Synchronous Bursting in a Subset of Interneurons Inhibitory to the Goldfish Mauthner Cell: Synaptic Mediation and Plasticity

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SUMMARY AND CONCLUSIONS

1. Presynaptic activity in the inhibitory network impinging on the Mauthner (M-) cell was investigated in the goldfish medulla in vivo using extra- and intracellular recordings. The inhibitory presynaptic volley elicited by stimulation of the contralateral vestibular nerve consisted of multiple successive peaks at high frequency (up to 1,000 Hz). Less pronounced multicomponent responses were recorded after antidromic activation of the M-cell. Such high-frequency “oscillatory” field potentials also occurred spontaneously.

2. In intracellular recordings, a subset of inhibitory interneurons showed evoked and spontaneous burst discharge. Burst action potentials were correlated with the peaks in the extracellular volley, suggesting that repetitive firing of these cells is synchronized. Nonbursting cells, on the other hand, fired single action potentials in response to vestibular stimuli and were not activated via the M-cell collateral network.

3. Bursting cells were determined morphologically to be part of the feedback inhibitory circuit. Their responses to stimulation of the contralateral vestibular nerve thus suggest the existence of a crossed excitatory pathway to these interneurons.

4. Vestibular-evoked excitatory postsynaptic potentials (EPSPs) in bursting interneurons had a short latency of 0.781 ± 0.08 ms (mean ± SD, n = 18) but reached threshold at 2.25 ± 1 ms (n = 21). These characteristics are suggestive of a chemically mediated EPSP. Indeed, the evoked synchronous repetitive activity of these cells was prevented by superfusion with excitatory amino-acid receptor antagonists.

5. Bursting neurons showed several characteristics that differentiate them from nonbursting cells, including brief action potentials, plateau responses, and intense spontaneous subthreshold activity.

6. With extracellular recordings, tetanization of contralateral vestibular primary afferents evoked a long-lasting potentiation of oscillatory population responses in 11 of 27 cases. Furthermore, in three experiments, the frequency of occurrence of spontaneous bursts was enhanced and a similar facilitation was detected at the intracellular level.

7. We conclude that a subset of interneurons in this inhibitory network is capable of repetitive discharges and that evoked as well as spontaneous firing in this population is synchronized. Although electrical coupling between interneurons may mediate synchronization and intrinsic membrane properties may promote burst activity, our data suggest strongly that repetitive firing requires chemically mediated transmission. Furthermore they indicate that the mechanisms underlying evoked as well as spontaneous bursting in this population show activity-dependent plasticity.

INTRODUCTION

The Mauthner (M-) cell is a paired medullary command neuron of telosts that is responsible for the initiation of the escape reflex, a vital and adaptive behavior (DiDomenico et al. 1988; Eaton and Bombardieri 1978). This neuron is subject to a strong synaptic inhibition mediated by two pathways (Fig. 1A), a feedback collateral network activated by an impulse in either M-cell (Furukawa and Furshpan 1963) and a bilaterally projecting set of commissural vestibular interneurons (Triller and Korn 1978).

Axons of both classes of inhibitory neurons terminate in the axon cap, a specialized neuropil surrounding the initial segment of the M-axon (Faber and Korn 1978). The high extracellular resistance of this region facilitates the recording of the inhibitory presynaptic activity that follows either antidromic stimulation of the M-cell axon in the spinal cord or a vestibular stimulus. Because presynaptic impulses propagate passively within the axon cap (Faber and Korn 1989), this volley is detected as a positive field potential, also called the extrinsic hyperpolarizing potential (EHP) (Furukawa and Furshpan 1963) because it electrically inhibits the M-cell.

This study was initiated by the observation that the EHP evoked by a contralateral vestibular stimulus can display multiple components that follow each other at high frequency (500–1,000 Hz). This waveform could in principle be due to variations in conduction velocity of the active fibers. However, it also could reflect synchronized repetitive activity in these processes. Thus we have used intracellular and extracellular recordings combined with morphological techniques to investigate the origin and electrophysiological characteristics of this presynaptic oscillatory activity. Our results show that, indeed, one subset of inhibitory interneurons displays both evoked and spontaneous high-frequency discharges. Repetitive burst-like action potentials occur in a variety of cell types in the central nervous system (Llinás 1990) and also have been observed in inhibitory neurons (Andersen et al. 1963; Jones and Buhl 1993; McCormick et al. 1985; Schwartzkroin and Mathers 1978; Willis 1971). However, synchronized bursting of inhibitory interneurons has not been described under normal conditions, although it may occur under conditions of heightened excitability (Michelson and Wong 1991). Although we are not able to accurately determine the mechanism of this synchronous activity, our data strongly suggest that burst firing depends on chemical excitatory neurotransmission. At the same time, the implicated neurons appear to have intrinsic properties facilitating repetitive firing, such as short action potentials and plateau potentials. Furthermore we present evidence that synchronous bursting can be potentiated, for long term periods, by a brief tetanization of the vestibular afferents, indicating that this phenomenon...
has plastic properties that may contribute to a persistent, activity-dependent enhancement of crossed inhibition.

