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The CA3 "Backprojection" to the Dentate Gyrus

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Abstract

The hippocampus is typically described in the context of the trisynaptic circuit, a pathway that relays information from the perforant path to the dentate gyrus, dentate to area CA3, and CA3 to area CA1. Associated with this concept is the assumption that most hippocampal information processing occurs along the trisynaptic circuit. However, the entorhinal cortex may not be the only major extrinsic input to consider, and the trisynaptic circuit may not be the only way information is processed in hippocampus. Area CA3 receives input from a variety of sources, and may be as much of an "entry point" to hippocampus as the dentate gyrus. The axon of CA3 pyramidal cells targets diverse cell types, and has commissural projections, which together make it able to send information to much more of the hippocampus than granule cells. Therefore, CA3 pyramidal cells seem better designed to spread information through hippocampus than the granule cells. From this perspective, CA3 may be a point of entry that receives information which needs to be "broadcasted," whereas the dentate gyrus may be a point of entry that receives information with more selective needs for hippocampal processing.

One aspect of the argument that CA3 pyramidal cells have a widespread projection is based on a part of its axonal arbor that has received relatively little attention, the collaterals that project in the opposite direction to the trisynaptic circuit, "back" to the dentate gyrus. The evidence for this "backprojection" to the dentate gyrus is strong, particularly in area CA3c, the region closest to the dentate gyrus, and in temporal hippocampus. The influence on granule cells is indirect, through hilar mossy cells and GABAergic neurons of the dentate gyrus, and appears to include direct projections in the case of CA3c pyramidal cells of ventral hippocampus. Physiological studies suggest that normally area CA3 does not have a robust excitatory influence on granule cells, but serves instead to inhibit it by activating dentate gyrus GABAergic neurons. Thus, GABAergic inhibition normally controls the backprojection to dentate granule cells, analogous to the way GABAergic inhibition appears to control the perforant path input to granule cells. From this perspective, the dentate gyrus has two robust glutamatergic inputs, entorhinal cortex and CA3, and two "gates," or inhibitory filters that reduce the efficacy of both inputs, keeping granule cells relatively quiescent. When GABAergic inhibition is reduced experimentally, or under pathological conditions, CA3 pyramidal cells activate granule cells reliably, and do so primarily by disynaptic excitation that is mediated by mossy cells. We suggest that the backprojection has important functions normally that are dynamically regulated by nonprincipal cells of the dentate gyrus. Slightly reduced GABAergic input would lead to increased polysynaptic associative processing between CA3 and the dentate gyrus. Under pathological conditions associated with loss of GABAergic interneurons, the backprojection may support reverberatory excitatory activity between CA3, mossy cells, and granule cells, possibly enhanced by mossy fiber sprouting. In this case, the backprojection could be important to seizure activity originating in hippocampus, and help explain the seizure susceptibility of ventral hippocampus.

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Introduction

Most explanations of hippocampal circuitry begin with the identification of the three major subfields and the trisynaptic circuit. As shown in Figure 1, the hippocampus of mammals is composed of three subfields: area CA1, containing primarily CA1 pyramidal cells, area CA3, also composed primarily of pyramidal cells, and the dentate gyrus, where the primary principal cell is the granule cell. The trisynaptic circuit is composed of three sequential glutamatergic synapses: perforant path axons of layer II neurons in entorhinal cortex project to the outer two-thirds of the dentate gyrus molecular layer, the location of the distal granule cell dendrites, mossy fiber axons of granule cells project to proximal dendrites of area CA3 pyramidal cells, and the Schaffer collateral axons of CA3 pyramidal cells project to stratum radiatum of CA3, where the apical dendrites of area CA1 pyramidal cells are located (Amaral and Witter, 1989).

This projection is clearly fundamental, and its importance to information processing in hippocampus is not to be doubted here. The point of emphasis here is, instead, that the trisynaptic pathway is not the only circuit to consider. One reason for hesitation is that a considerable number of studies have now shown that nonprincipal cells of hippocampus play critical roles in modulating the trisynaptic pathway. They are innervated by each of the fiber pathways mentioned above, and have diverse projections to all hippocampal principal cells. For example, within the dentate gyrus, the perforant path and the mossy fibers innervate numerous types of GABAergic neurons, as well as glutamatergic mossy cells of the hilus. The GABAergic “interneurons” and mossy cells project to granule cells at the level of the soma, axon hillock, and at every part of the dendritic tree. Indeed, it has been proposed that the primary targets of mossy fibers are GABAergic neurons, and the primary effect of mossy fiber transmission under normal conditions is inhibitory (Acsady, et al., 1998). This appears to be the case in recordings of CA3 neurons in slices, where they dominant response to mossy fiber stimulation is an IPSP (Scharfman 1993), despite the large unitary EPSP produced by granule cells (Scharfman, et al., 1990)(for review, see Jaffe and Gutierrez, this volume). It has been suggested that other characteristics of mossy fiber transmission may be even more important than their ability to depolarize postsynaptic pyramidal cells (Urban, et al., 2001).

