Presynaptic Frequency Filtering in the Gamma Frequency Band; Dual Intracellular Recordings in Slices of Adult Rat and Cat Neocortex

Using dual intracellular recordings in slices of adult rat and cat neocortex, the frequency-filtering characteristics of ‘depressing’ synapses made by pyramidal axons at interspike intervals between 5 and 50 ms were studied. At ‘depressing’ connections from excitatory cells to some inhibitory interneurons (n = 6), recovery from short interspike interval depression was near exponential. Extrapolation of exponentials fitted to this recovery demonstrated a residual 10–20% depression at intervals >50 ms. This slowly decaying component was larger for later excitative postsynaptic potentials (EPSPs) in trains which were typically more strongly depressed. At >80% of connections between spiny excitatory cells and at pyramid to parvalbumin-immunopositive interneuron connections, however, recovery exhibited a more complex time course. A narrow ‘notch’ (half-width 5 ms), peaking at intervals of 13–25 ms during which the EPSP was depressed further, interrupted recovery from short interval depression. This ‘notch’ was equally apparent for all EPSPs in brief trains and was mediated presynaptically.

Introduction

That local circuit activation in the neocortex is dramatically influenced by the frequency of afferent input is indicated by a paradoxical reduction in the lateral spread of activity, presumably via the axon collaterals of pyramidal and spiny stellate cells, when the frequency of stimulation is increased into the gamma range (≤25 Hz) (Contreras and Llinas, 2001). Although the synchronous firing of specific neuronal arrays time-locked to these oscillations may carry important information (Singer, 1999, 2001), the indiscriminate recruitment of many thousands of neurons into high-frequency oscillations would not only obliter-ate any information carried by this synchrony, but would cause excessive and potentially dangerous activation within the cortex. Mechanisms that suppress such indiscriminate recruit-ment are therefore likely to be of some importance.

Previous studies have demonstrated three presynaptic mechanisms that reduce the probability of transmitter release, or the number of available release sites during brief trains and was mediated presynaptically.

Materials and Methods

Dual/triple intracellular recordings (sharp microelectrodes filled with 2 M KMeSO4 and 2% biocytin, resistance 100–150 MΩ) were made from synaptically connected neurons in slices of adult rat (somatosensory, motor and visual) and cat (visual) neocortex. Methods for histological and immunofluorescent processing of biocytin-labelled cells are described elsewhere (Hughes et al., 2002). Male Sprague–Dawley rats (100–200 g) were anaesthetized with inhaled Fluothane and i.p. pentobarbitone sodium (60 mg/kg). Male cats (2.5–3.4 kg) were anaesthetized i.v. with a mixture of α-chloralose (70 mg/kg) and pentobarbitone sodium (6 mg/kg) for a different series of acute experiments (Wang and Ramage, 2001). Rats were perfused transcardially and cats via the carotid arteries (after an overdose of barbiturate), with ice-cold modified artificial cerebrospinal fluid (ACSF) with added pentobarbitone (60 mg/l) in which 248 mM sucrose replaced NaCl. Rats were decapitated and the brain removed. A block of brain including the visual cortex was removed from cats after a craniotomy. Slices of neocortex, 450–500 μm thick were cut (Vibrastat, Camden Instruments, UK) and transferred to an interface recording chamber where they were maintained for 1 h in sucrose-containing medium, before switching to standard ACSF containing (in mM) 124 NaCl, 25.5 NaHCO3, 3.3 KCl, 1.2 KH2PO4, 1.0 MgSO4, 2.5 CaCl2, 15 d-glucose equilibrated with 95% O2/5% CO2 at 35–36°C. All procedures were in compliance with UK Home Office Regulations for animal use.

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Data Synaptic neurons were depolarized with combinations of square-wave and ramped currents delivered once every 3 s to elicit square-wave and ramped APs with different patterns and at different frequencies; postsynaptic responses were recorded. Data were digitized (5–10 kHz, voltage resolution 0.005–0.01 mV) and analyzed off-line (Spike 2 data collection and in-house analysis software).

