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Context-Dependent Synaptic Action of Glycinergic and GABAergic Inputs in the Dorsal Cochlear Nucleus

Nace L. Golding and Donata Oertel

Department of Neurophysiology, University of Wisconsin, Madison, Wisconsin 53706

Cartwheel cells are prominent interneurons in the dorsal cochlear nucleus (DCN) that bear considerable homology to cerebellar Purkinje cells. They contact other cartwheel cells as well as fusiform cells, the principal cells of the DCN. In fusiform cells, the inhibition from cartwheel cells interacts with excitation mediated by granule cells and auditory nerve fibers, and shapes the output of the DCN in its ascent to the inferior colliculi.

With intracellular recordings from anatomically identified cells in slices, synaptic inputs to fusiform and cartwheel cells were analyzed pharmacologically. Shocks to the auditory nerve and granule cell domains evoked glutamatergic, glycinergic, and $GABA_A$ergic postsynaptic potentials (PSPs) in both cartwheel and fusiform cells. The temporal patterns of spontaneous and evoked glycinergic PSPs in fusiform and cartwheel cells were similar and mirrored the pattern of firing of cartwheel cells, probably reflecting the anatomical connections between these cell types and supporting the conclusion that cartwheel cells are glycinergic. In fusiform cells, glycinergic and $GABA_A$ergic IPSPs evoked with shocks reversed at −68 mV on average. In marked contrast, glycinergic and $GABA_A$ergic PSPs in cartwheel cells, as well as responses to exogenous application of 50–100 mM glycine or 100 μM muscimol, were depolarizing. Reversal potentials of PSPs and responses to glycine and muscimol were similar and averaged −52 mV. Glycinergic and $GABA_A$ergic PSPs could elicit firing from cartwheel cells at their resting potentials, but could also reduce rapid firing during strong depolarizations. Thus, the action of glycinergic and $GABA_A$ergic inputs on cartwheel cells depends on the electrophysiological context in which they occur.

Key words: auditory pathways; brain slices; cochlear nuclei; hearing; parallel fibers; glycine; GABA

Auditory and nonauditory inputs converge on a circuit in the superficial layers of the dorsal cochlear nucleus (DCN) that bears extensive homology to that of the overlying cerebellar cortex (Mugnaini et al., 1980a,b; Berrebi and Mugnaini, 1991; Zhang and Oertel, 1993; Manis et al., 1994). Auditory inputs comprise both ascending and descending projections, including unmyelinated, type II auditory nerve fibers, as well as projections from neurons in the cochlear nucleus, superior olivary complex, inferior colliculus, and auditory cortex (Brown et al., 1988a,b; Brown and Ledwith, 1990; Caicedo and Herbert, 1993; Feliciano et al., 1993; Berglund and Brown, 1994; Golding et al., 1995; Weedman et al., 1995). Nonauditory inputs include vestibular afferents and somatosensory afferents from the dorsal column and cuneate nuclei (Itoh et al., 1987; Weinberg and Rustioni, 1987; Burian and Gestoettner, 1988; Kevetter and Perachio, 1989; Wright et al., 1994; Kim et al., 1995).

The role of these multimodal inputs in the processing of auditory information is not well understood. However, recent evidence from single-unit recordings *in vivo* indicates that the circuitry that integrates these inputs ultimately produces inhibition in fusiform cells, the principal cells of the DCN that project to the contralateral inferior colliculus (Osen, 1972; Ryugo et al., 1981; Oliver, 1984). In cats, natural somatosensory stimuli, as well as electrical activation of somatosensory afferents, result in robust inhibition of DCN principal cells, which lasts in the range of tens of milliseconds (Young et al., 1995).

How is this inhibition of fusiform cells produced? Both auditory and nonauditory inputs converge on cochlear granule cells, which are present in several areas in both the ventral and dorsal cochlear nuclei, and frequently receive brainstem inputs from mossy endings (Osen and Roth, 1969; Kane, 1974; Mugnaini et al., 1980a,b; Osen et al., 1984). The axons of granule cells form parallel fibers, which contact multiple targets in a molecular layer, including fusiform cells and two types of local inhibitory interneurons: cartwheel and superficial stellate cells (Kane, 1974; Wouterlood and Mugnaini, 1984; Wouterlood et al., 1984). Both cartwheel and superficial stellate cells in turn contact fusiform cells, forming feedforward inhibitory circuits (Wouterlood et al., 1984; Berrebi and Mugnaini, 1991).

The present experiments show that the conversion of excitation from granule cells to inhibition of fusiform cells not only involves conventional glycinergic and GABAergic inhibition of fusiform cells, but also weak excitation of cartwheel cells. Because the reversal potential for glycinergic and GABAergic postsynaptic potentials (PSPs) is only slightly above the firing threshold of cartwheel cells, these PSPs may reduce the firing rate under conditions of strong depolarization. These "inhibitory" neurotransmitters can thus promote slow firing.

MATERIALS AND METHODS

Slice preparation. Slices of the cochlear nuclear complex were prepared from mice (CBA strain) ranging in age from 18 to 26 d after birth. Details of techniques have been published previously (Oertel, 1985). Animals

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Correspondence should be addressed to Donata Oertel, Department of Neurophysiology, University of Wisconsin, 275 Medical Sciences Building, 1300 University Avenue, Madison, WI 53706.

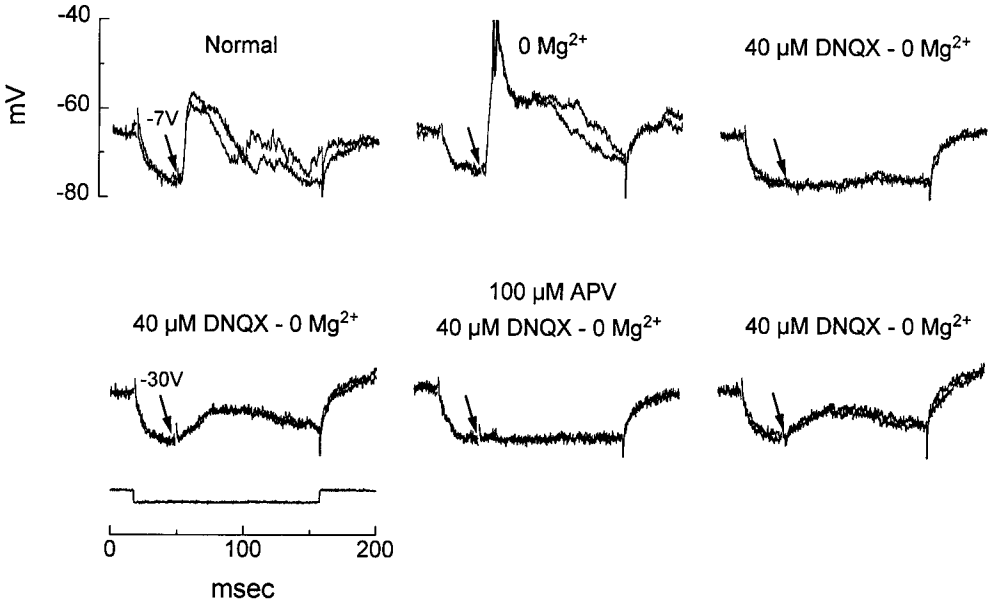

Figure 1. Shocks to superficial granule cells over the VCN activate both AMPA and NMDA receptors in a cartwheel cell. To reduce firing in the cell and to resolve underlying synaptic potentials, shocks were applied during a hyperpolarizing current pulse (−0.2 nA). Each panel shows two superimposed responses to identical shocks. In normal saline, the shock evoked a depolarizing response lasting ~100 msec (*upper left*). Removal of magnesium (Mg^{2+}) increased the size of the synaptic response (*upper middle*). The response was largely blocked by *DNQX* (*upper right*). A small residual depolarizing component remained, which was more apparent in response to stronger shocks (*lower left*). This component was reversibly eliminated by *APV* (*lower middle* and *right*). Strychnine (1 μM) was included in all magnesium-free solutions. *Arrows* indicate time of shock.

were decapitated and the brains were removed under oxygenated (95% O_2/5% CO_2) normal saline containing (in mM): 130 NaCl, 3 KCl, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 20 $NaHCO_3$, 3 HEPES, 10 glucose, 1.2 KH_2PO_4, pH 7.4. Parasagittal slices 200–350 μm thick were prepared using an oscillating tissue slicer (Frederick Haer, New Brunswick, ME). Slices were maintained in a chamber where they were submerged in rapidly flowing, oxygenated saline solution at ~34°C. Flow rates through the 0.3 ml chamber ranged from 9 to 12 ml/min.

For delivery of pharmacological agents during intracellular recording, the bathing solution was exchanged with the test solution without interruption of the flow. Strychnine, picrotoxin (PTX), DL-2-amino-5-phosphonovaleric acid (APV), 6,7-dinitroquinoxaline-2,3-dione (DNQX), tetrodotoxin (TTX), and bicuculline methiodide were obtained from Sigma (St. Louis, MO) and were dissolved in physiological saline.

