In the central nervous system, dendritic arborizations of neurons undergo dynamic structural remodelling during development. Processes are elaborated, maintained or eliminated to attain the adult pattern of synaptc connections. Although neuronal activity influences this remodelling, it is not known how activity exerts its effects. Here we show that neurotransmission-evoked calcium ($Ca^{2+}$) release from intracellular stores stabilises dendrites during the period of synapse formation. Using a ballistic labelling method to load cells with $Ca^{2+}$ indicator dyes, we simultaneously monitored dendritic activity and structure in the intact retina. Two distinct patterns of spontaneous $Ca^{2+}$ increase occurred in developing retinal ganglion cells—globular increases throughout the arborization, and local ‘flashes’ of activity restricted to small dendritic segments. Blockade of local, but not global, activity caused rapid retraction of dendrites. This retraction was prevented locally by local uncaging of caged $Ca^{2+}$ that triggered $Ca^{2+}$ release from internal stores. Thus, local $Ca^{2+}$ release is a mechanism by whichafferent activity can selectively and differentially regulate dendritic structure across the developing arborization.

To observe directly how neural activity dynamically regulates dendritic structure during development, we introduced $Ca^{2+}$ indicator dyes into retinal ganglion cells by a ballistic method that resulted in completefilling of the dendritic arborization (Fig. 1a). Using Oregon Green 488 BAPTA-1 dextran (OG1B; $k_d = 0.3–0.4 \mu M$), $Ca^{2+}$ levels were monitored across the dendritic arborisation ($266, 16.0 s$, occurring synchronously in neighbouring cells. In 9.6 $s$, 4 cells, 12 events) and lasted between 3.0 $s$ and 28.0 s ($8.9 \pm 2.5 s; 7 cells, 26 events$). The mean change in fluorescence ($\Delta F/F_o$ see Methods) of these events was $27.0 \pm 1.4%$ (12 cells, 129 events). With a lower affinity indicator, Fluo-4 dextran ($k_d = 4.1 \mu M, n = 3 cells, 5 events$; see Supplementary Information 2A), we observed local events with similar duration (10.2 $\pm 1.8 s$) and extent ($9.8 \pm 2.9 \mu M$), albeit at lower amplitude ($\Delta F/F_o = 13.2 \pm 1.9\%$). Local flashes appeared with a frequency of $5.2 \pm 0.7 min^{-1} per mm dendrite at E13. Qualitative comparison of the onset times of local events across the arborization did not reveal any strict temporal patterns in their occurrence, although they can occur repeatedly in the same locations (Fig. 1e).

In addition to local flashes, we observed $Ca^{2+}$ levels simultaneously increasing in the soma and throughout the arborization (Fig. 1b and c). Global activity was periodic at intervals of $19–29$ s in the intact retina. Two distinct types of $Ca^{2+}$ transients were observed (Fig. 1b and c; see Supplementary Information 1). First, local patches of dendrites showed transient increases in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) that extended from $5.0$ to $16.0 \mu M$ (mean $\pm s.e.m. = 9.6 \pm 0.9 \mu M; 5 cells, 12 events$) and lasted between 3.0 and $28.0 s (8.9 \pm 2.5 s; 7 cells, 26 events$). The mean change in fluorescence ($\Delta F/F_o$ see Methods) of these events was $27.0 \pm 1.4\%$ (12 cells, 129 events). With a lower affinity indicator, Fluo-4 dextran ($k_d = 4.1 \mu M, n = 3 cells, 5 events$; see Supplementary Information 2A), we observed local events with similar duration (10.2 $\pm 1.8 s$) and extent ($9.8 \pm 2.9 \mu M$), albeit at lower amplitude ($\Delta F/F_o = 13.2 \pm 1.9\%$). Local flashes appeared with a frequency of $5.2 \pm 0.7 min^{-1} per mm dendrite at E13. Qualitative comparison of the onset times of local events across the arborization did not reveal any strict temporal patterns in their occurrence, although they can occur repeatedly in the same locations (Fig. 1e).