During off-line analysis, paired recordings in which the first EPSP shape and amplitude and the postsynaptic membrane potential were stable over time and which spanned a suitable range of brief interspike intervals (<10 to >50 ms) were selected. Each single sweep was checked by hand to ensure that every presynaptic AP was recognized by the software and that the trigger points used for subsequent analysis were accurately aligned with the rising phase of each AP in each sweep. Sweeps including artefacts or large spontaneous events were excluded from averaged records.

To obtain measurements of averaged second EPSP amplitudes, subsets of sweeps were then selected on the basis of the interspike interval that preceded the second AP. Each subset included sweeps in which the second EPSP followed a given interspike interval. The width of the selection window was typically narrower for short interspike intervals (0.1–0.5 ms) than for broader intervals (up to 2–5 ms for the longest interspike intervals studied). The number of sweeps included in the average was partially determined by the coefficient of variation (CV) of the EPSP. Where this was high (>0.3), a larger number (20–50 sweeps) was required to provide an adequate estimate of average EPSP amplitude at that interval than when CV was low (<0.3). The second EPSPs occurring at each interspike interval were then averaged, using the rising phase of each second AP as the trigger. The averaged first EPSP for each of these data subsets (triggered from the rising phase of the first AP) was also computed to determine whether an adequate number of sweeps had been included in that subset. Only where the average of the first EPSP in this subset matched the average of a much larger sample of first, or single-spike, EPSPs in both shape and amplitude was that data subset used for measurements of averaged EPSP amplitude.

The averaged second EPSP was then superimposed on an average of all EPSPs elicited by single APs in that data set. The amplitude of the averaged second EPSP was measured from its peak to the appropriate point on the falling phase of the averaged single-spike EPSP (see Fig. 1). These averaged EPSP amplitudes were then plotted against the interval between the first and second spike. Single exponential curves were fit using PSI-Plot. Averaged EPSPs elicited by later APs in trains were analyzed similarly.

The averaged responses to trains of spikes illustrated here are composites of averaged EPSPs triggered by the first, second, third, etc., APs in trains. Since spike trains were elicited with a range of current pulses, a range of presynaptic firing patterns was often included in each paired recording. These varied from the adapting pattern typical of pyramidal cells activated with square wave pulses, to spike trains in which all intervals were of similar duration (typically those activated by combinations of square wave and ramped pulses). Separate composite averages were generated for each of these patterns and the average amplitude of the third, fourth, . . . , eighth EPSPs measured for each composite average. These amplitudes could then be plotted against the interspike interval that immediately preceded that EPSP (e.g. the interval between the third and the fourth AP) and against the interval between the first AP and the AP that triggered that EPSP in the train (see Fig. 2).

For recordings in which postsynaptic responses exhibited an adequate signal-to-noise ratio, single sweep events were measured by hand (with cursors, n = 8 paired recordings). For each paired recording, responses to single spikes were first averaged. This average was scaled to match the amplitude of the first EPSP in each single sweep and superimposed. The second (third, fourth, etc.) EPSPs were then measured from their peak to the appropriate point on the falling phase of the scaled, averaged first (second, third) EPSP. These points were plotted against the interspike interval that immediately preceded that EPSP. Running averages (including 3–10 points on either side of each point, the number again determined by the number of points within that interval range and the CV of the EPSP) were performed to reveal the trends within the normal spontaneous sweep-to-sweep fluctuations in EPSP amplitude.