In some experiments, glycine (free base, Sigma) or muscimol (Tocris Neuramin, Essex, UK) was applied exogenously with pressure pulses delivered through a picrospritzer (General Valve, Fairfield, NJ) via glass pipettes, the outer tip diameters of which ranged from 9 to 18 μm. The duration of pulses ranged from 3 to 800 msec at 15 psi. Glycine solutions were either 100 mM, dissolved in distilled water, or 50 mM, substituted in equimolar concentrations for sodium chloride in physiological saline. Muscimol was added to normal physiological saline solution at 100 μM.

Electrophysiological recordings. Intracellular recordings were obtained using sharp microelectrodes. Electrodes were filled with 1% biocytin (Sigma) in 2 M potassium acetate and had resistances of 120–200 MΩ. Membrane potential was monitored throughout the experiment on a chart recorder. Inputs to cartwheel cells were activated electrically by delivering shocks either to the cut end of the auditory nerve, the surface of the ventral cochlear nucleus (VCN), or the surface of the DCN dorsal to the recording site. Shocks (0.1–100 V amplitude, 100 μsec duration) were delivered through a pair of tungsten wires, insulated except at their 50 μm diameter tips. Synaptic responses were digitally sampled at 40 kHz, low pass-filtered at 10 kHz, and stored on computer using pClamp software (Axon Instruments, Foster City, CA). Responses to exogenously applied agonists were sampled between 0.67 and 1.3 kHz.

Morphological identification of recorded cells. To label cells during impalement, biocytin was ejected from the electrode using 0.5–2 nA depolarizing current pulses of 100 msec duration delivered at a rate of 2.5 pulses/sec for up to 5 min. Slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for periods ranging from 24 hr to 4 weeks. Slices were subsequently embedded in a mixture of gelatin and albumin and sectioned on a vibratome at 60 μm. The sections were reacted with avidin conjugated to horseradish peroxidase (Vector ABC kit, Vector Laboratories, Burlingame, CA) (Horikawa and Armstrong, 1988; King et al., 1989) and processed for horseradish peroxidase (Wickesberg and Oertel, 1988) using Co^{2+} and Ni^{2+} intensification (Adams, 1981). Sections mounted on coated slides were counterstained with cresyl violet.

All recordings shown in the figures are from separate, anatomically identified cells with one exception. The traces in Figures 4*A* and 6*A* are from the same cell.

RESULTS

The present results are based on intracellular recordings from 61 cartwheel cells and 23 fusiform cells that were labeled with biocytin and identified morphologically. Labeled cartwheel and fusiform cells resembled those described previously in slices (Oertel and Wu, 1989; Zhang and Oertel, 1993, 1994). Cartwheel cells exhibited resting potentials of −63 ± 5 mV (mean ± SD; n = 58). Input resistances, measured with 0.1 nA hyperpolarizing current pulses near rest, averaged 75 ± 22 MΩ (n = 59). Recordings lasted 1.41 ± 0.92 hr (n = 61). About half of the recordings were terminated by pulling the electrode out of the cell.

Direct glutamatergic inputs to cartwheel cells are probably from granule cells

Shocks to the auditory nerve, VCN, or dorsal DCN result in synaptic responses in cartwheel cells that elicit variable patterns of single and complex spikes lasting between tens and hundreds of milliseconds. Relatively weak synaptic currents trigger strong currents associated with the firing of cartwheel cells, which usually obscure underlying PSPs. Thus, the intrinsic electrical properties of cartwheel cells play a prominent role in shaping synaptic responses.

The parallel fibers are a major source of input to cartwheel cells (Wouterlood and Mugnaini, 1984). To examine the function of these inputs, shocks were applied to the surface of the dorsal VCN, which presumably activated superficial granule cells as well as granule cells in the lamina that separates VCN and DCN. One experiment illustrating a pharmacological dissection of responses to shocks of the VCN is shown in Figure 1. In normal saline, a cartwheel cell responded to the stimulus with depolarizing PSPs that lasted ~100 msec. Shocks were applied during a hyperpolarizing current pulse to resolve the underlying synaptic response by reducing the contribution of intrinsic, voltage-sensitive conductances. Strychnine, an antagonist of glycine receptors (Betz and Becker, 1988), was added to eliminate the contribution of glycinergic inputs in the following manipulations. Removal of magnesium from the bathing solution increased the magnitude of the

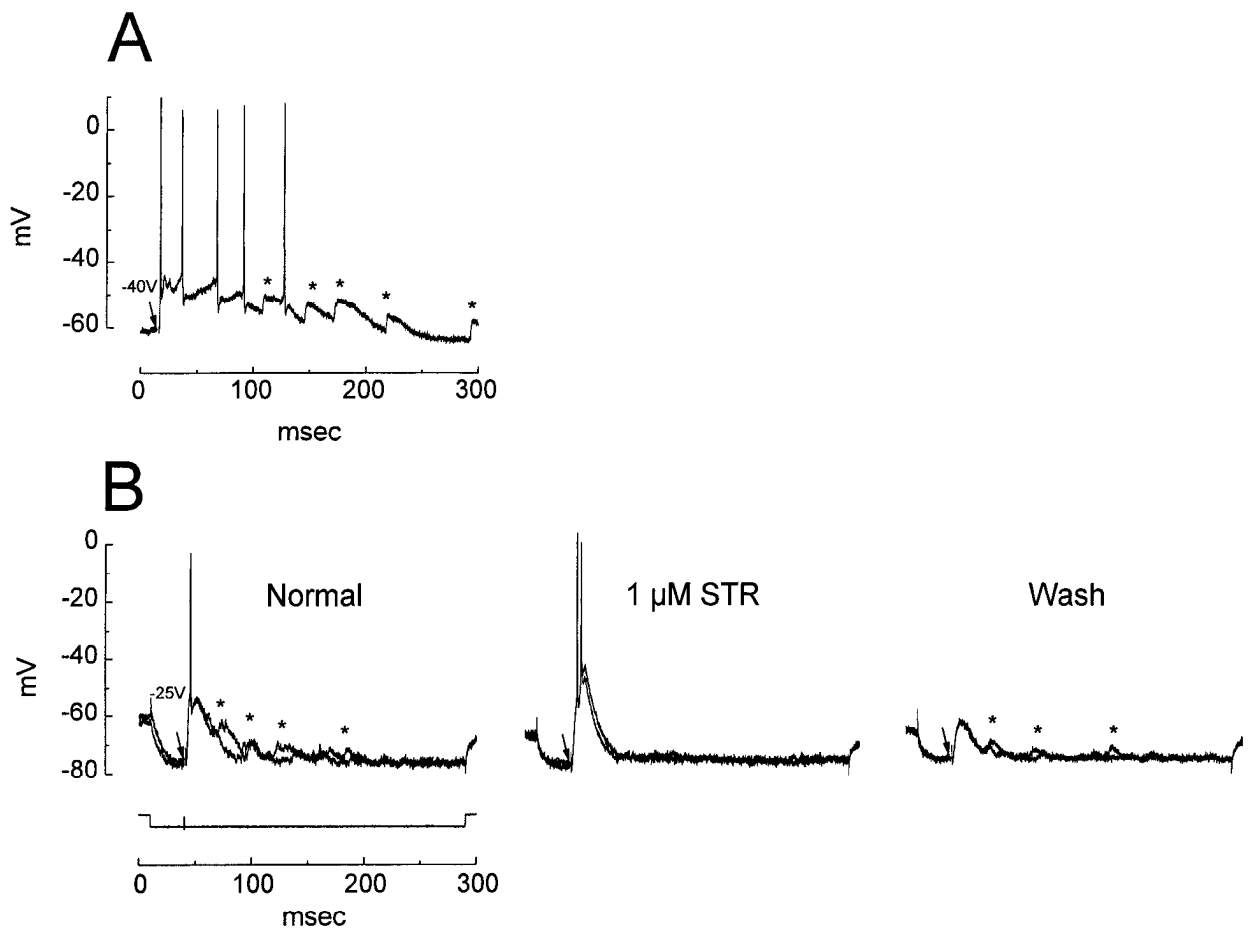

Figure 2. Shocks to the DCN evoke late, depolarizing PSPs in two cartwheel cells. *A*, Synaptic response of a cartwheel cell to a shock (*arrow*) applied to the DCN dorsal to the recording site. The cell responded with a train of action potentials ~100 msec long. The action potentials obscured underlying synaptic potentials, but after the firing stopped, late depolarizing PSPs were observed (*asterisks*). *B*, In another cartwheel cell, responses to shocks applied to the DCN were recorded while the cell was hyperpolarized with current (−0.2 nA) so that the PSPs could be resolved. In normal saline (*Normal*), an initial suprathreshold response was followed by trains of late PSPs (*left, asterisks*). Strychnine (*STR*) reversibly blocked the late PSPs and increased the amplitude of the initial response (*middle* and *right*).

synaptic response. The majority of the response in magnesium-free saline was mediated, monosynaptically or polysynaptically, by AMPA receptors, because most of the response was abolished by DNQX at 40 μM (Honoré et al., 1988). A small, DNQX-resistant, depolarizing component remained, which was more apparent in responses to stronger shocks. This component was mediated by NMDA receptors because it was reversibly eliminated in the presence of 100 μM APV (Mayer and Westbrook, 1985). All responses to shocks of the granule cells in the VCN were blocked by DNQX and APV even when strychnine was omitted (n = 3).