METHODS

Electrophysiological procedures

Experiments were performed in vivo on common goldfish 10–15 cm in length (*Carassius auratus*). The fish were anesthetized with MS 222 and immobilized with d-tubocurarine.

All recordings were obtained using either an Axoprobe-1A amplifier or an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) operated in the bridge mode. Recordings from the axon cap and intracellular recordings from the M-cell were obtained with either KCl (3 M)- or KAcetate (3 M)-filled microelectrodes of 2–4 and 10–20 MΩ resistance, respectively. Intracellular recordings of presynaptic interneurons were done using KCl-filled micro-electrodes of resistances 20–50 MΩ. Measurements were made at resting membrane potential, and impalements were discarded if spikes had amplitudes of <50 mV. Simultaneous recordings from inhibitory interneurons and the ipsilateral M-cell were obtained using the compensation circuit of the Axoprobe-1A amplifier adjusted to minimize capacitative and resistive coupling artefacts.

Initially, the M-cell axon cap was localized on the basis of the extracellular negative M-cell antidromic spike (Furshpan and Furukawa 1962) (Fig. 1). The negative focus was used as a reference point for finding the interneurons of interest and determining recording sites relative to the axon cap.

Antidromic activation of the M-cell was secured through a bipolar electrode placed on the posterior spinal cord. Vestibular stimuli were delivered at a frequency of 1–0.5 Hz to the posterior branch of the eighth nerve contralateral to the recording site via microstimulating bipolar steel electrodes that were insulated except at the tips. In conditioning experiments, tetani consisted of short trains of 10–20 pulses at 300–500 Hz applied at 2-s intervals for 1–3 min. The intensity of the conditioning stimulus was adjusted to be either just below or well above the threshold for the activation of the ipsilateral M-neuron, as determined at the onset of each experiment. In one experimental series, the excitatory amino-acid antagonists 6-cyano-7-nitroquinoxaline-2,3 dione) (CNQX, 50 μM) and D,L-2-amino-5-phosphonovleric acid (APV, 100 μM) were applied by superfusion in saline.

Numerical values are given as means ± SD. Significance levels were determined using Student’s *t* test.

Morphological methods

Microelectrodes (80–100 MΩ) were filled with biocytin (3%; pH, 7.3) dissolved in 0.5 M KCl. The dye was injected iontophoretically using positive-current pulses (400 ms, 2 nA) delivered at 0.5 Hz. After 2–8 min of injection, the preparation was left undisturbed for 15 min to allow for diffusion of the tracer. Fish were perfused intracardially with cold, freshly prepared 4% paraformaldehyde in 0.12 M phosphate-buffer for 20 min. The brains were removed, postfixed overnight in 4% paraformaldehyde and 40% sucrose in phosphate-buffered saline (PBS), and sectioned with a microtome. The slices (60 μm) were collected in 0.25% ammonium chloride in PBS, to block free aldehyde groups. After washing in PBS, the slices were allowed to react for 3 h at room temperature with streptavidin coupled to fluorescein isothiocyanate (Amersham) diluted in PBS (1/200) in the presence of the 0.12% of gelatin and 0.25% Triton X-100. The slices were washed extensively, mounted with citifluor (Amersham) and examined with a Zeiss microscope equipped for fluorescence.

RESULTS

Composite presynaptic volleys

As expected from the circuit diagrammed in Fig. 1A, an EHP is recorded in the right axon cap after activation of inhibitory neurons via either antidromic stimulation of the M-cell axon in the spinal cord (Fig. 1B) or a contralateral (left) vestibular stimulus (Fig. 1C). The superimposed recordings in Fig. 1C show that, depending on stimulus strength, the vestibular evoked EHP either consists of a single positive peak or displays a complex waveform with up to six successive peaks at intervals corresponding to frequencies of 500–1,000 Hz. Under control conditions (stimulation at 0.5 Hz), the number and amplitude of these peaks remain remarkably stable over long recording periods (up to 1 h). Similar oscillations usually were observed in the antidromically evoked EHP (Fig. 1B) but were of smaller amplitude and usually consisted of no more than three peaks.

In some experiments, oscillatory field potentials occurred spontaneously in the axon cap (Fig. 1D). These spontaneous events appeared at variable rates (up to 2/min) and showed frequencies similar to those of evoked fields, but they were smaller in amplitude than evoked oscillations.

