GABA Application to Hippocampal CA3 or CA1 Stratum Lacunosum-Moleculare Excites an Interneuron Network

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Perkins, Katherine L. GABA application to hippocampal CA3 or CA1 stratum lacunosum-moleculare excites an interneuron network. J Neurophysiol 87: 1404–1414, 2002; 10.1152/jn.00430.2001. Whole cell voltage-clamp recording and focal application of the neurotransmitter γ-aminobutyric acid (GABA) were used to investigate the ability of exogenous GABA applied to different locations within the guinea pig hippocampal slice to trigger a giant GABA-mediated postsynaptic current (GPSC) in pyramidal cells. A GPSC reflects the synchronous release of GABA from a group of interneurons. Recordings were done in the presence of 4-aminopyridine (4-AP) and blockers of ionotropic glutamatergic synaptic transmission. Spontaneous GPSCs occurred rhythmically in pyramidal cells under these conditions. Brief focal pressure application of GABA (500 μM; 30–200 ms) to CA3 stratum lacunosum-moleculare (SLM) or to the border between CA3 s. radiatum (SR) and SLM triggered an “all-or-none” GPSC in CA3 and CA1 pyramidal cells that looked like the spontaneous GPSCs. During the refractory period following a spontaneous GPSC, application of GABA could not trigger a GPSC. Both spontaneous GPSCs and GPSCs triggered by exogenous GABA were blocked by suppressing synaptic transmission with high Mg2+/low Ca2+ bath solution. On the other hand, focal application of GABA to CA3 s. oriens (SO) or to proximal SR did not trigger a GPSC in the CA3 pyramidal cell; instead it produced a graded response. Focal application of GABA to regions other than CA3 cells was also tested. Focal application of GABA to CA1 SLM always triggered a GPSC in the CA3 pyramidal cell. Focal application of GABA within the outer two-thirds of the dentate molecular layer often elicited a GPSC in the CA3 pyramidal cell. In contrast, focal application of GABA to CA1 SO, to CA1 SR, or to the hilus elicited no current response in the CA3 pyramidal cell. These data indicate that the GABA recorded in pyramidal cells that was triggered by focal GABA application resulted from the synchronous synaptic release of GABA from activated interneurons rather than from the binding of exogenous GABA to receptors on the pyramidal cell. Furthermore, the “all-or-none” nature of the response to SLM GABA applications of different durations indicates that the exogenous GABA was exciting (directly or indirectly) some members of a network of interneurons, which in turn recruited the rest of the network, rather than individually activating each interneuron that contributed to the GPSC. Interestingly, the effective sites of GABA application—CA3 SLM, CA1 SLM, and the outer two-thirds of the dentate molecular layer—are also the sites which receive direct innervation from the entorhinal cortex in an intact animal.

INTRODUCTION

Experimental work and simulation studies have shown that interneuron networks linked by synaptic connections and/or gap junctions are capable of generating several types of synchronous oscillatory activities in the brain (Galarreta and Hestrin 1999; Gibson et al. 1999; Michelson and Wong 1991, 1994; Skinner et al. 1999; Tamás et al. 2000; Traub 1995; Traub et al. 2001; Wang and Buzsáki 1996; White et al. 2000; Whittington et al. 1995; Zhang et al. 1998). Interneuron network-mediated oscillatory activity has been hypothesized to provide a rhythm which allows precise temporal coding, and ”super networks” of interneurons have been hypothesized to link different regions of the brain by providing a pattern against which other activity takes place (Buzsáki and Chrobak 1995).

One type of interneuron-generated activity occurs in the presence of 4-aminopyridine (4-AP) and blockers of ionotropic glutamatergic synaptic transmission in neocortical and hippocampal slices from adult guinea pigs and adult rats (Aram et al. 1991; Benardo 1997; Michelson and Wong 1991, 1994; Müller and Missfeld 1990; Perreault and Avoli 1992). In this condition, spontaneous giant GABA-mediated inhibitory postsynaptic potentials (GPSPs) occur rhythmically in principal cells. In combined entorhinal cortex-hippocampal slices, the spontaneous GPSPs occur nearly simultaneously in all regions of the slice—entorhinal cortex, CA1, CA3, and dentate gyrus (Avoli et al. 1996). This rhythmic activity in brain slices from adult animals is most robust when the slice is bathed in 4-AP but also occurs in certain conditions in the presence of blockers of ionotropic glutamatergic synaptic transmission without 4-AP: in high K+ solution (Michelson and Wong 1991), in solution containing zinc (Lambert et al. 1992), and in layer II neurons of entorhinal cortex in solution containing the muscarinic agonist carbachol (Dickson and Alonso 1997). GPSPs may also play a role in epilepsy: when glutamatergic transmission is intact, and in the presence of 4-AP, epileptiform ictal events originating in the entorhinal cortex and propagating to CA3 are closely preceded by a synchronous GPSP that appears to initiate the ictal discharge (Avoli et al. 1996).