These experiments support the conclusion that parallel fiber inputs to cartwheel cells are glutamatergic. Both AMPA and NMDA receptor subtypes contribute to glutamatergic responses, although the NMDA receptor-mediated component of the response was weak relative to that mediated by AMPA receptors. The experiments also indicate that if polysynaptic inputs contributed to the responses to shocks, those inputs must have been activated via glutamatergic excitation.

Context-dependent action of glycinergic and GABAergic excitatory inputs to cartwheel cells

Shocks to the auditory nerve, VCN, and DCN frequently gave rise to depolarizing, presumably polysynaptic PSPs, which occurred up to hundreds of milliseconds after the shock (Fig. 2*A*). At the resting potential, all PSPs in cartwheel cells were depolarizing (n = 61). Surprisingly, these late, depolarizing PSPs in cartwheel cells were blocked by strychnine (Fig. 2*B*) (n = 4). In normal saline, shocks to the DCN resulted in a long response that consisted of a series of depolarizing PSPs (Fig. 2*B*, *left trace*). Application of 1 μM strychnine eliminated late, depolarizing PSPs and increased the magnitude of the initial response. Whereas the increase in the early response can be readily explained by a disinhibition of an excitatory input to cartwheel cells, the elimination of late, depolarizing PSPs is difficult to reconcile with the blockade of inhibition.

To determine how nonglutamatergic inputs contribute to cartwheel cells' synaptic responses, the inputs had to be activated monosynaptically and separated pharmacologically. Monosynaptic glycinergic and GABAergic inputs were activated with shocks to the dorsal tip of the DCN while glutamatergic synaptic interactions were blocked pharmacologically. Figure 3 shows the pharmacological analysis of the synaptic responses of two cartwheel cells. In both cells (Fig. 3*A,B*), elimination of glutamatergic inputs with DNQX and APV revealed a nonglutamatergic, depolarizing PSP (Fig. 3, *second row*). Strychnine attenuated the PSP, indicating that the PSP was in part glycinergic (Fig. 3, *third row*). The remainder of the PSP was GABAergic, because it was eliminated

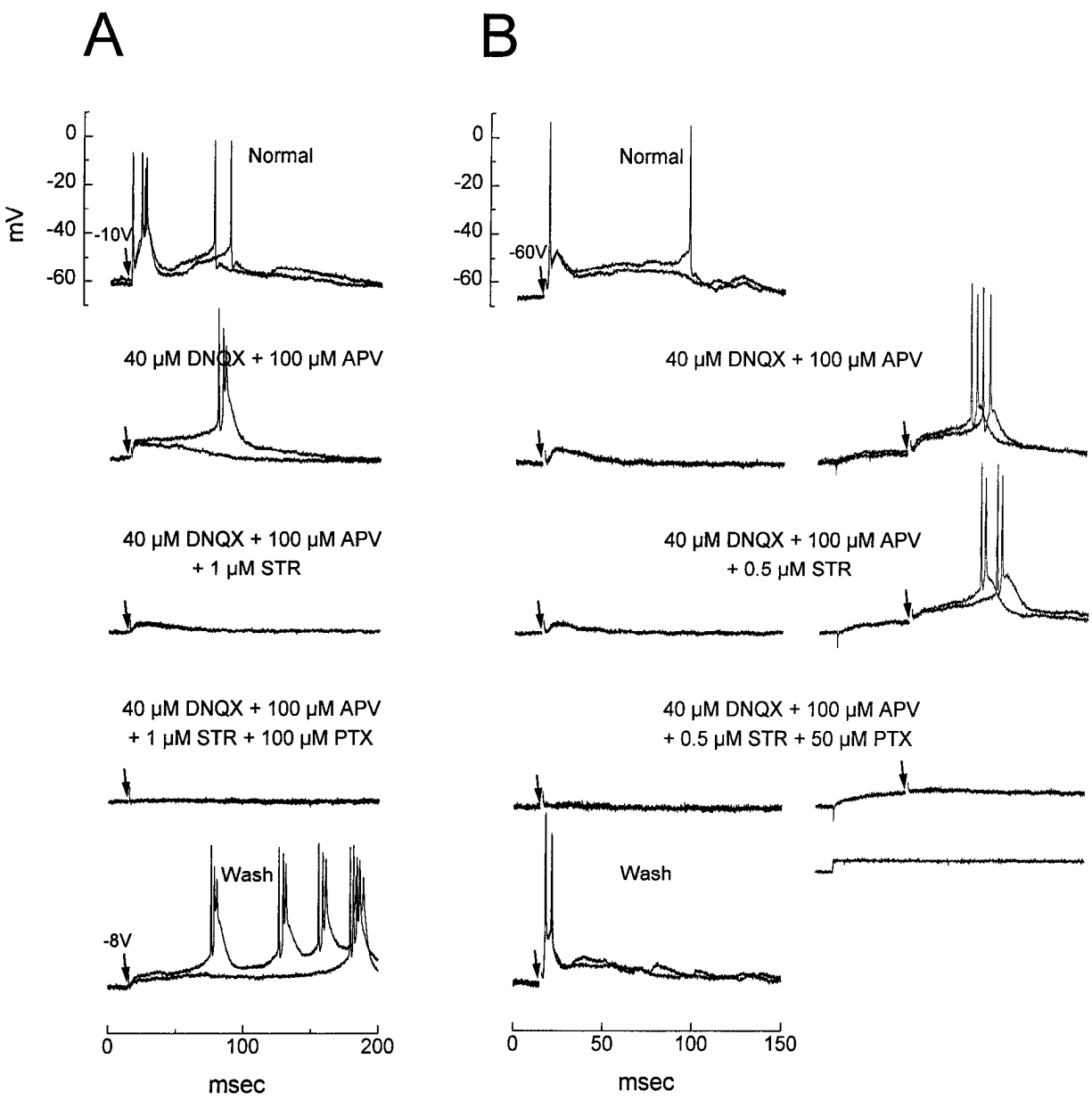

Figure 3. Glutamatergic, glycinergic, and GABAergic components were successively eliminated by the application of specific antagonists and accounted for the entire synaptic response in two cartwheel cells. Shocks to the DCN (*arrows*) dorsal to the site of impalement evoked long-lasting synaptic responses in normal saline in both cartwheel cells (*first row*). *DNQX* and *APV* eliminated the glutamatergic components of the synaptic responses (*second row*). The nonglutamatergic (combined glycinergic and GABAergic) PSP could be suprathreshold at the resting potential (*A, second row*) or when superimposed on a depolarization evoked with a current pulse of 60 pA (*B, second row*). Part of the nonglutamatergic PSP was glycinergic because it was blocked with strychnine (*STR, third row*). The nonglutamatergic, nonglycinergic (GABAergic) PSP also could be suprathreshold when it was superimposed on a depolarizing current pulse of 75 pA (*B, third row, right column*). The nonglutamatergic, nonglycinergic PSP was completely blocked by *PTX*, indicating that it was mediated by $GABA_A$ receptors (*fourth row*). In the absence of synaptic responses, a current pulse of 75 nA did not evoke an action potential (*B, fourth row, right column*). All manipulations were reversed (*fifth row*).

by 50 or 100 μM PTX, an antagonist of $GABA_A$ receptors (Fig. 3, *fourth row*) (Choi and Fischbach, 1981). The combined glycinergic and GABAergic response in *A* could be suprathreshold and was thus excitatory. The excitation was weak, because action potentials occurred 70 msec after the shock and presumably required the activation of weak, regenerative, intrinsic conductances. In the cell shown in *B*, glycinergic and GABAergic PSPs failed to elicit action potentials when evoked from the resting potential (Fig. 3*B*, *second* and *third rows, left*). However, when the same stimulus was imposed together with a small, subthreshold depolarizing current pulse, the combined glycinergic and GABAergic PSP, as well as the isolated GABAergic PSP, was suprathreshold (Fig. 3*B*, *right traces, second* and *third rows*). When the entire synaptic response was blocked pharmacologically, the current pulse itself did not elicit action potentials in the cell (Fig. 3*B*, *fourth row*). These results indicate that depolarizing, strychnine-sensitive PSPs are mediated by a direct glycinergic input to cartwheel cells. Thus, the late depolarizing PSPs that are blocked by strychnine in Figure 2*B* are also likely to be glycinergic. The experiment further demonstrates that not only glycinergic, but also GABAergic PSPs are weakly excitatory in cartwheel cells.

