Synaptic Connectivity of Distinct Hilar Interneuron Subpopulations

MATTEO FORTI AND HILLARY B. MICHELSON

Department of Pharmacology, State University of New York Health Science Center at Brooklyn, Brooklyn, New York 11203

Forti, Matteo and Hillary B. Michelson. Synaptic connectivity of distinct hilar interneuron subpopulations. J. Neurophysiol. 79: 3229–3237, 1998. Dual intracellular recordings of hilar interneurons and CA3 pyramidal cells were performed in transverse slices of guinea pig hippocampus in the presence of the convulsant compound 4-aminopyridine (4-AP) and ionotropic glutamate receptor antagonists. Under these conditions, interneurons burst fire synchronously, producing synchronized inhibitory postsynaptic potentials (sIPSPs) in pyramidal cells. Three different hilar interneuron subpopulations that contributed to the sIPSP were identified based on their projection properties and morphology. These three types were pyramidal-like stellate interneurons, spheroid interneurons, and oviform interneurons. Physiologically, pyramidal-like stellate interneurons could be differentiated from the other interneuron subpopulations because they generated short synchronized bursts of action potentials coincident with the hyperpolarizing and depolarizing \( \gamma \)-aminobutyric acid-A (GABA\(_A\))-mediated inhibitory postsynaptic potentials (IPSPs) recorded in pyramidal cells. The bursts in pyramidal-like stellate cells were abolished by the GABA\(_A\)-receptor blocker, bicuculline. In contrast, spheroid interneurons of the dentate-hilus (D-H) border and oviform hilar interneurons exhibited prolonged bicuculline-resistant bursts that occurred coincident with the GABA\(_B\) pyramidal cell sIPSPs. Pyramidal-like stellate interneurons likely did not contribute to the generation of synchronized GABA\(_B\) responses in hippocampal pyramidal cells. Spheroid interneurons were unique among these subpopulations of interneurons in that the bicuculline-resistant bursts in spheroid interneurons were sustained by a synaptic depolarization that persisted in the presence of antagonists of ionotropic glutamate, GABA\(_A\) and GABA\(_B\) receptors [6-cyano-7-nitroquinoxaline-2,3-dione, 20 \( \mu \)M; 3-(2-carboxyipiperazine-4-yl)propyl-1-phosphonate, 20 \( \mu \)M; bicuculline, 10–15 \( \mu \)M; CGP 55845A, 20 \( \mu \)M]. This novel depolarizing potential reversed between –30 and 0 mV. No noticeable synaptic depolarization sustaining burst firing could be isolated in oviform interneurons, suggesting that firing in this interneuron subpopulation was synchronized by nonsynaptic mechanisms. The results of the present study indicate that the hilar inhibitory circuit is composed of at least three different subpopulations of interneurons, distinguishable by their morphological characteristics and synaptic inputs and outputs. These findings give further support to the hypothesis that there are distinct populations of interneurons producing GABA\(_A\) and GABA\(_B\) responses with defined functional roles within the hippocampal inhibitory circuit. Notably, we found that spheroid interneurons were unique among the hiler interneurons studied, in that the synchronized bursts observed in these cells are sustained by a novel ionotropic glutamate and GABA receptor-independent synaptic depolarization.

INTRODUCTION

In the hippocampus, GABAergic interneurons play an essential role in the control of normal network activity (Buzsáki et al. 1992; Cobb et al. 1995; Miles and Wong 1987; Traub et al. 1996). Interneurons can influence the efficacy of principal cell excitation and thus modulate population activity within the hippocampal circuit. The dentate gyrus, in particular, plays an important role in information processing in the hippocampus. As the first step in the trisynaptic intrahippocampal loop, the dentate receives significant input from entorhinal afferents that it then relays to the CA3 subfield (Blakstad et al. 1970; Hjorth-Simonsen 1971). The dentate also functions to gate the propagation of epileptiform activity into the hippocampus (Paré et al. 1992).