**Figure 1.** Recovery from paired pulse depression in a pyramid-to-pyramid (MP –70 mV, A,B) and a pyramid-to-interneuron connection (C) in rat neocortex. Measurements of second EPSP amplitudes were obtained from averaged second EPSPs superimposed on averaged single-spike EPSPs (as shown by open arrows in insert C). (A) Averaged second EPSP amplitude plotted against interspike interval for the layer 4 pyramid to layer 3 pyramid connection in rat neocortex. (B) Spike pairs in the presynaptic pyramid and the (averaged) EPSPs they activated in the postsynaptic pyramid for some of these intervals. (C) A similar plot for a pyramid-to-interneuron pair. The insert shows averaged postsynaptic responses to single APs in trains. Since spike trains were elicited with a range of current pulses, a range of presynaptic firing patterns was often included in each paired recording. These varied from the adapting pattern typical of pyramidal cells activated with square wave pulses, to spike trains in which all intervals were of similar duration (typically those activated by combinations of square wave and ramped pulses). Separate composite averages were generated for each of these patterns and the average amplitude of the third, fourth, . . . , eighth EPSPs measured for each composite average. These amplitudes could then be plotted against the interspike interval that immediately preceded that EPSP (e.g. the interval between the third and the fourth AP) and against the interval between the first AP and the AP that triggered that EPSP in the train (see Fig. 2).

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Morphological Identification of Recorded Neurons
All recorded cells were filled with biocytin and processed histologically. All reported neurons were identified as pyramidal cells, spiny stellate cells or as aspiny (or sparsely spiny) inhibitory interneurons. Details of the processing procedures and the morphology of these neurons can be found elsewhere (Thomson et al., 2002).

Results
Paired and triple intracellular recordings were made in 23 experiments in rat and 8 in cat neocortex. Additional data from these experiments describing the morphology of the neurons, connectivity ratios, and the amplitudes and time courses of these synaptic events can be found elsewhere (Thomson et al., 2002).

The Early Phase of Recovery from Paired-pulse Depression
In 27 pairs of synaptically connected spiny excitatory cells (pyramidal and spiny stellate cells) in rat and 15 in cat, responses to pairs of presynaptic spikes at several different interspike intervals were studied. Paired-pulse depression was apparent at the shortest interspike intervals studied under our control conditions (Fig. 1A), even at those connections in which modest facilitation was apparent at longer intervals (e.g. Fig. 3). The second EPSP amplitude at an interspike interval of 5–6 ms was equivalent to 69.2 ± 16.1% (mean ± SD, n = 11) of the average first EPSP amplitude in rat and 60.4 ± 13.2% (n = 7) in cat.

In an additional 17 ‘depressing’ connections, the postsynaptic neuron was an inhibitory interneuron (10 pairs in rat and 7 in cat). For these pairs, the second EPSP amplitude at an interspike interval of 5–6 ms was equivalent to 55 ± 18.2% (n = 5) of the average first EPSP amplitude in rat and 72.4 ± 4.8% (n = 5) in cat (Fig. 1B).

A Simple Time Course for Recovery from Paired-pulse Depression at Some Connections
In 9 of the 28 ‘depressing’ connections studied in more detail, the early phase of recovery from paired-pulse depression appeared adequately described by a single exponential. In six of these nine pairs the postsynaptic neuron was an inhibitory interneuron (three in rat, three in cat, all parvalbumin-immunonegative, or not successfully tested with immunofluorescence, Fig. 1B). The remaining three pairs included two pyramid-to-pyramid (one in rat, Fig. 1A) and one spiny stellate-to-pyramid connection (in cat). Time constants for recovery from short interval paired-pulse depression ranged from 3.1 to 27.7 ms for these data sets (12.4 ± 8.1 ms, mean ± SD) and did not differ significantly between spiny excitatory and inhibitory neurons, or between rat and cat with this sample.

A contribution to the recovery from an additional, more slowly decaying component, which accounted for some 10–20% depression (relative to average first EPSP amplitude) at intervals >50 ms, was indicated by extrapolation of this simple curve (Fig. 1) in all nine connections. Facilitation was only rarely apparent at any interspike interval studied in these nine connections under our control conditions, and the third, fourth, . . ., seventh EPSPs in trains were generally more strongly depressed than second EPSPs at similar interspike intervals (Fig. 2). These later EPSPs exhibited a slower recovery at short interspike intervals and/or a larger contribution from the more slowly decaying component. With this increasingly more powerful and more slowly recovering depression as the spike train proceeds, EPSP amplitude continues to be powerfully depressed at lower frequencies than were required to produce the same degree of depression at the start of the train.