In this chapter an alternative to the trisynapto-centric view is emphasized. It involves the axons of CA3 pyramidal cells that project in the opposite direction of the trisynaptic circuit (“back projection”), into the dentate gyrus (Figure 1B).

I. Anatomical evidence for a “backprojection”

What is the evidence for a “backprojection?” First we will consider anatomical arguments and then physiological data. Historically, there have been only rare suggestions that CA3 pyramidal cells might project into the dentate gyrus (Schwerdtfeger and Sarvey, 1983;Zimmer, 1971). These did not seem to attract much attention, possibly because some of the experimental approaches had technical limitations. For example, the most common approach for tract tracing at the time relied on tracer injection, but CA3 is difficult to label selectively with this technique.

Newer approaches provided the requisite selectivity. These techniques involved dye injection, typically biocytin, into individual CA3 pyramidal cells using intracellular microelectrodes, followed by axon reconstruction to evaluate the axon arbor. Using this approach, Ishizuka et al. (Ishizuka, et al., 1990) filled individual CA3 neurons in hippocampal slices of the rat, and emphasized several aspects of the axon of CA3 neurons. They found evidence for axon collateralization within the hilus in many of their sampled cells, particularly those with a cell body in “proximal” CA3 (near the dentate gyrus, i.e., CA3b and CA3c). Li et al. (Li, et al., 1994) injected biocytin into CA3 pyramidal cells in vivo, also using adult rats, and found even greater evidence for a backprojection. They found that individual CA3 neurons had collaterals

in the hilus whether the cell body of origin was in CA3a, b, or c. Like Ishizuka et al. (1990), they found the most hilar collateralization in cells within CA3c, and they specifically identified the ventral portion of CA3c region as the area with the greatest collateralization in the hilus. Ventral CA3c pyramidal cells collateralized approximately 3–4x as much in the hilus as dorsal CA3c pyramidal cells, or CA3b cells.

Li and colleagues (1994) also noted that CA3 pyramidal cell axons entered the granule cell layer and collateralized in the inner molecular layer, and this was predominantly a characteristic of ventral CA3c pyramidal cells. These axons exhibited numerous varicosities, suggesting that they innervated dentate gyrus granule cells, and possibly other cell types. Therefore, the CA3c population of ventral hippocampus appears to be a subset of CA3 pyramidal cells with the highest potential for dentate gyrus interactions.

These studies were the first to define hilar collateralization of CA3 neurons unequivocally. However, the one caveat was that the sample sizes were small, and it remains unclear exactly how representative the data were of the entire CA3 population. It also is unclear whether the data from adult rat might vary across age and species. Due to the labor-intensive nature of the experiments, these questions remain unanswered.

Comparisons of CA3a, b, and c pyramidal cells are interesting to review in light of the findings that their axons appear to have a different preference for the dentate gyrus. Anatomically, CA3c pyramidal cells are heterogeneous neurons (Scharfman, 1993;Turner, et al., 1995), sometimes reflecting stereotypical pyramidal cell morphology, but in other cases they may appear more similar to nonpyramidal cells. Some of the electrophysiological characteristics of CA3c pyramidal cells appear to be distinct from CA3a and b, and this may be important because it could lead to unique physiological attributes to the backprojection. CA3c neurons are not as easily activated by mossy fiber stimulation as CA3b neurons in slices, suggesting that the GABAergic neurons which are targets of mossy fibers innervate CA3c more than CA3b. All else being equal, such preferential inhibition might quiet the backprojection normally, relative to other CA3 projection systems.

In comparisons of CA3b and CA3c pyramidal cells of the adult male rat, almost all CA3c neurons demonstrated burst (phasic) firing in response to current injection, whereas CA3b pyramidal cells were much more heterogeneous, often exhibiting trains of action potential (tonic firing) only. Variation in CA3a and b in this respect has been reported (Bilkey and Schwartzkroin, 1990). If CA3c neurons do have a greater percentage of neurons which intrinsically discharge in bursts, the information processed by backprojecting axons may differ in the way it influences granule cells from the way information is transferred to other targets of CA3 neurons.

