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Gap Junctions Compensate for Sublinear Dendritic Integration in an Inhibitory Network

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Abstract

Electrically coupled inhibitory interneurons dynamically control network excitability, yet little is known about how chemical and electrical synapses regulate their activity. Using two-photon glutamate uncaging and dendritic patch-clamp recordings, we found that the dendrites of cerebellar Golgi interneurons acted as passive cables. They conferred distance-dependent sublinear synaptic integration and weakened distal excitatory inputs. Gap junctions were present at a higher density on distal dendrites and contributed substantially to membrane conductance. Depolarization of one Golgi cell increased firing in its neighbors, and inclusion of dendritic gap junctions in interneuron network models enabled distal excitatory synapses to drive network activity more effectively. Our results suggest that dendritic gap junctions counteract sublinear dendritic integration by enabling excitatory synaptic charge to spread into the dendrites of neighboring inhibitory interneurons.

Inhibitory interneurons balance network excitation, enhance spike-time precision of principal neurons, and control synchrony within and across brain regions (1–4). Yet, little is known about how interneurons integrate chemical (2, 4–6) and electrical (7) synaptic inputs because of the inaccessibility of their dendrites. To investigate this, we studied dendritic integration in Golgi cells (GoCs), the main inhibitory interneurons in the input layer of the cerebellar cortex. GoCs receive excitatory mossy fiber (MF) (8) and parallel fiber (PF) (9) inputs onto their proximal and distal dendrites, respectively, and are predominantly interconnected by gap junctions (GJs) (10, 11). Two-photon uncaging of 4-methoxy-7-nitroindolyl glutamate (12) was used to mimic synaptic inputs at different dendritic locations (Fig. 1A). We tested the linearity of synaptic integration in a dendritic branch by comparing the arithmetic sum of individual photolysis-evoked excitatory postsynaptic potentials (pEPSPs), generated at different locations (section range 10 to 35 μm ; $18 \pm 5 \mu\text{m}$, 49 cells), with the response when all locations were activated synchronously (within a 3-ms window). For small pEPSPs, the synchronous response and arithmetic sum were similar, but for stronger pEPSPs, the integration became markedly sublinear (Fig. 1B). Sublinear integration was observed in apical and basolateral dendrites (0 to 261 μm from soma, $120 \pm 54 \mu\text{m}$, 65 apical and 16 basolateral branches) (fig. S1, A and B). However, for a given size of pEPSP recorded at the soma, sublinear dendritic integration became more pronounced with increasing distance from the soma (Fig. 1, C and D, and fig. S1C).

To investigate whether active conductances are involved in sublinear dendritic integration, we made paired whole-cell recordings from the dendrite and soma using two-photon guided