A More Complex Time Course for Recovery from Paired-pulse Depression
In 19 of the 28 ‘depressing’ connections studied in detail a simple exponential decay could not adequately describe the recovery from short interval depression (Figs 3–5). These connections included the majority (83%) of connections between spiny excitatory cells (12:14 pyramid to pyramid, 2:3 spiny stellate to pyramid and 1:1 spiny stellate to spiny stellate) (Figs 3 and 4) and all four pyramid-to-parvalbumin-immunopositive interneuron connections studied (one in cat, Fig. 5). At these 19 connections, the recovery from paired-pulse depression at short interspike intervals was interrupted by a ‘notch’ during which the second EPSP again decreased in average amplitude before recovering again. The maximum EPSP depression within this ‘notch’ was most commonly seen between 13 and 25 ms (n = 16) after the first EPSP (mean 20.3 ± 7.6 ms, range 11–40 ms, n = 19). The amplitude of the ‘notch’ was estimated by subtracting the
average EPSP amplitude at the peak of the notch from the amplitude predicted for that time bin from the single exponential fit to the points before and after the 'notch' (Fig. 3). This gave an amplitude for the 'notch' of between 10 and 50% of the first EPSP average amplitude (mean 23.3 ± 11.8%). The 'notch' was sharply tuned, with a mean width at half amplitude of 5.5 ± 2.6 ms (range 2–11 ms). In 15 of these 19 connections the third, fourth, and fifth EPSPs (Table 1, Figs 3A, B, 5B), and in one connection the fifth–seventh EPSPs in trains were also analysed (Fig. 5A). Although these later EPSPs in trains were typically more strongly depressed than second EPSPs and their recovery from depression was slower, they nevertheless exhibited a similar ‘notch’ which therefore represents a true frequency filter. Typically, as can be seen in Table 1, the latencies of occurrence increased and the ‘notch’ became broader as the spike train progressed (Fig. 5B).

**Table 1**

<table>
<thead>
<tr>
<th>EPSP in train</th>
<th>Interspike interval at the peak of the notch (ms)</th>
<th>notch width at half amplitude (ms)</th>
<th>notch amplitude as a percentage of average first EPSP</th>
</tr>
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<tbody>
<tr>
<td>Second EPSP (n = 19)</td>
<td>20.3 ± 7.6</td>
<td>5.5 ± 2.6</td>
<td>23.3 ± 11.8</td>
</tr>
<tr>
<td>Third EPSP (n = 15)</td>
<td>21.8 ± 6.2</td>
<td>8.2 ± 2.9</td>
<td>19.1 ± 6.1</td>
</tr>
<tr>
<td>Fourth EPSP (n = 6)</td>
<td>24.9 ± 5.9</td>
<td>8.1 ± 1.9</td>
<td>23.9 ± 8.9</td>
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**Non-linear Summation Does Not Appear to Contribute to the ‘Notch’**

To determine whether non-linear summation of postsynaptic events might have contributed to the expression of the ‘notch’, the following properties were compared for connections that displayed a ‘notch’ and those that did not. No significant difference was found in the postsynaptic membrane potential (~71.7 ± 4.9 versus ~67.5 ± 9.7 mV), the first EPSP average amplitude (2.1 ± 1.9 versus 2.7 ± 1.2 mV), 10–90% rise time (2.3 ± 1.1 versus 1.44 ± 0.7 ms), or the width at half amplitude (17.1 ± 11.3 versus 11.6 ± 6.2 ms) for those with and those without a ‘notch’. Nor was a correlation found between any of these parameters and the amplitude of the ‘notch’ or its width at half amplitude. In addition, the expression of the notch was not altered by changing postsynaptic membrane potential (n = 2), which at pyramid-to-pyramid connections dramatically alters EPSP time course and non-linear summation at short interspike intervals. These data suggest that non-linear summation did not contribute significantly to the expression of the ‘notch’.