CA3c neurons demonstrated the longest time constant, which is noteworthy because this characteristic might lead to a greater capacity for integration of synaptic inputs from distinct locations along the dendrites (Scharfman, 1993). However, CA3c neurons appear to have a relatively short electrotonic length, which would lead to rapid decay before integratation of dendritic inputs would reach the soma. Together the data would suggest a specialization of the dendrite as a separable processing unit, perhaps more than other CA3 neurons. It also would suggest that somatic inputs would have a relatively greater influence on action potential output than dendritic inputs.

CA3c is more vulnerable to certain types of excitotoxic stimulation than CA3a or b pyramidal cells (Chang and Dyer, 1985;Freund, et al., 1992;Haas, et al., 2001;Miettinen, et al., 1998). It was proposed that CA3 may be an area where burst generation occurs in models of epilepy, but others have suggested CA3a/b is that location (Colom and Saggau, 1994;Knowles, et al.,

1987). Selective lesions to CA3a vs. b vs. c are difficult to make without injuring adjacent areas, so the functional relevance of the differences between CA3a, b, and c remain unclear.

In summary, the anatomical data suggest that ventral hippocampus and CA3c neurons make the most robust backprojection in normal adult rats, and there is collateralization in the hilus, granule cell layer and inner molecular layer. Specific physiological characteristics of CA3c neurons may impart unique functional consequences to the backprojection.

II. Physiological evidence for a “backprojection”

Some of the first physiological evidence that CA3 axons innervated the dentate gyrus came from studies in hippocampal slices, which showed that a stimulating electrode placed in the hilus could activate an antidromic population spike, recorded extracellularly in the CA3 pyramidal cell layer.

Subsequent studies identified what cell types in the dentate gyrus could be influenced by the CA3 projection. Several approaches established that hilar neurons could be activated readily, but granule cells could not.

First, fimbria stimulation was used to activate CA3 pyramidal cells instead of the hilus, because antidromic action potentials are easily elicited in CA3 neurons by stimulation at this site, with no evidence for direct activation of hilar neurons and granule cells. Direct activation would be expected because hilar cells that project commissurally have an axon that descends into stratum orients of CA3b, presumably on its way to the fimbria, but in our slices, we have found that it is severed before reaching the fimbria. Direct activation of granule cells would only be possible if current spread entered stratum lucidum, which recordings show does not occur after fimbria stimulation using the methods we have employed.

Extracellular recordings showed that such fimbria stimulation led to antidromic and orthodromic activation of CA3 b and c pyramidal cells, followed by an extracellular negative wave in the granule cell layer and inner molecular layer that reversed polarity in the middle molecular layer (Figure 2). Due to the negative resting potential of granule cells, the negative potential could reflect an EPSP or depolarized IPSP, and it was shown subsequently by intracellular recording that it was a depolarized $GABA_A$ receptor-mediated IPSP (Figure 3; (Scharfman, 1994).

This fimbria stimulation site activated hilar mossy cells and hilar GABAergic neurons disynaptically, and was sensitive to the AMPA receptor antagonist CNQX but not the muscarinic antagonist atropine (Scharfman, 1993). At a longer latency, the granule cell IPSP began. These data suggesting that fimbria activation of CA3 pyramidal cells led to hilar neuron activation, presumably by hilar collaterals of CA3 pyramidal cells. Comparing the latency to the response of CA3 pyramidal cells and hilar cells supported this hypothesis, because CA3 pyramidal cells were activated at short latency and hilar cells approximately 2 ms later. The same stimulation evoked a long latency IPSP in granule cells, consistent with a CA3-hilar neuron-granule cell pathway. In the presence of bicuculline, a $GABA_A$ receptor antagonist, fimbria stimulation evoked EPSPs in granule cells, and did so with a similar latency as the IPSPs, suggesting a CA3-mossy cell-granule cell pathway is normally masked by the activation of GABAergic interneurons by CA3 hilar collaterals (Figure 3). Additional studies revealed that the GABAergic neurons may not only involve those in the hilus, but also the “basket cells” of the granule cell layer (Kneisler and Dingledine, 1995;Scharfman, 1994).