The spontaneous GPSPs in hippocampal pyramidal cells occur synchronously with bursts of action potentials in a subset of hilar interneurons and appear to result from the synchronous...
synaptic release of GABA from a group of interneurons (Michelson and Wong 1991, 1994; Müller and Misgeld 1990). In contrast to other proposed interneuron networks, in which the synaptic connections among interneurons are GABA-mediated inhibitory connections, the interneurons mediating the GPSPs are hypothesized to belong to a network of interneurons that synchronize their firing via GABA-mediated excitatory connections (Michelson and Wong 1991). Data suggest that interneuron-to-interneuron recurrent excitatory transmission mediated by the synaptic depolarizing GABA response (Perkins and Wong 1996) is necessary for the generation of spontaneous rhythmic GPSPs (Lamsa and Kaila 1997; Michelson and Wong 1991, 1994).

The experiments reported herein investigate whether focal application of the neurotransmitter GABA is capable of exciting a network of interneurons to generate a giant GABA-mediated postsynaptic current (GPSC) in the hippocampal pyramidal cell. In addition, these experiments investigate the most effective sites of this focal GABA application.

**METHODS**

**Slice preparation**

Experiments were done in guinea pig hippocampal brain slices from 14 to 30 days old animals. Unlike rats, guinea pigs are a precocial species whose brains (Altman and Das 1967) and, in particular, hippocampi (Nacher et al. 2000), are at an advanced stage of maturation at birth. Guinea pigs were decapitated with a guillotine. One hippocampus was removed, and the middle third was selected for slicing. Transverse slices (300 μM) were cut in oxygenated, ice-cold solution (solution composition same as bath solution listed in the following text, except 8 mM MgCl2 and 0.5 mM CaCl2) using a vibratome (Technical Products International, St. Louis, MO). Slices were transferred to the recording chamber (Fine Science Tools, Foster City, CA) where they were maintained at an interface between continuously perfusing oxygenated solution and humidified 95% O2-5% CO2 gas at 31°C until ready to record (≈1 h). The slices were submerged during recording.

**Solutions**

The bath solution contained (in mM) 125 NaCl, 25 NaHCO3, 2.5 KCl, 1.6 MgCl2, 2.0 CaCl2, and 11 d-glucose. During recording, the bath solution included 4-AP (50 μM), ionotropic glutamate receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM, Tocris Cookson, Ellisville, MO), 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 20 μM, Tocris Cookson), and the GABA receptor antagonist CGP 55845A (1 μM; gift from Ciba-Geigy, Basel, Switzerland). The high-Mg2+/low-Ca2+ bath solution, which was used to suppress synaptic activity in one set of experiments, was the same as that in the preceding text except the [Mg2+] was 12 mM and the [Ca2+] was 0.5 or 0.2 mM.

The GABA solution used in the pressure ejection pipette was the bath solution, including CNQX, CPP, 4-AP, and CGP 55845A, but containing 0.5 mM CaCl2 instead of 2 mM CaCl2 to avoid forming CaCO3 precipitate. The GABA solution also contained 500 μM GABA and 0.1% Fast Green dye. Control pressure ejection pipette solution was identical to the GABA solution except that it did not contain GABA.

Recording pipette solution containing greater than physiologic concentrations of HCO3- (potassium bicarbonate solution, see following text) was used in some recordings to accentuate the inward appearance of the late component of the GPSC at potentials near rest (Perkins and Wong 1996). Systematic variations of intracellular [HCO3-] have been explored in an earlier paper (Perkins and Wong 1996). Varying intracellular [HCO3-] in the recorded cell had no effect on the generation of GPSCs, only on the reversal potential of the late component of the GPSC (Perkins and Wong 1996).

The potassium bicarbonate recording pipette solution (used in the recordings in Figs. 2–6) contained (in mM) 102 KHCO3, 28 KOH, 5 NaCl, 5 TEA-Cl, 10 HEPES, 4 EGTA, 2.5–5.0 QX-314 (RBI, Natick, MA or Calbiochem, La Jolla, CA), and 4 Mg-ATP. The pH of the solution was adjusted to 7.0 (see Perkins and Wong 1996) with methanesulfonic acid while bubbling 95% O2-5% CO2 gas through the solution. In addition, 95% O2-5% CO2 gas was bubbled through the solution for ≈1 h immediately prior to filling the recording pipette.

The potassium gluconate recording pipette solution (used for the recordings illustrated in Fig. 8) contained (in mM) 119 K gluconate, 5 NaCl, 2 CsCl, 13 KCl, 10 HEPES, 2 EGTA, 5 QX-314 (Calbiochem), and 4 Mg-ATP. The pH of the solution was adjusted to 7.3 with KOH. Chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated.

As noted in the preceding text, the recording pipette solution contained QX-314. Intracellular QX-314 blocks voltage-dependent sodium currents (Connors and Prince 1982) and the hyperpolarization-activated current Ih (Perkins and Wong 1995). In addition, QX-314 blocks the GABA B component of the GPSC (Perkins and Wong 1996; see also Nathan et al. 1990) and the K+-dependent nonsynaptic depolarization that can follow the synaptic depolarizing GABA response (Smirnov et al. 1999).