PSPs also could reduce the firing of cartwheel cells under

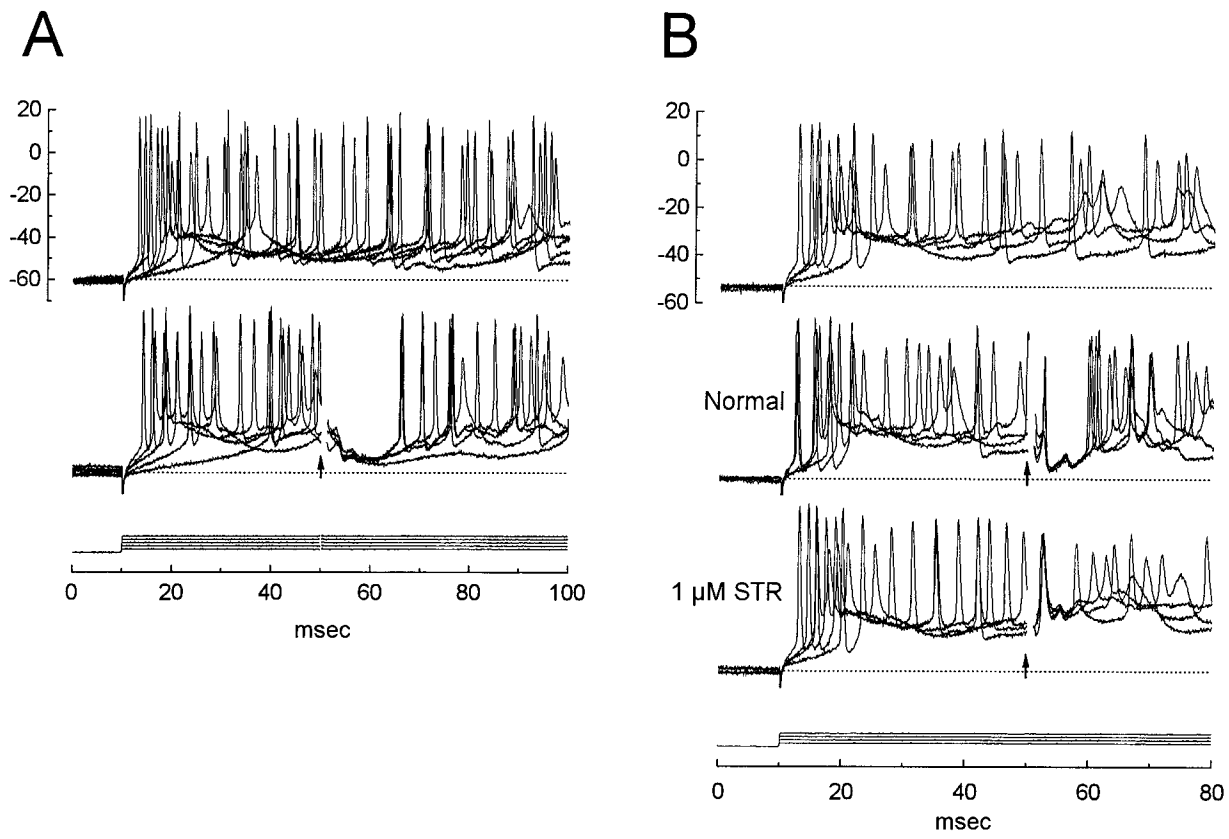

Figure 4. PSPs can suppress firing evoked with current pulses. *A*, PSPs transiently suppressed the firing of a cartwheel cell in response to current injection. Records were taken as 40 μM DNQX and 100 μM APV were being washed out (28 min) and as polysynaptic responses began to return. The contribution of polysynaptic inputs makes the synaptic action more obvious than when monosynaptic glycinergic and GABAergic inputs were isolated. Depolarization of cartwheel cells with a series of current pulses elicited trains of simple and complex spikes (*upper traces*). When a −40 V shock to the DCN was applied during these current pulses (*arrow*), the synaptic response hyperpolarized the membrane potential by between 5 and 14 mV, and suppressed firing in the cell for at least 15 msec (*lower traces*). Depolarizing current pulses were 0.1, 0.2, 0.3, 0.4, and 0.5 nA. *Dotted line* at −60 mV indicates the resting potential. *B*, In a different cartwheel cell, current pulses evoked similar tonic firing (*upper traces*). A −20 V shock to the DCN (*arrow*) evoked a simple spike followed by a complex PSP that transiently hyperpolarized the membrane to −50 mV and suppressed firing in the cell for 8 msec (*middle traces*). A large component of this suppression was mediated by a glycinergic component, because in the presence of 1 μM strychnine (*STR*), the depth of the PSP and the duration of the suppression of firing were reduced (*lower traces*). A small suppressive component remained in the presence of strychnine, but was not investigated further. Depolarizing current pulses were 0.1, 0.2, 0.3, and 0.4 nA. The *dotted line* at −53 mV indicates the resting potential.

conditions of strong depolarization (Fig. 4). Shocks to the DCN applied while the cell was driven with current suppressed firing for at least 15 msec (Fig. 4*A*). In another cell, shocks to the DCN during depolarizations elicited responses of both firing and suppression, probably reflecting the truncation of glutamatergic excitation by nonglutamatergic inputs (Fig. 4*B*). A large proportion of the suppression was glycinergic because it was blocked in the presence of strychnine. The remaining suppression of firing may have been mediated by GABAergic inputs, but the pharmacology was not explored. Thus, PSPs with large glycinergic, and possibly GABAergic, components could reduce firing in cartwheel cells.

Exogenous application of glycine and $GABA_A$ receptor agonists mimicked both the excitatory and suppressive effects of the synaptic inputs. For 11 cartwheel cells, glycine (50 or 100 mM) or 100 μM muscimol, an agonist of $GABA_A$ receptors, was pressure-ejected from a pipette placed onto the surface of the DCN within 300 μm of the impaled cell. The action of glycine and muscimol was depolarizing in each case, and elicited action potentials in 9 of 11 cells (glycine, 5 of 6 cells; muscimol, 4 of 5 cells). Figure 5*A* shows that a 200 msec pulse of muscimol elicited a depolarizing suprathreshold response that lasted ~8 sec. The excitatory action of muscimol was blocked by 10 μM PTX, indicating that the response was mediated by $GABA_A$ receptors. Correspondingly, a pulse of 50 mM glycine, 800 msec long, elicited a train of action potentials lasting ~2 sec. The antagonism of this response by 1 μM strychnine indicates that it was mediated by glycine receptors. (Fig. 5*B*).

Exogenous glycine and muscimol could also reduce the firing of cartwheel cells that were depolarized with current. Figure 5*C* shows that a pulse of 100 mM glycine suppressed the firing of a cartwheel cell elicited by a depolarizing, 0.1 nA current pulse. Comparable suppression of firing was also observed with muscimol application (not shown).

The finding that application of glycinergic and $GABA_A$ergic agonists can both evoke and prevent firing provides another indication that the interplay between synaptic and intrinsic currents is not simple in cartwheel cells. It is likely that the spatial and temporal activation of receptor-mediated and voltage-gated currents affects the generation of the firing pattern.

Estimate of the reversal potential for glycinergic and GABAergic PSPs

The suprathreshold (depolarizing) glycinergic and GABAergic PSPs were unexpected and suggest that the synaptic conductance has an unconventional reversal potential. The measurement of a discrete reversal potential was complicated by the presence of

