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Medial septal projections to the dentate gyrus of the rat: electrophysiological analysis of distribution and plasticity

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Summary. Previous electrophysiological experiments in rabbits have suggested that medial septal stimulation activates dentate granule cells and evokes an associated negative field response at the granule cell layer, without an associated "dendritic" response. Anatomical studies have suggested that septal input to the granule cells may be to stratum moleculare, or close to the cell layer, or may not exist at all. The present experiments confirmed in rats anaesthetised with urethane that medial septal stimulation elicits single action potentials from cells in the granule layer. The associated negative field potential was maximal in the granule cell layer and there was no sign of a separate dendritic potential. The fibres responsible for this potential travel to the dorsal hippocampus in the fornix superior rather than the fimbria, taking the same course as the fibres which contribute to the dense cholinesterase staining just above the granule cell layer. Stimulation at 100 Hz for 1 s of either medial septal, or perforant path, input to the dentate granule cell layer produced long term potentiation of the subsequent evoked field responses to the stimulated pathway. The responses to the non-stimulated pathway were unchanged. Paired pulse stimulation produced both homosynaptic and heterosynaptic potentiation. These data suggest that medial septal input synapses close to granule cell bodies and produces a negative field potential which is a combination of dendritic and population spike potentials. Medial septal input also appeared to produce direct activation of hilar neurones, some of which may be basket cells or other interneurones. The data also show that long term potentiation is specific to this input, perhaps dependent on presynaptic mechanisms. Paired pulse potentiation, at least in the heterosynaptic case appears to depend on postsynaptic mechanisms.

Key words: Medial septum – Hippocampus – Dentate gyrus – Evoked potentials

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Introduction

A number of authors have reported that stimulation of the septal area elicits field potentials in the dentate gyrus of the hippocampus (Andersen et al. 1961; Brust-Carmona et al. 1973; Krug et al. 1980; Wheal and Miller 1980; Robinson and Racine 1982). There are two reasons for thinking that the interpretation of the waveforms obtained may not be simple.

Firstly, Krug et al. (1980) present results which suggest that positive components of the waveform obtained on medial septal stimulation could be due to activation of the lateral septum.

Secondly, Andersen et al. (1961) present field potential depth profiles which suggest input from more than one class of fibre. They stimulated the septum in the rabbit and evoked a negative field potential, maximal in the granule cell layer of the dentate, which they attributed to summation of granule cell action potentials. This negative potential, occurred within a positive wave, but the latter did not reverse above the cell layer. So the data of Andersen et al. (1961) provide us with no indication as to where the input is terminating in the dentate region, and leaves open the question as to whether there is any functional relationship between the observed positive and negative waves.

This problem is highlighted by two papers. Fantie and Goddard (1982) observed septal modulation of perforant path evoked responses in the absence of septal evoked potentials. By contrast, Robinson and Racine (1982) report modulation accompanied by septally-evoked potentials. However, the potentials

observed were simple positivities with no sign of the type of negative spike described by other workers; and their electrode placements were 0.5 mm from the midline.

These data, taken together with that of Krug et al. (1980) and Andersen et al. (1961) suggest that there may be separable positive and negative potentials elicited from the septum, with the former containing predominantly lateral septal components and the latter medial septal components.

Since the medial septal input to the dentate may synapse very close to the granule cell layer (Mosko et al. 1973) it is possible that the negative potential described by Andersen et al. (1961) results from a combination of EPSP and spike responses. However, the exact anatomy of this projection is unclear (Ibata et al. 1971; Swanson and Cowan 1979).

The present experiments were undertaken to confirm, in the rat, the absence of a 'dendritic response' in stratum moleculare to medial septal stimulation; to confirm the presence of a negative wave maximal within the granule cell layer; and to investigate both the extent of the positive wave form within the dentate and its relation to lateral septal stimulation.

In addition, single unit recording was employed to demonstrate whether or not the negative potential recorded in the granule cell layer was in fact associated with the firing of granule cells, and to determine the extent to which hilar cells (Von Euler and Green 1960) as well as granule cells could be activated from the septum.

There have been previous reports of both paired pulse (Alvarez-Leefmans and Gardner-Medwin 1975; Fantie and Goddard 1982; Robinson and Racine 1982) and long-term (Robinson and Racine 1982) interactions of septal with perforant path input to the dentate. Those phenomena were also investigated.

Methods

Experiments were performed on 42 male Wistar rats acutely prepared under urethane anaesthesia (1.5 g/kg I.P.) and maintained at a body temperature of 37° C. A skull flap was removed extending 2 mm anterior and 5 mm posterior to bregma with a width of 3–5 mm on either side of the midline. The dura was excised to expose the underlying cortical tissue which was covered with warm saline throughout the experiments.

Concentric bipolar electrodes with a tip separation of 0.3–0.5 mm and a DC resistance in saline of 35 to 70 kΩ were used for stimulation. The electrodes were lowered stereotaxically and their position was then adjusted to produce maximal amplitude field potentials. With the nose bar set at 4.2 mm below the ear bars, coordinates used were: for medial septum, 0.0–0.5 mm anterior to bregma, on the mid-line, 3.0–6.0 mm below the surface of the cortex; for perforant path (angular bundle): 8.1 mm to posterior bregma, 4.3 mm lateral, 2.5 mm below cortex; for lateral septum, as for medial septum, but 1.0 mm lateral to the mid-line. In most cases lateral septal electrodes, when used, were held in the same micromanipulator as medial electrodes. Stimuli consisted of single monophasic square wave pulses of 0.1–0.5 ms duration and 5–50 V delivered through an isolation unit.

Extracellular unit activity and field responses were recorded using glass micro-pipettes filled with 4 M NaCl, having tip diameters of approximately 1 μm and DC resistance in saline of 1–5 MΩ. The electrode was stereotaxically positioned into the CA1 region of the hippocampus (3.5 mm posterior to bregma, 2.0–2.5 mm lateral, 2.0 mm below cortex) and then lowered into the dentate gyrus. Localization within this region was assessed during the experiment by the characteristic waveforms elicited by perforant path stimulation and by the increase in unit activity on entering the CA1 and dentate cell layers.

The signals were amplified, filtered (band-pass 0.1–3.0 kHz for field potentials, 1.0–3.0 kHz for unit activity and displayed on a storage oscilloscope for photography or passed through a PDP 11/10 computer for on-line averaging of field responses. Typically the average of 10–20 stimulus presentations with an interstimulus interval of 5 s was plotted.

Sites of stimulation were marked by passing a DC anodal current of 10–20 μA through the inner core of the electrodes with reference to the outer lead for 15–20 s. The animals were given an overdose of urethane and perfused through the heart with saline followed by potassium ferrocyanide-formalin solution. Histological verification of the electrode sites was made by locating the deposited blue spot in 50 μ frozen sections stained with thionine.

Fimbral lesions were performed by stereotaxically lowering a small knife into the brain 1.8 mm posterior to bregma at an angle of 25° from the vertical and 2.0 mm lateral to the midline until a change was observed in field potentials evoked from the medial and lateral septum. The knife was then moved through an angle as far lateral as possible. Fornix superior lesions were performed by lowering a 0.5 mm wide knife stereotaxically at the midline and at increasing distances from the midline until the medial septal response was substantially reduced. The extent of these lesions were verified histologically.