In addition to local flashes, we observed $Ca^{2+}$ levels simultaneously increasing in the soma and throughout the arborization (Fig. 1b and c). Global activity was periodic at intervals of $80.0 \pm 16.0 s$, occurring synchronously in neighbouring cells. In contrast to local flashes, global activity was shorter in duration ($2.0–7.0 s$, mean $3.9 \pm 0.7 s; 4 cells, 10 events$) and relatively larger in amplitude ($\Delta F/F_o = 53.0 \pm 4.8\%; 12 cells, 23 events$). The global activity resembled that of spontaneous bursting activity observed during propagation of retinal waves (8–10). Simultaneous calcium imaging and perfused patch recordings (5 cells) demonstrated
that global events were associated with depolarization and bursts of action potentials (24 events; Fig. 1d). Voltage changes were not detected during local events (15 events; Fig. 1d). All ganglion cells aged E13–19, and some before E13, exhibited global activity. Local flashes, however, occurred most frequently at E9–13 (Fig. 1f), during the period of extensive dendritic growth and remodelling\(^\text{b}\) that coincides with synaptogenesis in the inner retina\(^\text{a,11}\).

The mechanisms underlying local and global activities were distinct. We focused on E13, when local activity was at its peak. Ca\(^{2+}\) influx was necessary for both local and global activities. However, local but not global activity was significantly reduced by thapsigargin (Tha) and cyclopiazonic acid (CPA) which inhibit the endoplasmic reticulum Ca\(^{2+}\) ATPases (Table 1). Local activity was also diminished by 2-aminoethoxydiphenyl borate (2-APB) and ryanodine (Rya) which block InsP\(_3\)- and ryanodine-sensitive stores, respectively (Table 1). Neurotransmission contributes to evoking this release (Table 1). The neuronal nicotinic receptor antagonist dihydro-\(\beta\)-erythroidine hydrobromide (DH\(\beta\)E) reduced local but not global activity. Atropine, a non-selective antagonist of muscarinic receptors, modulated the frequency of both activities. Local and global activities were unaffected by blocking ionotropic glutamate receptors with 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[1]quinoxaline-7-sulphonamide (NBQX) and 2-amino-5-phosphonovaleric acid (APV), or with the metabolotropic glutamate receptor antagonist, methyl-(4-carboxyphenyl) glycine (MCPG). Although acetylcholine-mediated and not glutamate-mediated transmission evokes Ca\(^{2+}\) release at E13, it remains possible that the latter transmission plays a part at other ages. Local activity persisted in the presence of tetrodotoxin (TTX), which blocked global activity\(^\text{a,10}\). The persistence of local activity in TTX is not surprising because bipolar cell and most amacrine cell transmission are independent of action potentials. Together, these results suggest that at E13, nicotinic receptor-mediated transmission contributes significantly to evoking local release of Ca\(^{2+}\) from internal stores in the ganglion cell dendrites.

Because acetylcholine-mediated transmission arises from amacrine cells, it is possible that the local flashes occur near sites of amacrine cell contact. In one case where we fortuitously labelled a displaced amacrine cell and a nearby ganglion cell (Fig. 2a and b), we observed a potential contact form (Fig. 2c, and Supplementary Information 3). Upon initial contact, the dendrite of the ganglion cell did not show any change in [Ca\(^{2+}\)]\(i\) at the contact site. However, an hour later, the dendritic region adjacent to the site of contact exhibited spontaneous local increases in [Ca\(^{2+}\)]\(i\) (Fig. 2d and e). Although we do not know if acetylcholine-mediated transmission was involved, ionotropic glutamate-receptor-mediated transmission was not involved because the recording was performed in the presence of NBQX and APV. We thus suspect that local flashes are associated with sites at which stable contacts have formed with