Physiological identification of bursting neurons

The extracellularly recorded multiple components in the EHP could be a consequence of synchronous repetitive activity of inhibitory neurons terminating in the axon cap. In intracellular recordings, these neurons can be identified by the presence of a passive hyperpolarizing potential (PHP) (Fabér and Korn 1973; Korn and Fabér 1975), which is imposed by the current flowing in the axon cap during an action potential in the M-cell. Recordings were obtained from 80 PHP-exhibiting neurons located 50–100 μm anterior and 20–80 μm medial to the axon cap. Of these, 67 displayed burst-like repetitive action potential discharges in response to vestibular stimulation, with spike frequencies ranging from 570 to 1,000 Hz (772 ± 118, n = 17). The maximum number of action potentials elicited by strong vestibular stimuli ranged from three to nine. In a random sample of 18 cells, the mean number of spikes was 4.6 ± 1.46. Furthermore, in all cells that fired repetitively after a vestibular stimulus, one to three action potentials also were elicited by antidromic activation of the M-axon.

As illustrated in Fig. 2A, both after M-cell activation (A, 1) and after a vestibular stimulus (A, 2), the action potentials of individual bursting cells were well correlated with oscillations in the EHP recorded in the axon cap. The number of action potentials elicited showed the same dependence on stimulus strength as did the number of peaks in the EHP (not shown). Therefore, these cells discharge repetitively and synchronously, accounting for the burst-like extracellular volley observed in the axon cap.

In contrast, 13 of 80 PHP-exhibiting interneurons did not respond to a spinal stimulus at threshold intensity for antidromic activation of the M-cell (Fig. 2B, 1), and they fired only a single short-latency (from 0.2 to 0.5 ms) action potential in response to vestibular stimulation (Fig. 2B, 2).
FIG. 1. Oscillatory field potentials evoked in the Mauthner (M-) cell axon-cap by inhibitory networks. A: Experimental set-up and schematic diagram of the M-cell and its inhibitory afferents. Two classes of interneurons—collateral (coll.) and commissural (comm.)—terminate in the axon-cap (shaded area). The cell bodies of commissural cells are located in the vestibular nucleus and are synaptically driven by eighth nerve (VIII) stimulations. The collateral network can be activated disynaptically via the M-axon in the spinal cord. Extracellular potentials were monitored from the vicinity of the axon hillock, inside the axon cap. B: extracellular recording of the M-cell antidromic action potential (*) and the subsequent oscillatory extrinsic hyperpolarizing potential (EHP) evoked by a spinal stimulus. C: typical field potentials produced by stimulation of the contralateral eighth nerve at increasing stimulus strengths. A weak stimulation (2.5 V) produced a single short-latency positivity whereas after a stronger one (7 V), the EHP exhibited high-frequency multiple peaks (1,000 Hz) and an enhancement of the initial component (same experiment as in B). D: single spontaneous extracellular burst of spikes associated with a local field potential. Note the smaller amplitude than the evoked EHP. Traces in B and C are averages of 8 successive sweeps.

The latency of the single spike of these “nonbursting” cells corresponded well to the first peak of the vestibular-evoked EHP (Fig. 2B, 2).

It is interesting to note that the differences in firing behavior between bursting and nonbursting cells are matched by the duration of their action potentials. Measured from onset to return to baseline, those of bursting cells were significantly shorter (0.92 ± 0.07 ms, n = 11) than those of non-bursters (1.49 ± 0.42 ms, n = 11, P < 0.001).

Morphological identification of bursting interneurons

The fact that bursting neurons were activated after an antidromic M-cell action potential makes it likely that they belong to the recurrent collateral network. However, they also were activated by stimulation of the contralateral eighth nerve. Such responses are characteristic of commissural cells, which are second-order vestibular neurons (Triller and Korn 1978, 1981; Zottoli and Faber 1980) and have not been described for collateral inhibitory interneurons. Thus to obtain a more unequivocal identification, five bursting neurons were injected with biocytin and visualized using fluorescence staining. In all cases, their morphological characteristics were those of collateral interneurons (Fig. 3), that is, the cells remained entirely ipsilateral to the recording site, with round cell bodies situated 200–500 μm ventral to the M-cell and in lateral juxtaposition to the ventral dendrite. The neurons showed typical bipolar morphology, with a single dendritic process coursing in a ventral direction and a dorsally directed axon that terminated exclusively on the M-cell (Korn et al. 1978).

Synaptic mediation of bursting and synchronized activity in collateral interneurons

