinent to mention that the synapses in the cell body of the dentate granule cells are of the type 2 (Blackstad and Dahl 1962), thus being similar to inhibitory synapses identified in the hippocampus (Andersen, Eccles and Løyning 1964, Blackstad and Flood 1963, Hamlyn 1963) and on cerebellar Purkinje cells (Andersen, Eccles and Voorhoeve 1963, 1964, Palay *et al.* 1962).

Spike generation and propagation of the granular cells. The homogeneity of the dentate fascia with the vast majority of the cells being similar in shape and parallel to each other with the somata at the same depth, allows some conclusions to be drawn regarding the propagation of the spike recorded at various depths. The pure extracellular negativity of the spike in the granular layer and the first 100 μ of the molecular layer suggests that the spike is generated either in the soma, or more likely, in the proximal parts of the dendrites bordering the synaptically depolarized part of the cell. The progressive diphasicity and increasing peak latency of the spike with increasing distance from the soma suggest a somatofugal conduction of the spike. Conduction of a spike with the same slow velocity has been observed along the apical dendrites of hippocampal pyramidal cells (Cragg and Hamlyn 1955, Andersen 1960, Andersen and Jansen 1961, Fujita and Sakata 1962, Andersen, Holmqvist and Voorhoeve 1966).

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