Results

Dentate field potentials

Stimulation of the medial septum (10–30 V, 0.1–0.5 ms) evoked a characteristic triphasic waveform in the region of the granule cell layer. A laminar profile of medial septal evoked potentials within the dentate region is shown in Fig. 1. The initial positive deflection (P1) could be observed throughout the molecular layer and for some distance into the hilar zone with little change in peak latency or amplitude. The first negative component of this response (N1, peak latency 8–12 ms) showed a gradual increase in amplitude between the distal portions of the molecular layer and the granule cell layer, where it reached a maximum before apparently reversing to a positive waveform in the hilar region. A second, late, negative wave (N2) became evident between the granule and hilar zones reaching maximal amplitude in the latter. When the electrode

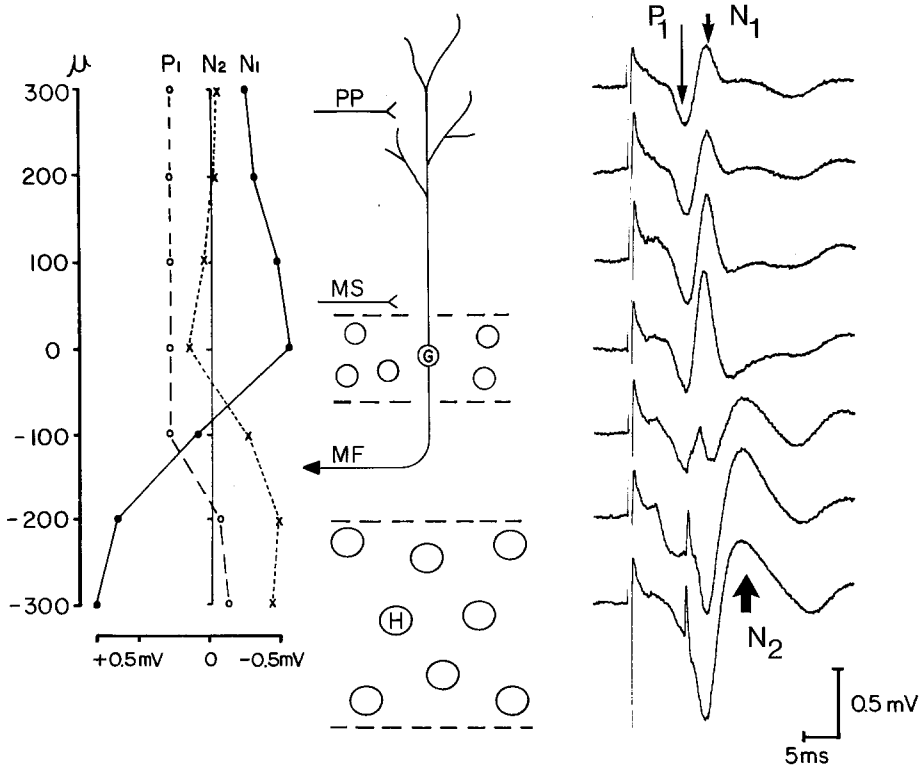

Fig. 1. Profile of averaged field responses evoked within the dentate gyrus by medial septal stimulation. In this and all subsequent figures negativity is upwards. The graph shows voltage plotted against depth at the peak latencies of the P1, N1 and N2 waves as defined in the region of the granule cell layer. PP indicates the approximate location of the perforant path synapses on granule cell dendrites. MS indicates the suggested location of medial septal synapses. MF indicates the approximate location of mossy fibres. G and H are the granule and hilar cell zones respectively

was well within the hilus an early sharp negative field appeared. This occurred at approximately the same latency as that of the P1 wave obtained in the molecular and granular layers and always had a latency 2–3 ms shorter than that of the N1 wave. It should be noted that in some animals a negative wave of slightly longer latency than N2 could be observed within the granule cell layer. This appeared to result form current spread to the lateral septum (see below).

Septal origins of dentate evoked responses

With the recording electrode located in the granule cell layer, movement of the stimulating electrode through the septal area generally elicited maximal N1 responses from two locations within each animal. Marker spots placed in the upper of the two foci appeared to be located in the region of the fornix superior (upper cluster Fig. 2A). The second focus was located within the dorsal aspect of the medial septal nucleus (lower cluster (Fig. 2A).

In some animals the two foci could not be clearly differentiated. The response profile from such an animal is illustrated in Fig. 2B. A maximal N1 response was recorded with stimulating electrode placements just dorsal to the medial septal nucleus, and this response remains large when evoked from positions within the more dorsal parts of the nucleus. The implication, that the relevant fibres from the medial septal nucleus (and possibly diagonal band nuclei) are travelling in the fornix superior, received some confirmation from the fact that a negative field response having the same configuration as the N1 component could be elicited in the granule cell layer by stimulating more posterior portions of the fornix superior at somewhat shorter latency than that from the medial septum (e.g. FS in Fig. 2A).

It should be noted that the MS and FS responses show only minimal signs of P1 and N2 waves. Potentials evoked from the lateral septum, by contrast appeared to show large positive and late negative waves in the virtual absence of N1 (Fig. 2A). However, the late negative wave to LS stimulation occurs later than would be expected of the N2 wave which makes it difficult to assess how far the waveform evoked by medial stimulation contains separable components with some due to current spread to the lateral septum. This question is addressed in the next section.

Location of pathways between the septum and dentate gyrus

The septal input to the dentate may be important for the generation of theta rhythm (Petsche et al. 1968; Macadar et al. 1970) and the fibres to the dorsal hippocampus controlling theta rhythm travel in the