The data presented above indicate that synchronized repetitive firing of collateral inhibitory neurons is particularly pronounced in response to stimulation of the contralateral eighth nerve. Although activation of these neurons via M-cell axon collaterals has been studied in some detail (Hackett and Faber 1983), their excitatory responses to contralateral vestibular stimuli represent a novel finding. It is important to note that these cells could be activated via the contralateral (left) vestibular nerve at stimulus strengths that were well below threshold for activation of the left M-cell, indicating that they were not being excited through its axon collaterals. For example, in the case of Fig. 4, the firing threshold of the M-cell ipsilateral to the stimulated vestibu-
FIG. 2. Electrophysiological identification of bursting inhibitory cells. Simultaneous responses were recorded extracellularly in the axon cap and from single inhibitory neurons identified by the presence of a passive hyperpolarizing potential (PHP). A: responses of a bursting neuron (bottom) to stimulations of the M-axon (1) and of the contralateral vestibular nerve (2). Both protocols induced repetitive firing (below) with action potentials appearing in phase with the oscillatory waves of the EHP (top). B: responses of a nonbursting neuron to identical stimuli as in A, but in a different preparation. Note that this cell did not fire in response to the antidromic stimulus (1) and that only a single action potential was triggered by a vestibular input (2) that was sufficient in intensity to provoke an oscillatory population response. Inset in A and B: superimposed averages (n = 8) are indicated of the intracellularly recorded PHP (*) and the corresponding field potential obtained immediately after leaving the cell. The recorded neuron responded to a 3-V stimulus with a complex depolarizing potential, the shape of which fluctuated to a great extent (Fig. 4A). This response is typical of that evoked in these neurons by weak stimuli. Overall, the latency of the first component, as determined in 18 neurons, was 0.78 ± 0.08 ms, ranging from 0.63 to 0.91 ms. The postsynaptic potential increased in amplitude with stimulation strength, eventually exceeding threshold for spike generation (Fig. 4B). In such bursting neurons, firing threshold was reached first late in the excitatory postsynaptic potential (EPSP), with fluctuating latencies of >1 ms (range 1.2 to 4.6 ms, 2.25 ± 1.11, n = 22). This is in contrast to nonbursting cells, which invariably discharged on an early electrically mediated EPSP component, with fixed spike latencies of <1 ms (cf. Fig. 2B).

With further increases in stimulus strength, multiple spikes could be discharged (Fig. 4, C and D). At the same time, the latency of the first spike decreased and the variation in latency of all discharged spikes was reduced. At high-stimulus strengths, the fluctuation in latency was smallest for the first spike and increased from the second to the last spike. This fact is in line with the decreasing amplitudes of successive peaks in the field potential oscillations, suggesting that the degree of synchrony of firing decreases for later peaks.

As shown in Fig. 5, neurons that burst to eighth nerve stimuli (5A, 1) spontaneously fired high-frequency action potential trains (5A, 2), with frequencies similar to those of spontaneous bursts observed extracellularly (cf. Fig. 1D). This bursting appeared linked to spontaneous brief depolarizations that resembled postsynaptic potentials and could reach action potential threshold (Fig. 5A, 3). Some of these events had rapid time courses, similar to those of electrotonically mediated potentials that can be observed in the M-cell dendrite (Lin and Faber 1988) and in vestibular second order neurons (Korn et al. 1977). Finally an interesting observation in spontaneously active cells (n = 6) was the occurrence of spontaneous (Fig. 5A, 4) or stimulus-evoked (Fig. 5A, 2) plateau potentials. As shown in Fig.

FIG. 3. Morphological characteristics of a biocytin-injected bursting interneuron. A: photographic reconstruction showing that this cell is a recurrent collateral interneuron. The soma (S) is located ventrally, the axon (Ax) runs dorsally, divides into a few branches (→) and impinges on the ipsilateral M-cell soma (M, — — —) and within the axon-cap (Ac, · · ·). A single dendrite (arrowheads) issued from this cell courses laterally and ventrally towards the edge of the brainstem. B: detailed view of the terminal portion of the stained dendrite showing its characteristic convoluted aspect in the vicinity of the brainstem edge (crossed arrows). This reconstruction was obtained at different depths of focus from 2 consecutive sections. Dorsal is up and medial is right. Calibration bars: 40 μm.
Synchronous bursting in inhibitory interneurons

**FIG. 4.** Vestibular activation of bursting cells. The contralateral eighth nerve was stimulated at the indicated intensities. A: subthreshold stimuli elicited fluctuating excitatory postsynaptic potentials (EPSPs), and spike threshold was reached on a late phase of the synaptic depolarization. In this high-gain recording, the spike is truncated. B-D: as the stimulus strength was increased slightly, the latency of the spike was reduced progressively. Increased strengths evoked repetitive firing (C) and high-frequency bursts of action potentials (D). This activity was associated with a reduction of the first spike's latency; note the stability of the first and second action potentials in the burst (9 V). Four successive responses are superimposed in each panel.

**FIG. 5.** Different patterns of activity of bursting collateral interneurons. A: spontaneous firing modes (except in A, 1) exhibited by a single collateral interneuron during an 8-min recording session. A, 1: repetitive discharge induced by vestibular stimulus; 2: spontaneous spike train; 3: single discharges (spikes truncated) and underlying subthreshold depolarization. Depolarizations (*) with rapid time courses are indicated (see text). 4: plateau potentials. Same voltage calibration in 1, 2, and 4. B: alternating firing patterns in response to a vestibular stimulus. This cell generated 3 action potentials and rapidly repolarized (1) or exhibited a plateau, shown superimposed with the former response in (2). Note the long-lasting after-hyperpolarization following the plateau.

