

Interneuron Diversity series: Containing the detonation – feedforward inhibition in the CA3 hippocampus

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Feedforward inhibitory circuits are involved both in the suppression of excitability and timing of action potential generation in principal cells. In the CA3 hippocampus, a single mossy fiber from a dentate gyrus granule cell forms giant boutons with multiple release sites, which are capable of detonating CA3 principal cells. By contrast, mossy fiber terminals form a larger number of Lilliputian-sized synapses with few release sites onto local circuit interneurons, with distinct pre-synaptic and postsynaptic properties. This dichotomy between the two synapse types endows the circuit with exquisite control over pyramidal cell discharge. Under pathological conditions where feedforward inhibition is compromised, focal excitation is no longer contained, rendering the circuit susceptible to hyperexcitability.

‘... one easily can see that these varicosities are triangular or stellate masses of cytoplasm with angles that give rise either to short, thick, diverging processes or to thin, rather long filaments ending in a varicosity... this appearance is definitely reminiscent of certain fibers in the cerebellum that we refer to as mossy fibers’ [Ramon y Cajal]

‘... in my thoughts I could not sufficiently wonder at the intrepidity of these diminutive mortals, who durst venture to mount and walk upon my body ... without trembling at the very sight of so prodigious a creature as I must appear to them.’ [Jonathan Swift, *Gulliver’s Travels*]

Feedforward inhibition in the hippocampus: emerging principles

Feedforward inhibition, a synaptic arrangement common to many brain structures, occurs when an afferent fiber excites both excitatory and inhibitory neurons [1] (Figure 1). Activation of inhibitory neurons then provides inhibition to the principal cell population to suppress excitability. Recent advances in interneuron research suggest this synaptic arrangement exists to exert a powerful regulatory control on the hippocampal network, but that the level of control is

dependent on the nature of transmission at the target neurons. Some inhibitory interneurons, for example those that target the soma of pyramidal cells, respond faster and more reliably to the same afferent than do excitatory neurons [2], even eliciting action potentials in inhibitory neurons [3]. As discussed in a later review in this series [4], some interneurons appear to be specialized at almost every level to sense incoming afferent input and rapidly convert it to an output. In the CA1 subfield, this built-in inequality in the circuit produces an inhibitory potential in the pyramidal cell that follows the monosynaptic excitatory potential with minimal disynaptic delay (Figure 1b), thereby greatly restricting spike generation in the pyramidal cell to a narrow window between the arrival of monosynaptic excitatory postsynaptic potentials (EPSPs) and disynaptic inhibitory postsynaptic potentials (IPSPs) [5,6] (Figure 1c–d,f). This minimization of the time window for temporal summation in the pyramidal cells creates a mechanism of precise coincidence detection. However, in other interneurons, particularly those targeting dendritic domains of the pyramidal cells, the nature of transmission at interneuron synapses is less precise, allowing a wider time window for coincidence detection [5,6] (Figure 1e). With the existence of numerous other feedforward circuits in the hippocampus, one must wonder whether such coincidence detection mechanisms are a general principle. This review will draw from the richness in detail of synaptic and cellular mechanisms to formulate a picture of feedforward inhibition in the CA3 network.

Structural features of the mossy fiber–CA3 network

One striking feature of the axons of dentate gyrus granule cells, the so-called mossy fibers (MFs), is that they innervate their principal cell and interneuron targets via anatomically distinct synapse types [7] (Figure 2). MF–principal cell synapses are formed by the well-described large complex ‘mossy’ terminals that comprise large numbers of release sites [8] (Figure 2b,c). By contrast, interneurons of the CA3 stratum lucidum are innervated by small *en passant* or filopodial MF synapses, which possess numerous anatomical properties distinct from synapses onto principal cells [7]. Giant MF terminals form up to 35 active zones [8,9] apposed to a single

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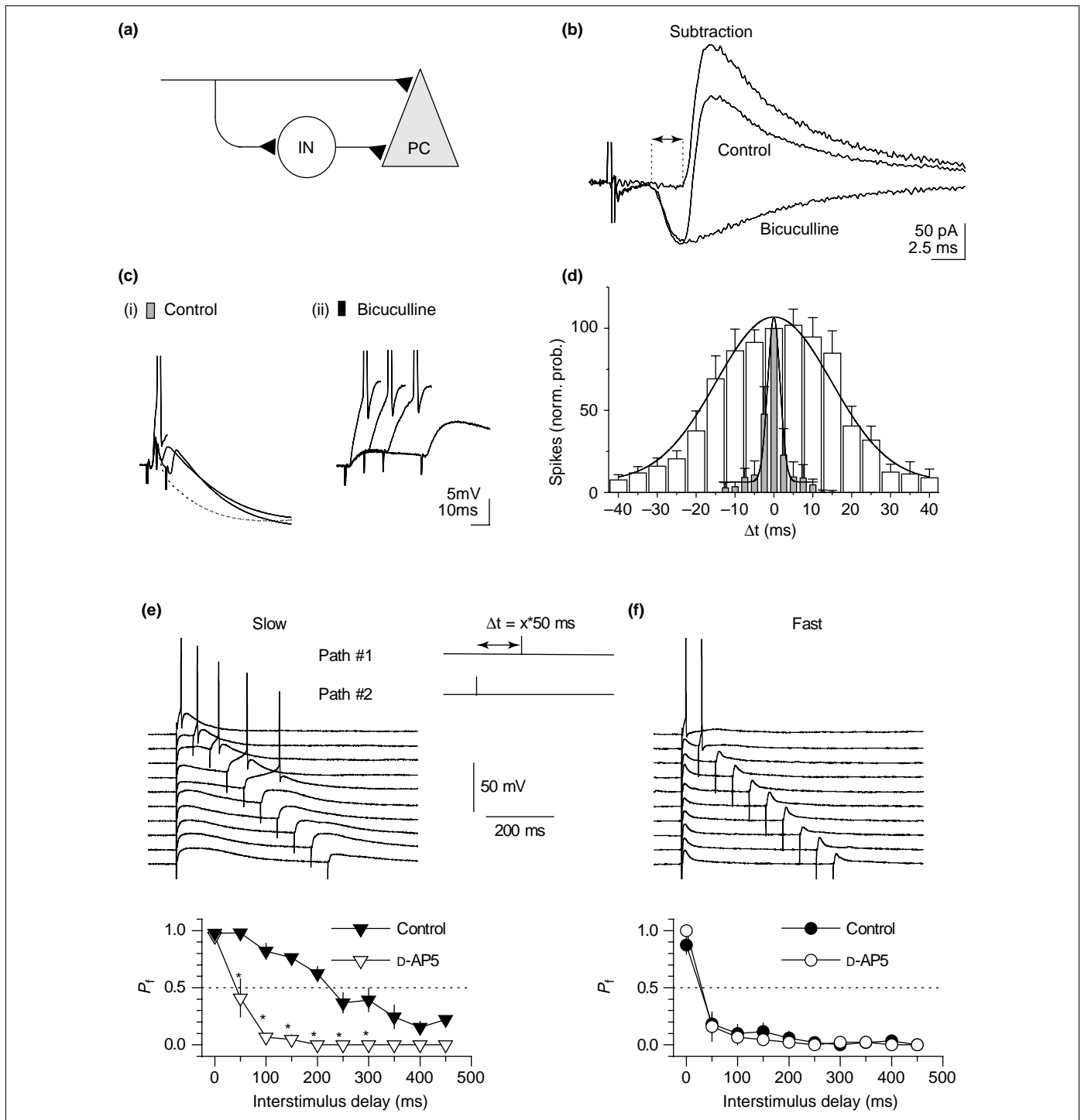