**Effect of First EPSP Amplitude on the ‘Notch’**

The effect of first EPSP amplitude on the ‘notch’ was examined in six pairs by comparing data subsets selected on the amplitude of the first EPSP in the train (Fig. 4). In one pair the effect of second and third EPSP amplitude on the ‘notch’ expressed during third and fourth EPSP recovery was also studied (Fig. 5B, insert). The ‘notch’ was equally apparent in all these data subsets, suggesting that its expression is not dependent on the amplitude of the preceding EPSP. Its precise timing could vary between these data subsets, suggesting that the amplitude of the first EPSP, or the factors that determine first EPSP amplitude may influence its rate of onset.

It was not possible to determine with certainty whether the ‘notch’ was absolutely dependent upon a release of transmitter occurring in response to the first AP. In none of the pairs in
which single-sweep EPSP amplitudes could be measured accurately were there enough total failures of transmission following the first AP at all the necessary interspike intervals to allow a full time course for recovery from depression to be plotted. It is only possible to state that data subsets that included both first spike failures and very small first EPSPs (<0.3 mV) exhibited a notch when the second EPSP amplitude for these sweeps was plotted against interspike interval. In two pairs, trains of EPSPs that followed brief and/or low-frequency spike trains were compared with those that followed longer, higher-frequency trains, i.e. those that exhibited post-tetanic potentiation. The appearance of the ‘notch’ was not affected by this potentiation.

All 19 connections displaying the ‘notch’ exhibited paired-pulse depression at the shortest intervals studied (<10 ms) under control conditions. In eight (including three of the pyramid-inhibitory interneuron connections), the second EPSP remained depressed relative to the first EPSP at all intervals studied (Fig. 3C) and for all EPSPs in trains (Fig. 5A). In four connections the second EPSP remained depressed until after the ‘notch’ when it became modestly facilitated (by 5–20% versus average first EPSP amplitude). Later EPSPs in trains were generally depressed at all intervals studied, however. While in the remaining seven connections (one pyramid-to-inhibitory interneuron connection), the earliest depression gave way to modest facilitation (5–20%) before the ‘notch’. The EPSP was then depressed during the ‘notch’, but facilitated again after. In two of these seven connections all EPSPs in brief trains exhibited a similar profile,
A Presynaptic Locus for the ‘Notch Filter’

To determine whether the mechanism underlying the notch was of pre- or of post-synaptic origin two tests were performed. Where sweep-to-sweep fluctuations in the second (third or fourth) EPSP amplitude included total apparent failures of transmission, a higher proportion of failures was found to occur during the ‘notch’ as well at the briefest intervals (corresponding to the earliest phase of depression), than at intermediate, or longer interspike intervals ($n \geq 3$ pairs, Fig. 6). This indicates a presynaptic locus for the expression both of the ‘notch’ and of the earliest phase of paired pulse depression. In addition, when normalized $CV^{-2}$ (coefficient of variation $^2 = npq/(1 – p)$ for a binomial distribution) (Clements, 1990; Faber and Korn, 1991) was plotted against normalized $M$ (mean EPSP amplitude = npq), the slope was equal to, or, more typically exceeded 1. This was the case when second EPSPs elicited at ‘notch’ frequencies were compared with second EPSPs elicited at shorter and at longer intervals (six pairs, 12 tests, Fig. 6). This indicates that a decrease in release probability ($p$) underlies the expression of the ‘notch’.

Discussion

The frequency-dependent properties of neocortical ‘depressing’ synapses over a range of relatively high frequencies (30–$\sim$80 Hz) corresponding to the gamma frequency band were examined using dual intracellular recordings in slices of adult rat and cat neocortex. Previous observations, that the majority of connections between spiny excitatory cells and those from pyramidal cells to parvalbumin immunopositive and to some other inhibitory interneurons exhibit paired-pulse and brief-train depression, while a separate group(s) of inhibitory interneurons receive strongly facilitating EPSPs (unpublished data), were confirmed. By measuring the EPSPs elicited by spike pairs and trains of spikes at a range of interspike intervals, however, it was possible to describe the time course of recovery from short interval depression more accurately and to demonstrate a complexity not apparent in previous studies. One of the more important outcomes of the present study is perhaps the demonstration of a need for more complete descriptions of the frequency-dependent properties of synapses if the functional significance of these properties is to be predicted. Any estimate of paired-pulse ratios, for example, should be taken as appropriate only for the interspike intervals studied and cannot, without further information, be used to predict synaptic efficacy at any other interval.