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**Table 1** Distinct mechanisms trigger local and global activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Local events</th>
<th>Global events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td></td>
<td>Amplitude</td>
<td>Amplitude</td>
</tr>
<tr>
<td></td>
<td>(n)</td>
<td>(n)</td>
</tr>
<tr>
<td>Zero calcium</td>
<td>–96 ± 2**</td>
<td>–100 ± 0**</td>
</tr>
<tr>
<td>Thapsigargin (1 (\mu)M)</td>
<td>–84 ± 6*</td>
<td>3 ± 13 – 1 ± 7</td>
</tr>
<tr>
<td>CPA (20 (\mu)M)</td>
<td>–88 ± 4*</td>
<td>– 15 ± 10 – 5</td>
</tr>
<tr>
<td>Ryanodine (10 (\mu)M)</td>
<td>–76 ± 8**</td>
<td>12 ± 23 – 5</td>
</tr>
<tr>
<td>2-APB (100 (\mu)M)</td>
<td>–70 ± 6*</td>
<td>16 ± 41 – 4</td>
</tr>
<tr>
<td>DHIE (100 (\mu)M)</td>
<td>–63 ± 9*</td>
<td>45 ± 33 – 17</td>
</tr>
<tr>
<td>Atropine (3 (\mu)M)</td>
<td>–27 ± 8*</td>
<td>–29 ± 11* – 6</td>
</tr>
<tr>
<td>NBQX (10 (\mu)M) + APV (100 (\mu)M)</td>
<td>–8 ± 15</td>
<td>9 ± 22 – 8</td>
</tr>
<tr>
<td>MCPG (1 (n)M)</td>
<td>–27 ± 12 – 7</td>
<td>– 7 ± 19 – 9</td>
</tr>
<tr>
<td>TTX (1 (\mu)M)</td>
<td>–15 ± 15 – 8</td>
<td>–85 ± 9* – 33</td>
</tr>
</tbody>
</table>

*See text for definitions of abbreviations in column 1. **P < 0.05; ***P < 0.01; n, number of cells.

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**Figure 1** Developing dendrites of retinal ganglion cells exhibit global and local spontaneous [Ca\(^{2+}\)]\(i\) increase. a, E13 cell filled with OGB1 dextran by ballistic delivery. b, Local (L1, L2) and global (G) Ca\(^{2+}\) increases at three times. Increases in [Ca\(^{2+}\)]\(i\), represented by ΔF/ \(F_0\), pseudocolour scale in c, e, Relative timing of the global and the two local events in b. Arrowheads mark extent of the measured dendritic segments. d, Simultaneous Ca\(^{2+}\) imaging and perforated-patch voltage recording (E13). Arrowhead, onset of local event. e, Distribution of local events of this cell. f, Local activity with age; error bars show ± s.e.m; number of cells shown in parentheses.
amacrine cells, possibly acetylcholine-containing amacrine cells, which constitute 90% of displaced amacrine cells in the chick retina. Because local signalling occurs during the period of dendritic development, we examined whether disruption of local activity altered dendritic organization. Dendrites retracted rapidly upon application of thapsigargin or 2-APB (Fig. 3a and b). Images of cells (n = 2) acquired every minute suggested that retraction started within a few minutes of thapsigargin application (Supplementary Information 4). Similar retraction occurred in the presence of all pharmacological agents that blocked local activity and Ca$^{2+}$ release from stores (Fig. 3c, Supplementary Information 2B). The retraction rate decreased with time, perhaps because the remaining larger proximal processes are more stable than the finer terminal dendrites. In contrast, dendrites were stable in the presence of pharmacological agents that preserved local activity, even when global activity was blocked (TTX, Fig. 3c). Indeed, in vivo blockade with TTX does not affect dendritic development of retinal ganglion cells. Together, these observations indicate that local, not global, activity sustains dendrites during development.