Recent studies have demonstrated that dentate gyrus interneurons have very extensive and regionally specific axonal arborizations, implying that single interneurons can exert widespread influence on the excitability of neurons throughout the hippocampus (Buckmaster and Schwartzkroin 1995a). Within the inhibitory circuit, interneurons generate chloride-dependent fast inhibitory postsynaptic potentials (IPSPs) in principal cells mediated by \( \gamma \)-aminobutyric acid-A (GABA\(_A\)) receptors, and potassium-dependent slow IPSPs mediated by GABA\(_B\) receptors (Alger and Nicoll 1982). However, it is not clear whether distinct subpopulations of interneurons differentially produce GABA\(_A\) or GABA\(_B\)-mediated IPSPs. Some investigators have suggested that, in the CA1 region of the hippocampus, separate groups of inhibitory cells are responsible for activating postsynaptic GABA\(_A\) and GABA\(_B\) receptors (Segal 1990; Samulack and Lacaille 1993; Williams and Lacaille 1990; Williams et al. 1993). No such evidence exists for interneurons in the hilus.

Several studies have described a wide variety of interneurons in the dentate gyrus, according to their morphological features (Amaral 1978; Lorente de Nó 1934; Ramón y Cajal 1893; Ribak and Seress 1983). More recent studies have identified different classes of interneurons according to their immunocytochemical properties (Baskt et al. 1986; Gulyás et al. 1991; Sloviter and Nilaver 1987) or their axonal target cell fields (Buckmaster and Schwartzkroin 1995a,b; Mitchell et al. 1997). Correlations of morphology with intrinsic properties have been performed on hilar interneurons (Buckmaster and Schwartzkroin 1995a,b; Mott et al. 1997); however, no studies have investigated correlations between the morphological heterogeneity of hilar interneurons and their recurrent connectivity or inhibitory projections onto pyramidal cells.

In the present study, we use the previously characterized 4-aminopyridine (4-AP) model of interneuronal synchronization (Aram et al. 1989; Michelson and Wong 1991, 1994; Muller and Misgeld 1990; Perrault and Avoli 1991) to examine the relationship between morphological and functional aspects of inhibitory neurons. Previous studies with 4-AP have demonstrated that interneurons can recurrently excite other interneurons via a GABA\(_A\)-mediated depolarizing response and that subpopulations of interneurons can be differentiated according to their responsiveness to the GABA\(_A\)
off-line digital analysis. All measurements of signal amplitude and duration are expressed as means ± SD with significance set at \( P < 0.05 \).

At the end of the experiments, the slices were fixed overnight with 4% paraformaldehyde for biocytin processing. The slices were incubated in ABC complex (Vectastain, Vector Laboratories, Burlingame, CA) and were processed with diaminobenzidine for visualization. Finally the slices were dehydrated and cleared in a series of alcohol rinses before being mounted and examined using a Nikon microscope with appropriate photographic accessories.

METHODS

Guinea pigs weighing 200–300 g were anesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane and rapidly decapitated. Transverse slices of hippocampus, 400 µm in thickness, were prepared using a vibratome according to standard procedures (Michelson and Wong 1994). Slices were transferred onto the nylon mesh of a gas-fluid interface recording chamber, maintained at 35°C (pH 7.4), and exposed to a warm, humidified atmosphere saturated with a 95% O₂-5% CO₂ gas mixture. The lower surfaces of the slices were in contact with a perfusion solution containing (in mM) 124 NaCl, 5 KCl, 2 CaCl₂, 1.6 MgCl₂, 26 NaHCO₃, and 10 d-glucose. Control solutions also contained 4-AP (75 µM); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 µM); and 3-3(2-carboxypiperazine-4-yl)propyl-1-phosphonate (CPP, 20 µM). Bicuculline methiodide (10–15 µM) and CGP 55845A (20 µM) were added to the solution in some experiments. CNQX and CPP were obtained from Tocris Cookson (St. Louis, MO), and CGP 55845A was kindly provided by CIBA-GEIGY (Basel, Switzerland). All other chemicals were obtained from Sigma Chemical (St. Louis, MO).