Figure 1. Feedforward inhibition and its role in coincidence detection. **(a)** The concept of disynaptic feedforward inhibition, illustrated for an interneuron (IN) and pyramidal cell (PC). **(b)** Feedforward inhibition observed in whole-cell recordings from CA1 pyramidal cells upon Schaffer collateral stimulation. Current traces are those recorded in control conditions and in the presence of the GABA_A-receptor antagonist bicuculline, and their algebraic difference ('subtraction'). The horizontal double arrow indicates a variable delay between onset of the excitatory and inhibitory postsynaptic currents. **(c)** Feedforward inhibition greatly narrows the window for spike generation in pyramidal cells. Voltage traces for current-clamp recordings from CA1 pyramidal cells upon stimulation of two Schaffer collateral pathways. (i) Control conditions. The dotted line is the average response to stimulation of one pathway. Continuous lines represent single responses to three different inter-stimulus intervals. (ii) Four superimposed responses to four inter-stimulus intervals in the presence of bicuculline. Spikes were truncated. **(d)** Normalized probability of spike generation plotted against the inter-stimulus interval (Δt). **(e)** NMDA-receptor-dependent integration of synaptic input. Integration of synaptic input is different in interneurons with slow versus fast excitatory postsynaptic potential (EPSP) kinetics. The probability of firing (P_f) was calculated after two independent pathways were stimulated at different interstimulus delays (Path #1 0–450 ms, in 50 ms increments; Path #2 fixed at 0 ms). Upper panel: single traces for increasing interstimulus intervals. Lower panel: summary plot showing the integrative properties of slow interneurons. Integration is NMDA-receptor dependent, as shown using the NMDA-receptor antagonist D(-)-2-amino-5-phosphopentanoate (D-AP5). Dotted line indicates P_f at the 0.5 level. Asterisks indicate significance of $P_f < 0.05$ over the 50–300 ms interval. **(f)** Integration properties of fast interneurons. Upper panel: single traces for each interval tested [scale bars as in upper panel of (e)]. Lower panel: summary plot showing that the integrative properties of fast interneurons are NMDA-receptor independent ($P_f > 0.05$ over the entire 0–450 ms interval). Data in (a–d) reproduced, with permission, from Ref. [5], © (2001) American Association for the Advancement of Science (<http://www.sciencemag.org>); data in (e) and (f) reproduced, with permission, from Ref. [6], © (2002) Society for Neuroscience.

pyramidal cell. Historically, the large number of release sites suggested that the strength of excitation was sufficient to cause action potentials in the CA3 pyramidal cells. This peculiar anatomy initially led to the concept of the MF synapse onto pyramidal cells as a 'teacher' or 'detonator' [10]. By stark contrast, the synaptic arrangement of MF synapses onto interneurons is more diffuse. MF synapses onto CA3 interneurons typically form one or a few active zones, through both *en passant* boutons or filopodial extensions radiating from the main MF terminal [7] (Figure 2b,c). Moreover, interneurons in the hilus and CA3 receive approximately ten times more synapses from MFs than do principal neurons [7], with a single MF innervating typically 40–50 inhibitory targets in CA3 alone. From an anatomical perspective, MF–interneuron synapses appear ideally positioned for feedforward inhibition given that each inhibitory interneuron can contact several hundred principal cell targets. Can the plethora of Lilliputian-sized MF–interneuron synapses compete with the prodigious size of the MF terminal, seemingly poised to detonate pyramidal cells?

Mechanisms underlying spike transmission at MF–CA3 interneuron synapses

Although it has been known for over a decade that MF stimulation can elicit action potentials in interneurons *in vitro* [3], the contribution that individual dentate granule cells make towards interneuron excitation has been elucidated only recently [11,12]. By combining intracellular recordings of dentate gyrus granule cells with single unit recordings from CA3 targets *in vivo*, Henze *et al.* [11] demonstrated that in response to initial spikes from granule cells, putative CA3 pyramidal cells only rarely elicited monosynaptic action potentials, indicating that MF synapses do not always act as a detonator *in vivo*. However, the probability of spike transmission increased during the train of action potentials, owing to the high degree of facilitation and summation of MF excitatory postsynaptic currents (EPSCs) characteristic of these synapses *in vitro* [13]. In addition, a significant increase in the probability of spike transmission during the train was also observed at putative CA3 interneurons. Given the anatomical evidence that MF–CA3 interneuron synapses typically comprise a single active zone [7] this observation is striking, and suggests that excitation of interneurons is somehow accomplished despite the severely limited capacity for multi-site release [7] and short-term facilitation [14] at interneuron synapses. Do the quantal parameters at interneuron synapses bear this out? Variance–mean analysis at MF–CA3 interneuron synapses [12] reveals that the initial release probability is in the low-mid range (0.1–0.5) at near-physiological levels of external Ca^{2+} (Figure 3). By contrast, on a per-release-site basis, release probability is probably an order of magnitude lower at MF–pyramidal cell synapses (~ 0.01 – 0.05) [15,16]. The quantal amplitude is also larger at interneuron synapses; the mean unitary quantal amplitude at MF–interneuron synapses is ~ 27 pA (however, some interneuron synapses exhibit quantal amplitudes of up to 80 pA), more than three times the somatic quantal amplitude observed at pyramidal cell synapses when