Use of $GABA_A$ receptor antagonists also led to another approach to examine the effects of CA3 on the dentate gyrus, one that involved no stimulation to activate CA3 neurons. In the presence of GABAergic receptor antagonists such as penicillin, picrotoxin, or bicuculline, CA3

neurons discharge rhythmically in bursts of action potentials (Knowles, et al., 1987;Scharfman, 1994;Schwartzkroin and Prince, 1977). Therefore, one can examine simultaneously any other cell type in the slice to determine when and if they are concurrently influenced. Simultaneous recordings showed that immediately after the onset of spontaneous bursts in CA3 pyramidal cells, hilar mossy cells and GABAergic interneurons also demonstrated bursts (Scharfman, 1994). Granule cells depolarized and could reach threshold, but did so at a greater delay from the CA3 burst than hilar cells. The hilar activity and granule cell excitation, but not CA3 bursts, were blocked by transecting the border between the dentate gyrus and CA3b, or by application of CNQX (Figure 4; (Scharfman, 1994). When the timing of the burst discharges was examined more closely, the onset of the depolarization of CA3 neurons immediately preceded the onset in the mossy cells and GABAergic neurons, and the onset of hilar neuron bursts immediately preceded granule cell depolarization, suggesting single synaptic delays of a CA3-hilus-granule cell pathway (Scharfman, 1994). The most parsimonious explanation was that CA3 pyramidal cells innervated both types of hilar neurons, and evidence was obtained for this by simultaneous intracellular recordings from monosynaptically-connecctedpyramidal cells and mossy cells (Scharfman, 1994). The same approach showed that mossy cells innervated granule cells and interneurons in the hilus (Scharfman, 1995), suggesting that multiple polysynaptic circuits could be set in motion by CA3 pyramidal cells, and ultimately influence dentate granule cells. Current source density has also provided evidence of CA3-dentate gyrus interactions similar to those discussed above (Wu, et al., 1998).

In summary, there is strong evidence from studies in hippocampal slices that the CA3 backprojection innervates hilar neurons, both mossy cells and GABAergic neurons. Currently there is no physiological evidence that CA3 directly innervates the granule cells of the dentate gyrus, but absence of evidence is not proof of absence.

At the present time, the evidence suggests that the granule cells, even if they receive a direct projection from CA3 neurons, are primarily inhibited by the backprojection, not activated. However, once they are relieved of $GABA_A$ receptor-mediated inhibition, a strong excitatory circuit is unmasked. The excitatory circuit appears to primarily be derived from the mossy cell projection to the inner molecular layer.

III. Functional implications of the CA3 backprojection

A. Hippocampal information processing

1. Is CA3 the point of entry to hippocampus? Given the projection of CA3 pyramidal cells innervates CA1, the dentate gyrus, other CA3 neurons by recurrent collaterals, and sends information both ipsilaterally and contralaterally, the argument can be made that it is a cell type that may be central to hippocampal information processing (Figure 1B). In light of this, it is interesting that the general assumption is “trisynapto-centric,” that granule cells receive the primary input, and the mossy fiber control how the hippocampus processes the incoming input. This view might be correct in cases that involve layer II of entorhinal cortex specifically, but it may be rare that layer II is ever activated in isolation, given its broad input from other layers within the entorhinal cortex, and the diverse inputs to entorhinal cortex. In actuality, it is highly likely that the sensory and other cortical input that comes to entorhinal cortex would also involve subcortical structures that at the same time might reach CA3 through the fimbria. Furthermore, the temporoammonic pathway may activate CA3 neurons at lower threshold than granule cells (see Chapter by Derrick, this volume), and this further supports the tenet that incoming information might first activate the CA3 region of hippocampus, rather than do so via dentate granule cells. Given the data that mossy fiber primarily inhibit CA3 pyramidal cells, the dentate may actually function to keep CA3 from being activated excessively by the concomitant inputs from the cortex and subcortical zones.

2. The dentate gyrus has two "gates" rather than one Another implication of the discussion above, particularly the physiological studies, is that the dentate gyrus actually has two "gates" (Figure 5). The first is a gate that prevents activation by the entorhinal cortex (see Chapter by Hsu, this volume). This may not be an all-or-none function, so the word "filter" may be better (see Chapter by Dudek, this volume). However, the concept is appropriate regardless of the semantics: cortical input is not very effective in activating granule cells above their threshold.

It appears that a second "gate" or "filter" also exists, from CA3 excitation back to the dentate gyrus. Thus, the backprojection does not appear to be effective in depolarizing granule cells, and it appears that the reason is the divergence of CA3 hilar collaterals to both excitatory hilar mossy cells, and inhibitory GABAergic interneurons. Evidently, the inhibitory neurons exert a stronger net influence, and it is important to note that in vivo it may be different, because mossy cell axons are truncated in the slice much more than GABAergic neurons. In vivo, one would predict that CA3 would inhibit granule cells within the same lamella, but activate them in distal sections of hippocampus because of the long translamellar mossy cell axon projection.