Dual intracellular recordings were performed using glass micropipettes (40–100 MΩ resistance) filled with 2% biocytin in 1 M potassium acetate. Only one interneuron per slice was recorded in order to avoid multiple intracellular staining. Signals were amplified by a dual-channel Neurodata amplifier and stored on tape for off-line digital analysis. All measurements of signal amplitude and duration are expressed as means ± SD with significance set at \( P < 0.05 \).

The results presented have been limited to include only cells in which data was obtained continuously before and throughout all drug administrations. Dual intracellular recordings (\( n = 15 \)) were obtained from hilar interneurons and CA3 pyramidal cells in the presence of the convulsant compound 4-AP (75 µM), CNQX (20 µM) and CPP (20 µM) also were added to the bath to block ionotropic glutamatergic excitatory inputs (Honore et al. 1988). Under these conditions, synchronized IPSPs (sIPSPs) were generated in pyramidal cells at a frequency of 0.125–0.25 Hz, which occurred simultaneously with spontaneous bursting activity in interneurons (Fig. 1A). The triphasic sIPSPs in pyramidal cells were composed of a GABA₅ hyperpolarizing phase and a GABA₆ depolarizing phase which were both blocked.

**FIG. 1.** Synaptic inputs and morphology of pyramidal-like stellate interneurons. A: hyperpolarization and depolarization of a pyramidal-like stellate cell by DC current injection in control conditions, with 4-aminopyridine (4-AP, 75 µM); 3,3(2-carboxypiperazine-4-yl)propyl-1-phosphonate (CPP, 20 µM), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 µM) in the bath. All events were spontaneously occurring. Resting membrane potential was \(-60 \text{ mV}, \) input resistance was \( 44 \text{ MΩ}, \) PYR, pyramidal cell. \( ± \), hyperpolarizing \( γ\)-aminobutyric acid-A (GABA₅) input. \( ± ± ± \), depolarizing GABA₆ input. Note that the peak of the depolarization shifted toward the peak of the GABA₅ hyperpolarizing component in the pyramidal cells when the interneurons where hyperpolarized beyond the CI⁻ reversal potential, suggesting that the pyramidal-like interneurons received also a GABA₅ hyperpolarizing input. Also note that electrical capacitance transients associated with the firing of simultaneously recorded cells are visible in most traces in this and subsequent figures. B: hyperpolarization of the same cell after bicuculline wash-in. Note that a GABA₅-mediated hyperpolarization was apparent in 2 of 5 pyramidal-like stellate cells examined. \( ± ± ± \), GABA₅ input. C and D: camera lucida drawings of pyramidal-like stellate cells stained with biocytin. D is a composite of cells taken from 5 different slices. All the cells had functional properties similar to those shown in the figure. \( ± ± ± \), granule cell layer; a, axon. Calibration bars, C: 15 µm; D: 90 µm.
by bicuculline (see Fig. 2A), followed by a late GABA$_B$ component blocked by CGP 55845A (see Fig. 6). Previous studies have demonstrated that sIPSPs are population events that occur simultaneously in all pyramidal cells and are generated by the synchronized discharge of interneurons (Aram et al. 1991; Michelson and Wong 1991, 1994).

Some of the recorded interneurons were stained with biocytin, and the morphology of each cell was correlated with its physiological properties. Three types of interneurons were identified following the classification of Amaral (1978): pyramidal-like stellate cells, spheroid cells of the dentate-hilus (D-H) border, and oviform cells.

Pyramidal-like stellate cells

Pyramidal-like stellate interneurons (n = 5) were morphologically identified as cells with large triangular somas and three to five primary dendrites (Fig. 1, C and D). Spines were not apparent on the dendrites of these interneurons.