5 B, 1 versus 2, a fixed vestibular stimulus could alternately evoke normal repetitive discharges or the plateau response.

Electrotonic coupling is known to mediate vestibular excitation of commissural inhibitory neurons (Triller and Korn 1981; Zottoli and Faber 1980). However, the time course of the vestibular-evoked EPSP and the latency of the spike at threshold in bursting neurons are both suggestive of chemically mediated synaptic transmission. This hypothesis was tested using the oscillatory field potential as an indication of the activation of bursting neurons.

As shown in the example of Fig. 6, superfusion of the preparation with saline containing CNQX (50 μM) and APV (100 μM) suppressed population oscillations recorded in the axon cap in 9 of 12 experiments, indicating that burst firing in this population is dependent on glutamatergic synaptic transmission. In five of nine cases this effect was reversible after perfusion with normal saline for ~1 h.

**Plasticity of vestibular-evoked bursting responses**

It has been demonstrated recently that tetanization of ipsilateral vestibular afferents induces long-term potentiation (LTP) of the mixed excitatory synaptic transmission to the M-cell lateral dendrite (Yang et al. 1990). We therefore have tested the effect of tetanic stimulation (see METHODS) of the contralateral posterior vestibular nerve on bursting activity of collateral inhibitory interneurons.

In a first series of experiments, the synchronized activity of this population was monitored by recording the eighth-nerve-evoked EHP in the axon cap. In 11 out of 27 cases, tetanization induced a long-lasting potentiation of peaks in the EHP elicited by a given stimulus intensity. When tetani were successful, the degree of potentiation depended on the stimulation strength used for conditioning. The maximal increase in amplitude obtained in seven experiments using subthreshold tetanization (see METHODS) averaged 24.3% (range 6.2–82.6%) and 43.0% (range 6.1–143.5%) for the first and second peak, respectively. In four instances, suprathreshold stimulation yielded mean increases in the corresponding peaks of 68.1% (range 25.4–114.6%) and 215.1% (range 35.0–436.6%). After successful conditioning, the EHP remained potentiated in the manner described above as long as the recording conditions remained stable, that is, for up to 90 min. In the remaining 16 conditioning experiments, 12 subthreshold and 4 suprathreshold tetani did not cause detectable potentiation of the EHP. In the example shown in Fig. 7, three additional peaks appeared late in the EHP whereas the amplitude of the preexistent...
Potentiation of oscillatory field potentials by tetanization of contralateral vestibular afferents. A: averaged (n = 8) extracellular field potentials recorded in the contralateral axon cap in response to eighth nerve stimulation at constant strength, obtained before (-10') and after (+32') a tetanus. B: time course of change in the amplitude of the first (●) and second (●) peaks, and the previously absent third (●) and fourth (●) ones. Note the rapid and persistent potentiation of all components after conditioning. The amplitude of each evoked response was measured from the baseline to its peak, and each data point represents the average of 28 traces. The period of the suprathreshold tetanization (trains of 10 pulses at 500 Hz, every 2 s during 2 min) is indicated by the shaded box.

Components 1 and 2 increased. A similar emergence of one to three additional peaks was observed in all successful "suprathreshold" tetanization experiments. These findings indicate that synchronized repetitive firing can be enhanced by tetanic stimulation of primary vestibular afferents, albeit with great variability and a limited success rate. In confirmation, Fig. 8 illustrates results of recording intraxonally from two different bursting neurons. In Fig. 8 A, a test stimulus to the contralateral eighth nerve elicited a biphasic, subthreshold EPSP in the control (A, 1), whereas 7 min after a tetanus this stimulus produced one or two action potentials (A, 2). The same cell responded to a stronger stimulus with maximally two action potentials before (Fig. 8 B, 1) and three action potentials after (Fig. 8 B, 2) tetanization. Thus in this case, tetanization led to increased repetitive firing, associated with a lowering of the minimal stimulus strength for action-potential generation. Table 1 summarizes comparable results obtained from six PHP-exhibiting neurons that could be recorded stably for sufficient time to test for the presence or absence of facilitation after a vestibular tetanus. All four bursting neurons showed enhanced responses after a train. Of the two nonbursting neurons tested, one was recruited with a previously subthreshold stimulus. No facilitation was observed, however, for suprathreshold test stimuli in nonbursting cells, that is, they did not fire repetitively after conditioning.