When inhibition is reduced experimentally, CA3 discharge is extremely robust in depolarizing granule cells, and it appears to be primarily due to inner molecular synapses of mossy cells. Thus, the hilar neurons modulate the CA3 backprojection under normal conditions, and any condition that will reduce GABAergic inhibition would be expected to facilitate CA3 activation of dentate granule cells.

When this occurs, it would be predicted that associative processes would be enhanced. In other words, not only are there recurrent excitatory circuits among CA3 pyramidal cells that perform autoassociative function, but rich polysynaptic autoassociation would potentially develop between CA3 and the granule cells when GABAergic inhibition is weak. One argument that makes this even more compelling is that granule cells do not necessarily need to reach threshold in order for mossy fiber transmission to occur (Alle and Geiger, 2006). Subthreshold depolarizations in granule cells can lead to effects in granule cell targets. An important implication is that autoassociative networks, particularly the complex CA3-dentate network discussed here, is likely to be controlled by GABAergic hilar neurons and mossy cells.

B. Pathological conditions—The CA3 backprojection may subserve an important role in conditions like ischemia or epilepsy, when hilar GABAergic neurons are injured or killed (Johansen, et al., 1987;Sloviter, 1987); see Chapter by Tallent, this volume). In both conditions, the somatostatin-immunoreactive hilar neurons that project preferentially to the outer molecular layer are lost. In animal models of epilepsy that use status epilepticus as the initial experimental manipulation to induce an epileptic state, there are many types of GABAergic neurons that may be affected, but most laboratories suggest a relative preservation of parvalbumin-immunoreactive basket cells. The animal models of epilepsy are also interesting because both the granule cell axons and the pyramidal cell axons may sprout new collaterals in response to injury adjacent to them. Granule cell axons sprout into the inner molecular layer and hilus, as well as stratum oriens in CA3. Pyramidal cell sprouting is less well established, and would be of interest to determine whether the backprojection increases in response to injury.

Regardless of these changes, it does appear that the backprojection is robust under epileptic conditions. The data are from hippocampal slices from animals that had status epilepticus and became epileptic, i.e., they developed recurrent spontaneous seizures for the rest of their lifespan. In most of these animals, CA3 pyramidal cell burst discharges occur when slices are prepared, even when bicuculline is absent (Scharfman, et al., 2001). This affords an opportunity to examine the backprojection by simply examining concurrent activity in hilar neurons and

granule cells. When this has been done, it is clear that the backprojection to mossy cells still exists, because mossy cells exhibit synchronized burst discharges with CA3 pyramidal cells in slices from rats with recurrent seizures (Scharfman, et al., 2001). Interneurons in the hilus do as well, although the population of both mossy cells and interneurons is reduced relative to the normal rat, and it is not yet clear which GABAergic neurons are involved of the many types that exist. Granule cells appear to be relatively quiet when CA3 burst discharges occur (Scharfman and McCloskey, 2007) but this is not always the case: some granule cells have depolarizations during the CA3 burst discharges - and do so at a long latency. The long latency suggests that CA3 neurons activate granule cells via mossy cells, similar to the normal rat. In this case, bicuculline may not be required because of a loss of GABAergic inhibition when hilar inhibitory neurons are killed after status epilepticus.

This pathway may be relevant to the seizures that occur in the whole animal, because the CA3 discharges appear to generate reverberatory activity between the dentate and CA3 pyramidal cells (Figure 6). During burst discharges in CA3, we have found that the onset of the burst discharge occurs in pyramidal cells first, and then in granule cells, but during afterdischarges that follow the primary burst, granule cell discharge may precede the pyramidal cells. These data suggest CA3 may be a generator of activity initially, but granule cells can initiate further excitation. In the whole animal, such reverberatory excitation could lead to substantial network activity of CA3/dentate neurons, and possibly trigger seizures after a certain threshold of activity is reached. One would predict that this might occur in more ventral hippocampus than dorsal, given that the backprojection is more prevalent in ventral hippocampus. This prediction is supported by findings in slices of temporal hippocampus, where seizure activity is much more robust (Borck and Jefferys, 1999;Bragdon, et al., 1986;Scharfman, et al., 2002).