Pyramidal-like stellate interneurons could be distinguished from the other interneuron populations by their bursting characteristics in the presence of 4-AP and ionotropic glutamate receptor blockers. All pyramidal-like stellate interneurons exhibited short burst discharges with a duration of 336.7 ± 16.8 ms (n = 60 in 3 cells) and a latency to peak of 152.1 ± 10.4 ms. These bursts occurred coincident with the GABA$_A$ components of the spontaneous IPSPs recorded in pyramidal cells (Fig. 1A). The short synchronized bursts in all pyramidal-like stellate cells were sustained by a GABA$_A$ depolarizing input that was abolished by bicuculline (10–15 μM; Fig. 1B). This depolarizing input had a reversal potential more positive than −45 mV (Fig. 1A). A hyperpolarizing GABA$_A$ event was revealed when the cells were sufficiently depolarized (Fig. 1A, −50 mV). Two of five pyramidal-like stellate cells received a GABA$_A$ input, which became evident after blockade of the bursting activity with bicuculline. Under these conditions, monophasic GABA$_A$ IPSPs with a reversal potential around −90 mV occurred in the interneurons simultaneously with the sIPSPs in the pyramidal cells (Fig. 1B). These GABA$_B$ events were blocked by CGP 55845A (not shown).

Although bicuculline blocked all bursting activity in pyramidal-like stellate cells, synchronized GABA$_B$ events in pyramidal cells persisted in the presence of bicuculline, presumably sustained by synchronized activity in other interneurons. We therefore performed experiments to evaluate whether pyramidal-like stellate cells participate in the generation of the synchronized GABA$_B$ event before bicuculline administration. These experiments compared the amplitude of the GABA$_A$ sIPSPs before and after bicuculline-induced blockade of bursting activity in pyramidal-like stellate interneurons (Fig. 2). The amplitude of the GABA$_B$ sIPSP was significantly larger after bicuculline wash-in when measured near the peak of the synchronized event in control conditions (at 375 ms: control = −6.1 ± 0.3 mV; bicuculline = −9.1 ± 0.5 mV, P < 0.05). However, when measured at a delay of 525–725 ms from the onset of the sIPSP, the amplitude of the GABA$_A$ event was not significantly different before and after bicuculline wash-in (at 700 ms: control = −2.2 ± 0.5 mV; bicuculline = −2.4 ± 0.8 mV, P > 0.05).

Because the early phase of the sIPSP in control conditions is a mixed event, with substantial overlap between the GABA$_A$ depolarizing component and the GABA$_B$ component, the amplitude of the late phase of the sIPSP, beyond the peak of the depolarizing GABA event, reflects a more reliable measurement of the pure GABA$_A$ response (Perkins and Wong 1996). If pyramidal-like stellate interneurons contributed to the synchronized GABA$_B$ response in pyramidal cells, the amplitude of the GABA$_B$ event would be expected to be significantly smaller after bicuculline administration. Thus the lack of a significant change in the amplitude of the GABA$_B$ response after bicuculline, measured at later latencies during the event, suggests that pyramidal-like stellate cells did not contribute significantly to the generation of GABA$_B$ responses in pyramidal cell sIPSPs.

Oviform cells

Oviform cells (n = 5) were identified morphologically as cells with one or two large spiny dendrites emerging from oviform somas on the opposite side of the axon. These cells also had three to four smaller primary dendrites that ramiﬁed extensively within the hilus (Fig. 3, C and D).