**Whole cell recording**

Electrophysiological recordings were carried out in the whole cell voltage-clamp configuration (Hamill et al. 1981) on CA3 and CA1 pyramidal cells using a List EPC-7 patch-clamp amplifier (List Electronic, Darmstadt, Germany) and pClamp software (Axon Instruments, Foster City, CA). Whole cell electrode resistances ranged from 3 to 7 MΩ when filled with intracellular recording solution. Seals were established using the patch-slice method of Blanton et al. (1989). No series resistance or slow capacitance compensation was used during the experiment.

The charging current response to a 5-mV voltage step (ΔV) was recorded in all cells and periodically retested during the experiment. When measuring the charging current for purposes of estimating the access resistance (R ACCESS), the current was filtered at 10 kHz by a three-pole Bessel filter and sampled at 70 kHz. The RACCESS was estimated using the equation \( R_{ACCESS} = \Delta V / I \) (Jackson and Hsu 1994), where A is the amplitude of the charging current, R ACCESS ranged from 6 to 35 MΩ.

The liquid junction potential (V LJ ) between the whole cell pipette solution and the bath solution was determined experimentally using the procedure of Neher (1992). Series resistance error (V S ) was calculated after the experiment using the equation \( V_S = R_{ACCESS} \times I \). The I used in the calculation was the baseline holding current at a given command potential, V COM. All holding potentials (V COM) reported in this paper have been corrected for V LJ and V S using the equation \( V_{COM} = V_{COM} - V_L - V_S \).

4-AP (50 μM) was used to elicit giant, rhythmic GABA-mediated postsynaptic currents (GPSCs) in hippocampal pyramidal cells. 4-AP is particularly useful for this purpose because the GPSCs it elicits in a given cell type have a consistent time course (Perkins and Wong 1996). The GPSCs elicited by 4-AP occurred spontaneously and could also be triggered with pressure application of GABA to a specific area of the slice (see following text). When comparing the amplitude of two biphasic responses, the peak amplitude of the early (outward) component of the responses was measured. The duration of the response was measured as the time between the initial rise and the point at the end of the entire response at which the current had returned to baseline.

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Focal application of GABA

To selectively apply GABA, a pressure ejection pipette was positioned just below the surface of the slice in the selected area. The GABA solution was delivered by applying a brief pulse of pressure (5 psi) to the pressure ejection pipette using a multi-channel picospritzer (General Valve). Pipettes were pulled from 1.5-mm-diam glass and had resistances of 6–9 MΩ when filled with GABA solution. In experiments comparing the response to GABA applied focally to two different areas in the CA3 region, two different pressure ejection pipettes were used. The pressure ejection pipettes were placed before obtaining the whole cell recording. When applying GABA in the CA3 region and recording from a CA3 pyramidal cell, the pressure ejection pipettes and whole cell recording electrode were placed in a row along the presumed axis of the pyramidal cell (Fig. 1). When applying GABA to regions other than CA3 while recording from a CA3 pyramidal cell, only one pressure ejection pipette was used; this pipette was moved from location to location while maintaining the electrical recording. The GABA solution (composition in the preceding text) contained Fast Green to allow visual confirmation that the GABA solution was ejecting properly, to ensure proper placement of the pipette, and to allow visualization of the affected area. With each placement of the pipette and following each response failure, the Fast Green dye was visualized to confirm proper ejection. GABA applications were always ≥30 s apart and ≥30 s after the previous GPSC (except when specifically investigating the refractory period, see RESULTS).

Pressure ejection pipette placement was chosen by visualizing through a dissecting microscope a transverse slice which was illuminated from above. Figure 1 is a photograph of a hippocampal slice in the recording chamber as it appears illuminated from above. Labels identify the locations of recording electrodes and of pressure ejection pipettes in the CA3 region. The stratum lacunosum-moleculare (SLM) region of CA3 is delineated as shown by Blackstad (1956, 1958).

RESULTS

Focal application of GABA to the SR/SLM border or to SLM evoked a GPSC-like response in CA3 pyramidal cells

As described previously (Perkins 1999; Perkins and Wong 1996, 1997), hippocampal pyramidal neurons display rhythmic, spontaneous giant GPSCs in the presence of 4-AP and ionotropic glutamate receptor blockers. At holding potentials near rest when GABA_B receptors are blocked, the GPSC in CA3 pyramidal cells consists of an early outward current mediated by α1 followed by a late inward current mediated in large part by HCO_3 (Perkins and Wong 1996). In addition to the GPSCs, spontaneous small IPSCs also occur. These IPSCs typically have a reversal potential similar to that of the early component of the GPSC (Perkins 1999).