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patching (13) (Fig. 2A). Although GoC dendrites are thin (diameter $0.9 \pm 0.1 \mu\text{m}$, $n = 19$), recordings could be made up to $184 \mu\text{m}$ from the soma (range 19 to $184 \mu\text{m}$; $88 \pm 47 \mu\text{m}$). Resting potentials at the soma and dendrite were similar ($P > 0.05$) [see supporting online material (SOM)]. Current injections into the dendrite produced voltage changes that were maintained for the duration of the pulse (distance $116 \pm 37 \mu\text{m}$, eight cells) (Fig. 2B) and had a linear current-voltage relation [correlation coefficient (r) = 0.99] (Fig. 2C). In contrast, somatic current injections produced hyperpolarizing voltage responses with a sag and a nonlinear current-voltage relation [recorded in tetrodotoxin (TTX)] (Fig. 2, B and C). Injection of artificial excitatory postsynaptic current (EPSC)-shaped currents (aEPSCs) (fig. S2) into the dendrites produced EPSP-like local voltage responses (distance $103 \pm 28 \mu\text{m}$, five cells) (Fig. 2D). The waveform of these aEPSPs was invariant, irrespective of the size of the depolarization (Fig. 2D, inset, and fig. S3, A to C), which indicated linear dendritic behavior. In contrast, aEPSC injections into the soma produced aEPSPs with decays that accelerated with depolarization (eight cells, in TTX) (Fig. 2E), which indicated the presence of perisomatic active conductances. The integrals of the dendritic and somatic responses were markedly different (Fig. 2F). Cell-attached recordings from the soma confirmed the presence of depolarization-activated outward currents (12 patches), consistent with K^+ conductances in GoCs (9, 14), but negligible inward or outward currents were observed in dendritic patches (15 patches) (Fig. 2, G and H). Any functional voltage-activated Na^+ , Ca^{2+} , and K^+ channels present on the dendrite are, therefore, below our detection limit and do not contribute substantially to the electrical properties of GoCs. Seven additional observations confirmed that few intrinsic voltage-gated channels are present on GoC dendrites: (i) the K^+ channel antagonists tetraethylammonium and 4-aminopyridine had no effect on pEPSPs (fig. S3, D and E); (ii) the SK-channel blocker apamin had no effect on PF EPSPs (fig. S4); (iii) there was little or no hyperpolarization-activated current (h current)-dependent sag (9) in dendritic recordings (Fig. 2B and fig. S5A) or in somatic recordings when the axon was cut (fig. S5B); (iv) immunolabeling for the HCN1 subunit, which mediates the h current, was strong in the axon, but no detectable immunoreactivity was found in dendrites (fig. S5, C to E); (v) differentiating the pEPSP rise showed no evidence of dendritic spikes (12) (fig. S1, D to G); (vi) the persistent Na^+ current was blocked by local TTX application to the axon initial segment (AIS) (fig. S6); and (vii) immunolabeling for Na^+ channel subunits was strong in the AIS but not detectable in GoC dendrites (fig. S7, E and F).

Distal synapses can have a larger conductance than proximal inputs (15). To examine whether this counteracts dendritic attenuation in GoCs, we made dual soma-dendrite recordings and compared the properties of fast-rising spontaneous EPSPs (sEPSPs), arising close to the dendritic pipette (13, 16), and aEPSPs (15). The amplitude of dendritic sEPSPs increased with distance from the soma, whereas their amplitude at the soma decreased (Fig. 3, A and B). The effective space constants for dendrite-to-soma attenuation of sEPSPs and aEPSPs were comparable (Fig. 3C), which suggests that synaptic conductance does not compensate for dendritic attenuation. N-Methyl-D-aspartate (NMDA) receptors made only a small contribution to PF responses (fig. S8, A to C), similar to that observed for MF inputs (8).

For some inhibitory interneurons, the axon arises from the dendrite (2, 5, 6) rather than from the soma. Because this affects how synaptic inputs are integrated, we made paired soma-dendrite recordings to determine the spike initiation site in GoCs. Current injection into the dendrite or soma evoked an action potential (AP) that always occurred first at the soma ($n = 19$ cells, $r = 0.83$; $P < 0.001$) (Fig. 3, D and E). Moreover, the rapid decay of the AP amplitude with distance from the soma (Fig. 3F) is consistent with a passive spread of voltage into the dendrites (5, 6). To distinguish whether the AP arose in the soma or axon, we made whole-cell recordings from putative axons (29 to $57 \mu\text{m}$ from soma, $43 \pm 10 \mu\text{m}$, n

= 6) (fig. S7, A to C). Current injections into the axon or soma triggered APs that always occurred first in the axon. Immunolabeling with the GoC-specific marker metabotropic glutamate receptor 2 (mGluR2) (17) and the AIS markers ankyrin-G, pan- Na_v , or $\text{Na}_v1.6$ confirmed that a single AIS arose $3.7 \pm 1.5 \mu\text{m}$ ($n = 12$) from the soma and that it expressed a high density of Na^+ channels (fig. S7, D to F).