The synchronized bursts in the oviform interneurons shared some common features with bursts occurring in spheroid interneurons (see further). They were signiﬁcantly longer than synchronized bursts recorded in pyramidal-like stellate cells (626.3 ± 27.4 ms; n = 60 in 5 cells; P < 0.05) and exhibited a longer rise time (293.7 ± 43.7 ms, P <
FIG. 3. Synaptic inputs and morphology of oviform interneurons. A: hyperpolarization and depolarization of an oviform interneuron in control conditions. Resting membrane potential was \(-65\,\text{mV}\), input resistance was \(86\,\text{M} \Omega\). – – – hyperpolarizing GABA\(_A\) input. Synchronized bursts in these cells were either monophasic and synchronous with the GABA\(_B\) input in pyramidal cells or biphasic. ± ± ±, biphasic burst in the interneuron corresponding to GABA\(_A\) and GABA\(_B\) components in the pyramidal cell (PYR) sIPSPs. B: hyperpolarization and depolarization of the same cell after bicuculline wash-in. – – –, GABA\(_B\) input. ± ± ±, GABA\(_B\) sIPSPs in the pyramidal cell and corresponding bicuculline-resistant burst in the interneuron. Note the nonlinear voltage dependence of the small depolarization underlying the burst. C and D: camera lucida drawings of oviform cells stained with biocytin. All the cells had functional properties similar to those shown in the figure. – – –, granule cell layer; a, axon. Calibration bars, C: 15 \(\mu\text{m} \); D: 90 \(\mu\text{m}\).

0.05). Unlike pyramidal-like stellate cells, the bursts in oviform interneurons were resistant to bicuculline (Fig. 3B, middle and right, \(n = 8\)) and occurred coincident with the pure GABA\(_B\) component of the sIPSP in pyramidal cells.

All oviform cells received both GABA\(_A\) inputs and a GABA\(_B\) input (Fig. 3, A and B, –→). The bicuculline-resistant component of the synchronized burst in oviform interneurons usually originated abruptly from the baseline at resting membrane (Fig. 4B, \(-65\,\text{mV}\)). When the cells were hyperpolarized, small truncated spikes occurred originating directly from the baseline (not shown). These characteristics were consistent with a nonsynaptic origin of the burst discharges, as previously described in hilar interneurons (see Michelson and Wong 1994).

Dye coupling of interneurons, an indication of the presence of electrotonic junctions, has been demonstrated previously (Michelson and Wong 1994) and was found again among oviform interneurons in one instance (\(n = 1\) of 5). Figure 4 shows three oviform interneurons stained together during a single recording. The dye coupling among oviform cells suggests that electrical connections among oviform interneurons may play a role in their synchronization.

Spheroid cells of the D-H border

Spheroid cells (\(n = 3\)) were located on the border between the hilus and the granule cell layer. These cells had small round somas with six to nine long primary dendrites showing few ramifications (Fig. 5, C and D).

All spheroid cells of the D-H border received both a hyperpolarizing and depolarizing GABA\(_A\) input and a GABA\(_B\) input (Figs. 5, A and B, and 6A, –→). Addition of bicuculline (10–15 \(\mu\text{M}\)) and CGP (20 \(\mu\text{M}\)) into the bath revealed a depolarization that occurred coincident with the monophasic GABA\(_B\) event in pyramidal cells (Figs. 5B and 6A). This depolarization increased linearly in amplitude when the cells were hyperpolarized, consistent with a chemical synaptic origin of the event (Fig. 6B).

The reversal potential of the excitatory synaptic input sustaining the prolonged burst in spheroid interneurons was estimated by measuring the amplitude of the depolarization at different membrane potentials in the presence of CGP 55845A (\(n = 2\)). CGP 55845A effectively blocked the GABA\(_B\) input in both pyramidal cells and interneurons and, in spheroid interneurons, further pharmacologically isolated...
the depolarizing potential. The monophasic depolarization exhibited a linear voltage dependence and showed an extrapolated reversal between −30 and 0 mV, suggesting the involvement of a mixed cationic current in the generation of the event (Fig. 6C). As this excitatory synaptic input occurred with CNQX, CPP, and bicuculline in the perfusion solution, it clearly was not mediated by ionotropic glutamate or GABA\textsubscript{A} receptor activation.