Under the same conditions, pressure application of GABA (500 μM; 30–200 ms duration) to the border between CA3 s. radiatum (SR) and SLM (see ○ in Fig. 1) always (unless the GABA was delivered during the refractory period, see following text) resulted in a biphasic current which looked like a GPSC in the CA3 pyramidal cell (Fig. 2A, n = 35 cells in 30 different slices). On the other hand, focal application of control solution (no GABA) to the border between CA3 SR and SLM elicited no response in the pyramidal cell (n = 3 cells). Application of GABA to SLM (see Fig. 1, ○) rather than at the SR/SLM border was also tested. Pressure application of GABA (50-ms duration) in CA3 SLM always triggered a GPSC (n = 5 cells in 5 different slices). Systematic variation of the holding potential over a 20- to 30-mV range revealed that the spontaneous GPSC and the current elicited by the GABA application at the SR/SLM border looked virtually identical at all potentials (Fig. 2A, n = 4 cells).

The amplitude and duration of the response to focal GABA application at the CA3 SR/SLM border did not change with increasing GABA application durations in the range of 30–200 ms (Fig. 2B; n = 11). As GABA application duration was decreased <30 ms, a threshold application duration was reached at which the CA3 pyramidal cell response decreased abruptly to <10% of the amplitude of the GPSC (Fig. 2B; n = 7). At threshold application duration, the application of GABA sometimes resulted in a GPSC-like response and sometimes in a dramatically smaller (<10%) response (Fig. 2B, n = 6). Figure 2B shows an example of a threshold duration of 15 ms. It also illustrates the increasing delay between the GABA application and the GABA_A ex (exogenous GABA)-triggered GPSC-like response as the GABA application duration was decreased from 50 to 15 ms. This inverse relationship between GABA application duration and latency to response was consistent (n = 5 cells). To ensure valid comparison between responses to GABA application, GABA was applied at a set interval following the previous spontaneous GPSC or GABA_A ex-triggered GPSC-like response.

![FIG. 1. Photograph of hippocampal slice indicating pressure ejection pipette and recording electrode positions. Photograph of submerged slice in recording chamber was taken with a Leica digilux 4.3 digital camera with macro attachment. The 3's indicate the boundaries of the area of CA3 from which CA3 pyramidal cell recordings were made. ○, the location of a pressure ejection pipette placed in stratum lacunosum-moleculare (SLM). ●, the location of a pressure ejection pipette placed at the s. radiatum/SLM (SR/SLM) border. ▲, the location of a pressure ejection pipette placed in proximal SR. △, the location of a pressure ejection pipette placed in the distal third of s. oriens (SO). •, the approximate area of green seen in the microscope with a 50-ms pressure application of GABA solution containing Fast Green. The P indicates how the CA3 recording electrode would be positioned with respect to pressure ejection pipettes. (Only 1 or 2 pressure ejection pipettes were in place at a given time.) When recording from a CA1 pyramidal cell, the recording electrode was placed in the CA1 pyramidal cell layer in the location indicated on the figure by CA1.](http://jn.physiology.org/DownloadedFrom)
FIG. 2. Focal GABA application to the CA3 SR/SLM border triggered an all-or-none biphasic current in the CA3 pyramidal cell that looked like the spontaneous GPSC in the same cell. A: comparison of spontaneous GPSCs (left, spontaneous) with the current responses triggered by 50-ms applications of GABA to the CA3 SR/SLM border (right, puff SR/SLM) at 3 different membrane potentials in the same cell. $V_m$ is listed to the left of each row. $R_s = 6 \, \text{M} \Omega$ for the $-61 \, \text{mV}$ traces and $10 \, \text{M} \Omega$ for the $-51$ and $-37 \, \text{mV}$ traces. Traces offset for display. B: comparison of the current elicited in a CA3 pyramidal cell by GABA applications of different duration (puff duration) applied to the CA3 SR/SLM border. Note the increasing delay between the application and the peak outward current with decreasing application durations. Note also that the 15-ms application of GABA sometimes elicited a "giant" current and sometimes a tiny outward current, demonstrating the "all-or-none" nature of the response. Each application was delivered 60 s following the previous GPSC. Time of GABA application indicated by the small horizontal line under each trace. A 5-mV hyperpolarizing voltage step preceded each GABA application. Different cell from that in A. $V_{\text{com}} = -48 \, \text{mV}; V_H = -50$ to $-55 \, \text{mV}; R_S = 6$–$9 \, \text{M} \Omega$. Traces offset for display.

GPSC-like response to focal GABA application was dependent on synaptic transmission

To suppress synaptic transmission, the bath solution was switched to the high-Mg$^{2+}$/low-Ca$^{2+}$ bath solution. As a result of the solution change, both the GPSC-like response to the focal application of GABA at the CA3 SR/SLM border and the spontaneous GPSCs were gradually blocked (Fig. 3, n = 3 of 3). Following the block of synaptic transmission, focal GABA application to the SR/SLM border continued to elicit a small outward current in the pyramidal cell in two of three recordings (Fig. 3, 56 min); this current was presumably the direct response of the pyramidal cell dendrite to the exogenous GABA. The GPSC-like response to the GABA application and the spontaneous GPSCs returned on return to normal solution (Fig. 3, n = 2 of 2). These data suggest that the GPSC-like response to GABA application was not the direct response of the pyramidal cell dendrite to the exogenous GABA but was rather the response of the pyramidal cell to synaptic transmission of GABA. The GPSC-like response to focal GABA application will thus be termed a GABA$_{\text{ex}}$-triggered GPSC.