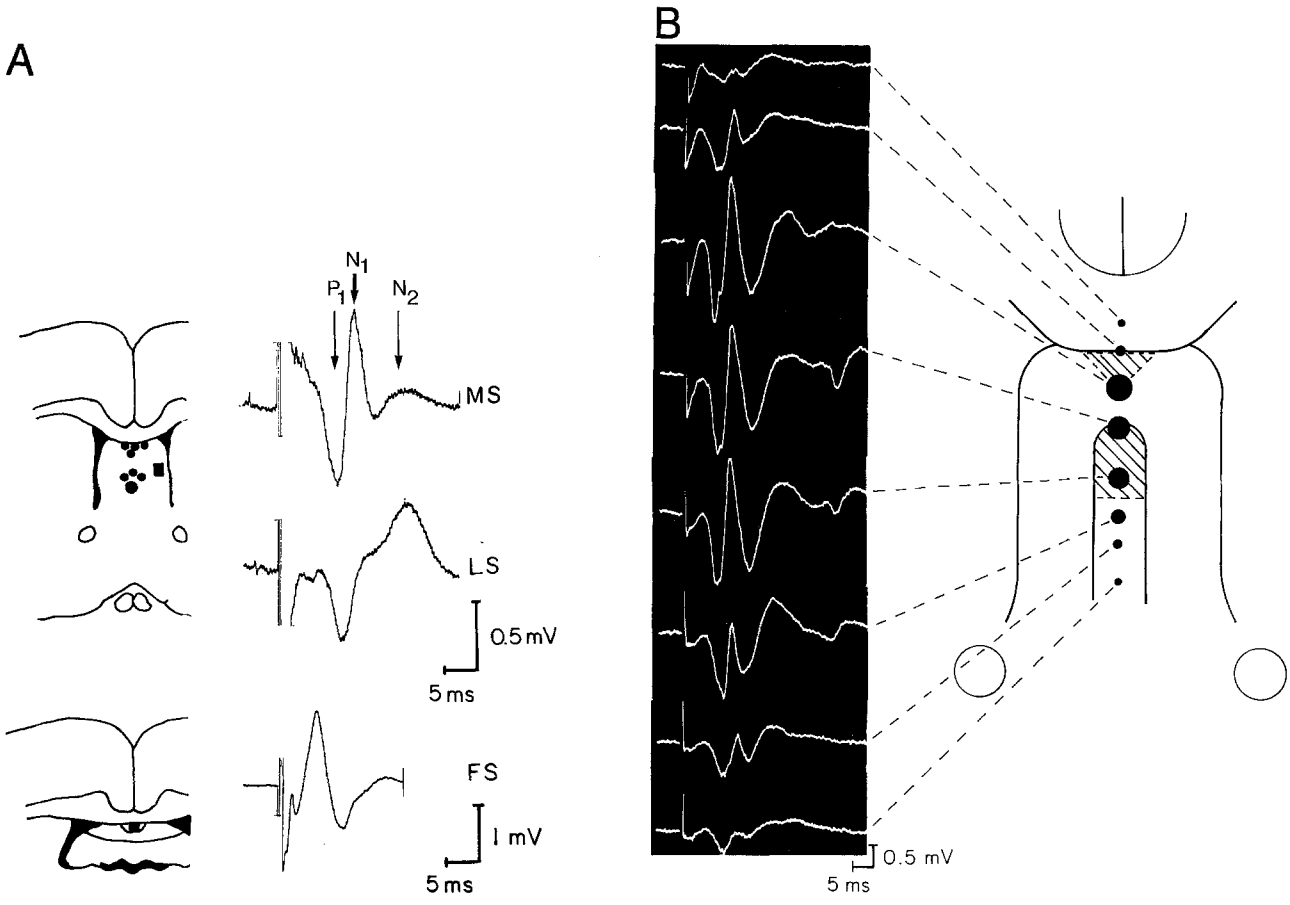

Fig. 2A. Localisation of stimulation site within the septum when recording at the granule cell layer. Most animals had two optimal sites, one in the region of the fornix superior and one in the head of the medial septal nucleus. Sites of marker lesions made in 4 animals with the electrode in the upper focus and 4 with electrodes in the lower focus are shown by filled circles. The large filled circle and large filled square in the septal section show locations from which medial and lateral septum were stimulated with identical voltage to obtain the field potentials indicated by MS and LS. The two stimulating and the single dentate recording electrode remained stationary during collection of these potentials. Below is shown a site located more posteriorly where a maximal negative field potential (FS) was obtained in another animal. The site is again located n the fornix superior, however the potential observed cannot unequivocally be identified with the negativity obtained to medial septal stimulation. **B** In some animals two separate maxima could not be identified. The profile shown is of single, unaveraged, potentials from such an animal. The size of the negative potentials obtained at different points in relation to the two expected maxima (cross hatched areas) is indicated by the size of the filled circles plotted

fornix superior rather than the fimbria (Rawlins et al. 1979). We therefore used lesions of the fornix superior and fimbria to determine whether the septal-dentate input takes the same course as the fibres controlling theta rhythm.

Figure 3 shows responses in two animals before and after lesions of the fimbria or of the fornix superior. In the first case medial and lateral septal responses recorded from a single electrode in the granule cell layer before and after fimbrial section showed total abolition of the positive and late negative components. The lesion essentially spared the fornix superior and produced only slight reductions in the size of the N1 component with both medial and lateral responses. This latter reduction was observed immediately after insertion of the knife and no further reduction was observed as the lesion was extended laterally.

In the second case shown in Fig. 3, one stimulating electrode (MS) was in the medial septum but just contralateral to the midline with respect to the recording electrode, while the second (MS/LS) was located approximately 0.5 mm from the midline in the region of the border between the medial and lateral nuclei. As can be seen this MS/LS placement produced, clear positive N1, and late negative components. Lesion of the fornix superior produced a large reduction in the size of the N1 component but did not change the positive and late negative waves to any great extent.

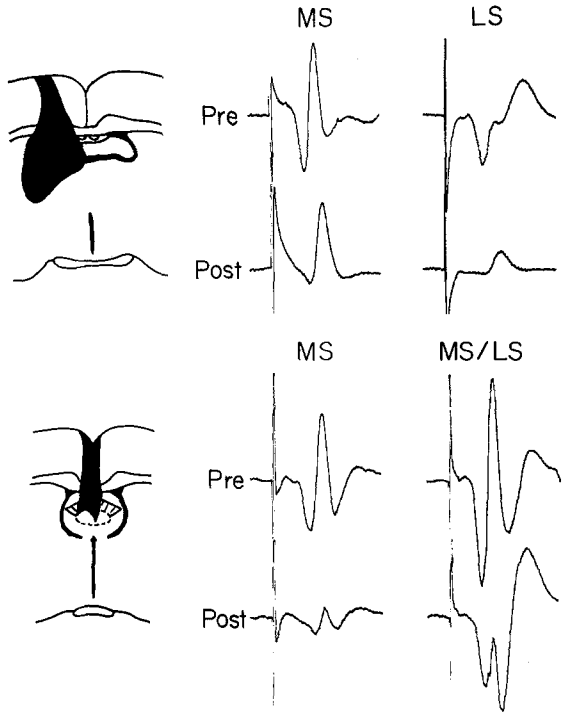

Fig. 3. Effects of lesions of the fimbria (above) or the fornix superior (below). Medial and lateral septal stimulating electrodes were implanted in all cases at the same depth as each other, held in the same electrode carrier. The recording electrode was in the granule cell layer. Pre: indicates the response immediately prior to insertion of the knife. Post: indicates the response at least 20 min after the final knife cut which produced the lesions shown. Note that in the lower panel the responses labelled MS were obtained by stimulation of the contralateral medial septum, while the response labelled MS/LS were obtained while stimulating the border between the medial and lateral septum

Three points should be noted with respect to variations across animals in the effects of fimbrial lesion. Firstly, in cases where the lesion damaged both fornix superior and fimbria *unilaterally* all responses were abolished. Secondly, in 4 animals, the lesion abolished the positive and late negative components but did not damage extreme lateral portions of the fimbria. This is consistent with the organisation of the fimbria as reported by Andersen et al. (1973). Thirdly, while the positive and late negative waves observable at the cell body layer was abolished, the P1 and N2 responses from the hilar region (see Fig. 1) were not abolished.

The pattern of control responses in relation to electrode placement and the effects of fornix and fimbria lesions in these animals strongly suggest that the N1 component arises in the medial septum and travels in the fornix superior. When recording in the granule cell layer, positive and late negative components can be obtained which arise in the lateral septum and travel in the fimbria. These components can clearly be confused with the P1 and N2 waves to medial septal stimulation if inappropriate stimulating electrode positions or voltages are used.

Responses of single cells to medial septal stimulation

Figure 4 shows an example of a single cell found in the dorsal part of the granule cell layer of the upper blade of the dentate gyrus. MS stimulation activated this cell within the latency of the N1 field close to the time at which the amplitude of the field was maximal. Perforant path stimulation also elicited a single action potential from the same cell with a latency corresponding to the perforant path population spike. This suggests that the cell was a granule cell. Fifty-one cells were identified in this way. In response to medial septal stimulation they all produced single action potentials with a latency between 7 and 12 ms (mean 9.76) followed by a period of silence lasting between 70–260 ms. These latency values compare with the 8–11 ms range of latencies obtained with the N1 field. The maximum variation in latency of any individual cell at constant stimulating voltage was 1.5 ms with a mean (21 cells) of 0.83 ms. Increased stimulating voltage could decrease the latency of firing of individual cells by as much as 2 ms, increasing the overall within-cell range of observable latencies to 3.5 ms. The maximum variation in latency observed between cells within any one animal (8 cases) was 3.5 ms. The variation in latency observed between animals, as noted above, is 5 ms. This larger value appears to be due to the latency of the associated N1 field (range 8–11 ms, variation 3 ms). The latter may be attributed to variation in either conduction velocity or distance.