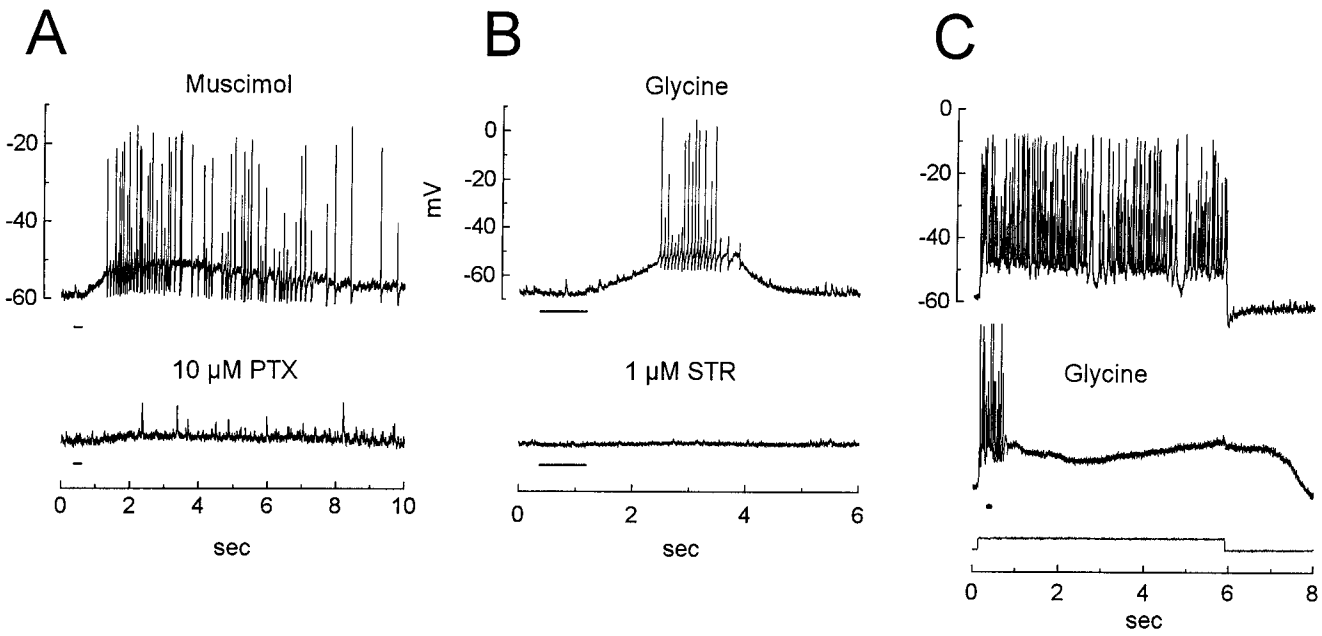

Figure 5. Context-dependent action of exogenously applied glycine and muscimol. *A*, Exogenous application of muscimol, an agonist of $GABA_A$ receptors, with a pressure pulse (*bar*) elicited firing in a cartwheel cell (*upper trace*). The low sampling rate makes some action potentials appear attenuated. The response to muscimol was blocked by 10 μM *PTX*, although frequent, spontaneous depolarizing PSPs remained (*lower trace*). *Bar* represents duration of the pressure pulse used to apply 100 μM muscimol (200 msec, 15 psi). *B*, Exogenous application of glycine (*bar*) depolarized a cartwheel cell to threshold. In normal saline, glycine depolarized the cell and caused it to fire (*upper trace*). Strychnine (*STR*; 1 μM) blocked the response to glycine and reduced the spontaneous PSPs (*lower trace*). *Bar* represents duration of the pressure pulse used to apply 50 mM glycine (800 msec, 15 psi). *C*, A depolarizing current pulse (0.1 nA) evoked steady firing in another cartwheel cell (*upper trace*). A pressure pulse of 100 mM glycine [30 msec, 15 psi (*dot*)] suppressed firing evoked by current (*lower trace*).

strong intrinsic, voltage-sensitive currents that often obscured synaptic responses. Measurement of reversal potentials of evoked PSPs was impossible in many cells, because shocks near enough to the recorded cell to activate local interneurons caused cartwheel cells to fire directly in the presence of depolarizing current pulses, obscuring synaptic responses. In the presence of DNQX and APV, the glycinergic and GABAergic components were generally too weak for a consistent reversal potential to be measured. It was possible, however, to measure reversal potentials of combined glycinergic, GABAergic, and glutamatergic PSPs, a large proportion of which have been shown to be glycinergic (Fig. 4*B*). Reversal potentials of PSPs were measured in five cartwheel cells; traces from one of these are shown in Figure 6*A*. A shock to the DCN was applied at the cell's resting potential as well as during a depolarization elicited with current injected through the electrode. At rest, the shock resulted in a depolarizing response to −52 mV that triggered a complex spike after ~70 msec. When the same shock was given when the cell was depolarized, the membrane potential hyperpolarized to approximately −52 mV and transiently suppressed firing. The average reversal potential measured in five cells was −53 ± 4 mV (mean ± SD) (Fig. 6*B*).

The reversal potential of exogenously applied glycine and muscimol was measured in seven cartwheel cells when voltage-gated Na^+ conductances were blocked pharmacologically with TTX (Fig. 6*C–F*). Pressure ejection of glycine (50 or 100 mM) or 100 μM muscimol depolarized all cartwheel cells from their resting potentials. In the cell shown in Figure 6*C*, responses to glycine applied at the cell's resting potential initially depolarized it to approximately −55 mV, with the entire response lasting ~2 sec. When the same stimulus was given during a depolarization elicited by a 60 pA current pulse, the membrane potential transiently hyperpolarized to approximately −55 mV. The slow depolarization late in the response probably reflects the interaction of responses to glycine with the intrinsic conductances of the cell. The cell shown in Figure 6*E* responded to a pressure pulse of muscimol with a 9 sec depolarization that reversed at approximately −54 mV. The amplitudes of the initial responses are plotted as a function of the membrane potential immediately before the application of the agonist in Figure 6, *D* and *F*. Reversal potentials of responses to glycine and muscimol averaged −52 ± 3 mV and −50 ± 5 mV, respectively.

A comparison of Figure 6, *B*, *D*, and *F*, shows that the reversal potentials of PSPs and the responses to extrinsically applied glycine and muscimol are remarkably similar. All lie in the range between −45 mV and −60 mV with the means between −50 mV and −53 mV. Reversal potentials of PSPs and responses to glycine and muscimol were not correlated with the age of the animals over the range 18 to 26 d. The most negative of the reversal potentials, −60 mV (Fig. 6*B*, *diamonds*), was measured in the youngest animal (18 d), and the most positive, −45 mV (Fig. 6*F*, *filled boxes*), from a slightly older animal (20 d).

Glycinergic and GABAergic inputs to fusiform cells are inhibitory

While fusiform cells also received glycinergic and GABAergic inputs, the action of both of these inputs was inhibitory, with a reversal potential of −68 ± 4 mV (mean ± SD; $n = 5$). Synaptic responses evoked by shocks to the DCN were pharmacologically dissected in five fusiform cells. In normal saline, these shocks evoked a mixture of excitation and inhibition lasting ~100 msec (Fig. 7). In the presence of DNQX and APV, all excitation was eliminated and the full extent of monosynaptic inhibition was revealed (Fig. 7, *second trace*). The residual IPSP contained both glycinergic and GABAergic components, which could be elimi-