differences in experimental conditions are taken into account (Figure 3). What is the basis for the larger quantal amplitudes at interneuron synapses? First, the larger and more variable quantal amplitudes at interneuron synapses are consistent with the longer and more variable postsynaptic densities that are apposed to filopodial extensions and *en passant* boutons [7]. This is based on the assumption that the size or area of the postsynaptic density is directly correlated with AMPA-receptor content. However, MF synapses onto many stratum lucidum interneurons also possess Ca^{2+} -permeable AMPA receptors [14,17], an indication that these AMPA receptors lack the subunit GluR2 [18]. AMPA receptors lacking GluR2_{flip} not only speed the time course of channel deactivation [18] but also enhance the single-channel conductance [19], contributing to large, rapidly decaying EPSCs [20,21]. Consistent with this idea, at interneuron synapses there is a correlation between the time course of the synaptic conductance and inhibition by philanthotoxin, an agent that blocks GluR2-lacking AMPA receptors [21]. Finally, the proximal dendritic location of MF synapses [21], higher average input resistance [22–24] and more compact structure than CA3 pyramidal neurons [25] suggest less attenuation in the amplitude of EPSCs from the site of synapse to the action potential initiation site. These specializations, together with a more depolarized interneuron membrane potential [15,22,26], could account for the robust spike transmission observed *in vivo*. However, whether active properties in stratum lucidum interneuron dendrites, as with other interneuron dendrites [27,28], might also contribute to an enhanced postsynaptic sensitivity remains an open question.

NMDA-receptor-dependent control of spike timing and integration in interneurons

Is the time course of the AMPA-receptor-mediated EPSP the sole determinant of synaptic integration at stratum lucidum synapses? At CA1 interneuron synapses, the NMDA-receptor content is vitally important in controlling the integrative properties of interneurons [6]. MF–interneuron synapses that comprise Ca^{2+} -permeable AMPA receptors possess only a small NMDA-receptor component that contributes minimally to the decay of the EPSP [29,30]. In this case, feedforward inhibition onto CA3 pyramidal cells will be shaped largely by the fast AMPA-receptor-mediated EPSCs, causing precise timing of action potentials and fast disynaptic inhibition, yielding a narrow integration window in CA3 pyramidal cells. By contrast, other MF–interneuron synapses feature a prominent NMDA-receptor component and Ca^{2+} -impermeable AMPA receptors [29]. Synapses in which the EPSP decay is shaped substantially by the NMDA component show a much wider window for synaptic integration [6], which translates to a wider temporal window for integration in CA3 pyramidal cells. However, it is unclear whether the two MF-synapse types populate different interneuron types or whether they coexist on the same stratum lucidum interneuron. Experiments in area CA1 suggest a division of labor among interneurons [5,6]. Interneurons participating in fast disynaptic inhibition tend to synapse more proximally on the dendritic tree of