Potentiation of spontaneous burst firing

In the axon cap, unprovoked extracellular bursts were observed in six experiments before conditioning. Peaks in these oscillations occurred at high frequency (>1,000 Hz, i.e., higher than that observed in single cells) and had variable amplitudes. It is thus unlikely that these events reflect activity of a single unit. Rather, they appear to be produced by an assembly of neurons discharging simultaneously. In three preparations, an important background of such bursting activity was present under control conditions, as exemplified in Fig. 9 A. In this experiment, the spontaneous bursts occurred at a frequency of 8.8 ± 3.5/minute. After conditioning, this value increased by 61% to 14.2 ± 5.8 (P < 0.05, unpaired t test). Changes in the form of these bursts were analyzed using 10 events selected at random before and after tetanization. The mean number of detectable peaks per burst increased by 80% (from 9.0 ± 4.69 to 16.2 ± 8.75) whereas the mean burst duration increased by 57.5% (from 4.7 ± 2.13 to 7.4 ± 3.74 ms). Similar results were found in the other two experiments. In the longest one, spontaneous bursting remained potentiated for 50 min after the conditioning train.

The longer duration of bursts could be explained by prolonged repetitive firing in single cells. The increased intra-burst frequency, however, points to the possibility that additional neurons were recruited into the spontaneously active population. This hypothesis could be confirmed by one intraaxonal recording, which is illustrated in Fig. 9 B. This cell, which is the same as that shown in Fig. 8, was not spontaneously active before tetanization. However, there were small subthreshold depolarizations that presumably

![FIG. 7. Potentiation of oscillatory field potentials by tetanization of contralateral vestibular afferents. A: averaged (n = 8) extracellular field potentials recorded in the contralateral axon cap in response to eighth nerve stimulation at constant strength, obtained before (-10') and after (+32') a tetanus. B: time course of change in the amplitude of the first (●) and second (●) peaks, and the previously absent third (●) and fourth (●) ones. Note the rapid and persistent potentiation of all components after conditioning.](http://jn.physiology.org/)
represent spontaneous postsynaptic potentials (Fig. 9 B, I). Their frequency and amplitude were increased after tetanization. At the same time, the cell became spontaneously active, with action potentials appearing in bursts, or as isolated, single events (Fig. 9 B, 2). Thus after the tetanus, this cell was both recruited into the active population after a weak vestibular stimulus (Fig. 8 A) and converted into a spontaneously bursting neuron. The augmentation of spontaneous subthreshold events was observed in two other neurons that also displayed spontaneous bursting activity after conditioning.

**Synaptic efficacy of bursting cells**

To confirm the inhibitory function of this subpopulation of interneurons, we performed simultaneous intracellular recordings from them and the M-cell.

As shown in Fig. 10, after iontophoretic injection of chloride into the postsynaptic cell, antidromic stimulation of the M-axon (A), and stimulation of the contralateral eighth nerve (B) both produced depolarizing inhibitory postsynaptic potentials (IPSPs) in the M-cell, reflecting activation of the collateral and commissural networks, respectively. The presynaptic cell exhibited repetitive discharges that began just before the collateral and vestibular IPSPs (Fig. 10, A and B, bottom). Direct activation of the presynaptic neuron produced a typical unitary IPSP (Fig. 10 C) (cf. Korn and Faber 1976). The amplitude of unitary responses to activation of single bursting cells averaged 5.6 ± 3% (ranging from 1.5 to 7.5%, n = 9) of the full collateral IPSP. These values are comparable with those previously obtained (Faber and Korn 1982) suggesting that there may not be a fundamental difference in synaptic strength of unitary IPSPs produced by the bursting neurons and other PHP cells.

**TABLE 1. Changes in responses of inhibitory interneurons after tetanic stimulation**

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Indicated responses are maximal responses observed after stimulation of the contralateral posterior VIIIth nerve. Tetanus (TET) strength was adjusted to be either supra- or subthreshold for activation of the ipsilateral M-cell. RMP and spike amplitude values expressed as mV. EPSP, excitatory postsynaptic potential; RMP, resting membrane potential.

**FIG. 9.** Enhancement of spontaneous burst activity after afferent tetanization. A: frequency histogram of spontaneous extracellular bursts (per 5-min interval) showing an enhancement after the tetanus (>). The mean frequencies and standard deviations are as indicated (tot.: 10 pulses, 500 Hz, 1/2 s, 2 min, I = 0.7 T); sample trace shown (inset) of discharge recorded during the control session. B: potentiation of spontaneous discharge in a single bursting cell. 1: 3 periods of spontaneous activity recorded before conditioning; 2: this cell exhibited bursts of action potentials (20 min after the tetanus) associated with an increased frequency of the subthreshold depolarisations. Recordings are from the same cell as that of Fig. 8.
We identified with intracellular recordings a subset of inhibitory interneurons that, upon stimulation of contralateral vestibular afferents, showed repetitive discharges correlated with the peaks in the extracellular presynaptic volley. These cells were also capable of repetitive discharge in response to antidromic activation of the M-cell, with action potentials again correlated with the peaks in the EHP. The electrophysiological findings are complemented by morphological data that indicate that the bursting neurons belong to the ipsilateral collateral network described previously (Korn et al. 1978). We therefore conclude that the oscillatory waves in the presynaptic volley or EHP are due to synchronous bursting of collateral inhibitory interneurons.