Conclusions

The CA3 region is often considered to be the second subfield that receives information from extrinsic afferents to hippocampus, while the dentate gyrus is the initial entry point. Here we suggest that CA3 may be a major gateway to hippocampus, particularly with respect to subcortical input. One reason to think of CA3 as a central access point is that it has a projection that reaches the vast majority of hippocampal neurons, both ipsilaterally and contralaterally, including both CA1 and the dentate gyrus. The projection to the dentate gyrus, a ‘backprojection,’ has not been studied in as much detail as the Schaffer collateral system, leading to appreciation of CA1–CA3 interactions when CA3-dentate networks may be just as important to hippocampal function. Physiological data suggest that the CA3 backprojection engages intermediary hilar neurons to exert its primary effect on granule cells, although direct projections to granule cells in a subset of CA3 neurons in ventral hippocampus have been shown by anatomical methods. GABAergic inhibition appears to regulate the ability of CA3 pyramidal cells to exert a primarily inhibitory or excitatory effect on granule cells, and to control the possible reverberatory activity between the two subfields. CA3-dentate interactions may subserve an important associative function in the behaving animal, influenced by factors that depress GABAergic neuron activity or GABAergic transmission; they may also play a role in pathological conditions when GABAergic neurons are damaged or lost, such as temporal lobe epilepsy.

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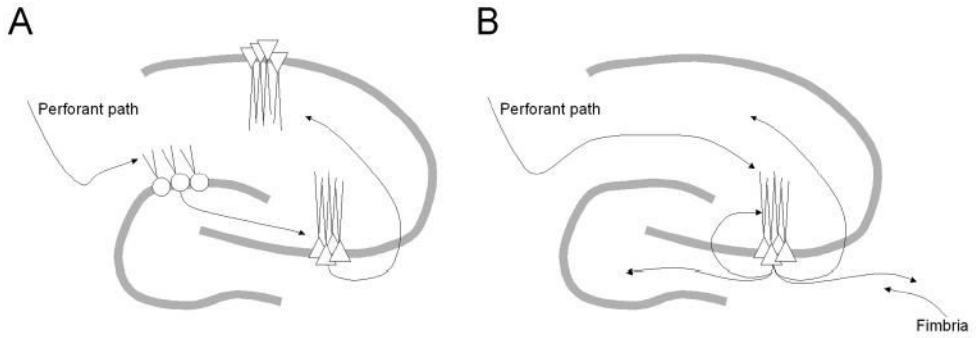

Figure 1. The "trisynapto-centric" vs. "CA3-centric" view of hippocampal information processing

A. The trisynaptic circuit is diagrammed schematically for a horizontal section through the adult rodent hippocampus. Cell layers are in gray.

B. The axonal arbor of CA3 pyramidal cells is schematically presented to illustrate the perspective that these neurons may be a central point of information processing in the hippocampus.