Focal application of GABA to SO did not trigger a GPSC

To investigate whether the location of the focal GABA application was crucial to the triggering of a GPSC in the pyramidal cell, GABA was applied to other locations in CA3. Pressure application of GABA (50- to 200-ms duration) to the distal third of stratum oriens (SO, see Fig. 1, $\triangle$) never resulted in a GPSC-like response in the CA3 pyramidal cell even though 30- to 200-ms GABA application at the SR/SLM border or in SLM resulted in GABA$_{\text{ex}}$-triggered GPSCs in those same cells in all cases (Fig. 4A; n = 11 cells in 11 slices). The response to GABA application (50 ms) to SO at holding potentials near $-50 \, \text{mV}$ was a biphasic outward-inward current or a monophasic outward current. The outward current amplitude of the response to a 50-ms GABA application to SO

FIG. 3. The giant biphasic current triggered by focal GABA application to CA3 SR/SLM border was dependent on synaptic transmission. Solution in bath reservoir was changed to one containing 0.2 mM Ca$^{2+}$ and 12 mM Mg$^{2+}$ at 0 min. Response in CA3 pyramidal cell to 50-ms GABA application changed gradually from a giant biphasic GPSC-like current (2 min) to an outward current with a slower rise time (50 min) to individual GABA$_{\text{ex}}$-triggered inhibitory postsynaptic currents (IPSCs) riding on a small outward current (51 min) to a small outward current alone, which was presumably a direct action of the exogenous GABA on the pyramidal cell dendrite (56 min). Note that the peak of the small outward current elicited by GABA application to the SR/SLM border at 56 min occurred sooner than the peak of the GPSC-like response recorded at 2 min. After 54 min, solution in reservoir was changed back to normal bath solution. The GPSC-like current response to the GABA application had partially recovered already at 8 min of wash. Time of 50-ms GABA application indicated by the small horizontal line under each trace. Each GABA application was preceded by a 5-mV hyperpolarizing voltage step to monitor $R_s, V_{\text{com}} = -48 \, \text{mV}; V_H = -50$ to $-55 \, \text{mV}; R_S = 9$–$29 \, \text{M} \Omega$. Traces offset for display.
was 25 ± 6% (mean ± SE, n = 12 cells) of the mean outward current amplitude of the spontaneous and/or GABA$_{ex}$-triggered GPSCs in the same cell at the same membrane potential. Most importantly, in contrast to the all-or-none GPSC triggered by an application of GABA to the SR/SLM border, incremental increases in the duration of the GABA application to SO did cause incremental increases in the duration of the response (Fig. 4A; n = 8 of 8). On the other hand, increasing application duration to >50 ms did not increase the amplitude of the outward portion of the response (Fig. 4A; n = 8). Two cells showed what appeared to be prominent, individual, GABA$_{ex}$-triggered inhibitory postsynaptic currents (IPSCs) riding on the outward current (e.g., Fig. 4A).

Focal application of GABA to proximal SR usually did not trigger a GPSC

Pressure application of GABA (50–100 ms duration) to proximal SR in the CA3 region (see square in Fig. 1) usually did not trigger a GPSC in the CA3 pyramidal cell. Application of GABA to SR triggered a GPSC in the CA3 pyramidal cell in only one cell out of 5. In 4 out of 5 cells, the response to GABA application in SR was either a monophasic current whose direction depended on the holding potential, or a biphasic outward-inward current at potentials near −50 mV. For these four cells, the amplitude of the monophasic response or the amplitude of the outward component of the biphasic response to a 50 ms GABA application was 32 ± 8% (mean ± SE) of the amplitude of the spontaneous GPSCs or GPSCs triggered by an application of GABA to the SR/SLM border recorded at the same membrane potential in the same cell. Increasing the duration of GABA application to SR to >50 ms did not increase the amplitude of the outward component of the biphasic response (Fig. 4B; n = 3 of 3). Most importantly, like the response to GABA focally applied to SO and in contrast to the “all-or-none” GPSC triggered by focal application of GABA to the SR/SLM border, incremental increases in the duration of the GABA application to SR did cause incremental increases in the duration of the response (Fig. 4B; n = 3 of 3). One cell showed what appeared to be prominent, individual, GABA$_{ex}$-triggered IPSCs riding on the outward current (Fig. 4C).