**DISCUSSION**

Two important conclusions can be drawn from the results of the present study. First, it is clear that the inhibitory circuit is composed of at least three different subpopulations of interneurons, each sharing common morphological characteristics, functional connectivity and synaptic inputs. These findings give further support to the hypothesis that there are distinct populations of interneurons producing GABA\textsubscript{A} and GABA\textsubscript{B} responses. Second, one subpopulation of hilar interneurons, the spheroid cells, are unique among the interneurons studied because the synchronized bursts generated in these cells in 4-AP appear to be sustained by an ionotropic glutamate and GABA receptor-independent synaptic depolarization.

The experimental conditions, using 4-AP and ionotropic glutamate receptor antagonists, allowed for the characterization of spontaneous bursting activity of different interneuron subpopulations, and its correlation with the synchronized inhibitory responses produced in pyramidal cells in the absence of fast excitatory glutamatergic activity. Our experiments support the findings of others who have demonstrated that interneurons within the hilus exhibit physiological and morphological heterogeneity (Buckmaster and Schwartzkroin 1995a,b; Buhl et al. 1994; Han et al. 1993; Mott et al. 1997). These previous studies, however, could only speculate on the functional role of interneuron subpopu-
lutions based on their axonal arborization. The conditions of the present study allowed for the first time some insight into the functional role of different interneurons subtypes within the inhibitory circuit.

To morphologically classify the interneurons in the present study, we used the classification system of Lorente de Nó (1934) and Amaral (1978); this classification system correlated very well with the differences in synaptic connectivity observed in our experiments. This morphological classification also has been used recently by other investigators to classify hilar interneurons (Buckmaster and Schwartzkroin 1995a,b). The main morphological differences observed in the present study among these interneuron subpopulations were in the shape and dimension of the soma and the number of primary dendrites. Other differences were found in the degree of arborization, the length of the dendrites, and the spine density. Another recent classification, based mainly on the axon projections of interneurons, has been used by Han et al. (1993) and others (Mott et al. 1997; also see Freund and Buzsáki 1996). We did not perform an extensive study on the axon ramifications to compare the recorded interneurons with the subtypes proposed by these authors.

Because each morphologically identified interneuron subpopulation also could be differentiated by its synaptic connectivity, it is possible to speculate on functional roles of each interneuron subtype within the inhibitory circuit. With our experimental paradigm, we found that pyramidal-like stellate interneurons generated only GABA\textsubscript{A} receptor-mediated responses in CA3 pyramidal cells, whereas GABA\textsubscript{B} responses were generated only by spheroid and oviform interneurons.

Table 1 summarizes the characteristics of the three subtypes of interneurons. Pyramidal-like stellate cells were defined as GABA\textsubscript{A} interneurons because synchronized firing of the population elicited only the GABA\textsubscript{A} components in pyramidal cell sIPSPs. In addition, pyramidal-like stellate cells do not appear to participate in producing the GABA\textsubscript{B} response because the amplitude of the GABA\textsubscript{B} component of the sIPSP was not affected when the synchronized bursts in these cells were blocked by bicuculline.

Spheroid cells of the D-H border and oviform cells are likely candidates for the generation of GABA\textsubscript{B} events because their prolonged burst corresponded to the GABA\textsubscript{B} component in sIPSPs and because the bicuculline-resistant bursts were correlated temporally with pure GABA\textsubscript{B} sIPSPs in the pyramidal cells. These results are suggestive of a distinct population of GABA\textsubscript{B}-producing cells; however, because none of the pairs of interneurons and pyramidal cells

![Figure 5](http://jn.physiology.org/DownloadedFrom)
recorded in this study were connected monosynaptically, these results are not unequivocal. Recording directly from monosynaptically connected pairs of spheroid or oviform interneurons and pyramidal cells should confirm this hypothesis. In addition, because it is not possible to pharmacologically isolate depolarizing and hyperpolarizing GABA_A-mediated responses in interneurons, the present data cannot exclude the possibility that the GABA_A oviform or spheroid interneurons also contribute to the generation of the hyperpolarizing GABA_A response.