When the recording electrode was situated well within the hilar region, medial septal stimulation elicited single or double action potentials (7 cells) with latencies between 6–8 ms (mean 6.71 ms). These cells were all associated with the early sharp negative field recorded from the hilus (see Fig. 4). The range of latencies of these cells as a group overlaps that recorded for the granule cells. However, there are three reasons for supposing them to be a distinct class of neurones. Firstly, the location from which they were recorded is well below the granule cell layer; secondly, none of them could be elicited by perforant path stimulation at intensities which easily elicited granule cells; thirdly, in four animals from which both types of neurones were recorded the latest hilar cell was at least 1 ms earlier than the earliest granular cell (mean minimum se-

paration for the 4 animals was 1.88 ms). Again comparing between animals the longer latency cells were found in animals in which the sharp negative hilar field occurred at a longer latency. Within animals this hilar potential preceded the granule cell N1 wave fairly consistently by 1–2 ms.

Three cells recorded just below the granule cell layer showed a bursting discharge in response to medial septal stimulation. They were only held for a short time. In two of these the discharge consisted of 3 spikes associated with the N2 wave. In the remaining case (Fig. 4) a single spike was elicited with a latency of 4 ms and was followed by a longer duration burst. As with the other two cells, the burst was associated with the N2 wave. As far as could be told from size and shape of action potential both single spike and burst were from the same cell. In all three cases the first three spikes of the burst were initiated at 7–8 ms and completed at 10–11 ms; and in all three cases perforant path stimulation caused the cell to fire in a burst associated with the late negative field potential to stimulation of this input (see Fig. 4).

Paired pulse potentiation

If an initial (conditioning) pulse to MS was followed after a short (e.g. 60 ms) interval by a second (test) pulse the evoked response to the test pulse was potentiated (Fig. 5A). The amount of potentiation of the initial negative (N1) field potential, expressed as a percentage, depended on how the amplitude of this field was measured. Measurement from maximum positive deflection to maximum subsequent negative deflection gave values which appeared more reliable, and were smaller as percentages than those obtained by measurement from maximum negativity to maximum subsequent positivity. In the remainder of this paper, except where specifically indicated, measurement of the N1 field elicited by medial septal stimulation was taken from maximum initial positivity to maximum subsequent negativity.

A single MS conditioning pulse was also found to potentiate the granule cell population spike elicited by a subsequent perforant path (PP) stimulus. Similarly, PP could produce potentiation of the MS N1 wave. Percentage potentiation for MS–MS, MS–PP

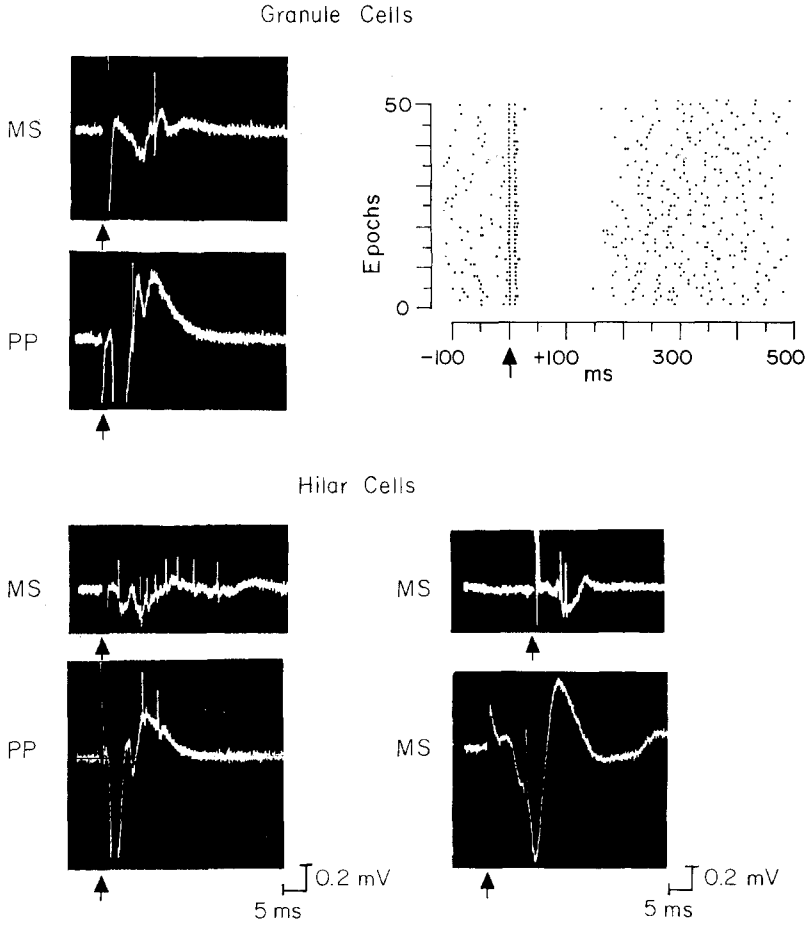

Fig. 4. Examples of the responses of single cells in the dentate gyrus to medial septal and perforant path stimulation. Upper panel, left; a single cell recorded in the granule cell layer activated by both medial septal and perforant path stimulation at latencies corresponding to the relevant "population spike" responses. Upper panel, right: raster display of firing of such a cell generated with a zero crossing detector. In this and other panel arrow indicates delivery of stimulus. The complex of dots at this point in the raster includes the stimulus artefact, firing of the cell and occasional zero crossing producted by the field potential. Lower panel, left: a single cell recorded just below the granule cell layer showing bursting discharge to both medial septal and perforant path stimulation at latencies corresponding to the relevant late negative potential fields. This particular cell also showed direct activation prior to the burst discharge. Lower panel, right: two cells recorded within the hilus of the dentate showing single and double spike activation in response to medial septal stimulation at latencies corresponding to the early sharp negative field recorded in the hilus. The lower example was recorded with filters set at 0.1–3 KHz, as opposed to 1.0–3.0 KHz in the rest of the figure

Table 1. Mean values (with range in brackets) across animals for the maximum potentiation (%), condition-test interval (ms) for peak potentiation and the shortest and longest intervals over which potentiation could be obtained, with a combination of medial septal (MS) and perforant path (PP) conditioning and test pulses.

	%	PEAK	shortest	longest
MS–MS	270 (160–410)	57.5 (30–80)	20.0 (10–30)	215.0 (150–400)
MS–PP	246 (126–590)	61.5 (40–90)	25.0 (10–30)	139.0 (90–200)
PP–MS	201 (124–300)	62.9 (30–90)	21.7 (10–30)	140.0 (100–160)

and PP–MS paradigms tested within a single animal are plotted as a function of condition-test interval in Fig. 5. Table 1 shows the maximum potentiation obtained in the 3 paradigms in a number of animals, together with the shortest and longest condition-test intervals at which potentiation could be obtained, and the interval at which the maximum potentiation could be obtained, and the interval at which the maximum potentiation was found. MS–MS pairing generally appeared most effective both in terms of the maximum potentiation produced and the extent of the condition-test interval over which potentiation was observed.