GABA$_{ex}$-triggered GPSCs could not be elicited during a refractory period following a spontaneous GPSC

Applying GABA at varying intervals following a spontaneous GPSC revealed that SR/SLM border application triggered a GPSC only if sufficient time had passed since the previous GPSC. Applying GABA at the SR/SLM border 4–15 s after the previous GPSC failed to trigger a GPSC; instead, the response to the GABA application was a small monophasic current or a small biphasic outward-inward current or nothing.
at all (Fig. 5A; \(n = 10\) cells). In contrast, the response to GABA applications to SO that were delivered 8–15 s after the previous spontaneous GPSC were of similar appearance to the responses from GABA delivered there 40–90 s after the previous spontaneous GPSC (Fig. 5B, \(n = 3\) cells). Figure 5C illustrates the mean amplitudes of the outward current responses to focal applications of GABA at the SR/SLM border and in SO delivered 40–90 s after the previous spontaneous GPSC versus 8–15 s after the previous spontaneous GPSC.

**Focal application of GABA to the CA3 SR/SLM border triggered a GPSC in CA1 pyramidal cells**

To determine whether a focal application of GABA to the CA3 SR/SLM border can trigger a GPSC in pyramidal cells that are farther away from the site of the GABA application, recordings were obtained from CA1 pyramidal cells that lay approximately midway between the CA3 region and the subiculum. In all cells, a 50-ms GABA application at the CA3 SR/SLM border triggered a GPSC in the CA1 pyramidal cell that was indistinguishable from the spontaneous GPSCs occurring in the same cell (Fig. 6A; \(n = 4\) cells in 4 different slices). The response showed refractoriness: whereas application of GABA 45–90 s after the previous GPSC elicited a GPSC in the CA1 pyramidal cell, application of GABA within 20 s of the previous GPSC elicited no response in the CA1 pyramidal cell (Fig. 6B; \(n = 3\) of 3 cells). Applying GABA 20–45 s after the previous GPSC elicited a GPSC in some slices and no response in others, apparently depending on the spontaneous rate of GPSCs in that slice.

**Focal application of GABA to CA1 SLM triggered an all-or-none GPSC in CA3 pyramidal cells**

To further investigate the areas of the hippocampal slice in which focal application of GABA would evoke a GPSC in the CA3 pyramidal cell, focal application of GABA to CA1 SLM was tested. The GABA pressure application pipette was placed in CA1 SLM at a spot anywhere between mid CA1 and the CA1/subiculum border. In all cases, a 50-ms GABA application to CA1 SLM triggered a GPSC in the CA3 pyramidal cell that was indistinguishable from the spontaneous GPSCs occurring in the same cell (Figs. 8 and 9; \(n = 12\) cells in 9 different slices). The CA3 pyramidal cell’s response to GABA application to CA1 SLM displayed a threshold duration at which the application of GABA sometimes resulted in a GPSC and sometimes in no response. Threshold duration measured in three recordings from three different slices was 10 ± 6 ms (mean ± SD; range, 6.4–16.5 ms).

**Focal application of GABA to CA1 SR or SO elicited no response in CA3 pyramidal cells**

To further investigate the areas of the hippocampal slice in which focal applications of GABA would evoke a GPSC in the CA3 pyramidal cell, focal applications of GABA to CA1 SO and SR were tested. The GABA pressure application pipette was located in SR or SO in the middle third of the CA1 region. Focal application of GABA (50 or 100 ms) to either CA1 SO (\(n = 4\) cells in 4 slices) or CA1 SR (\(n = 5\) cells in 5 slices) failed to elicit any response in the CA3 pyramidal cell (Figs. 8 and 9; \(n = 3\) cells). Applying GABA 20–45 s after the previous GPSC elicited a GPSC in some slices and no response in others, apparently depending on the spontaneous rate of GPSCs in that slice.
and 9). In all cases, 100-ms applications were tested when 50-ms applications failed to elicit a response. In most cases, the pipette was moved deeper into the slice or to another spot in the same region for additional testing after the first application site failed to elicit a response to three separate applications. In all cases, a focal GABA application to CA1 SLM did elicit a GPSC in the CA3 pyramidal cell in the same slice which failed to respond to a CA1 SO or SR GABA application.

**Focal application of GABA to the hilus elicited no response in CA3 pyramidal cells**

Earlier studies identified a group of interneurons which rhythmically fire bursts of action potentials in the presence of 4-AP (Forti and Michelson 1998; Michelson and Wong 1991, 1994). These interneuron recordings were made in a specific region of the hilus that is adjacent to the upper blade of the granule cell layer. It is within this region of the hilus (see Fig. 7) that GABA was applied in this portion of this study. Focal application of GABA (50 or 100 ms) to the hilus failed to elicit any response in the CA3 pyramidal cell (Figs. 8 and 9, n = 6 cells in 5 slices). In all cases, 100-ms applications were tested when 50-ms applications failed to elicit a response. In most cases, the pipette was moved deeper into the slice or to another spot in the same region for additional testing after the first application site failed to elicit a response to three separate applications. In all cases, a focal GABA application to CA1 SLM or to the dentate molecular layer (see following text) did elicit a GPSC in the CA3 pyramidal cell in the same slice that failed to respond to a hilal GABA application.