To address directly whether dendrites can be selectively stabilized by local Ca$^{2+}$ release, we attempted to differentially ‘rescue’ dendrites from retracting when spontaneous local flashes were blocked. Caged Ca$^{2+}$ (o-nitrophenyl EGTA, NP-EGTA) was introduced together with OGB1 into single cells using the ballistic method. In this way, Ca$^{2+}$ release was restricted to the labelled cell. Co-labelling with the Ca$^{2+}$ indicator allowed us to estimate the spread of Ca$^{2+}$ that was uncaged within the dendritic arborization Before uncaging, the retinae were placed in zero Ca$^{2+}$ solution (Ringer’s solution containing 0 mM Ca$^{2+}$, 4 mM Mg$^{2+}$ and 1 mM BAPTA) to induce net retraction of dendrites. Ca$^{2+}$ was then released locally by focusing an ultraviolet (UV) laser beam upon part of the arborization (Fig. 4a). Uncaging caused a local but relatively sustained elevation in [Ca$^{2+}$]i when the stores were not pharmacologically blocked. In contrast, in the presence of ryanodine and 2-APB, laser uncaging evoked a localized but transient increase in [Ca$^{2+}$]i (Fig. 4b). These observations suggest that uncaging in the absence of store blockers may have caused Ca$^{2+}$-induced Ca$^{2+}$ release (CICR). Terminal dendrites that experienced CICR after photo-uncaging did not retract, whereas unstimulated processes retracted.
as expected (Fig. 4c and d, Supplementary Information 2c). Continual activity may be required for stabilizing dendrites, because dendrites within the stimulated region retracted somewhat after photo-uncaging ceased (Fig. 4d). When uncaging occurred in the presence of ryanodine and 2-APB, both stimulated and unstimulated dendrites retracted (Fig. 4e). Thus, CICR is required to locally stabilize dendritic processes.

What regulatory signals could local activity provide that differ from those of global activity? Differences in amplitude, temporal and spatial properties of the Ca\textsuperscript{2+} signal can account for the enormous diversity of actions by this ion\textsuperscript{15,16}. The local and global activities differ in all these characteristics, and in the mechanisms by which [Ca\textsuperscript{2+}], is increased within the dendrite. CICR, rather than Ca\textsuperscript{2+} influx alone, is the key mechanism stabilizing dendrites: the localized nature and/or sustained increase in [Ca\textsuperscript{2+}], of CICR may be the important factors. Despite the large increase in [Ca\textsuperscript{2+}], during a global event, [Ca\textsuperscript{2+}], rises due to influx might be limited to regions close to the cell membrane, and do not effectively unload the stores. Alternatively, global events may trigger Ca\textsuperscript{2+} release, but opening of voltage-gated Ca\textsuperscript{2+} channels concurrent with nicotinic receptor activation may suppress the downstream effects of CICR, as previously observed\textsuperscript{17}. Local release of Ca\textsuperscript{2+} in cultured cells\textsuperscript{18} has been shown to regulate spine structure\textsuperscript{19} and synaptic plasticity\textsuperscript{20-22}. The spatial confinement of Ca\textsuperscript{2+} increase in spines\textsuperscript{22} and small segments of dendrites enables local regulation of synaptic strength\textsuperscript{23,24}. Likewise, localized Ca\textsuperscript{2+} release may dynamically and selectively regulate dendritic structure across the arborization during development by maintaining only dendrites that receive active inputs\textsuperscript{25-28}, thus enabling an efficient distribution of postsynaptic material. Local control of dendritic stability may also be a means for selecting between competing inputs; inputs that trigger CICR may be maintained whereas those that fail to do so may be eliminated by dendritic retraction. The patterning of synaptic inputs onto a dendritic tree may thus be sculpted not only by selective withdrawal or maintenance of afferent terminals, but also by local stabilization or destabilization of dendrites.

