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Control of Interneuron Firing by Subthreshold Synaptic Potentials in Principal Cells of the Dorsal Cochlear Nucleus

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SUMMARY

Voltage-gated ion channels amplify, compartmentalize, and normalize synaptic signals received by neurons. We show that voltage-gated channels activated during subthreshold glutamatergic synaptic potentials in a principal cell generate an excitatory→inhibitory synaptic sequence that excites electrically coupled interneurons. In fusiform cells of the dorsal cochlear nucleus, excitatory synapses activate a TTX-sensitive Na^+ conductance and deactivate a resting I_h conductance, leading to a striking reshaping of the synaptic potential. Subthreshold voltage changes resulting from activation/deactivation of these channels subsequently propagate through gap junctions, causing slow excitation followed by inhibition in GABAergic stellate interneurons. Gap-junction-mediated transmission of voltage-gated signals accounts for the majority of glutamatergic signaling to interneurons, such that subthreshold synaptic events from a single principal cell are sufficient to drive spikes in coupled interneurons. Thus, the interaction between a principal cell's synaptic and voltage-gated channels may determine the spike activity of networks without firing a single action potential.

INTRODUCTION

Inhibitory interneurons sharpen the temporal integration of excitatory inputs and control spikes in principal cells during afferent activity, thereby tempering network excitation and enhancing the signal-to-noise of information relayed to downstream brain regions (Cruikshank et al., 2007; Santamaria et al., 2007; Isaacson and Scanziani, 2011; Haider et al., 2013). Interestingly, interneurons in many brain regions are electrically coupled via gap junctions, allowing multiple interneurons to synchronize their spiking (Mann-Metzer and Yarom, 1999; Dugué et al., 2009). Although gap junctions act as low-pass filters and preferentially transmit slow signals such as subthreshold synaptic events rather than fast, suprathreshold spikes (Bennett and Zukin, 2004), many previous studies investigating gap junctions focused primarily on transmission of action potentials (Mann-Metzer and Yarom, 1999; Christie et al., 2005; Dugué et al., 2009; Apostolides and Trussell, 2013; but see Zsiros et al., 2007; Lamotte d'Incamps et al., 2012). How subthreshold depolarizations propagate through electrical networks, and whether they represent a physiologically relevant signal, is unclear.

In the cerebellum-like dorsal cochlear nucleus (DCN), granule cell parallel fibers relaying sensory information form sparse excitatory synapses with projection neurons (fusiform cells) and inhibitory molecular layer interneurons. This organization implies that neighboring cells are activated by different sets of parallel fibers and that local inhibition is not recruited in a traditional "feedforward" manner (Roberts and Trussell, 2010). However, fusiform cells are electrically coupled to small, high-impedance GABAergic interneurons (stellate cells; Apostolides and Trussell, 2013, 2014). Do subthreshold synaptic inputs in fusiform cells excite stellate cells? If so, this would allow the lateral activation of stellate cells across divergent parallel fibers, ensuring recruitment of local inhibition despite sparse convergence from excitatory synapses. Using patch-clamp recordings in acute slices of mouse DCN, we show that parallel fiber synapses onto fusiform cells activate a long-lasting Na^+ conductance at subthreshold voltages and deactivate a hyperpolarization-activated, cyclic-nucleotide-gated (HCN or I_h) conductance. Transmission of this synaptic and voltage-gated waveform through electrical synapses profoundly alters the time course and amplitude of excitatory transmission onto stellate cells and drives spikes in an inhibitory network.

RESULTS

Glutamate Release Generates a Noncanonical Excitatory→Inhibitory Sequence in Stellate Cells

Stellate and fusiform cells receive excitatory synapses from parallel fibers in the DCN molecular layer (Figure 1A) (Wouterlood et al., 1984). In stellate cells voltage-clamped at −67 mV, single shocks to parallel fibers (see Supplemental Experimental Procedures available online) caused a multiphasic response with an