Single cells recorded in the granule cell layer were presumed to be granule cells on the basis of their response to perforant path stimulation. An action potential could be reliably elicited by the second stimulus of an MS–MS pair when none was elicited by the first stimulus (Fig. 5B) provided that stimulating volatage was correctly adjusted.

In a few cases when the recording electrode was moved slightly deeper a field potential resembling a population spike could be observed in response to the test pulse (Fig. 5C). Also shown in Fig. 5 is an example of MS potentiation of the PP population spike (Fig. 5D, E).

Since it is possible that potentiation of the perforant path response could arise from afferents

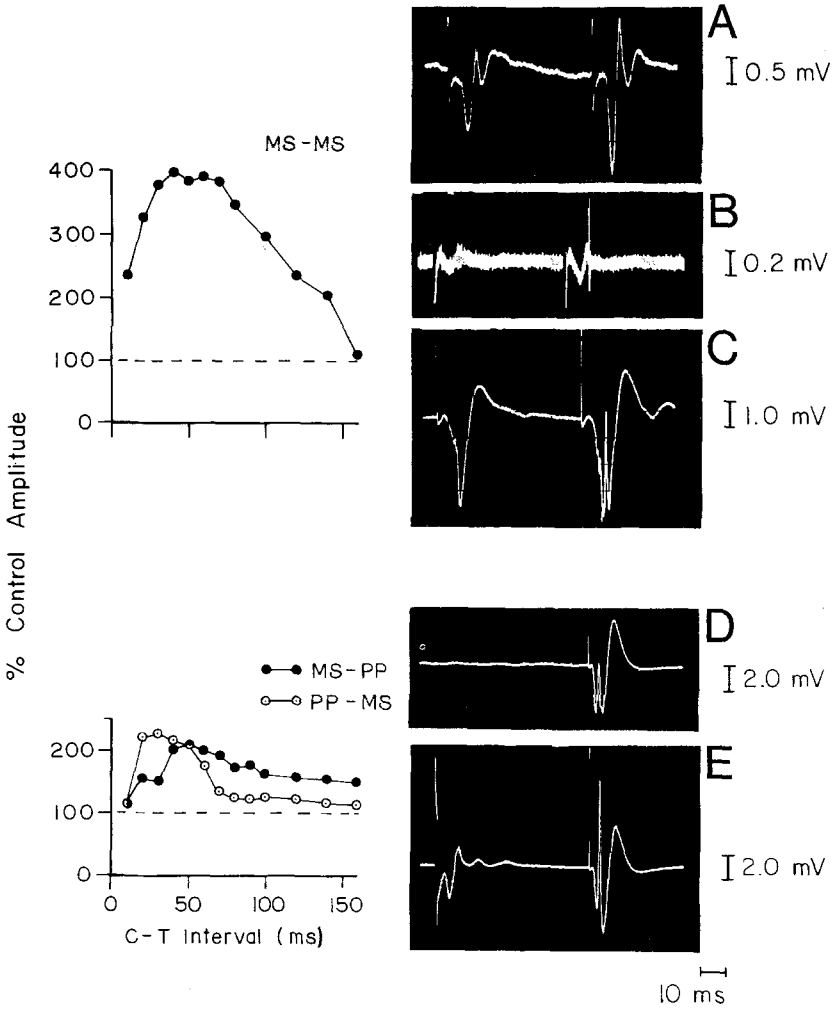

Fig. 5A–E. Effects of paired pulse stimulation of medial septal and perforant path input to the dentate gyrus. Left hand panels: size of field potential evoked by a test (T) response as a percentage of the response to a single pulse control, after delivery of a prior conditioning (C) pulse. The C–T input combinations are indicated by MS for medial septal stimulation and PP for perforant path stimulation. Right hand panels are single sweep examples of, from top to bottom: **A** MS–MS stimulation recording in the granule cell layer; **B** MS–MS stimulation with voltage adjusted just below threshold for activation of a single unit by the C pulse. In this example filters were set at 1.0–3.0 KHz, and several sweeps were collected, in all other examples the filters were set at 0.1–3.0 KHz and only one sweep recorded; **C** MS–MS stimulation recording just below the granule cell layer. In this and a few other subjects the T pulse evoked a negative potential which may have been a pure population spike; **D** control response to PP stimulation; **E** MS–PP stimulation showing potentiation of PP response in comparison to **D**

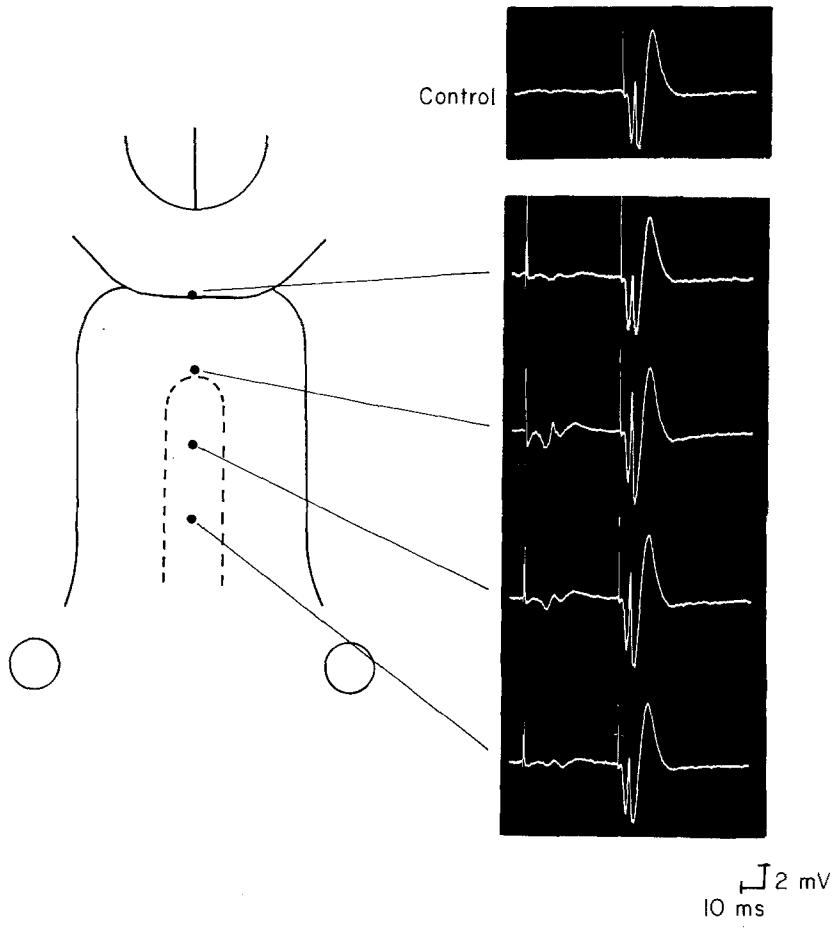

Fig. 6. Localisation of paired pulse potentiation to parts of the septum producing an evoked field potential to medial septal stimulation. Stimulation voltages were kept constant and the medial septal electrode lowered to the indicated points in the septum (dotted line indicates the approximate border of the medial nucleus). Test pulses were delivered to a fixed perforant path electrode

other than medial septal, localisation of the MS–PP potentiation within the septum was examined. As can be seen in Fig. 6 the degree of MS–PP potentiation varied with the amplitude of the MS response and was localised within the dorsal part of the medial septum.