**Focal application of GABA within the outer two-thirds of the dentate molecular layer triggered a GPSC in CA3 pyramidal cells**

It was noted that the effective GABA-application sites are also the areas that are innervated by fibers from the entorhinal cortex in an intact animal (Steward 1976). To test the hypothesis that all regions directly innervated by the entorhinal cortex would be sites at which GABA application triggered a GPSC in the CA3 pyramidal cell, GABA was focally applied to the remaining region directly innervated by the entorhinal cortex, the outer two-thirds of the dentate molecular layer (ML). The pressure application pipette was positioned in the outer two-thirds of either the upper or lower blade of the dentate ML. Focal application of GABA either elicited a GPSC in the CA3 pyramidal cell that was indistinguishable from spontaneous GPSCs occurring in the same cell or no response. Focal application of GABA (50 or 100 ms) to the upper blade of the dentate ML elicited a GPSC in the CA3 pyramidal cell in four of five slices (7 of 8 cells; Figs. 8 and 9). Focal application of GABA (50 or 100 ms) to the lower blade of the dentate ML elicited a GPSC in the CA3 pyramidal cell in two of six slices (5 of 10 cells; Figs. 8 and 9). In all cases, 100-ms applications were tested if 50-ms applications failed to elicit a response. In most cases, the pipette was moved deeper into the slice or to another spot in the same region for additional testing if the first application site failed to elicit a response to three separate applications. In all cases in which a focal GABA application to the dentate ML failed to elicit a GPSC in the CA3 pyramidal cell, a focal GABA application to CA1 SLM in the same slice did elicit a GPSC in the CA3 pyramidal cell.

**DISCUSSION**

There are two main findings in this report. The first is that focal application of GABA in 4-AP evoked an interneuron-network-mediated synaptic response in hippocampal pyramidal cells. The second is that the effective GABA-application...
suggests that the same interneurons involved in generating the refractory period following a spontaneous GPSC in which a pressed using a high-Mg²⁺ application was blocked when synaptic transmission was sup-
pered rather than displaying a “threshold” application duration. The likelier scenario is that the exogenous GABA was activating only a subset of the members of the interneuron network and that these then activated other members of the network. The greater delay to peak response with shorter GABA applications (Fig. 2B) may have been the result of a longer waiting time for recruitment of the network when starting with a smaller number of interneurons initially directly activated. Imaging voltage-sensitive dye under the same conditions used in this study (excitatory amino acid receptor antagonists and 4-AP), Sinha and Saggau (2001) observed a spontaneous GABA-dependent depolarization moving in a wave across the hippocampal slice and found that the spontaneous depolarization was equally likely to move from the subicular end of the CA1 region toward the CA3 end of the CA1 region as it was to travel in the opposite direction. This imaging observation supports the bidirectional nature of the triggering of GPSCs observed in this study—GABA application to CA1 SLM evoked a GPSC in the CA3 pyramidal cell, and, conversely, GABA application to CA3 SLM evoked a GPSC in the CA1 pyramidal cell. These findings support the notion that the depolarizing event spreads across the slice traveling from one subgroup of interconnected interneurons to another.

Focal GABA application evoked an interneuron-network-mediated population response in pyramidal cells

The data indicate that the GPSC evoked by focal GABA application was not the result of the exogenous GABA acting on pyramidal cell receptors. The GPSC evoked by focal GABA application was blocked when synaptic transmission was sup-
pressed using a high-Mg²⁺/low-Ca²⁺ bath solution. In addi-
tion, focal application of GABA was able to evoke a GPSC in the pyramidal cell located >2 mm away. The existence of a refractory period following a spontaneous GPSC in which a GPSC could not be triggered by focal application of GABA suggests that the same interneurons involved in generating the spontaneous GPSC were involved in generating the GABA_{ex}-
triggered GPSC.

The data indicate that the GABA_{ex}-triggered GPSC was the result of the activation of a network of interneurons rather than a result of the direct excitation of every individual interneuron that contributed to the GPSC in the pyramidal cell. If the GPSC was simply the result of direct activation of all of the inter-
neurons contributing to the GPSC, then the response would have gotten smaller and smaller with shorter and shorter application durations as fewer and fewer interneurons were acti-
vated rather than displaying a “threshold” application duration. The greater delay to peak response with shorter GABA applications (Fig. 2B) may have been the result of a longer waiting time for recruitment of the network when starting with a smaller number of interneurons initially directly activated. Imaging voltage-sensitive dye under the same conditions used in this study (excitatory amino acid receptor antagonists and 4-AP), Sinha and Saggau (2001) observed a spontaneous GABA-dependent depolarization moving in a wave across the hippocampal slice and found that the spontaneous depolarization was equally likely to move from the subicular end of the CA1 region toward the CA3 end of the CA1 region as it was to travel in the opposite direction. This imaging observation supports the bidirectional nature of the triggering of GPSCs observed in this study—GABA application to CA1 SLM evoked a GPSC in the CA3 pyramidal cell, and, conversely, GABA application to CA3 SLM evoked a GPSC in the CA1 pyramidal cell. These findings support the notion that the depolarizing event spreads across the slice traveling from one subgroup of interconnected interneurons to another.
GABA application was effective at sites that receive direct entorhinal innervation