Methods

Tissue preparation and dye-filling

Embryonic chick retinas were prepared as previously described\textsuperscript{10}. Before Ca\textsuperscript{2+} imaging, the retina was shot with tungsten particles coated with a Ca\textsuperscript{2+} indicator using the ‘Calistic’ method\textsuperscript{7}. Briefly, 1.5 mg of OGB1 (Molecular Probes, no. O-6798) or Fluo-4 dextran (Molecular Probes, no. F-14240) was dissolved in H\textsubscript{2}O and then mixed with 25 mg of 1.7-μm-diameter tungsten particles (BioRad). The mixture was spread thinly on a glass slide, allowed to dry completely, and transferred into polypropylene tubing (BioRad) that was precoated with 1% (w/v) polyvinylpyrrolidone (Sigma) solution. For the uncaging studies, the particles were co-coated with 0.5 mg NP-EGTA (Molecular Probes, no. N-6802) and 1.5 mg OGB1. Particles were delivered at 80 p.s.i. through a culture insert membrane (Falcon, no. 35-3092) using the BioRad Helios gene gun\textsuperscript{27,28}. Cells labelled by Calistics maintained their endogenous content and activity over several hours. Cells were not damaged using the ballistic method because (1) they exhibited spontaneous Ca\textsuperscript{2+} activity resembling retinal wave activity\textsuperscript{7}, (2) their dendritic filopodia moved at rates similar to that previously observed in cells expressing green fluorescent protein\textsuperscript{5} and (3) synaptic excitatory potentials were observed.

Imaging

Recordings were performed using a cooled CCD (SensiCam, Cooke) or a silicon-intensifier-target tube (SIT) camera (C2400-08, Hamamatsu), controlled with MetaFluo or MetaMorph (Universal Imaging Inc.). Images were acquired at 0.3–3 Hz (CCD) or 7.5–30 Hz (SIT), for continuous recording over 1–10 min (CCD) or 0.5–2 min (SIT). Integration time for each image was 0.2–2.5 s (CCD). To release Ca\textsuperscript{2+} locally, a UV laser beam (Stabilite 2017, Spectra Physics) was focused onto a selected region of the dendritic arborization using the 400× water immersion objective. The half-maximal width of the spot was 32–36 μm. The power of the UV beam at the sample was less than 1 mW. (Ca\textsuperscript{2+}), levels were monitored during photo-uncaging of NP-EGTA. A dichroic mirror placed in front of the mercury lamp housing reflected the UV laser beam into the objective, and enabled blue excitation light to pass through during Ca\textsuperscript{2+} imaging. Typically, seven consecutive pulses (10–40 ms duration, approximately 8–32 μl per pulse) were delivered at 3-min intervals...this was the minimal stimulus that produced reliable uncaging with each pulse. Pharmacological agents were obtained from Sigma Chemicals unless stated otherwise.

Patch recording

Perforated-patch voltage recordings (gramicidin D, 100 mg l\textsuperscript{-1}, Sigma) were carried out in current-clamp mode, with no current injected\textsuperscript{27}. Excitatory postsynaptic potentials (EPSPs) were identified using Minianalysis (Synaptosoft)\textsuperscript{29}. EPSPs were defined as events with rise times of <1 ms (typically 0.3 ms), and amplitudes that exceeded the root mean square of the baseline noise (typically around 0.08 mV) by threefold. No EPSPs were detected within 1 s before or 0.5 s after the onset of the local Ca\textsuperscript{2+} events.