Long term potentiation

Delivery of a single high frequency volley (100 Hz, 1 s) to MS caused a long lasting (at least 30 min, and in some animals up to 5 h) potentiation of the MS response in a majority of animals. In a few animals a second volley was required before potentiation was observed, and in one animal (possibly because of a greater depth of anaesthesia) no potentiation could be produced. With the exception of this latter animal, the increase in the N1 response was between 50–200% of control (100–600% if N1 was measured from maximum negativity to subsequent positivity). The perforant path population spike recorded in the same animals was unaffected. One animal showed an increase in the perforant path population spike to 150% of control in the first minute after the MS volley only, the maximum increase in any other case was to 126% (30 min reading in Fig. 7). In one animal, the perforant path response appeared to be depressed (greatest depression was to 50% of control), but this occurred gradually. For all animals in which MS and PP responses were alternately monitored at a number of time intervals after MS potentiation there was no overlap in the MS and PP scores. Examples of the field potentials and values for potentiation at a range of time intervals in an individual animal are shown in Fig. 7).

To show that the lack of potentiation produced by PP in response to high frequency stimulation of MS was not due to an inability of this pathway to show potentiation, all animals which had shown potentiation of MS subsequently received a high frequency volley delivered to PP (100 Hz, 1 s). This stimulation produced potentiation of the PP population spike in all cases, the resultant response being between 139–421% of the control value in each case. The amplitude of the MS response was unchanged (98–106%) relative to the period immediately before high frequency stimulation of PP. In a small number of animals the effect of a volley to PP was investi-

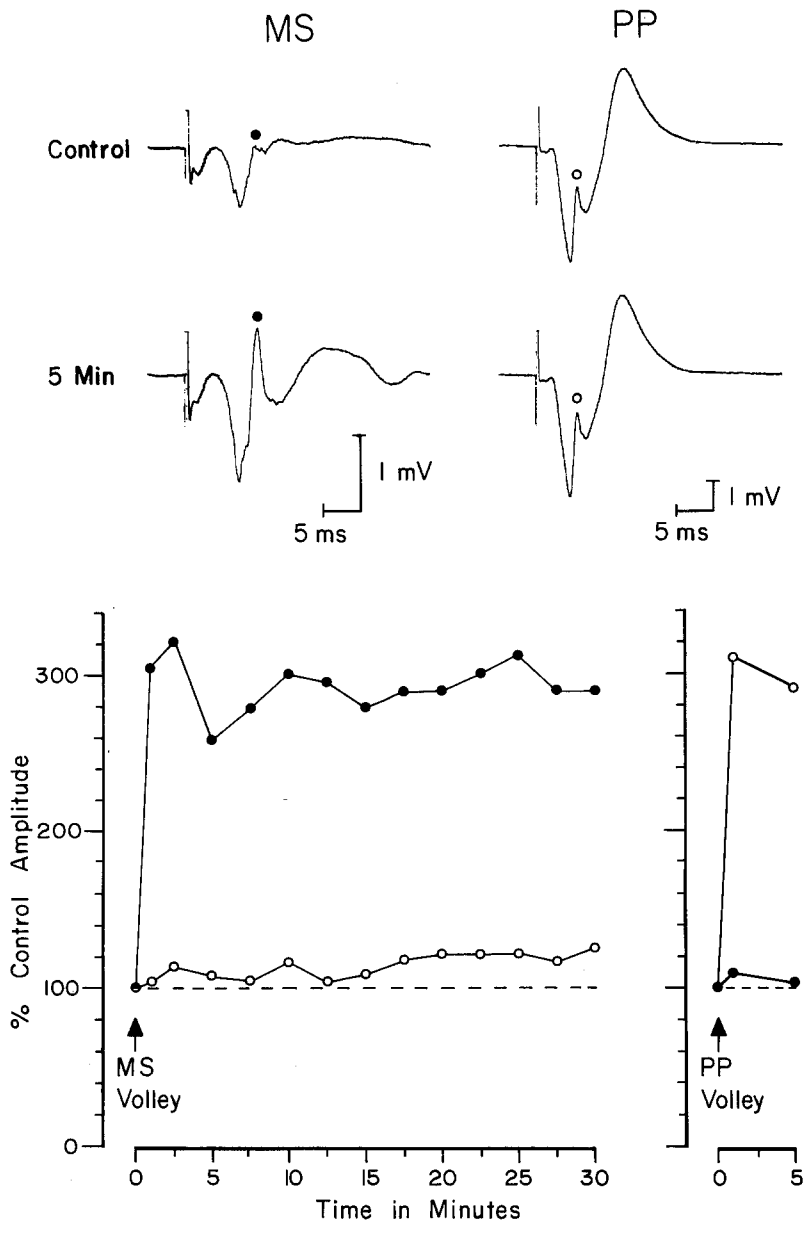

Fig. 7. Effects of stimulation at 100 Hz for 1 s on size of field potentials evoked in the granule cell layer of the dentate gyrus. Above: field potentials to medial septal (MS) and perforant path (PP) stimulation before and 5 min after delivery of a volley to the medial septum. Below: amplitude of MS (filled circles) and PP (open circles) negative field potentials plotted against time following either an MS volley or a PP volley. The values after MS volley are taken from the same test as the evoked potentials shown above. The values after PP volley are taken from a second animal which had shown prior MS potentiation. The potentiation of the stimulated pathway is large and of approximately similar magnitude in the two cases. The change in the unstimulated pathway is in both cases negligible

gated without delivery of any prior volley to MS. In these animals also the PP response was potentiated while the MS response remained unchanged.

In the above experiments voltage was set so that before high frequency stimulation a small N1 response was obtained. In a few rats voltage was initially set below threshold for an observable N1 response. In these rats high frequency stimulation of MS resulted in a N1 response (filled bars, Fig. 8). A second burst of high frequency stimulation of MS produced potentiation which could be, in different animals, negligible, moderate or extensive (open bars, Fig. 8). When paired pulse stimulation is delivered after long term potentiation the resultant response (N1) is larger than that produced by either alone (Fig. 8: field responses and right hand histograms). There are two ways in which this interaction is not linear. Firstly, the decrement over time in the N1 response to the conditioning stimulus after long term potentiation is not necessarily accompanied by a decrement in the N1 response to the test stimulus; secondly, long term potentiation, at least with a second volley, of the conditioning N1 response need not be accompanied by any increase in the test response (Fig. 8).

Finally, it should be noted that, despite these nonlinearities, the conformation of the potentiated N1 response is essentially the same whether the potentiation is achieved by paired pulse or long term manipulations.