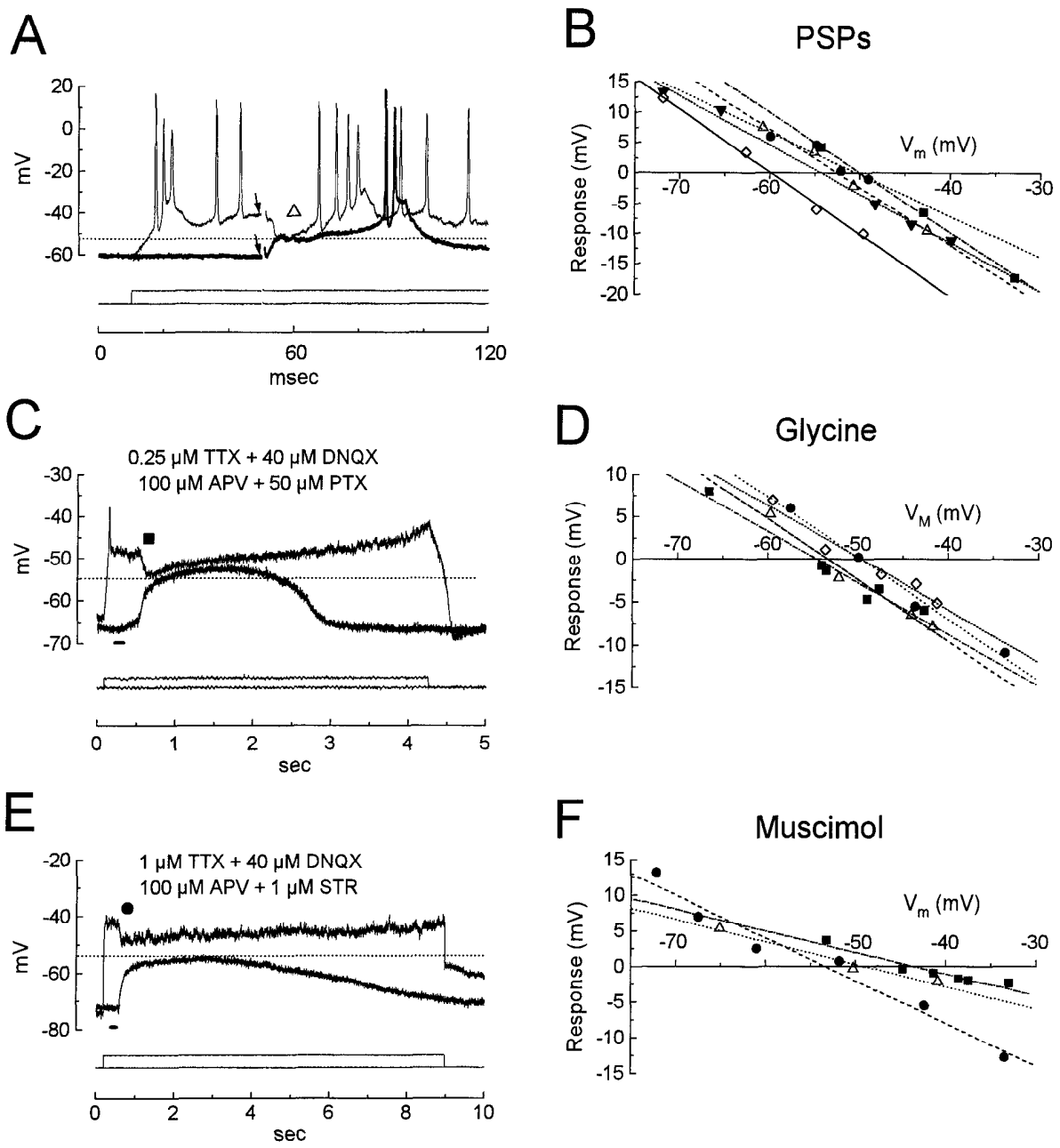

Figure 6. Reversal potentials for PSPs are similar to the reversal potentials of responses to exogenously applied glycine and muscimol. *A*, At the resting potential, a −40 V shock to the DCN (*arrow*) evoked a suprathreshold response (*thick trace*). When the same shock was applied during a 0.4 nA depolarizing current pulse, firing in the cell was transiently suppressed (*open triangle*). The synaptic response reversed at −52 mV (*dotted line*). *B*, The reversal potential of the cell in *A* (*open triangles*) and of four other cells are shown graphically. The average reversal potential for PSPs was −53 ± 4 mV (mean ± SD). Ages of animals in days after birth: *diamonds*, 18; *filled triangles*, 19; *open triangles*, 21; *boxes*, 20; *filled circles*, 23. *C*, Glycine at 100 mM was applied with pressure pulses (*bar*: 140 msec, 15 psi) at the resting potential and while the cell was polarized with current. Earliest responses to glycine (*box*) reversed at approximately −55 mV (*dotted line*). *D*, The amplitude of the response to pressure pulses of glycine relative to the membrane potential just preceding the response of the cell in *C* (*boxes*) and of three other cartwheel cells are shown graphically. Cell indicated by *circles* is in the presence of 0.25 μM TTX; cells indicated by *boxes* and *triangles* and *diamonds* are in the presence of 0.25 μM *TTX*, 40 μM *DNQX*, 100 μM *APV*, and 50 μM *PTX*. The reversal potentials for all three cells lie between −50 and −55 mV (−52 ± 3 mV, mean ± SD). Ages of animals in days after birth: *filled boxes*, 22; *open triangles*, 26; *diamonds*, 20; *filled circles*, 24. *E*, Muscimol at 100 μM was applied with pressure pulses (*bar*: 150 msec, 15 psi) at the resting potential and while the cell was polarized with current. Earliest responses to muscimol (*circle*) reversed at approximately −54 mV (*dotted line*). *F*, The amplitude of the response to muscimol relative to the membrane potential just preceding the response of the cell in *E* (*circles*) and of two other cartwheel cells are shown graphically. All measurements were made in the presence of 1 μM *TTX*, 40 μM *DNQX*, 100 μM *APV*, and 1 μM strychnine (*STR*). The reversal potentials for all three cells lie between −45 and −54 mV (−50 ± 5 mV, mean ± SD). Ages of animals in days after birth: *filled circles*, 22; *open triangles*, 23; *filled boxes*, 20.

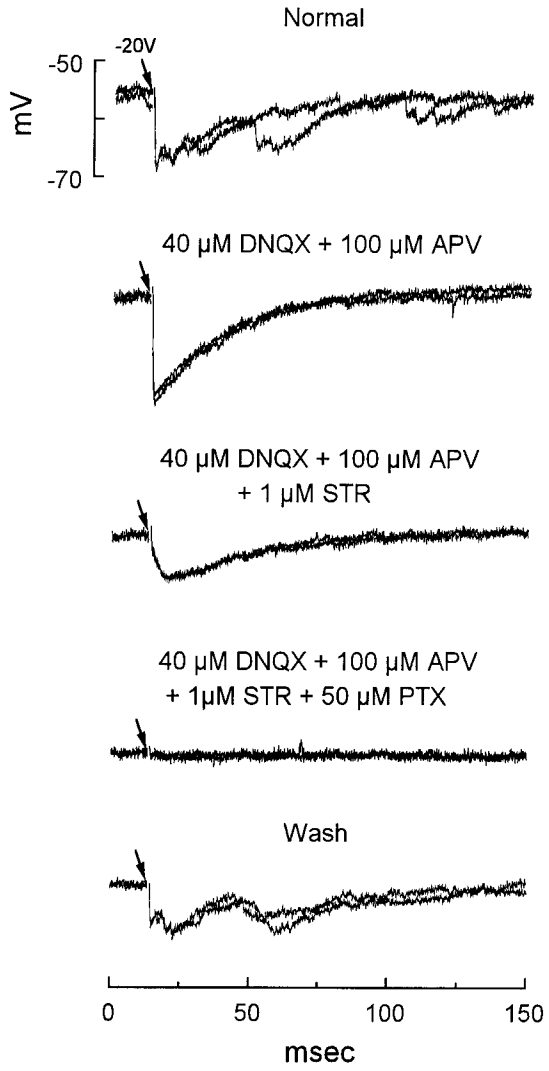

Figure 7. Glutamatergic, glycinergic, and GABAergic components were successively eliminated by the application of specific antagonists in a fusiform cell. Glycinergic and GABAergic PSPs in fusiform cells were conventionally inhibitory. Shocks to the DCN evoked a complex synaptic response of which two are shown superimposed (*upper traces*). Excitation was blocked by *DNQX* and *APV* (*second row*). Some of the inhibition was glycinergic because it was blocked by strychnine (*STR; third row*). The remainder of the inhibition was mediated by $GABA_A$ receptors because it was blocked by *PTX* (*fourth row*). The action of all drugs was reversible (*bottom traces*).

nated by strychnine and PTX, respectively (Fig. 7, *third* and *fourth traces*).

Cartwheel and fusiform cells receive similar patterns of spontaneous synaptic activity

Because cartwheel cells provide a significant projection to both fusiform cells and other cartwheel cells (Mugnaini et al., 1987; Berrebi and Mugnaini, 1991), the synaptic responses in both of these targets should reflect the firing of cartwheel cells. The long-lasting firing in responses to shocks and the bursts of action potentials, both spontaneous and evoked, that are characteristic of cartwheel cells should be reflected in PSPs in their targets. Both cartwheel cells and fusiform cells received spontaneous glutamatergic, glycinergic, and GABAergic PSPs (Fig. 8). In the cartwheel cell and fusiform cell shown, these spontaneous PSPs were numerous and occurred both singly and in bursts (Fig. 8, *asterisks*). Few of these PSPs were glutamatergic because their rate was not dramatically altered in the presence of DNQX and APV. In some other cartwheel and fusiform cells, spontaneous glutamatergic PSPs were more frequent. Most of these PSPs were glycinergic, because the majority of them were eliminated by strychnine. The few remaining spontaneous PSPs were GABAergic because they were eliminated by PTX. In both cartwheel and fusiform cells, spontaneous GABAergic PSPs occurred infrequently, if at all. The cells illustrated in Figure 8 were selected because they received spontaneous GABAergic PSPs; in three of five cartwheel cells and three of six fusiform cells, no GABAergic PSPs were detected. As expected, 10 μM bicuculline, an antagonist of $GABA_A$ receptors, did not detectably affect the amplitude or frequency of spontaneous PSPs ($n = 2$). In contrast, frequent, spontaneous glycinergic PSPs that occurred singly or in bursts were a consistent feature of all cartwheel cells and fusiform cells whose synaptic activity was dissected pharmacologically ($n = 13$). Spontaneous glycinergic and GABAergic PSPs were always depolarizing in cartwheel cells and hyperpolarizing in fusiform cells.

The timing of glycinergic IPSPs and EPSPs in cartwheel and fusiform cells is correlated with the timing of firing of cartwheel cells

In responses to shocks, the timing of glycinergic, but not GABAergic, PSPs in cartwheel and fusiform cells resembled the timing of firing of cartwheel cells. Shocks to the nerve or to the DCN evoked trains of glycinergic PSPs lasting ~100 msec and occurring in bursts (Figs. 2, 7). Figure 9*A* shows responses to similar shocks in a fusiform cell (*upper trace*) and a cartwheel cell (*lower two traces*) recorded sequentially in the same slice. The fusiform cell responded to shocks of the auditory nerve with a mixture of excitation and inhibition, including many late IPSPs. In fusiform cells, IPSPs evoked by shocks to the auditory nerve have been shown to be glycinergic (Zhang and Oertel, 1994). Similar shocks evoked an excitatory response in the cartwheel cell (Fig. 9*A, middle trace*). The timing of late EPSPs in this cell was more apparent when firing was suppressed with a hyperpolarizing current pulse (Fig. 9*A, bottom trace*). The results of the present study indicate that these late PSPs in cartwheel cells are glycinergic as well and support the conclusion that cartwheel cells are the source of the glycinergic PSPs.