In vivo, GoCs receive both synchronous (18) and slowly modulated asynchronous synaptic inputs (19) that are spatially distributed on the dendritic tree. Because it is difficult to study this experimentally, we developed experimentally constrained multicompartment models of GoCs that matched our measurements (Fig. 3, C, E, and F) and reproduced a wide range of firing behavior (10, 20). Synaptic inputs with identical conductance waveforms (fig. S2D) were randomly distributed over the dendritic tree: Those in the granule cell layer were defined as MFs, whereas those in the molecular layer were defined as PFs (Fig. 3G, inset). Synchronous activation of subsets of six randomly selected MF inputs was required to fire the model GoC from -60 mV (Fig. 3G), as found experimentally (8). In contrast, 11 PF inputs were required to fire the model cell, which demonstrated that distal inputs are less efficient (Fig. 3G). Sustained asynchronous MF inputs were also more effective than PF inputs, as they produced a higher firing rate (Fig. 3H). Similar results were obtained for two other morphologically reconstructed GoCs (fig. S9). During asynchronous PF input, dendrites in the molecular layer depolarized more than dendrites in the granule cell layer during MF input, because the latter were more hyperpolarized by perisomatic pacemaker conductances (9, 14, 20) (Fig. 3I). The reduced driving force in distal dendrites limited the synaptic current generated, which made PFs less effective than MFs and explained the distance-dependent sublinear summation of pEPSPs (Fig. 1). Indeed, converting synaptic conductances to currents (which are independent of driving force) in our model reduced the difference in efficacy between MFs and PFs during both synchronous and asynchronous input (Fig. 3, G and H).

As for many types of inhibitory interneurons in the brain (7), GoCs are coupled through electrical synapses, mediated by GJs composed of connexin-36 (Cx36) subunits (10, 11). We examined their impact on input resistance (R_{input}) by applying $20 \mu\text{M}$ mefloquine, a Cx36-specific concentration that blocks $\sim 70\%$ of the GJ conductance (21). This increased R_{input} in wild-type mice by 48% ($P = 0.0002$; $n = 13$ cells), without having an effect on GoCs from Cx36 knockout mice (22) ($P = 0.2$) (Fig. 4A). This difference was significant ($P = 0.01$). Moreover, GoCs from Cx36 knockout mice ($n = 8$) had a 77% higher R_{input} than those from wild-type mice ($P = 0.007$, $n = 7$) (Fig. 4A). To examine how GJs are distributed, we counted Cx36-positive puncta on mGluR2-positive GoCs. This revealed a nonuniform GJ density, which peaked in the dendrites located in the inner molecular layer (Fig. 4B) and provided no evidence of axonal GJs (fig. S10). Consistent with this distribution, cutting off the molecular layer dendrites markedly reduced the effect of mefloquine on R_{input} ($P = 0.003$, $n = 5$) (Fig. 4A and fig. S11). Using the density of Cx36 puncta and mean dendritic surface area from reconstructions ($n = 5$), we estimate that a GoC forms 35 GJs.

To explore how electrical synapses and passive dendritic properties affect chemical synaptic integration at the network level, we built experimentally constrained GoC network models (10, 23) (Fig. 4C). Electrical coupling was implemented probabilistically, with discrete GJ conductances, using the nonuniform GJ density (Fig. 4B) and distance-dependent (soma-soma) coupling probabilities (10) (see SOM). Removing GJs from the models increased GoC R_{input} , as found experimentally (Fig. 4A). Without GJ coupling, network models exhibited different mean input-output relations for MF-only and PF-only excitation (Fig. 4D), as for the single GoC models. By contrast, when GJs were included, synaptic excitation was enhanced, and the difference between MF- and PF-driven network excitation was reduced (Fig. 4D). Separate analysis of innervated and noninnervated GoCs (Fig. 4, E and F)