On the other hand, we recorded from inhibitory neurons that only fired single action potentials in response to vestibular stimulation, and these cells were not activated by stimulation of the M-cell axon. This fact, together with the short latency of the impulse evoked by vestibular stimuli, makes it likely that nonbursting neurons are identical to the second order commissural inhibitory neurons described by Zottoli and Faber (1980) and Triller and Korn (1981). The fact that oscillatory components are never observed in the axon cap ipsilateral to the stimulated vestibular nerve (see also Furukawa et al. 1963) reinforces the conclusion that these commissural cells do not fire repetitively, as they have been shown to terminate in both axon caps (Triller and Korn 1981) (Fig. 11). The single action potential discharged by these cells after contralateral vestibular stimulation coincides with the first peak of the EHP and will thus contribute to its amplitude.

DISCUSSION

Origin of presynaptic oscillatory activity

The organization of inhibitory afferents to the M-cell soma is unique in that it allows detection of presynaptic population activity as well as electrophysiological identification of single presynaptic neurons in vivo. This report is concerned with the origin and significance of oscillatory high-frequency waves in the presynaptic volley (or EHP). In the present experiments, oscillations already described by Furukawa and Furshpan (1963) were particularly prominent in response to stimulation of the contralateral vestibular nerve at higher intensities than used in other studies, where stimuli had been deliberately adjusted to give a single peak in the EHP (Korn et al. 1992; Mintz and Korn 1991).
Activation of collateral interneurons via contralateral vestibular afferents

Excitation of collateral inhibitory neurons via contralateral vestibular afferents, although presumed in earlier discussions of the circuit (Faber and Korn 1978), has not been demonstrated. Although primary saccular fibers have been reported to cross the midline in one teleost family (Mormyridae) (Bell 1981), vestibular nerve fibers are assumed generally to remain ipsilateral (McCormick 1981). Thus direct excitatory connections between contralateral primary vestibular afferents and the collateral interneurons are unlikely.

Vestibular-evoked EPSPs in collateral cells, in fact, did not resemble those found in the M-cell lateral dendrite (Lin and Faber 1988) or in secondary vestibular interneurons (Korn et al. 1977; Zottoli and Faber 1980), which are directly contacted by the primary afferents. In these latter neurons, vestibular stimulation gives rise to a biphasic electrical-chemical EPSP, and spike threshold is generally reached on the electrical "short-latency depolarization," resulting in a fixed, fast-occurring impulse (cf. Fig. 2B, 2). In contrast, the EPSPs set up by contralateral vestibular stimuli in collateral bursting neurons, exhibit, albeit a short-onset latency, a complex and varying waveform that reaches firing threshold after a slow rising phase. This synaptic response has a significantly longer latency than the short-latency depolarization observed by Zottoli and Faber (1980) in commissural second order neurons. It is therefore likely that it is a chemically mediated EPSP. The suppression of vestibular-evoked synchronized population discharges after block of excitatory amino acid receptors further indicates that glutamatergic transmission is a necessary component of the pathway mediating this response. Given the anatomic constraints mentioned above, this network is likely to be di- or polysynaptic, as indicated in the schematic representation in Fig. 11. To explain the short latency to onset of vestibular-evoked EPSP, however, one or more connections involved would have to be electrical or mixed. Such crossed vestibular pathways have been described in other vertebrates (Shimazu and Smith 1971).

Synchronization of collateral interneurons

A straightforward explanation of evoked synchronous discharge in these neurons is that with increasing stimulus strengths, a progressively larger long-lasting chemical EPSP is generated to which a homogeneous population of neurons responds in a similar way. In line with this assumption, synchrony of firing was most pronounced early in an evoked repetitive discharge, as evident from the decreasing amplitude of successive extracellular peaks and fluctuations in spike latency. The bursting response pattern then would be due to postsynaptic intrinsic conductances common to these interneurons, which, for instance, appear to keep action potentials short compared with those of non-bursting cells, thus conveying the ability to discharge at high rate. In addition, the plateau-like potentials we observed in bursting cells may be indicative of slow inward conductances, which have been shown to play important roles in burst firing of other neurons (Conway et al. 1988; Hounsgaard et al. 1988; Linås 1990). In fact, collateral interneurons have been shown to exhibit anodal break depolarizations, which could be due to a persistent sodium conductance (Faber and Korn 1983). The importance of intrinsic properties is underscored by the fact that synchronous repetitive firing of collateral cells also is observed in response to antidromic stimulation of the M-cell.

By direct current injection, we have, on the other hand, not been able to provoke plateau potentials or discharges at frequencies comparable with those induced by synaptic activation. This finding may be interpreted as evidence for a decisional role of synaptically gated conductances in burst firing. However, because in these experiments impalements may have been made at an axonal site, their somadendritic membrane may not have been sufficiently depolarized by injected current to trigger an intrinsic bursting mechanism.