DISCUSSION

In cartwheel cells, glycinergic and GABAergic inputs are weakly excitatory, in contrast to neighboring fusiform cells, in which such inputs are conventionally inhibitory. The reversal potentials of glycinergic and GABAergic PSPs lie above the firing threshold in cartwheel cells, whereas in fusiform cells they lie below rest and well below the firing threshold, possibly reflecting differences in the chloride electrochemical gradient in these cells. Glycinergic and GABAergic inputs promote firing when cartwheel cells are at rest, but can also reduce rapid firing when cartwheel cells are strongly depolarized. Thus, in cartwheel cells the action of glycinergic and GABAergic activity on firing rate depends on the electrophysiological context in which the synaptic input occurs.

Weakly excitatory action of glycinergic and GABAergic inputs

The reversal potential for glycinergic and GABAergic PSPs is approximately −53 mV in cartwheel cells, which is depolarized with respect to the firing threshold of these cells. Although depolarizing GABAergic responses have been demonstrated in several types of mature central neurons, the reversal potentials for such

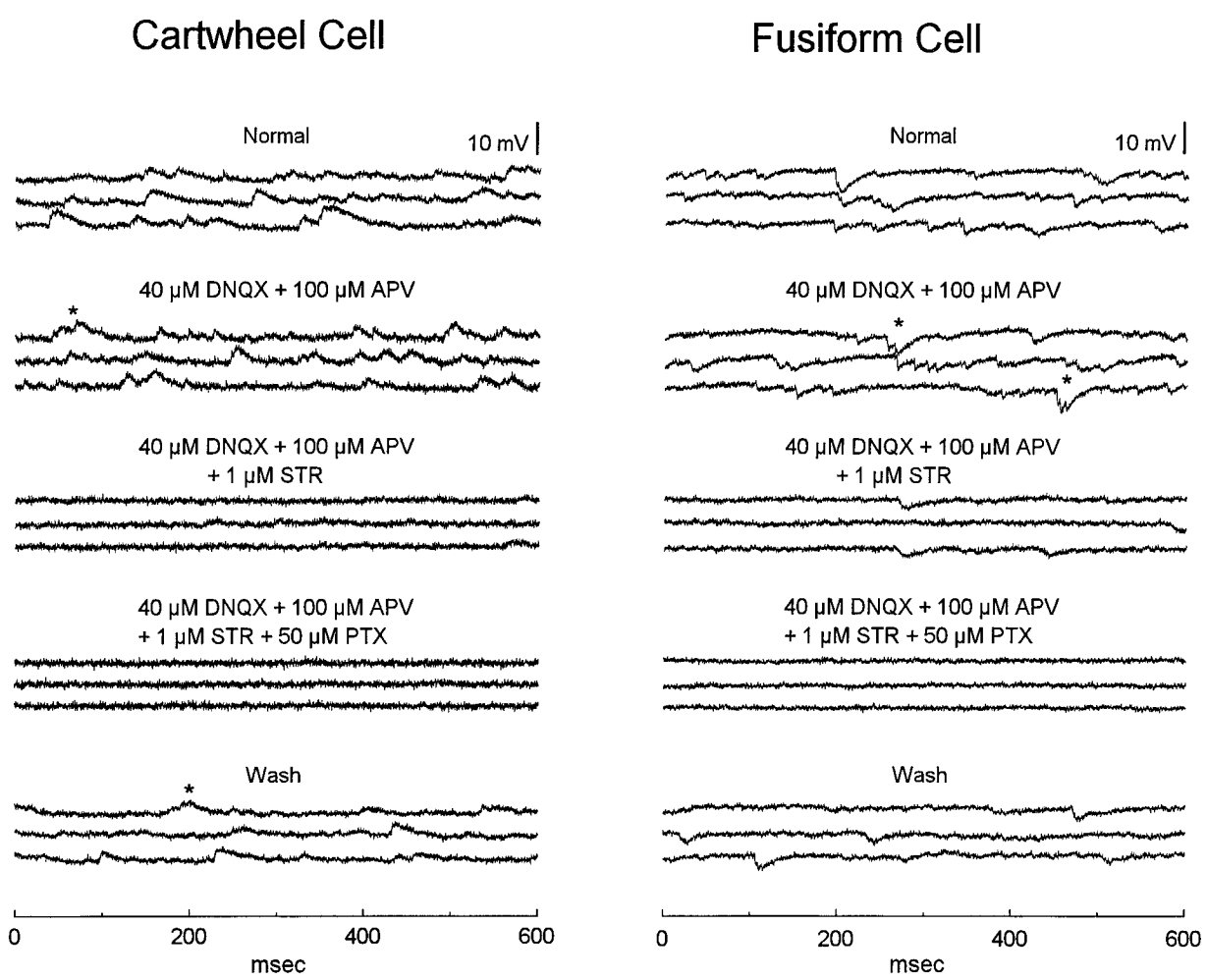

Figure 8. Cartwheel and fusiform cells receive similar temporal patterns of spontaneous glycinergic and GABAergic PSPs. Glycinergic and GABAergic PSPs were always depolarizing in cartwheel cells and hyperpolarizing in fusiform cells. Spontaneous PSPs were observed in both cells in normal saline (*upper traces*). Spontaneous PSPs in both cells, some of which occurred in bursts (*asterisks*), remained in the presence of glutamate receptor antagonists (*second row*). Most of these spontaneous PSPs in these cells were glycinergic, because they were eliminated by strychnine (*STR; third row*). In both cells, all remaining PSPs were mediated by $GABA_A$ receptors, because they were blocked by *PTX* (*fourth row*). The effects of blockers were reversible (*bottom row*). Resting potentials: −62 mV, cartwheel cell; −56 mV, fusiform cell.

responses generally lie below the firing threshold, and thus mediate inhibition (Misgeld et al., 1986; Tseng and Haberly, 1988; Hyson et al., 1995). However, indirect evidence for an excitatory effect of exogenously applied glycine and GABA has been reported in cerebellar neurons (Kaneda et al., 1995).

An unusually high intracellular chloride concentration could account for the weakly excitatory action of both glycinergic and GABAergic inputs on cartwheel cells. Both glycine and $GABA_A$ receptors are highly permeable to chloride and less permeable to bicarbonate (Hamill et al., 1983; Bormann et al., 1987; Kaila and Voipio, 1987; Kaneda et al., 1995; Staley et al., 1995). The reversal potential of $GABA_A$- and glycine-mediated synaptic responses would thus be expected to be similar and to lie close to the chloride equilibrium potential. If cartwheel cells were to have an intracellular chloride concentration of ~19 rather than ~11 mM, the chloride equilibrium potential in cartwheel cells will be expected to be −53 mV rather than −68 mV as in fusiform cells. The reversal potential of glycinergic PSPs in fusiform cells of the DCN resembles that measured in cells of the VCN in similar preparations, −67 mV (Wu and Oertel, 1986). High intracellular chloride concentrations in cartwheel cells might be maintained by an active inward transport of chloride ions. A role of inward chloride transport has been suggested for the granule cells of the dentate gyrus, the GABAergic inputs of which mediate depolarizing inhibition (Misgeld et al., 1986).

It seems unlikely that the different synaptic actions of glycine and GABA in cartwheel and fusiform cells result from differences in the ionic selectivity of both glycine and $GABA_A$ receptors across the two cell types. There is little, if any, evidence that ion selectivity varies among glycine or $GABA_A$ receptor subtypes in central neurons. It seems especially unlikely that two different receptor classes have parallel differences in ion selectivity.

A final consideration is that the weakly excitatory action of glycinergic and GABAergic inputs to cartwheel cells may represent ongoing developmental changes in the DCN. In neonatal neurons of the hippocampus, brainstem, and spinal cord, responses mediated by glycine and $GABA_A$ receptors are depolarizing and excitatory during the first postnatal week; it has been suggested that the electrochemical gradient for chloride changes with development (Cherubini et al., 1991; Wu et al., 1992; Kandler and Friauf, 1995). In a brainstem auditory nucleus, the lateral superior olivary nucleus, the reversal potential of glycinergic PSPs