revealed GJ-mediated excitation of the latter population. We tested this model prediction with GoC paired recordings. These showed that depolarization of a single GoC could “wake up” neighboring GoCs and cause them to fire APs (four pairs) (Fig. 4G). Simulations revealed that the efficacy with which MFs and PFs excite noninnervated GJ-coupled cells is switched compared with the efficacy of excitation of innervated cells (Fig. 4, E and F). Moreover, inclusion of synaptic NMDAR components further enhanced the efficacy of PFs over MFs in driving noninnervated GJ-coupled cells (fig. S8, F and I). Two factors augmented the spread of excitatory charge from PF synapses to neighboring GoC dendrites: colocalization of PF synapses and GJs in the molecular layer minimized dendritic attenuation of current (Fig. 4H), and GJ driving force was larger because distal dendrites depolarized more during synaptic input (Fig. 3, A and I). GJ-mediated enhancement of synaptic excitation was robust across a wide range of GJ conductances and densities (Fig. 4I and fig. S12), was robust in the presence of chemical inhibitory conductances, (fig. S13), and was strongest when the GoC population was sparsely activated by chemical excitatory synaptic inputs (fig. S14).

Our results show that the passive properties of GoC dendrites confer distance-dependent sublinear chemical synaptic integration. This weakens the impact of distal excitatory inputs. However, the high density of dendritic GJs in the molecular layer enables PF synaptic charge to flow into the dendrites of neighboring GoCs. This GJ-mediated lateral excitation counteracts the effects of sublinear dendritic behavior by enabling distal inputs to drive network activity more effectively. Dendritic GJs therefore counteract the problem of dendritic saturation (24) without the need to boost electrically remote synaptic input with active dendritic conductances (25). A key role of interneurons is to counteract and balance network excitation. The combination of passive dendrites and dendritic GJs facilitates this by enabling a larger fraction of interneurons to respond to localized patches of synaptic excitation. Our results reveal how GJs on inhibitory interneuron dendrites could contribute to spatial averaging, which has been proposed in the retina (26) and excitatory olfactory neurons in insects (27), and to the broad tuning of inhibitory interneurons in cortex (28). These mechanisms are also likely to contribute to gain control in the granule cell layer through PF-mediated feedback (29), and it seems likely that interneurons in cortical and subcortical structures (7) use similar mechanisms. Our results suggest that interneurons do not operate as fully independent neuronal units but share charge during chemical synaptic excitation and thus exhibit features of a syncytium.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Distance-dependent sublinear dendritic integration in GoCs. **(A)** (Left) GoC filled with Alexa-594 dye: granule cell layer (GCL), Purkinje cell layer (PCL) (dashed circles), and molecular layer (ML). (Right) Glutamate uncaging locations for dendrite 2 on left and individual pEPSPs at about -70 mV for 10 locations; average in gray. **(B)** (Top) Mean arithmetic sum of individual pEPSPs (five locations) and mean pEPSP evoked by synchronous uncaging at the same locations. (Bottom) Uncaging at 10 locations and the effect of 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX). **(C)** Synchronous pEPSP and arithmetic sum for each dendritic branch indicated in (A); number of uncaging locations and laser power were adjusted to evoke similar somatic pEPSP amplitudes. **(D)** Ratio of synchronous pEPSP amplitude to arithmetic sum versus branch-soma distance. Only synchronous pEPSPs with similar amplitude (3 to 5 mV) were used (averaged over 40- μm bins).

Fig. 2.