Averaging of Data

Most of the data presented here are averages obtained either by averaging single-sweep responses digitally, or calculated from measurements of single-sweep EPSP amplitudes. In many pairs it was possible to see the patterns described here by observing sequential original sweeps, but the large spontaneous sweep-to-sweep fluctuations in amplitude, particularly with EPSPs in inhibitory interneurons, often obscured the trends when only a few sweeps were observed. Visualization of the ‘notch’ and of some of the other components of depression and facilitation often required data to be averaged. This does not, however, indicate an insignificant role for these mechanisms. In vivo, several presynaptic neurons will be activated near simultaneously by afferent input and/or in association with, for example, gamma oscillations. The postsynaptic neuron will ‘average’ these several inputs and be most effectively excited by coincident inputs that follow appropriate interspike intervals.

Brief Time Constants for Recovery from Short Interval Depression

When a range of short interspike intervals were used to activate EPSPs, time constants for recovery from short interval paired-pulse depression were found to be one–two orders of magnitude lower than previous reports (Markram et al., 1998b). One explanation for this difference might be that the underlying mechanisms are slower to decay in immature brain. The other possibility is that many previous studies utilized a smaller range
of rather longer interspike intervals and investigated therefore the more slowly decaying components of depression (equivalent to the residual depression apparent at 50–100 ms in the present study).

Caution should be exercised in attempting to put specific values on single mechanisms within a complex system, unless they can be isolated experimentally. This is almost impossible to achieve with synaptic transmission since many presynaptic mechanisms are affected by the same conditions, such as \([\text{Ca}^{2+}]_i\). To put an absolute value on the recovery from paired-pulse depression is problematic. All connections studied appropriately to date have exhibited facilitation to some degree. This is often masked by the relatively high probability of release at ‘depressing’ connections, but can be revealed when only those second EPSPs that follow failures of transmission in response to the first AP are measured (Thomson and Bannister, 1999). This facilitation is sufficient to confound assessment of the precise time course of recovery from paired-pulse depression (Markram et al. 1998a,b, Tsodyks et al., 1998). In addition, there are several mechanisms that can contribute to depression (Thomson, 2000). The range of time constants reported here (3.1–27.7 ms) for recovery from short interval depression may therefore reflect differential expression of these various mechanisms.

**Complexity in the Time Course for Recovery from Paired-pulse Depression**

Some of the complexity described here has been predicted by modelling studies (Markram et al. 1998a; Tsodyks et al., 1998). The present observation that very short interval paired-pulse depression can give way to modest facilitation at slightly longer interspike intervals (at some pyramid-to-pyramid and a few pyramid-to-inhibitory interneuron connections) is predicted by phenomenological models that include both facilitating and depressing components, provided that appropriate time constants for decay/recovery are utilized. The main difference between the previous model and the present experiment is the faster time course of events in the current work. What such models do not predict, however, is the ‘notch’ as described here. Attempts were made to fit the present data with curves that combined one or two exponentially decaying facilitating and two or three depressing components, but they gave poor correlations when all points were included and failed to fit the ‘notch’ in the data. Careful selection of variables in these models could generate a ‘notch’ that interrupted the recovery from short interval depression, or one that interrupted a phase of facilitation following brief interval depression, but this ‘notch’ either occurred at a much shorter interspike interval than in the data (e.g. 5 ms), or was more broadly tuned (half width >20 ms) (also A. Destexhe, personal communication). This suggests that a combination of previously described mechanisms cannot entirely account for the complex recoveries observed here, unless a previously unreported delay to the onset of one or more of the components is introduced into the model.