A striking property of collateral interneuron activity is the occurrence of spontaneous population bursts, which indicate that the neurons can fire simultaneously in the absence of a large synchronous input. Peak amplitudes of spontaneous bursts varied between 5 and 50% of those of oscillations evoked by strong vestibular stimuli, but their individual components were not wider than those in the evoked EPSP, indicating that synchrony is achieved in a number of cells. Thus this synchrony may well be relevant with more natural activation of the circuit than electrical stimulation of the afferent nerve. Such spontaneous synchrony could be generated in a manner intrinsic to the neuronal population or be due to transmission of activity synchronized elsewhere. In the hippocampal slice preparation, for instance, spontaneous bursts generated by local excitatory connections between CA3 pyramidal cells (Miles et al. 1984) can be transmitted synaptically to other regions.

Although local excitatory interactions between the inhibitory neurons via Cl-mediated postsynaptic potentials are not inconceivable (Michelson and Wong 1991), they are unlikely in this situation, because collateral interneurons terminate exclusively on the M-cell (this report, and see Korn et al. 1978). Gap junctional contacts, however, may well exist, and rapid depolarizations reminiscent of junctional potentials were sometimes seen in these cells. Furthermore in this preparation, field effects are known to play a prominent role (Faber and Korn 1989). Inhibitory interneurons are weakly coupled through the extracellular space, allowing for excitatory field interactions that can contribute to synchronization of firing (Faber and Korn 1984). Burst firing mediated by field effects has been proposed in the hippocampus in vitro (Dudek et al. 1988; Haas and Jefferys 1984).

On the other hand, excitatory interactions are likely to exist between the cells of origin of the crossed excitatory network mediating vestibular excitation of collateral interneurons. Second-order vestibular neurons are connected through gap junctional contacts with primary afferent fibers (Korn et al. 1977). Spontaneous activity therefore could be synchronized in the presynaptic excitatory network and transmitted to collateral interneurons as a long-lasting EPSP giving rise to the observed repetitive discharge. In fact, preliminary data (not shown) indicate that burst discharges can be coincident with slow negative field potentials in the contralateral vestibular nucleus.
Potentiation of the presynaptic inhibitory network

It recently has been demonstrated that tetanization of the vestibular afferents induces LTP of the mixed synaptic transmission to second-order vestibular inhibitory neurons (Korn et al. 1992) as well as to the M-cell lateral dendrite (Yang et al. 1990). In this study, we found that the evoked and spontaneous discharges of bursting interneurons can be potentiated by a similar protocol applied to the contralateral eighth nerve. Intracellular recordings showed that both enhanced recruitment and increased repetitive firing of individual bursting neurons underlie the potentiated EHP. Activation of nonbursting (i.e., commissural) cells could be facilitated; however, these neurons did not show repetitive firing after conditioning. These findings are consistent with the hypothesis that the crossed connection between primary vestibular afferents and collateral inhibitory interneurons expresses use-dependent enhancement of synaptic efficacy. One likely site of potentiation is the first order synapse, analogous to that at the M-cell (Yang et al. 1990). However, we cannot exclude the possibility that modifiable synapses exist later on in a pathway that, following the anatomic constraints discussed above, should be at least disynaptic.

Functional implications of synchronous burst firing in inhibitory interneurons

Repetitive firing in response to synaptic activation is widespread in inhibitory neurons of many regions in the central nervous system, including spinal cord (Willis 1971), thalamic reticular nuclei (Steriade and Llinaš 1988), and various cortical areas (Andersen et al. 1963). The fact that collateral inhibitory neurons discharge repetitively at high frequencies raises the question of whether inhibitory synaptic transmission is efficient throughout such bursts and/or undergoes fatigue. A frequency-dependent decrease in quantum content has been previously observed at inhibitory synapses to the goldfish M-cell (Korn and Faber 1984). This decrease was apparent already at moderate frequencies (20–33 Hz). However, these measurements were obtained during continuous stimulation of the presynaptic neuron at a test frequency. The synaptic fatigue observed in this steady-state condition may take some time to develop. In fact, our data seem to indicate that transmission remains potent at least during the first few action potentials evoked at frequencies >250 Hz, while failing later on.

The interspike intervals observed in burst discharges of collateral interneurons are shorter than the time constant of decay of unitary IPSPs. Repetitive discharge thus will lead to effective summation of inhibitory conductances and increase the maximal conductance change undergone by the postsynaptic membrane. This summation will guarantee the persistence of a sustained inhibition. Furthermore, at the level of receptor interaction, high frequency synchronous repetitive release from adjacent boutons may, because of accumulation of transmitter, promote repeated receptor binding and, by broader diffusion, increase the recruitment of receptors located beyond the postsynaptic density (Faber et al. 1985). Both factors would further enhance the duration of the inhibitory response.

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