An unexpected finding is that spheroid interneurons received a chemical excitatory synaptic input that was not mediated by ionotropic glutamate or GABA receptors. The chemical nature of the synaptic depolarization sustaining the bicuculline-resistant burst in spheroid interneurons is demonstrated by its linear voltage dependence. This input is sufficiently large at resting potential to generate suprathreshold firing and therefore is likely to be an important factor in the synchronization of these interneurons. The nature of the excitatory input is still under investigation, but neither metabotropic glutamate receptors nor β-adrenergic or muscarinic cholinergic receptors appear to be involved, as (S)-(−)-methyl-4-carboxyphenylglycine, propranolol, and atropine did not affect the GABA_A component of the sIPSPs.

**TABLE 1. Summary of morphological and physiological properties of hilar interneurons subtypes**

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<thead>
<tr>
<th>GABA_A Interneurons</th>
<th>GABA_B Interneurons</th>
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<tr>
<td><strong>Pyramidal-like stellate cells</strong></td>
<td><strong>Spheroid cells of the D-H border</strong></td>
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<tr>
<td>Event related with sIPSPs in CA3 pyramidal cells (control)</td>
<td>Short burst (coincident with GABA_A components)</td>
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<tr>
<td>Event related with GABA_B sIPSPs in pyramidal cells (bicuculline)</td>
<td>No Burst</td>
</tr>
<tr>
<td>Synaptic inputs</td>
<td>GABA_A</td>
</tr>
<tr>
<td>Morphology</td>
<td>Large triangular soma</td>
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<tr>
<td></td>
<td>Stellate cells with few primary dendrites</td>
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GABA_A, γ-aminobutyric acid-A; D-H, dentate-hilus; sIPSP, synchronized inhibitory postsynaptic potential.
(unpublished observations). We recently have described a similar glutamate and GABA-independent synaptic depolarization in dentate granule cells which appears to be generated by the synchronous firing of interneurons (Forti and Michelson 1997). Because the experimental conditions in the present study were identical to those used in the previous study on dentate granule cells (Forti and Michelson 1997), it is likely that the synchronized firing of spheroid and/or oviform cells contribute to the generation of this novel synaptic depolarization in the granule cells. In addition, because the granule cell depolarization and spheroid interneuron depolarization are elicited under identical conditions, it is probable that the glutamate and GABA-independent depolarizations in these two cell populations either share a common input or are generated by a similar mechanism.

In contrast to spheroid interneurons, the synchronized bursts in oviform interneurons occurred within a limited range of membrane potentials and were likely to be driven by nonsynaptic mechanisms. The observation of dye coupling selectively among this interneuron subtype suggests that these cells may be coupled electrotonically and is consistent with previous findings demonstrating dye coupling among interneurons with picrotoxin-resistant bursts that were not sustained by an underlying synaptic event (Michelson and Wong 1994).

In conclusion, three subpopulations of hilar interneurons have been identified in the present study, each with distinct morphological properties and synaptic connectivity. These findings indicate that hilar interneuron subpopulations are differentially recurrently connected via various excitatory pathways, including depolarizing GABA synaptic pathways and nonsynaptic pathways. Uniquely, spheroid interneurons are interconnected via a novel, and as yet, unidentifed, excitatory synaptic mechanism that is not mediated by either glutamate or GABA. Together, the intrinsic and projectional differences among these interneuron subtypes suggest that each subpopulation of interneuron plays a specific physiological role within the hippocampal circuit.

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Address for reprint requests: H.B. Michelson, Dept. of Pharmacology, Box 29, State University of New York Health Science Center at Brooklyn, 450 Clarkson Ave., Brooklyn, New York 11203.

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