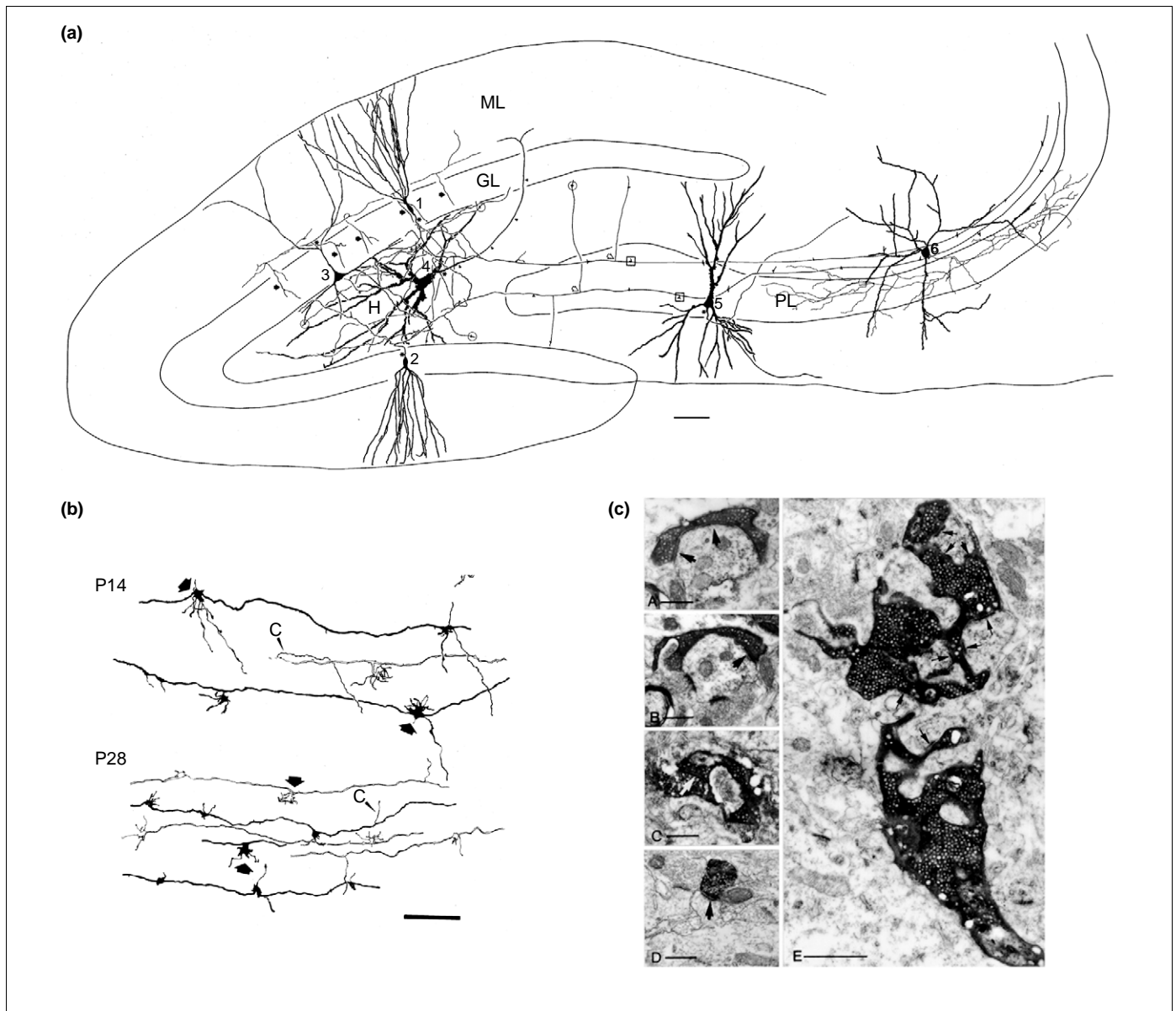


Figure 2. Basic anatomical elements of mossy fiber-CA3 circuitry. (a) The dentate gyrus and proximal portion of the hippocampus, summarizing the major synaptic relationships of mossy fibers (MFs). Granule cells (labeled 1 and 2) give rise to extensive collateral plexuses that are distributed throughout much of the hilus (H) and make synaptic contact with both dentate gyrus basket cells (3) and mossy cells (4). As the parent MF approaches the CA3 pyramidal cell layer (PL), the large presynaptic MF expansions begin to appear and are typically 140–200 μm apart for the entire length of the axon. These expansions form complex synapses on the proximal apical dendrites of pyramidal cells (5). The small filopodial or *en passant* terminals make synapses onto local circuit interneurons primarily located within the stratum lucidum (6). Abbreviations: GL, granular layer; ML, molecular layer. Scale bar, 100 μm . (b) Camera lucida drawings of Golgi-impregnated MFs illustrate that the filopodial extensions emerging from the large complex MF terminal (large arrows). By postnatal-day (P) 14, mature MF expansions are common. At P28 there is an increase in the number of MF expansions that have reached an adult shape and size and the lengths of the individual expansions have decreased to adult levels. C indicates thin collaterals emanating from the main MF axon. Scale bar, 50 μm . (c) Electron micrographs of different terminal types along MFs in the CA3 region (A–C,E) and of a CA3 pyramidal cell terminal (D). All electron micrographs have the same magnification, for comparison of the relative size of the terminals. (A,B) A small *en passant* terminal establishes a single asymmetrical synapse on a dendritic shaft with long perforated postsynaptic density (arrows). (C) A filopodial extension of a mossy terminal forms a synapse (arrow) with a substance-P-receptor-immunoreactive interneuron. (D) The postsynaptic target of a pyramidal cell terminal is a simple spine of a CA1 pyramidal neuron. (E) A large, double-headed mossy terminal forms multiple contacts (arrows) with thorny excrescences of a CA3 pyramidal cell. The individual release sites are short. Scale bars, 0.5 μm (A–D) and 1 μm (E). Panel (a) reproduced, with permission, from Ref. [63], © (1986) Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.; the stratum lucidum inhibitory interneuron (cell 6) was added to the original figure by J.J. Lawrence and C.J. McBain. Panel (b) reproduced, with permission, from Ref. [64], © (1981) Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Panel (c) reproduced, with permission, from Ref. [7], © (1998) the Society for Neuroscience.

CA1 pyramidal cells, whereas interneurons associated with a slower time course for disynaptic inhibition synapse more distally. There is reason to suspect that there might also be a similar division of labor in stratum lucidum interneurons. Many stratum lucidum interneurons form a dense network of inhibitory terminals within stratum pyramidale and stratum lucidum [24], providing the physical basis for extensive lateral inhibition. Stratum

lucidum interneuron activation results in large, fast inhibitory postsynaptic currents (IPSCs) to CA3 pyramidal cells [24]. It is tempting to speculate that this interneuron type is poised for fast disynaptic inhibition following MF excitation of CA3 pyramidal cells. However, there also appear likely to be populations of stratum lucidum interneurons that synapse more distally on CA3 pyramidal cells, as suggested by the more diffuse arborizations of