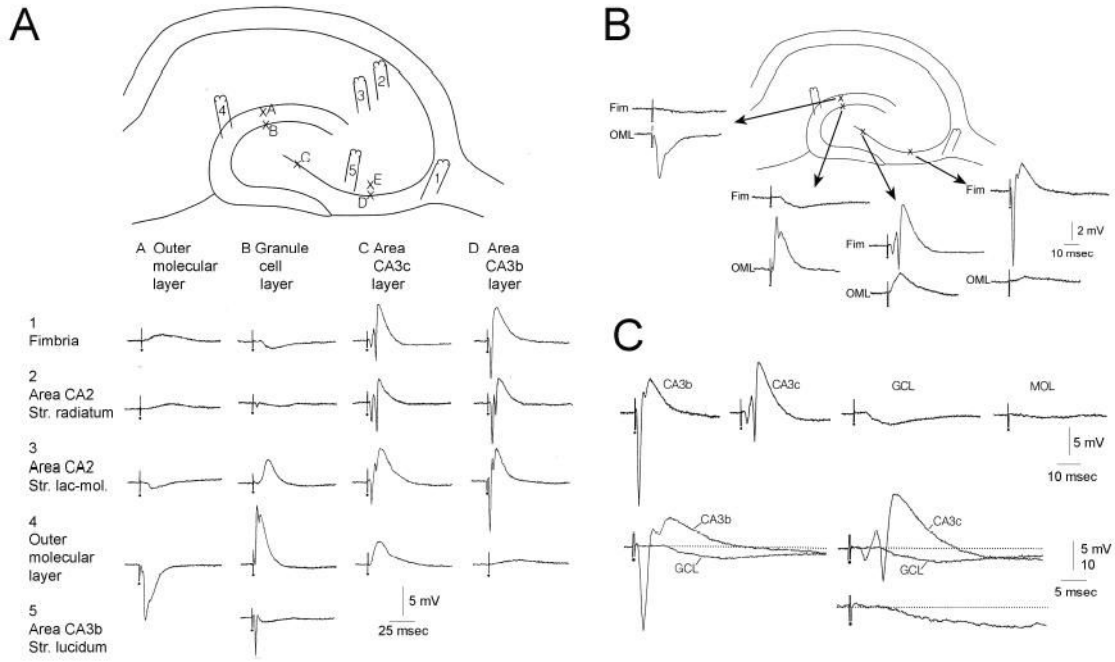

Figure 2. Recordings in hippocampal slices show evidence of trisynaptic and backprojecting pathways

A. Extracellular recordings of evoked responses to 5 stimulation sites (1–5) and 4 recording sites (A–D). Recordings were made sequentially in the same slice in response to a fixed stimulus for each stimulus site.

B. A schematic is used to allow better comparisons of fimbria-evoked and outer molecular layer-evoked responses recorded at four sites in the same slices: the outer molecular layer, granule cell layer, CA3c cell layer, and CA3b cell layer. X marks recording site locations.

C. Comparison of evoked responses to fimbria stimulation in the same slice demonstrate the responses in the granule cell layer following pyramidal cell activation by the fimbria, but do so with a delay. Superimposition of responses illustrates that the onset of the granule cell layer field potential begins approximately 1–2 ms after the antidromic population spike recorded in area CA3b. This delay suggests an intermediary synapse, probably in CA3c or the hilus, between CA3b and granule cells. Indeed, the granule cell field potential begins immediately after the CA3c orthodromic population spike, which probably is due to CA3b recurrent excitation of CA3c pyramidal cells. At the same time, hilar cells are also activated and innervate granule cells (see later figures), although CA3c pyramidal cells could innervate granule cells also (Li et al., 1994). The bottom trace is an IPSP recorded intracellularly from a granule cell in the same slice in response to the same stimulus. It shows that the onset of the IPSP is similar to the onset of the granule cell layer field potential, which likely reflects the average of many IPSPs in granule cells situated around the extracellular electrode.

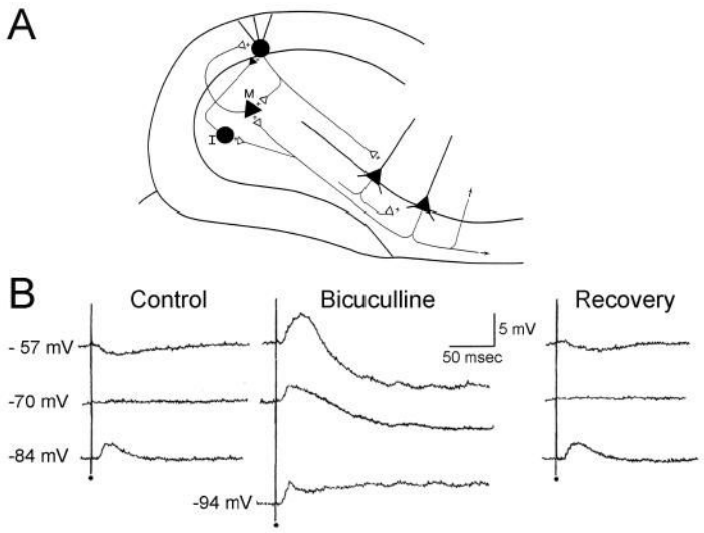

Figure 3. Physiological evidence for a CA3 backprojection mediated by hilar neurons

A. A schematic illustrates the backprojection supported by physiological evidence collected to date. It shows that CA3 pyramidal cells innervate GABAergic and glutamatergic mossy cells of the hilus, which in turn innervate granule cells.

B. Recordings illustrate the physiological correlates of the schematic in A. Intracellular recordings from granule cells in response to fimbria stimulation in an adult male rat slice illustrate an evoked IPSP that reverses at −70 mV, indicated a $GABA_A$ receptor-mediated IPSP is primarily evoked under normal conditions. After the $GABA_A$ receptor antagonist bicuculline was bath-applied, the evoked response was an EPSP followed by an IPSP that reversed at approximately −80 mV, suggesting an EPSP followed by a $GABA_B$ receptor-mediated IPSP is normally masked by $GABA_A$ receptor-mediated inhibition. From Scharfman (1994a).