Data analysis

Changes in [Ca\textsuperscript{2+}], are given by \(\Delta F/F\), where \(F_0\) is the baseline fluorescence level, \(\Delta F\) is the
change intensity from this value. Care was taken to omit all changes that were due to focal shifts or sample movement in any dimension. The spatial extent (half-maximal width) of focal flashes measured from line scans of ΔF/F0 at the time of maximal intensity change. To determine the effects of the pharmacological agents, we followed and compared the summed length of the same processes in control Ringer’s solution and after applying the agents. Because the distribution of neuronal processes is largely two-dimensional in the retina, complete representations of the majority of dendrites could be obtained in single planes. Comparison of the total lengths of the processes in control solution at two time points, 20 min apart, indicated that addition and retraction of processes were balanced, with no net change in total length25. The total length was then measured 25–30 min after the drug was introduced. Changes in total length upon drug treatment were expressed as a percentage of baseline length. The rate of change was calculated by dividing this number by the time between measurements (% change per min).

In the uncaging experiments, we measured the total length of all terminal processes in focus that were at least partially located within 20 μm around the centre of the laser spot, before and after stimulation. All other terminal dendrites outside this region of maximal stimulation served as controls. The average initial lengths of individual terminal dendrites were similar in both groups (inside and outside the stimulated area). All statistical analyses: t-test, paired or unpaired.

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Competing interests statement
The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to R.O.L.W. (e-mail: wong@pcg.wustl.edu).

TRPV3 is a calcium-permeable temperature-sensitivity cation channel

Haoxing Xu1, I. Scott Ramsey2, Suhas A. Kotecha1,‡, Magdalene M. Moran3, Jayhong A. Chong4, Deborah Lawson1, Pei Ge5, Jeremiah Lilly†, Inmaculada Silos-Santiago6, Yuxie Liu7, Peter S. DiStefano8,9, Rory Curtis8 and David E. Clapham*1

*Howard Hughes Medical Institute, Children’s Hospital, Harvard Medical School, Enders 1309, 320 Longwood Avenue, Boston, Massachusetts 02115, USA
‡Program in Neuroscience, Harvard Medical School, Goldenson Building B2-228, 220 Longwood Avenue, Boston, Massachusetts 02115, USA
§Millennium Pharmaceuticals Inc., 75 Sidney Street, Cambridge, Massachusetts 02139, USA
†These authors contributed equally to this work.

Transient receptor potential (TRP) proteins are cation-selective channels that function in processes as diverse as sensation and vasoregulation. Mammalian TRP channels that are gated by heat and capsaicin (>43°C; TRPV1 (ref. 1)), noxious heat (>52°C; TRPV2 (ref. 2)), and cooling (<22°C; TRPM8 (refs 3, 4)) have been cloned; however, little is known about the molecular determinants of temperature sensing in the range between ~22°C and 40°C. Here we have identified a member of the vanilloid channel family, human TRPV3 (hTRPV3) that is expressed in skin, tongue, dorsal root ganglion, trigeminal ganglion, spinal cord and brain. Increasing temperature from 22°C to 40°C transmits in mammalian cells transfected with hTRPV3 elevated intracellular calcium by activating a nonselective cationic conductance. As in published recordings from sensory neurons, the current was steeply dependent on temperature, sensitized with repeated heating, and displayed a marked hysteresis on heating and cooling8–10. On the basis of these properties, we propose that hTRPV3 is thermosensitive in the physiological range of temperatures between TRPM8 and TRPV1.

Survival depends on the precise regulation of body temperature11. Certain dorsal root ganglia (DRG) neurons detect temperature changes between 32°C and 43°C (warmth)12 and respond with calcium influx processes13. Recordings of a nonselective ion channel activated by temperatures from 25°C to 49°C (ref. 6), as well as temperature-activated cationic currents in both central and sensory neurons14–18 are indicative of an involvement of TRP ion channels. Activation of TRP channels depolares cells from the resting membrane potential and shortens action potential duration. Most TRP channels are cation-nonselective and permeant to the signal transduction element Ca2+. Although all channels and enzymes are inherently temperature-sensitive, the discovery of ‘hot pepper’, noxious heat* and menthol-sensitive† TRP channels with high 10-degree temperature coefficients (Q10 values (ref. 12)) motivates the search for other ion channels in the mammalian TRP superfamily.