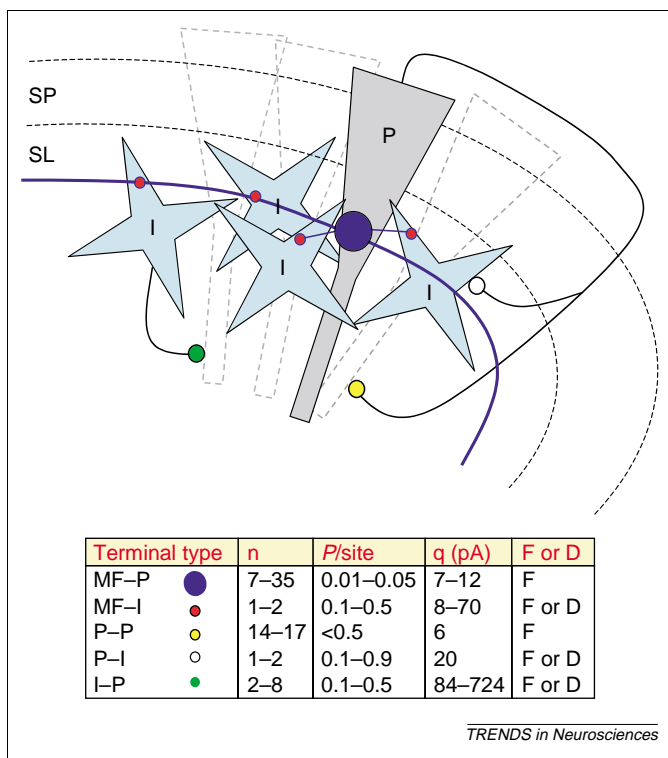


Figure 3. Mossy fiber circuitry in CA3 and table summarizing quantal parameters associated with the CA3 network. Mossy fibers (blue fiber) innervate pyramidal cells via giant boutons (large blue terminal) and innervate interneurons via small terminals either radiating from the giant bouton or from *en passant* synapses (red terminals). Pyramidal cells also form a feedback network with each other (yellow terminal) and with interneurons (white terminal). Finally, stratum lucidum interneurons innervate pyramidal cells (green terminals). Abbreviations: D, depressing; F, facilitating; I, stratum lucidum interneuron; MF, mossy fiber; n, putative number of release sites; P/site, initial release probability per release site; P, pyramidal cell; q, quantal amplitude; SL, stratum lucidum; SP, stratum pyramidale. Parameters were taken or estimated from Refs [7,8,12-15,24,64-68].

some stratum lucidum interneurons [17,22,24,31] and diversity of unitary IPSC kinetics onto CA3 pyramidal cells [32]. Moreover, there is a population of calretinin-positive stratum lucidum interneurons that project to the septum [31] and contact only other interneurons [31,33], suggesting a complicated hierarchy of interactions in CA3 upon MF activation. Finally, there is the possibility that fast and slow synapse types coexist on the same stratum lucidum interneuron [17,21]. For example, MF activation of synapses possessing a high Ca^{2+} -permeable AMPA-receptor and low NMDA-receptor content could create a narrow window for disynaptic inhibition following excitation of CA3 pyramidal cells by MFs. By contrast, activation of synapses possessing slower AMPA receptors and/or a higher NMDA-receptor content would result in a wider window of disynaptic inhibition of CA3 pyramidal cells. Thus, stratum lucidum interneurons could be efficient multi-taskers, varying the length of the window for disynaptic inhibition depending on the glutamate-receptor content of the active synapse.

Differential synaptic plasticity at MF synapses

Although much of the preceding discussion focuses on the nature of point-to-point transmission within the MF-CA3 dialog, it is important not to overlook the striking mechanisms of synaptic short-term and long-term plasticity

that exist within this network and that must undoubtedly be a major determinant of the feedforward mossy fiber excitatory drive.

Long-term potentiation (LTP) at MF-CA3 pyramidal neurons has a presynaptic expression locus [34,35] and involves a long-lasting alteration in the presynaptic release mechanism controlled by the interplay between cAMP-dependent cascades and the proteins Rab3A and Rab3A-interacting molecule α (RIM1 α) [36,37]. Given that the filopodial extensions originate from the large MF terminal, it was originally assumed that a change in presynaptic release probability would distribute evenly to all synapses, resulting in long-term changes at both MF-pyramidal cell and MF-interneuron synapses indiscriminately. In hindsight this was a naïve hypothesis and the reality was far more interesting. At MF-CA3 stratum lucidum interneuron synapses the same high-frequency protocol that induces LTP at principal cell synapses induces two forms of long-term depression (LTD) at interneuron synapses [29,38,39] (Figure 4c). Induction of both forms of LTD requires a postsynaptic elevation of Ca^{2+} , whereas the expression loci are on opposite sides of the synapse. At MF-interneuron synapses bearing Ca^{2+} -permeable AMPA receptors, expression is presynaptic and involves a reduction in neurotransmitter glutamate release [40]. By contrast, at Ca^{2+} -impermeable AMPA-receptor synapses, expression is entirely postsynaptic and involves translocation of AMPA receptors in a manner similar to that seen for LTD at Schaffer collateral-CA1 pyramidal cell synapses [41]. Of particular interest is that whereas MF-pyramidal cell LTP involves cAMP dependent cascades, transmission at either form of MF-interneuron synapse is insensitive to manipulations that elevate cAMP levels [38]. This compartmentalization of the biochemical machinery highlights one potential functional utility of the filopodial versus large terminal arrangement. How the cAMP-dependent cascade is segregated across the inter-terminal compartments is not understood at this time.

Presynaptic forms of plasticity that alter transmitter-release probability often affect synaptic response to brief episodic activity. Following LTP at MF-pyramidal synapses, despite a potentiation of synaptic responses evoked early in response to trains of stimuli, events occurring later in the train are reduced [14,34,42]. This has been referred to as a 'redistribution' of synaptic efficacy [43] and in the hippocampus it appears unique to MF synapses [44]. The opposite is true at MF-interneuron Ca^{2+} -permeable AMPA-receptor synapses following LTD induction. Although the synaptic response amplitude is reduced at low basal frequencies, the degree of facilitation observed in response to short trains of activity is significantly enhanced; moreover, synapses that originally demonstrated short-term depression can be converted to short-term facilitation [14]. By contrast, LTD occurring at MF synapses onto interneurons with Ca^{2+} -impermeable AMPA receptors does not involve changes in either presynaptic release properties or a redistribution of synaptic strength.