GoC dendrites behave as linear cables. (A) GoC filled with Alexa-594 dye and pipette locations. (Inset) Superimposed Dodt-contrast and fluorescence images of the patched dendrite. (B) (Top) Dendritic voltage responses to dendritic current pulses of equal magnitude. (Bottom) Somatic voltage responses to somatic current pulses (in 1 μ M TTX). (C) Steady-state current-voltage relations. (D) Dendritic voltage responses to dendritically injected aEPSCs (red) of increasing amplitude. (Inset) Seven responses normalized to peak. (E) Somatic voltage responses to somatic aEPSCs in 1 μ M TTX. (Inset) Nine responses normalized to peak. (F) Relation between aEPSP area and aEPSC amplitude, with linear extrapolations to the initial two responses. (G) Cell-attached voltage-clamp recordings; current responses to 20-mV steps from -80 mV to $+40$ mV. (H) Amplitude of transient outward currents (red arrow in G), sustained outward currents (black arrow in G), and inward currents versus distance to soma.

Fig. 3.

Passive dendrites weaken distal synaptic inputs. **(A)** Simultaneous somatodendritic recordings of fast-rising (<0.5 ms) sEPSPs originating locally in the dendrite. Pipette locations are shown. **(B)** Amplitudes of sEPSPs in the dendrite and soma versus soma-dendrite distance. **(C)** sEPSP and aEPSP attenuation from dendrite to soma, with exponential fits and model prediction from morphologically realistic GoC model. **(D)** Simultaneously recorded APs in soma and dendrite, evoked by current injection (50 pA, 200 ms). **(E)** Latency between somatic and dendritic APs. **(F)** Attenuation of back-propagating APs with soma-dendrite distance. **(G)** (Inset) Reconstructed GoC morphology for modeling [red, granule cell layer (GCL), blue, molecular layer (ML)]. (Main) Relations between spike probability and number of inputs for synchronous MF and PF synaptic conductance and current (10 simulations). **(H)** Mean input-output relations for asynchronous MF and PF synaptic inputs (four simulations). **(I)** Simulated somatic and dendritic voltage traces for granule cell layer and molecular layer dendrites [arrowheads in (G) indicate locations] during MF and PF inputs (at ~230 Hz).

Fig. 4. Dendritic gap junctions enhance the efficacy of distal synaptic input. **(A)** (Left to right) Mean ($\pm$ SEM) increase in GoC input resistance (R_{input}) with 20 μ M mefloquine for wild-type (WT), WT dendrotomy, and $Cx36^{-/-}$ mice, respectively [in 10 μ M NBQX, 50 μ M D-(-)-2-amino-5-phosphonopentanoic acid (AP5), and 10 μ M SR95531]; between $Cx36^{-/-}$ mice and $Cx36^{+/+}$ littermates; and when GJs were removed from GoC network models. **(B)** Cx36 immunopuncta (red) on mGluR2-positive dendrites (green) in the granule cell layer (GCL) and the inner-, middle-, and outer molecular layer (ML). Scale bar, 50 μ m. (Inset) Cx36 punctum between two mGluR2-positive dendrites, Scale bar, 5 μ m. Bar graph indicates density of Cx36 puncta. **(C)** Network model with 45 GoCs; 15 were innervated by MF or PF inputs. **(D)** Network input-output relations showing the mean spike frequency across all GoCs during asynchronous MF (red) or PF input (blue) without (dashed lines) and with (solid lines) GJs between cells (mean $\pm$ SD, four networks). (Inset) Top view showing innervated (yellow) and noninnervated (purple) GoCs. **(E and F)** Input-output relations for innervated (E) and noninnervated (F) GoCs only. **(G)** Voltage responses of a GoC pair to hyper- and depolarizing current pulses in the prejunctional cell [cell 2, filled with a quaternary lidocaine derivative (QX314) to block spiking]. **(H)** Spike frequency increase of all GoCs for networks with measured dendritic GJ distribution (left), with a homogeneous dendritic GJ distribution (middle), or with GJs restricted to the soma (right). **(I)** Spike frequency increase versus GJ conductance. **(J)** Proximal MF inputs strongly activate

innervated GoC, but spread of synaptic charge to GJ-coupled GoCs is weak. Distal PF inputs weakly activate innervated GoC, but synaptic charge spreads efficiently to GJ-coupled GoCs, increasing their firing.