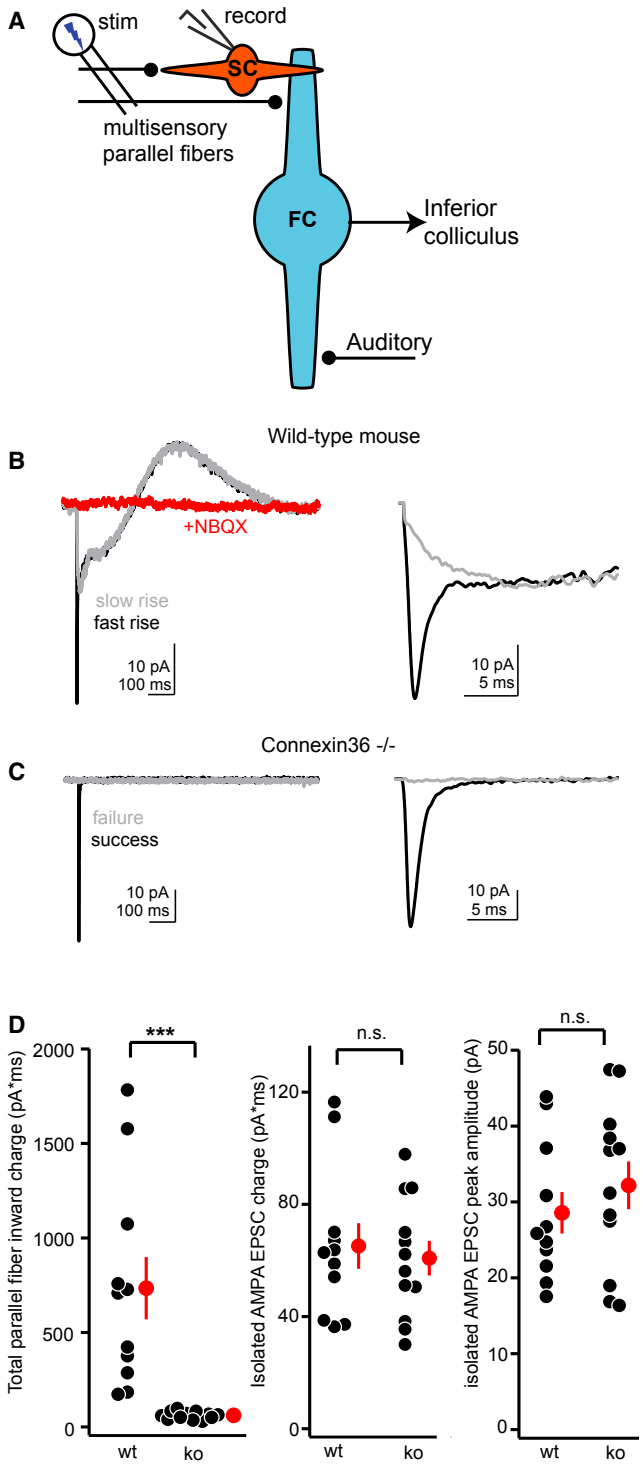

Figure 1. Parallel Fiber Transmission Generates an Excitatory→Inhibitory Sequence Mediated by AMPA Receptors

(A) DCN circuit and experimental setup. Projection neurons (fusiform cells [FC]) integrate excitatory synapses from auditory nerve and granule cell parallel fibers. The latter also contact the stellate interneurons that are electrically coupled to fusiform cells. A bipolar stimulating electrode is placed in the molecular layer to activate parallel fibers. (B) A single parallel fiber shock generates an excitatory→inhibitory sequence in stellate cells that is blocked by the AMPA receptor antagonist NBQX (10 μM; red trace). Black trace is an average of events that had an initial rapidly rising component, whereas the gray trace is an average of events lacking the fast component. The right panel shows the two types of events on a faster time base to highlight the drastic difference in rise times. (C) Parallel fiber EPSCs in *Cx36*$^{-/-}$ mice lack the slow inward and outward components. Black and gray traces represent averages of trials in which the fast component either succeeded or failed, respectively. (D) Quantification of EPSCs from wild-type and *Cx36*$^{-/-}$ mice. Left panel: the total inward charge transfer of parallel fiber EPSCs in wild-type mice was on average −734 ± 164 pA•ms (n = 11), whereas it was only −62 ± 6 pA•ms in the knockout (KO) mice (n = 12). Middle and right panels: the inward charge transfer and peak amplitude of the fast rising AMPA EPSC from parallel fiber synapses directly on stellate cells was not significantly different between wild-type and KO mice (WT charge and peak amplitude: −65 ± 8 pA•ms, −29 ± 3 pA; KO charge and peak amplitude: −60 ± 6 pA*ms, −32 ± 3 pA; p = 0.67 and 0.39, respectively; unpaired t test). The fast rising AMPA EPSC was isolated by subtracting the average of slowly rising events from the average of fast rising events (e.g., black trace minus gray trace). Errors given as ±SEM.

early inward and a late outward component (Figure 1B). The outward component was not due to glycine or $GABA_A$ receptors, as all experiments were performed in the presence of strychnine (1–2 μM) and SR95531 (10 μM). Interestingly, NBQX (10 μM) abolished both inward and outward components (n = 7), indicating that AMPA receptor activation triggered excitatory and inhibitory responses (Figure 1B, red trace).

Close inspection of the traces revealed trial-to-trial variation in the responses to parallel fiber stimulation. Some events had an initial rapidly rising and decaying inward component that resembled a fast AMPA receptor-mediated excitatory postsynaptic current (EPSC) and were followed by the slow inward and outward phases. Other events lacked the initial fast phase, yet maintained identical slow inward and outward components (n = 11) (Figure 1B, right, black and gray traces, respectively). This slow component accounted for 75% ± 7% of the total inward charge transfer. The stimulus intensity was adjusted to evoke initial fast-rising currents in approximately half of the trials; we thus interpret the variable appearance of the fast inward component as reflecting quantal fluctuation of release from single parallel fiber synapses on the recorded stellate cells. Given that AMPA receptors in DCN stellate cells display rapid rise and decay kinetics (Apostolides and Trussell, 2014), the drastically slower kinetics and comparatively invariant nature of the later components shown in Figure 1B suggest that these "synaptic" currents originate in fusiform cells electrically coupled to the voltage-clamped stellate cell.

Accordingly, stellate cell EPSCs recorded in slices from mutant mice lacking electrical coupling in the DCN (*Connexin36*$^{-/-}$ mice; *Cx36*$^{-/-}$) fluctuated between a purely fast inward current and no current at all (n = 12 cells) (Figure 1C). The slow outward component was also absent in *Cx36*$^{-/-}$ mice, indicating that it similarly originated from prejunctional fusiform cells. The total inward charge transfer of EPSCs from *Cx36*$^{-/-}$ mice was much smaller than EPSCs of WT mice, suggesting that parallel fiber inputs passing through fusiform cells represent the majority of synaptic excitation for stellate cells in WT mice under these stimulus conditions (Figure 1D, left panel). However, the charge and peak amplitude of the fast AMPA component (representing parallel fiber transmission directly onto the voltage-clamped stellate cell) was similar across