What is the advantage of possessing a presynaptic form of plasticity rather than the more 'conventional'

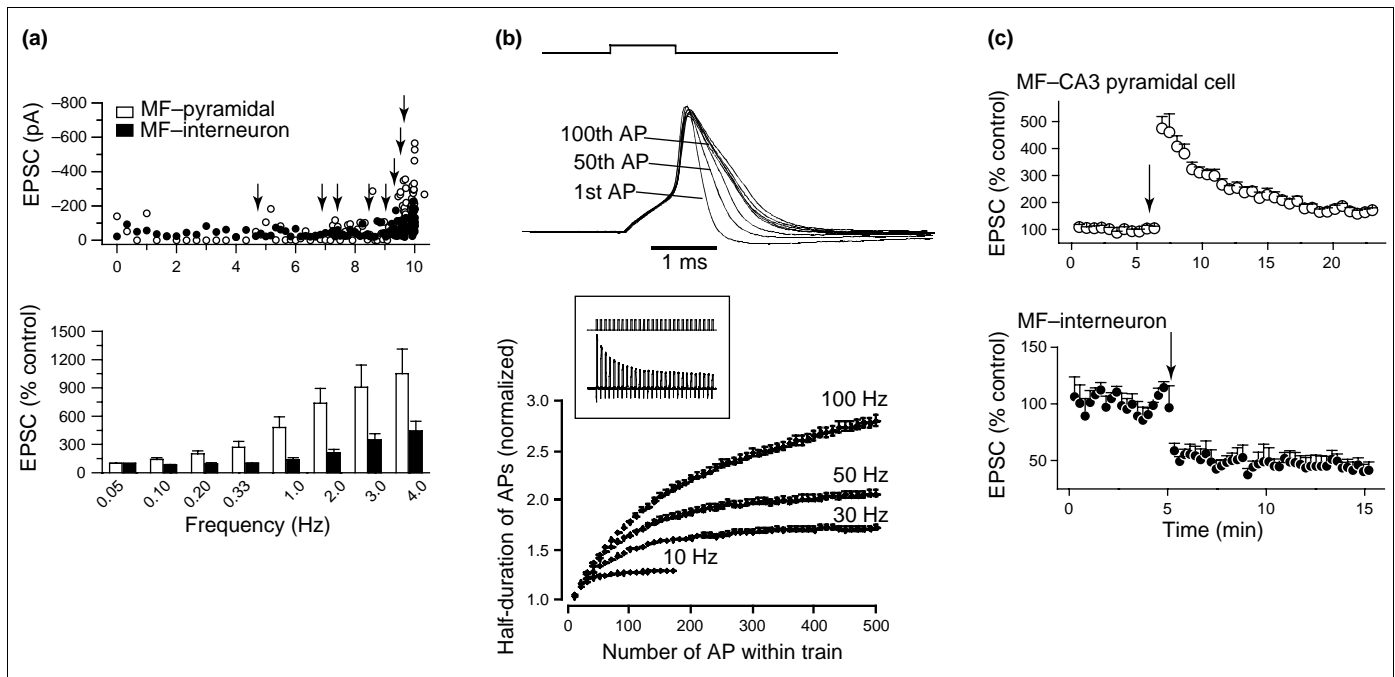


Figure 4. Plasticity at mossy fiber synapses. **(a)** A broader dynamic range of synaptic transmission exists at mossy fiber (MF)–pyramidal neuron synapses than at MF–interneuron synapses. Excitatory postsynaptic currents (EPSCs) were evoked at a starting frequency of 0.05 Hz (indicated by arrows). The stimulation frequency was then increased to 0.10, 0.20, 0.33, 1.0, 2.0, 3.0, and 4.0 Hz. The lower panel shows that at all frequencies tested MF–pyramidal neuron facilitation greatly exceeds that of the MF–interneuron synapse. At 4 Hz, MF–pyramidal EPSC amplitude is > 10 times that observed at 0.05 Hz, compared with only a fourfold increase at MF–interneuron synapses. **(b)** The strong degree of frequency-dependent facilitation observed at MF–pyramidal neuron synapses results in part from a marked spike broadening and voltage-gated K^+ -channel inactivation in single MF boutons. A recording from a presynaptic MF bouton illustrates marked spike broadening in response to a train of depolarizing stimuli delivered at 50 Hz. The upper panel shows every 50th action potential (AP) superimposed on the first action potential in the train. The lower panel illustrates a plot of duration of action potentials at half maximal amplitude in whole-cell recorded MF boutons against the number of action potentials within the train for the frequencies indicated. Inset shows a cumulative inactivation of K^+ channels responsible for the observed spike broadening induced by repetitive activation recorded in a MF bouton. Traces are K^+ currents activated by a series of pulses to +20 mV (holding voltage = –90 mV, 3 ms duration, 7 ms inter-pulse interval). **(c)** Differential long-term synaptic plasticity at MF–pyramidal neurons versus MF–interneuron synapses. Identical high-frequency stimulation (100 Hz; 1 s three times at 10 s intervals, at time indicated by the arrows) induces long-term potentiation at MF–pyramidal neuron synapses but long-term depression at MF–interneuron synapses. Data in (a) and (c) reproduced, with permission, from Ref. [14], © (2000) the Society for Neuroscience. Data in (b) reproduced, with permission, from Ref. [54].

NMDA-dependent postsynaptic forms of plasticity, in which temporal fidelity is maintained following potentiation or depression? The answer to this is not straightforward but could suggest that a balanced interplay between feedforward excitation onto both pyramidal cells and interneurons is required for efficient MF–CA3 dialog. Mechanisms that alter the mean EPSC amplitude with a concomitant change in the variance (presynaptic LTD) will have a profoundly different impact on the circuit from conventional forms of depression, in which changes in mean EPSC amplitude are not accompanied by change in variance (postsynaptic LTD) [45]. Furthermore, it is well established that the nature and strength of inhibitory control of the CA3 pyramidal cell network is crucial for the emergence of excitatory networks and propagation of excitatory activity within the CA3 hippocampus [46,47].

One lingering puzzle has been the apparent lack of LTP at MF–stratum lucidum interneuron synapses. It is not an unreasonable expectation that any synapse equipped with machinery to undergo LTD would have a mechanism to de-depress or potentiate transmission [48,49]. Although LTP has been reliably described at MF synapses onto dentate gyrus inhibitory interneurons and shares many of the properties of cAMP-dependent LTP at MF–pyramidal cell synapses [50], it has not been observed at other MF–interneuron synapses [14,29,38]. Future experiments

will undoubtedly reveal whether these interneuron synapses also show bi-directional plasticity.