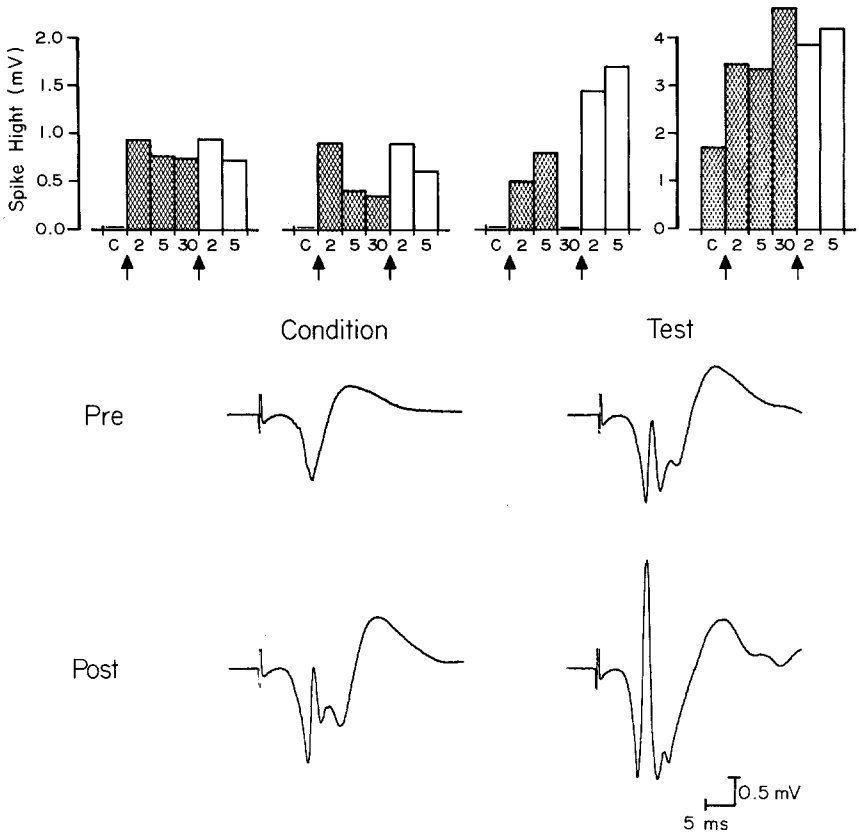

Fig. 8. The effects of an 100 Hz stimulation of MS for 1 s in the absence of an initially measurable N1 response to septal stimulation at the same voltage; the effects of a second MS volley; and the interaction of long term and paired pulse potentiation. Above: The three left hand histograms show the amplitude of the N1 wave in three separate animals at various times (minutes) in relation to a volley to MS. The volley is indicated by the arrows. The right hand histogram shows the response of the third of these animals to a test MS pulse delivered 50 ms after an MS conditioning pulse. Below: Examples of responses to conditioning and test MS pulses (C–T 50 ms) delivered before (pre) and after (post) high frequency stimulation of MS. Voltage was constant throughout the experiment. In this example recordings were made slightly below the granule cell layer of the dentate gyrus. Note the similar conformation of the response to the test pulse before, and to the conditioning pulse after, high frequency stimulation

Discussion

Responses to single pulse stimulation

The present study has demonstrated that stimulation of the medial septum in urethane-anaesthetised rats elicits evoked potentials in the dentate region with a number of components: a negative field potential with a latency of 8–11 ms which is maximal in the granule cell layer; one shorter and one longer latency negative field potential which both maximise within the hilar zone; and a positive field also in the hilus of the dentate. When recording at the granule cell layer additional positive and late negative components can be obtained especially with more lateral placements in the septum and with high voltages of stimulation. The following discussion attributes the principal negativity in the granule cell layer to the direct activation of granule cells; the early hilar negativity to direct activation of hilar neurones; and the late hilar negativity to activation, direct or indirect, of interneurones. The nature of the depth profiles and the complexity of the waveforms make it difficult to determine whether the critical aspect of activation, resulting in the negativitites, reflects synaptic excitation, cell firing or both combined.

The positive wave recorded in the hilus has a conformation which could be attributed to volume conduction of a passive (sink-source) relationship with the presumed granule cell wave, or could be due to the summation of independent positive events in the hilus. The complexity of the observed waveforms makes it difficult to separate these two possibilities.

The activation of single units in the granule cell layer at latencies corresponding to the N1 potential might suggest that N1 is a population spike. However, there is no discrete dendritic potential – equivalent to that obtained from perforant path for example – which can be dissociated from the N1 wave. Such a discrete potential would be expected if the septal input terminated in stratum moleculare (Ibata et al. 1971). It is possible therefore that the N1 wave results in part from a dendritic input closely associated with the cell layer and in part from the summation of action potentials, the two types of event being so close together that they cannot be separated by the microelectrode. In a few cases paired pulse potentiation produced what appeared to be a discrete population spike slightly below the granule cell layer. It may be that in this case the population spike is sufficiently large to be recorded at some distance from the cells and hence discriminated from EPSPs.

Input close to the cell layer is supported to some extent by anatomy. Mosko et al. (1973) report a pattern of degeneration after medial septal lesions which matches the dense cholinesterase staining seen just above the cell layer (e.g. Rawlins et al. 1979) and shows no signs of degeneration in stratum moleculare. This degeneration was greatest at the longest survival time they used – 13 days. They did obtain degeneration in stratum moleculare, with survival times as short as 4 days, if they lesioned the contralateral hippocampus; and they point out that septal lesions can easily damage the ventral hippocampal commissure. Ibata et al. (1971) used survival times between 2 and 7 days and did not present the histology of their lesions. It is possible, therefore, that their results are due to somewhat posterior lesions damaging the ventral hippocampal commissure with insufficient recovery time to demonstrate degeneration of septal neurones. It might be suggested, on a similar basis, that the stimulation in the present experiments could have spread to the ventral hippocampal commissure. However, there are a number of facts which render this possibility unlikely.

Firstly, there is the data from medial and lateral septal stimulation. The shift from medial to lateral electrodes produced a large change in the observed waveforms while the shift is essentially parallel to the closest portion of the commissure. Further, the N1 potential is virtually abolished by a shift of approximately 1 mm (Fig. 2A) lateral to the midline suggesting that current spread is in the region of 1 mm. Yet this stimulation site (0.5 mm anterior to bregma) is more than 1 mm from the ventral hippocampal commissure.

Secondly, there are the previously reported properties of the commissural input to the dentate. Deadwyler et al. (1975) report that stimulation of this input produces a negative field potential in the inner third of stratum molecular; while Douglas et al. (1983) report that the interaction of this input with perforant path input to the dentate is essentially inhibitory. Neither of these observations matches in any way the effects of septal stimulation observed in the present paper.

Subject to further anatomical work, it seems reasonable to assume that the N1 wave represents input from cholinesterase containing, if not cholinergic, cells of the medial septum arriving on or just above the granule cell layer and discharging granule cells. This may not be the only input from the medial septum to the granule cells. The optimal stimulation site for production of the N1 wave was in the most dorsal portion of the medial septal nucleus. This contains a relatively large proportion of cholinesterase containing cells compared with slightly more ventral parts of the nucleus (Lynch et al. 1978). However, abolition of theta rhythm is most extensive, in free moving rats, when lesions of the medial septum include this more ventral area (Rawlins et al. 1979). It may be that pacemaker cells for the noncholinergic production of theta rhythm are located in this area and also project to the dentate granule cells. Since the noncholinergic theta rhythm is blocked by the anaesthetic used in the present study, urethane, (Kramis et al. 1975) such cells would not be expected to contribute to the evoked potentials observed here. Experiments with unanaesthetised animals treated with a cholinergic blocker could resolve this point.

About the N2 wave which was maximal just below the granule cell layer, less can be said. Its conformation could result from summed EPSPs, relatively asynchronous single action potentials from very large numbers of units, or burst firing of synchronously activated units. Three units were recorded in the infragranular region which fired bursts, and these bursts were of appropriate latenc and duration to correspond with the N2 wave. The same neurones fired bursts in response to perforant path stimulation. It seems likely therefore that the N2 wave results from synchronous bursting activity in a population of infragranular neurones which may well be interneurones. Further work is needed to show whether the firing pattern and distribution of these cells genuinely corresponds to the N2 wave, or whether the latter may in part reflect summed EPSPs.