To explain the ‘notch’, therefore, a presynaptic mechanism is required that has a delayed onset — some 12–15 ms after a presynaptic action potential — and decays rapidly thereafter, with little desensitization or inactivation, since all EPSPs in brief high-frequency trains exhibited a similar ‘notch’. None of the manipulations tested removed the ‘notch’, so at this stage we can only exclude some possible mechanisms, such as non-linearities in postsynaptic responsiveness, or mechanisms that are strongly dependent upon the release that occurred in response to the preceding AP, such as release site refractoriness or presynaptic autoreceptor activation.

**High-frequency Tuning in the Neocortical Circuit**

The complexity of the time course of recovery from presynaptic depression described here complements the inherent firing characteristics of the presynaptic pyramidal cells. At the high instantaneous firing frequencies (>100 Hz), achieved during pyramidal spike-pair, or burst firing, paired-pulse depression can be powerful, but summation of successive EPSPs can maintain or increase the depolarization achieved by the first EPSP. This is particularly effective in pyramidal cells and some regular-spiking inhibitory interneurons in which EPSPs are broader than in fast-spiking inhibitory interneurons. At slightly lower frequencies (70–100 Hz) summation is reduced, but recovery from early depression and the modest facilitation seen here at some connections can compensate. Even modest facilitation may be significant since at these intervals the spike accommodation and the prolonged spike afterhyperpolarization (AHP) in regular spiking cells increase postsynaptic spike threshold. A further small decrease in frequency brings the majority of local circuit excitatory inputs onto pyramidal cells into the ‘notch’ filter range (40–70 Hz). Temporal summation is further reduced at these intervals, while in regular- and burst-firing postsynaptic cells, spike accommodation and the AHP persist, so that even a relatively weak ‘notch’ could reduce recruitment at these frequencies. At longer intervals, recovery from all forms of depression is more advanced and, with the concomitant decline in spike accommodation and in the spike AHP, pyramidal cells are again more excitable. The gradual increase in the onset and duration of the notch with later EPSPs in the train matches the adapting firing patterns typical of pyramidal cells.

Fast-spiking inhibitory interneurons generate much briefer EPSPs than pyramidal cells and temporal summation is only significant at extremely brief interspike intervals. Since at these intervals paired-pulse depression is profound, these interneurons rarely fire in response to a second, very short interval EPSP. If they fire at all in response to the beginning of a presynaptic spike train, therefore, the timing of that AP is extremely accurate, in striking contrast to the responses of pyramidal cells and regular-spiking interneurons. If the interneuron fires in response to the first EPSP, the deep-spike AHP typical of these neurons will preclude activation by a second very short interval AP. The depth and rapid onset of these AHPs prevents significant spike accommodation, however, and their brief duration allows the interneuron to be readily excitable again within 7–15 ms. At intervals >7–15 ms therefore, whether the interneuron can be repetitively activated by a pyramidal spike train at a given frequency will depend on the degree of EPSP depression and the rate of recovery. This depression is typically greater and its recovery slower for later EPSPs in trains, matching the frequency adaptation typical of pyramidal cell discharge and a relatively stable (depressed) EPSP amplitude is often achieved early in an adapting train before the pyramidal cell achieves a stable firing rate. The ‘notch’ adds additional detail to these responses and during a single adapting spike train the profound depression seen at the shortest intervals and during the ‘notch’ can sometimes be seen to alternate with less profound depression, or with modest facilitation at intermediate and longer intervals.

**Concluding Remarks**

A powerful ‘notch’ dramatically reduces efficacy in a narrow frequency band, selectively reducing efficacy of EPSPs at those intervals that fall within it, without affecting those that fall at shorter, or longer, intervals. The expression of the ‘notch’ at connections between excitatory cells and at specific pyramid-to-
inhibitory interneuron connections further supports the suggestion that very high-frequency oscillations are a property of fast-spiking interneuronal circuits (Traub et al., 1998) and that the frequency is independent of local excitatory drive. Given the density of local and long-range connections between pyramidal (and spiny stellate) cells and h, as proposed, synchronous firing of neuronal arrays time-locked to specific cycles of the gamma rhythm underlies recognition of salient features of a stimulus (Singer, 1999, 2001), suppression of indiscriminate synchrony within this population would be an essential feature of the circuit.

Notes
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