Metabotropic-glutamate-receptor-dependent regulation of MF transmission

Metabotropic glutamate receptors (mGluRs) are expressed at numerous presynaptic and postsynaptic locations throughout the mammalian CNS. The roles played by mGluRs are well established at both MF–principal cell and inhibitory interneuron synapses. Within the CA3 hippocampus, group II mGluRs are expressed exclusively on MFs but not on CA3 collaterals [17,38,51–53]. mGluR2 is distributed along the MF axon and is not associated with presynaptic terminals *per se*; consequently, activation of these receptors reduces the probability of transmitter release at synapses onto all targets [12]. By contrast, mGluR7 expression is restricted to the presynaptic grid (the site of synaptic vesicle fusion) of MF terminals onto interneurons but not pyramidal cells. The affinity of glutamate for recombinant mGluR7 is exceptionally low at ~1 mM (c.f. 10 μ M for mGluR2), suggesting that concentrations of glutamate high enough to activate this receptor occur only during periods of intense stimulation. Although unclear at this time, one would expect from this anatomical arrangement that activation of mGluR7 would modulate synaptic transmission at synapses onto

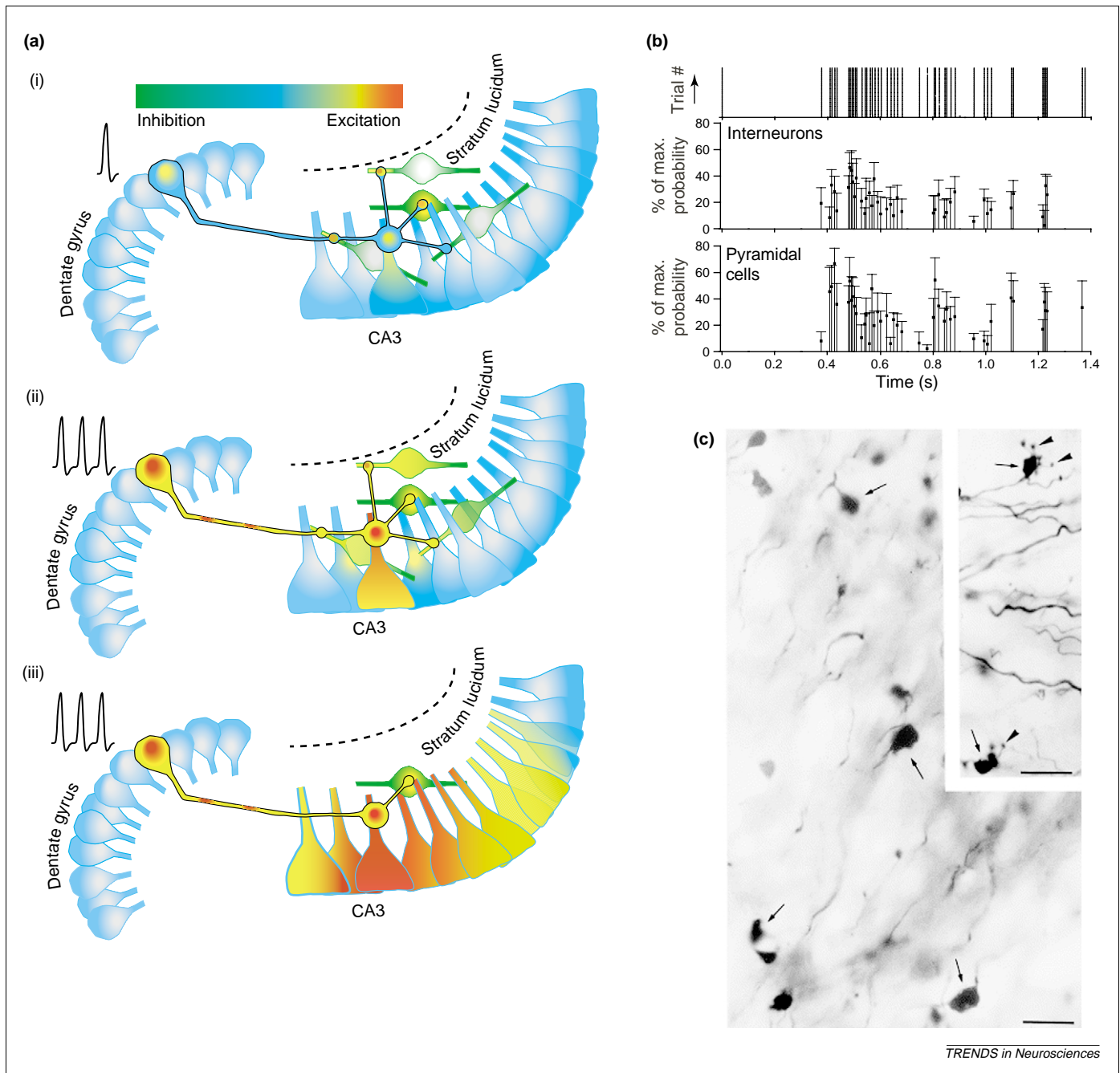


Figure 5. The consequences in the CA3 network of firing of a single dentate granule cell. **(a)** In response to single action potentials at low frequencies (i), transmitter release probability is higher at mossy fiber (MF) synapses onto interneurons than onto pyramidal cells, creating a net inhibition in the CA3 network. In response to a high-frequency train of action potentials (ii), the high degree of short-term facilitation at MF–pyramidal cell synapses causes spike transmission in the pyramidal cell. However, the ‘detonation’ is focal and abbreviated owing to significant spike transmission in interneurons and consequent feedforward inhibitory control (b). (iii) Under pathological conditions in which feedforward inhibition has been compromised, a high-frequency train of action potentials is expected to result in excitation of the pyramidal cell and improper spread of excitation to other CA3 pyramidal cells, owing to recurrent feedback circuitry. **(b)** *In vivo* recordings of spike transmission in CA3 targets during physiological granule cell firing patterns. Shown are a rastergram of 36 trials of granule cell action potentials using a template of a spike train recorded during exploration while the animal traversed the place field of the granule cell (top), and mean percentage of maximum spike transmission probability onto interneurons (middle) or pyramidal cells (lower) as a function of each spike in the granule cell spike train. **(c)** Ischemic insult results in a dramatic decrease in the number of MF terminal (arrows) filopodia (arrowheads) compared with normal animals (inset). Scale bars, 10 μ m. Data in (b) reproduced, with permission, from Ref. [11] © (2002) Nature Publishing Group (<http://www.nature.com/>); data in (c) reproduced, with permission, from Ref. [62].

interneurons but not principal cells. Such a differential regulation of presynaptic release mechanisms might be an adaptive process that minimizes activity through either the inhibitory or the excitatory arm of the network under intense activity, thereby shifting the level of feedforward excitation to a particular target.