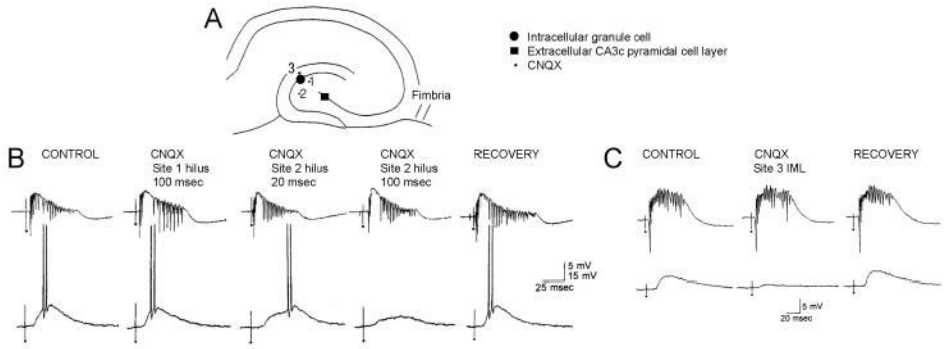

Figure 4. The CA3 excitatory backprojection is dependent on hilar mossy cells

A. Schematic illustrating the experimental approach for results shown in B–C. A site in CA3c was used for extracellular recording while recording intracellularly from a granule cell. Pressure application of CNQX was used at two distinct sites in the hilus or in the inner molecular layer. CNQX was applied in microdrops that were barely detectable by eye, allowing preferential application to select areas of the slice.

B. In the presence of bicuculline, CA3 epileptiform discharges were evoked by fimbria stimulation, and following the onset of the burst discharge, a granule cell depolarized and discharged 2 action potentials. After CNQX was pressure-applied to the hilus, the granule cell response decreased but the CA3 discharge remained unaffected.

C. In a different slice, CNQX pressure-application to the inner molecular layer, near the recorded granule cell, reversibly decreased the EPSP of the granule cell, suggesting an inner molecular layer glutamatergic synapse was necessary for the EPSP. From Scharfman (1994a).

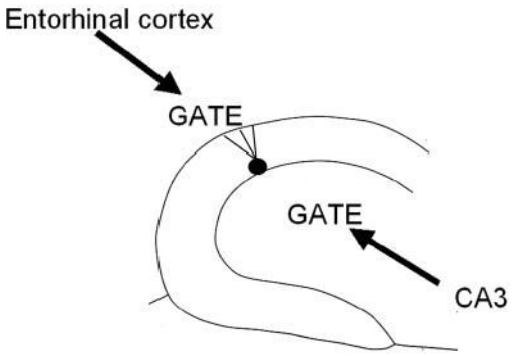

Figure 5.
The trisynaptic circuit and CA3 backprojection supports a bi-directional gate to the dentate gyrus granule cells.

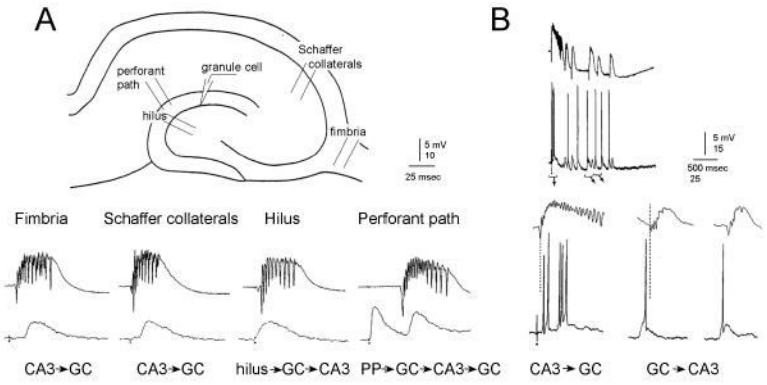

Figure 6. Evidence for associative networks and reverberatory circuits in the dentate gyrus under control of GABAergic inhibition

A. A schematic illustrates electrode locations for the recordings shown in B.

B. Stimulation of specific sites in the slice evoked bursts of action potentials in CA3 pyramidal cells, and simultaneous intracellular recordings from a granule cell show bidirectional activation of CA3 and the granule cell.

C. In a different slice from B where CA3 epileptiform bursts were followed by numerous afterdischarges, CA3 appeared to precede activation of the simultaneously-recorded granule cell, but during the afterdischarges, the converse appeared to develop. From Scharfman (1994a).