Focal GABA applications to CA3 SLM, CA1 SLM, and the outer two-thirds of the dentate ML evoked GSFCs in CA3 pyramidal cells. These areas are the same locations that have been shown both with anatomical data (Blackstad 1958; Hjorth-Simonsen 1972; Hjorth-Simonsen and Jeune 1972; Steward 1976; Witter 1993) and with current-source density analysis (Berzhanskaya et al. 1998; Empson and Heinemann 1995; Wu and Leung 1998; Yeckel and Berger 1990) to be directly innervated by fibers from the entorhinal cortex.

There are several different classes of interneurons that have cell bodies or dendrites residing in CA3 SLM (Freund and Buzsáki 1996; Jongen-Relo et al. 1999; Kiss et al. 1996; Misgeld and Frotscher 1986), CA1 SLM (Ácsády et al. 1996a,b; Buhl et al. 1994; Freund and Buzsáki 1996; Hájos and Mody 1997; Halasy et al. 1996; Lacaille and Schwartzkroin 1988a,b; Vida et al. 1998) or the outer two-thirds of the dentate ML (Buckmaster and Schwartzkroin 1995; Buhl et al. 1994; Ceranik et al. 1997; Freund and Buzsáki 1996; Han et al. 1993). Several of these interneuron types can be monosynaptically discharged by perforant path stimulation: CA1 SLM interneurons, whose cell bodies reside in SLM or on the border of SLM and SR (Lacaille and Schwartzkroin 1988a; Williams et al. 1994); HICAP interneurons, hilar interneurons with commissural-associational pathway-associated terminals (Buckmaster and Schwartzkroin 1995; Sik et al. 1997); and axo-axonic cells that have cell bodies residing in CA1 or the hilus (Buhl et al. 1994). In addition, immunohistochemical studies indicate that parvalbumin-immunoreactive interneurons whose cell bodies reside just outside the principal cell layer but whose dendrites extend into CA3 or CA1 SLM (basket or chandelier cells) (Kiss et al. 1996) or the outer two-thirds of the dentate ML (Zipp et al. 1989) receive perforant path synapses. In the hippocampal imaging study described above (Sinha and Sag-gau 2001), the spontaneous depolarizations occurring in 4-AP and excitatory amino acid receptor blockers were strongest in CA1 SLM, suggesting the possibility that the interneurons responsible for the rhythmic depolarizations were located in CA1 SLM. One type of interneuron that is particularly intriguing in the context of this paper is the IS-2 neuron (Freund and Buzsáki 1996), which is a vasoactive intestinal polypeptide (VIP)-positive GABAergic neuron that has its cell body at the SR/SLM border. The dendritic arbors of IS-2 neurons lie exclusively in SLM, which means that they may receive synapses from entorhinal afferents, and IS-2 neurons synapse extensively onto other interneurons, which may allow them to recruit an interneuron network response (Ácsády et al. 1996a,b).

Focal application of GABA to the area of the hilus adjacent to the upper blade of the granule cell layer did not evoke a GSFC in CA3 pyramidal cells. This area was specifically chosen because interneurons that have somata residing in this location fire bursts of action potentials synchronous with the GSFCs in pyramidal cells (Forti and Michelson 1998; Michelson and Wong 1991, 1994) and can respond to exogenous GABA application by firing a burst of action potentials (Michelson and Wong 1991). There are two possible explanations for the apparent discrepancy between the earlier reports and the findings presented here. The first possibility is that the hilar interneurons may be “follower” cells that fire in response to GABA application and that can be recruited by members of the interneuron network but that are unable to themselves initiate a GSFC. The same may be true of some interneurons in CA3 SO and CA3 SR in this report; these interneurons apparently fired in response to GABA application as evidenced by GABA-evoked IPSCs recorded in the pyramidal cell but were unable to recruit the network to generate a GSFC. The second possibility is that the only hilar interneurons that fire in response to GABA are those that have dendrites extending into the outer two-thirds of the dentate ML. In this scenario, the distal dendrites, but not the soma, of these cells would respond to GABA with a depolarization capable of making the cell fire.

In conclusion, the interneurons responsible for generating the GSFC in pyramidal cells in response to focal application of GABA would need to have at least two features: 1) respond (directly or indirectly) to a puff of GABA with a depolarization and 2) excitation of one interneuron would lead to excitation of other members of the network. It is not known whether the interneurons that responded to the focal GABA applications by recruiting a network response are ones that have somata that reside in the SLM layer or the dentate ML, or whether these neurons are ones that have somata that reside elsewhere but that have dendrites in SLM or the dentate ML that depolarize in response to GABA. Regardless of where the somata reside, this interneuron network may be designed to be recruited by input from the entorhinal cortex.

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FOCAL GABA APPLICATION EXCITES INTERNEURON NETWORK