The ability to integrate granule cell spiking activity over a broad dynamic range of frequencies sets MF–pyramidal neuron transmission apart from other cortical synapses (Figure 4a). Facilitation of MF transmission occurs at presynaptic stimulation frequencies as low as 0.025 Hz and can reach ~600% of control at 0.33 Hz [13]. This great

degree of frequency-dependent facilitation results primarily from large rises in intra-terminal Ca^{2+} concentration and activation of Ca^{2+} /calmodulin-dependent kinase II [13], cumulative K^+ channel inactivation, and concomitant broadening of the presynaptic action potential [54] (Figure 4b). Unlike MF–pyramidal cell synapses, transmission at MF–interneuron synapses possess only moderate frequency-dependent facilitation [14]. Whether similar mechanisms of K^+ channel inactivation and spike broadening underlie frequency-dependent facilitation at MF–interneuron synapses is at present unknown. Activation of presynaptic mGluR by released glutamate decreases transmission at synapses onto both MF–interneuron and MF–pyramidal cell synapses [14] in a frequency-dependent manner. However, although blocking presynaptic mGluR enhances frequency-dependent transmission at interneuron synapses, they are still unable to match the large dynamic range of MF–pyramidal cell synapses.

Functional implications of the CA3 circuit

How do different firing frequencies of dentate granule cells shape the dynamics of excitation and inhibition in CA3? Coupled with the higher probability of release and higher potency of each release site in driving spike transmission, the extensive interconnectivity of MF synapses should create a lateral inhibition surrounding connected pyramidal cells at low (< 0.5 Hz) *in vivo* discharge rates of dentate granule cells [55]. Taken together, the anatomical and physiological evidence points to a strong feedforward network resulting in a net inhibition in CA3 (Figure 5a,i), a concept underscored by the paradoxical observation that synchronous activation of dentate granule cells *in vivo* during dentate spikes results in a net inhibition of CA3 pyramidal cell firing [56–58]. Thus, the higher release probability of MF–interneuron inputs [12], higher interneuron innervation [7], higher initial probability of interneuron spike firing [11], and great divergence in inhibitory projections [22,24] will tend to favor feedforward inhibition at low firing frequencies of dentate granule cells. However, *in vivo* and *in vitro* experiments suggest that the relative release dynamics will change at higher frequencies of dentate granule cell firing [11,13,14]. When the animal moves into the place field of a dentate granule cell, the cell can fire in short, high-frequency bursts during the in-field discharge [11] (Figure 5b). *In vitro* experiments indicate that repetitive activation of MFs induces short-term depression at roughly half of interneuron synapses but induces massive facilitation at pyramidal cell synapses. Although *in vivo* experiments indicate that repetitive activation of the dentate granule cell increases the probability of spike transmission at both CA3 targets, the much larger frequency-dependent facilitation in the pyramidal cell causes a much higher spike-probability increase in these cells, allowing relief from inhibition, focal discharge of the pyramidal cells, and transient expansion of the sphere of excitation (Figure 5a,ii, b). Thus, the peculiar presynaptic specializations of the MF synapses endow the circuit with a way of shifting the dynamics of excitation and inhibition within CA3 depending on the firing

frequency of the dentate granule cell. It is worthwhile pointing out that, although Figure 5 illustrates this for a single MF complex and its associated filopodia, how the dynamics of excitation and inhibition are coordinated at each and every MF terminal along a the axon remains largely unexplored.

Implications for pathological states

In experimental models of stroke and ischemia, there is evidence that this powerful feedforward network in CA3 is severely compromised. Spiny stratum lucidum interneurons that are immunopositive for calretinin are among the most vulnerable in diverse types of insults [59–61]. In experimental models of stroke, stratum lucidum interneurons and hilar neurons of the dentate gyrus are the first to die [59–61], cell death occurring within 12–24 h. Because the death of CA1 pyramidal neurons follows, stratum lucidum interneurons could participate in the cascade leading to hippocampal cell death at later stages [60]. A similar vulnerability of stratum lucidum interneurons has been described in experimental models of epilepsy [61]. The death of this population is suggested to cause the extensive remodeling of MF axons within CA3, in which a large proportion of MF filopodial extensions are permanently reduced, while excitatory connections to CA3 pyramidal cells remain largely intact [62] (Figure 5c). This long-term synaptic reorganization in CA3 suggests a profound shift from feedforward inhibition towards direct excitation, possibly leading to an excessive recruitment of CA3 pyramidal cells by dentate granule cells and predisposition to epileptiform activity (Figure 5a,iii). Any pathological increase in excitability in the dentate propagating to CA3 will be amplified by the diminished network of inhibition, persistence of frequency-dependent facilitation at MF–CA3 pyramidal cell synapses, and recurrent excitation in CA3. Under these conditions, the detonator will be unleashed, causing a juggernaut of excitation in CA3.

‘... supposing these people had endeavoured to kill me ... I should certainly have awaked with the first sense of smart, which might so far have roused my rage and strength, as to have enabled me to break the strings wherewith I was tied; after which, as they were not able to make resistance, so they could expect no mercy.’ [Jonathan Swift, *Gulliver’s Travels*]

Acknowledgements

We thank everyone whose data contributed to this review. In addition we thank all of the authors of the articles in the Interneuron Diversity series for their valuable time and for providing such high quality reviews. We are especially grateful to Sian Lewis and the staff of TINS for indulging our suggestion for the review series and for their endless support in managing all of the invited articles.

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