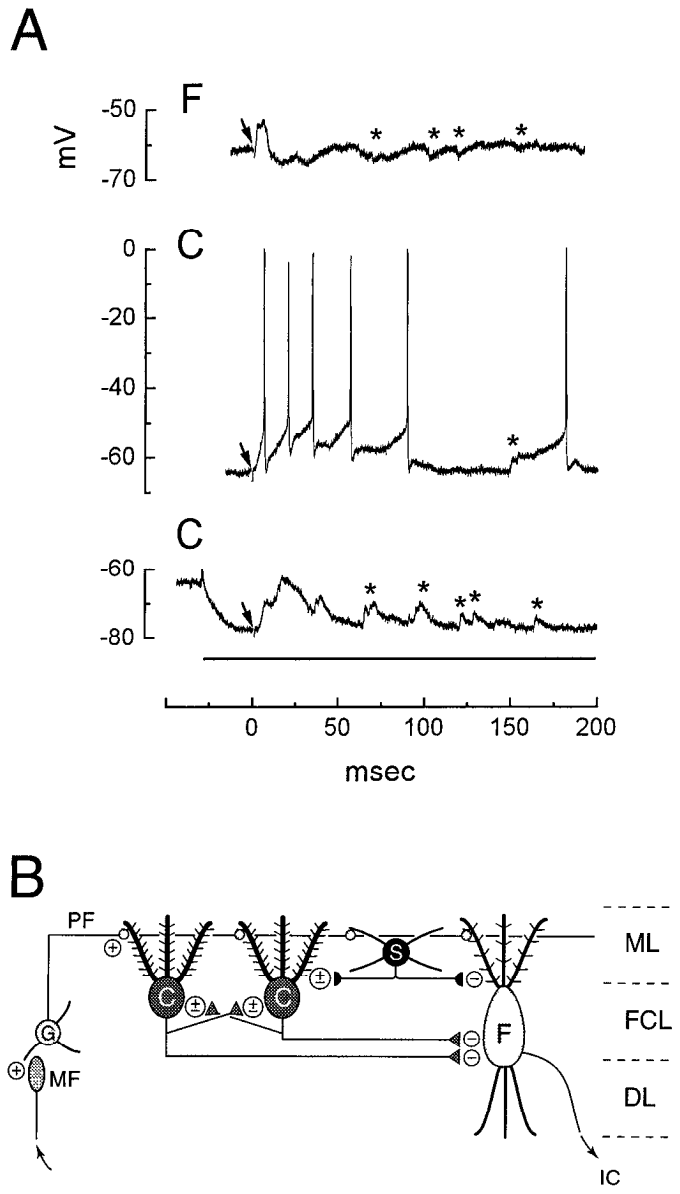

Figure 9. Shocks to the auditory nerve evoke late action potentials in cartwheel cells as well as late PSPs in cartwheel and fusiform cells. *A*, *Top trace*, Responses of a fusiform cell to a −7 V shock of the auditory nerve involved the summing of EPSPs and IPSPs. Some IPSPs (*asterisks*) occurred late over approximately the same time as action potentials occurred in the cartwheel cell. *Middle trace*, Response of a cartwheel cell, recorded later in the same slice, to a −6 V shock to the auditory nerve shows the temporal firing pattern of a cartwheel cell under similar conditions. The cartwheel cell responded with simple spikes that lasted >100 msec. The action potentials obscured underlying PSPs. This cell responded to stronger shocks with early complex spikes and later simple spikes (not shown). *Bottom trace*, To examine the temporal pattern of PSPs in response to an identical shock in the same cartwheel cell, the cell was hyperpolarized. The cell received both early and late PSPs (*asterisks*). The PSPs occurred over approximately the same time as action potentials in the same cell. *F*, Fusiform cell; *C*, cartwheel cell. *B*, Diagram of the neuronal circuitry in the superficial DCN. Granule cells of the VCN and DCN are driven by mossy fiber inputs. The parallel fibers of granule cells in turn provide glutamatergic excitation to cartwheel, superficial stellate, and fusiform cells in the DCN. Superficial stellate cells provide GABAergic input and cartwheel cells provide glycinergic input to cartwheel and fusiform cells. Excitatory inputs are marked with a +, inhibitory inputs are marked with a −, and context-dependent inputs are marked with a ±. *MF*, Mossy fiber; *G*, granule cell; *PF*, parallel fiber; *C*, cartwheel cell; *S*, superficial stellate cell; *F*, fusiform cell; *IC*, inferior colliculus; *ML*, molecular layer; *FCL*, fusiform cell layer; *DL*, deep layer.

has been shown to shift from −30 mV on the day of birth to −70 mV at 10 d after birth in rats (Kandler and Friauf, 1995). In the cochlear nuclei, such changes have not been measured systematically, but hyperpolarizing IPSPs have been recorded from ventral cochlear nuclear neurons in mice at 7 and 9 d (Wu and Oertel, 1987). In the present study, no correlation between reversal potential and age was observed over 18 to 26 d after birth in cartwheel cells (Fig. 6*B,D,F*). Furthermore, reversal potentials were conventional in the fusiform cells with which the cartwheel cells are intermingled. Therefore, if the weakly excitatory action of glycine and GABA in murine cartwheel cells 3 to 4 weeks after birth reflects ongoing developmental processes, those processes must, in mice, be late and specific to cartwheel cells. Spontaneous, hyperpolarizing PSPs have been observed in cartwheel cells in DCN slices of guinea pigs over 1 month old, but little is known about these PSPs (Manis et al., 1994).

Neuronal circuitry of the molecular layer of the DCN

The present results support the scheme of connections in the molecular layer of the DCN proposed by Osen et al. (1990) based on anatomical and immunocytochemical studies (summarized in Figure 9*B*). Granule cells provide glutamatergic excitation to cartwheel, superficial stellate, and fusiform cells. The cartwheel and superficial stellate cells in turn provide feedforward inhibition to fusiform cells through glycinergic and GABAergic synapses, respectively. Cartwheel and superficial stellate cells influence the strength of cartwheel cells' inhibitory input to fusiform cells in a context-dependent manner.

Granule cells contact the spines of cartwheel and fusiform cells, as well as the dendritic shafts of superficial stellate cells. The small, clear round vesicles and asymmetric synaptic densities are consistent with their being excitatory (Kane, 1974; Wouterlood and Mugnaini, 1984; Wouterlood et al., 1984). Biochemical evidence supports the conclusion that the granule cells release a glutamate-like neurotransmitter (Godfrey et al., 1977; Oliver et al., 1983). In addition, cartwheel, superficial stellate, and fusiform cells express mRNAs for AMPA receptor subunits (Hunter et al., 1993). In the present experiments, shocks to granule cells in the VCN resulted in glutamatergic responses in cartwheel cells that were mediated primarily by AMPA receptors and secondarily by NMDA receptors (Fig. 1).

Cartwheel cells have been shown to contact other cartwheel cells and fusiform cells (Mugnaini et al., 1987; Berrebi and Mugnaini, 1991). The present results confirm the earlier suggestion that these synaptic interactions are likely to be glycinergic (Zhang and Oertel, 1994). Cartwheel cells fire spontaneous action potentials singly and as complex spikes (Manis et al., 1994; Waller and Godfrey, 1994). These two types of action potentials are also observed in cartwheel cells' responses to shocks, which last up to 500 msec (Zhang and Oertel, 1993; present results). The known targets of cartwheel cells receive glycinergic PSPs with this same distinctive pattern. Both fusiform and cartwheel cells receive frequent, spontaneous PSPs. In responses to shocks, trains of PSPs often occur in bursts and last for up to hundreds of milliseconds after the shock. Because these late PSPs in both cell types are blocked by strychnine, cartwheel cells are likely to be glycinergic (Zhang and Oertel, 1994; present results). This conclusion is supported by immunohistochemical evidence that shows that cartwheel cells, like other neurons that are demonstrated to be glycinergic, exhibit strong glycine-like immunoreactivity (Wenthold et al., 1987; Osen et al., 1990; Oertel and Wickesberg, 1993; Wickesberg et al., 1994).

Superficial stellate cells could be the source of GABAergic PSPs in both cartwheel and fusiform cells. Superficial stellate cells, like their counterparts in the cerebellum, have dendrites and axonal arbors that extend broadly in the plane of the molecular layer, allowing them to be activated directly by shocks in the present experiments (Zhang and Oertel, 1993). Consistent with the pattern of GABAergic PSPs observed in the present experiments, superficial stellate cells fire spontaneously and, in response to shocks to granule cells, fire one or a few action potentials (Zhang and Oertel, 1993; Golding, unpublished recording from one anatomically identified superficial stellate cell). These cells are GAD- and GABA-immunopositive (Wouterlood et al., 1984; Mugnaini, 1985; Adams and Mugnaini, 1987; Osen et al., 1990). The only other GABA- and GAD-positive cochlear nuclear neurons that terminate in the molecular layer are Golgi cells and cartwheel cells. Golgi cells are thought to innervate granule cells rather than cartwheel or fusiform cells (Mugnaini et al., 1980a; Mugnaini, 1985; Osen et al., 1990). The present results are inconsistent with cartwheel cells being the source of GABAergic input to cartwheel and fusiform cells, because their firing patterns do not match the temporal patterns of GABAergic PSPs.

The influence of the molecular layer on the output of the DCN through the fusiform cells is intriguing. Although parallel fibers excite fusiform cells directly, they also inhibit fusiform cells through cartwheel cells. The inhibition signals somatosensory (Young et al., 1995) or auditory (Ding et al., 1994; Parham and Kim, 1995) sensory stimuli. This study shows that the inhibition through cartwheel cells is shaped by a mechanism that promotes or suppresses firing, depending on what other concurrent events take place in the cell. If the cell is depolarized either synaptically or by intrinsic, voltage-dependent currents, glycinergic and GABAergic input suppresses firing. If the cell is at rest, however, those same inputs can lead to the cell's firing. These inputs also affect the timing of firing by delaying action potentials.

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