There were single cell responses associated with the early sharp negative potential obtained in the hilus of the dentate. This implies a direct input from the medial septum to the hilus (Swanson and Cowan 1979; Mosko et al. 1973) projecting through the fornix superior (Von Euler and Green 1960). These cells could be being activated *en passage* by fibres which also activate the infragranular neurones and perhaps the granule cells. However, in the latter case it would be difficult to account for the difference in latency of activation. It is more likely as suggested by Mosko et al. (1973) that separate fibre bundles are involved in each case.

The present data in rats shows substantial agreement with the results obtained in rabbits by Andersen et al. (1961). However, the data of Andersen et al. do not distinguish between granule cell and early hilar negativities. It is possible that in the rabbit the septal-granular and septal-hilar pathways have similar conduction times. Alternatively the septal-hilar input, which produces only a small sharp negative response in the present experiments, may produce a negligible negative response in rab-

bits. Given the extent of the positivity observed in the hilus by Andersen et al. (1961) and the early occurence of their dentate granule cell response, the former cannot be explained in terms of a passive (sink-source) relationship with the latter. This suggests that the positivity observed in the hilus both in Andersen's experiments and the present experiments may represent summed IPSPs or summed after hyper-polarisations.

These results suggest that there are at least 3 separate inputs from the medial septum to the dentate gyrus and hilus as shown in Fig. 9: a direct input to the granule cells terminating mainly close to the cell body layer and perhaps to a smaller extent more distally; a direct input to the cells of the hilus; and, speculatively, a direct input to interneurones located just below the granule cell layer. Such interneurones may depend less for their activation on direct septal input than on collateral input from the granule cells.

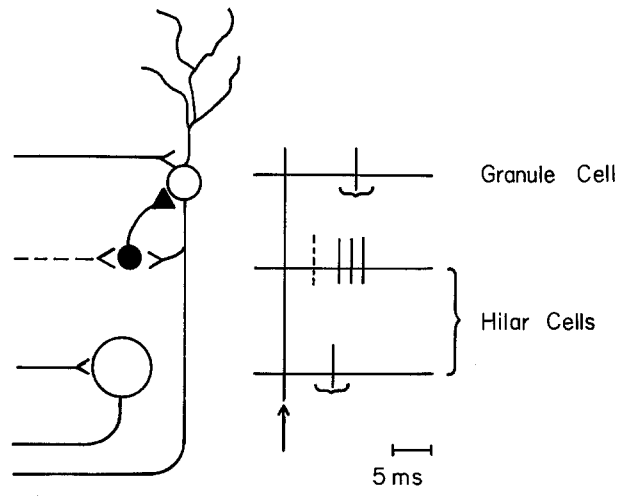

Fig. 9. Diagrammatic representation of proposed medial septal input to the dentate region: Input arriving just above the granule cell layer and producing single spike activation of granule cells; collateral and perhaps direct activation of what are likely to be interneurones producing a bursting discharge; and direct single or double spike activation of hilar neurones. The right hand side of the figure shows the proposed firing patterns and latencies to medial septal stimulation delivered at the point indicated by the arrow. The brackets under the single spikes drawn for granule cell and hilar cell responses indicate the range of latencies observed in the present experiments. The above proposals in relation to neurones showing a bursting response are highly speculative as only three such cells were observed. The suggested inputs are not intended to be exhaustive and it is likely that additional medial septal input to the dentate could be demonstrated in unanaesthetised animals

Responses to repeated stimulation

It has been suggested that the medial septum and perforant path supply separate kinds of information about events of importance to the animal which are integrated by the hippocampus (Vinogradova 1975). The present experiments with paired pulse stimulation provide some support for such suggestions. They show that inputs from either pathway which are closely correlated in time with input on the other will result in an increased granule cell response. This phenomenon could form the basis of the changes in excitability of the dentate gyrus seen with variation in phase of theta rhythm (Rudell et al. 1980). It is also clear that both short term and long term increases in response can be obtained homosynaptically.

Comparison of the single cell and field responses in the MS–MS paired pulse paradigm suggests that the increase in the size of the N1 component of the response to MS stimulation recorded at the granule cell layer is related to the activation of increased number of granule cells (bursting was never observed in response to such stimulation). This could result from an increased likelihood of activation of cells by an unchanged afferent input or it could arise from an increased input to the dendrites. Because the conformation of the septal response does not allow separation of dendritic and population spike responses it is unclear which of these possibilities is the case and both may be combining to produce the observed effects.

The localisation of the paired pulse potentiation in this experiment to stimulation of parts of the medial septum which produce an evoked potential in the dentate is in contrast to results reported by Alvarez-Leefmans and Gardner-Medwin (1975) and by Fantie and Goddard (1982). They reported that septal stimulation produced increased perforant path responses in the absence of any septal evoked potential. Alvarez-Leefmans and Gardner-Medwin (1975) do not report what anaesthetic they used. Fantie and Goddard (1982) used barbiturate anaesthetised rats and obtained clear potentiation of perforant path responses with medial septal stimulation which produced no observable septal response even with amplification set at 50 times that for perforant path responses. While, theoretically, the absence of an evoked potential in this experiment could be due to incorrect placement of recording electrode in relation to stimulating electrode (cf. Brust-Carmona et al. 1973), in practice the method used by Fantie and Goddard to map the septum rules out this possibility. It appears, then, that under barbiturate anaesthesia *medial* septal modulation of perforant path responses can be obtained in the absence of evoked potentials, while under urethane

this modulation is accompanied by, and appears attributable to, evoked potentials.

Robinson and Racine (1982) also report septal modulation of perforant path responses under barbiturate anaesthesia. However, in this case evoked potentials were observed. Two points should be noted. Firstly, the evoked potential constituted a simple positivity, lacking any negativity of the type which would be expected of medial septal responses in the dentate gyrus. Secondly, while it is unclear as to whether Robinson and Racine were stimulating in the septum or in the fornix-fimbria, they state that their electrode placement was 0.5 mm lateral to the midline. Both of these points when taken together with the data presented above, suggest that Robinson and Racine (1982) were observing modulation of perforant path responses by stimulation of lateral rather than medial septum. This point is backed up particularly by the careful histology presented by Fantie and Goddard (1982) in what, except for strain of rat, appears to be an essentially similar preparation to that of Robinson and Racine.

The medial septal and perforant path afferents to the dentate gyrus show input specific long term potentiation. They also show clear heterosynaptic interactions with paired pulse paradigms. While it might be argued that the lack of interaction in the long term case is due to lack of convergence onto common granule cells, it should be noted that all of 51 cells in the granule cell layer activated by medial septal stimulation were also activated by perforant path stimulation. The present data, then, strongly suggest that paired pulse and long term potentiation depend on different mechanisms. Paired pulse potentiation, at least in the heterosynaptic case, is likely to depend on post-synaptic mechanisms which may include local circuitry. Long term potentiation on the other hand is likely to depend on presynaptic mechanisms, or if postsynaptic changes occur they must be limited to immediately postsynaptic elements rather than being generalised to the whole dendritic field.

In conclusion, stimulation of the dorsal part of the medial septal nucleus activates a pathway which travels in the fornix superior and makes excitatory contact with granule cells of the dentate gyrus in the dorsal hippocampus. Discharge of the granule cells is accompanied by a negative evoked potential which is maximal in the region of the granule cell layer. This potential appears to contain contributions from both EPSPs and action potentials which are difficult to separate because the input terminates close to the granule cell layer. The medial septal and perforant path inputs to the dentate show long term potentiation which is input specific and also paired pulse potentiation which is heterosynaptic.

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