In a small fraction of *Xenopus* tadpoles, a single retinal ganglion cell (RGC) axon misprojects to the ipsilateral optic tectum. Presenting flashes of light to the ipsilateral eye causes that ipsilateral axon to fire, whereas stimulating the contralateral eye excites all other RGC inputs to the tectum. We performed time-lapse imaging of individual ipsilaterally projecting axons while stimulating either the ipsilateral or contralateral eye. Stimulating either eye alone reduced axon elaboration by increasing branch loss. New branch additions in the ipsi axon were exclusively increased by contralateral eye stimulation, which was enhanced by expressing tetanus neurotoxin (TeNT) in the ipsilateral axon, to prevent Hebbian stabilization. Together, our results reveal the existence of a non–cell-autonomous “Stentian” signal, engaged by activation of neighboring RGCs, that promotes exploratory axonal branching in response to noncorrelated firing.

The experimental paradigm consisted of 1 h of darkness followed by 2 h of monocular light flashes (10 ms at 0.5 Hz) to either the contralateral (contra) or ipsi eye (Fig. 1C). Stimulating the contra eye alone activates many axons, and postsynaptic partners, around the single ipsi axon being imaged, and thus tests how activity of surrounding inputs modulates axonal growth and dynamics. Conversely, ipsi eye stimulation activates just the single axon without its neighbors. Ipsi eye stimulation resulted in simpler arbors (Fig. 1 D–H), with fewer branch tips (Fig. 1 F) and reduced branch density (Fig. 1 H) after 2 h compared to contra eye stimulation. While stimulation of either eye reduced the rate of axon branch accumulation compared to RGC axon growth in darkness, activation of the ipsi eye caused a greater reduction in branch elaboration, resulting in a loss of branch tip number over time (Fig. 1 I).

While visual stimulation caused no significant differences in branch addition (mean number of new branch tips every 10 min, normalized to 1 h of darkness) or elongation (mean length added every 10 min on growing branches, normalized to 1 h of darkness) (Fig. 1 J and K), visual stimulation through either eye significantly increased rates of branch loss and retraction relative to baseline in darkness (Fig. 1 L and M). Thus, activating neighboring inputs increased addition and loss rates comparably, maintaining arbor complexity, whereas stimulation of the axon of interest reduced arbor complexity by shifting the balance to favor branch loss over addition (Fig. 1 F and H).

In prior studies, synaptic activation of N-methyl-D-aspartate receptors decreased axon branch addition rates, suggesting Hebbian stabilization (7, 10). Expressing tetanus neurotoxin (TeNT) to block transmission from RGC axons eliminated this branch suppression and unmasked an activity-dependent up-regulation in branch additions (7, 11). To remove confounding influences of the stabilization signal, we tested the effects of ipsi versus contra eye stimulation on TeNT-expressing axons.

When blocking vesicular release in the ipsi axon, contra eye stimulation increased the rate of new branch additions and elongation compared to darkness, whereas ipsi eye stimulation had little impact (Fig. 2 A–F). Branch loss was significantly up-regulated with contra eye stimulation, and, to a lesser extent, with ipsi eye stimulation (Fig. 2 G and H), which enhanced branch retraction (Fig. 2 I and J). In summary, axon branching and elongation were up-regulated by stimulating the surrounding contra eye axons and not by stimulating the ipsi axon. Branch elimination was increased by stimulating either eye, with retractions significantly enhanced by ipsi eye stimulation.

Stentian structural plasticity in the developing visual system

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Edited by Yuh Nung Jan, Howard Hughes Medical Institute and University of California, San Francisco, CA, and approved April 16, 2020 (received for review January 22, 2020)
These experimental findings support a model in which firing up-regulates branch loss in general, but activity of nearby axons also generates a signal that promotes new branch formation in noncoactive axons. Thus, axons exhibit exploratory branch dynamics (both formation and elimination of branches) under conditions of uncorrelated firing.

In an earlier study when contra and ipsi eyes were synchronously stimulated, synaptic strength was maintained and exploratory branch remodeling was down-regulated, indicative of a Hebbian stabilization mechanism (7). In contrast, asynchronous stimulation of the eyes weakened synaptic inputs and destabilized axon branches. Such findings support Stent’s corollary that synaptic efficacy may be reduced when there is postsynaptic activity without concurrent presynaptic firing (6, 12, 13). Our results provide direct evidence for an intercellular “Stentian” signal that promotes an axon’s elaboration when other inputs repeatedly and persistently fire without it, forcing it to seek out more appropriate contacts elsewhere through exploratory growth.

**Methods**

Animals were maintained at 18 °C to 21 °C with a 12-h:12-h light–dark cycle. We performed plasmid electroporation as described previously (7), using 1.5 μg/μL to 2 μg/μL pEGFP-N1 or 5UAS-TeNT-Lc:EGFP (TeNT) + pbGAL4-VP16
Fig. 2. TeNT expression in ipsi RGC axon enhances dynamic branch additions in response to contra, but not ipsi, eye stimulation. (A and B) TeNT-expressing ipsi RGC axons undergoing (A) contra eye stimulation or (B) ipsi eye stimulation. Reconstructed arbors show cumulative changes during 1 h in darkness and the last hour of stimulation. (C and D) Mean branch additions every 10 min. (C) normalized to mean additions in darkness and (D) binned by hour. (E–J) Corresponding graphs for (E and F) branch elongation, (G and H) losses, and (I and J) retraction. All graphs present TeNT-expressing ipsi axons from contra-eye-stimulated (n = 7, dark blue) and ipsi-eye-stimulated animals (n = 9, light blue). Mixed-design two-way ANOVA to compare hours is indicated above bar graphs, and interaction of time vs. eye stimulated is indicated to the right of time plots (*P < 0.05, **P < 0.01).

injected into the eyes of stages 39 to 40 albino X. laevis tadpoles. Animals were anesthetized in 0.02% MS-222 before electroporation. Electroporated tadpoles were screened for single ipsi RGC axons 3 d to 4 d later around stages 46 to 48 and immobilized for imaging by intraperitoneal injection of d-tubocurarine (2.5 mM). They were placed into a custom polydimethylsiloxane chamber or embedded in 1.8% [wt/vol] low-melt agarose under a coverslip and perfused with oxygenated 0.1× modified Barth’s solution with Hepes. Animals stabilized for 30 min in darkness before imaging. A BFL48-400 optical fiber (Thorlabs) was used to stimulate the ipsi or contra eye with Red Rebel LEDs (Luxeon Star) controlled by a STG4002 stimulus generator (Multichannel Systems). Multiphoton z-stacking was achieved using a Spectra Physics Maitai BB Ti:sapphire pulsed laser. Statistical tests were performed using Prism 7.0 (Graphpad). Normality of the data distributions was confirmed using the Shapiro–Wilk test. Error bars indicate SEM. All animal experiments were approved by the Animal Care Committee of the Montreal Neurological Institute-Hospital.

Data Availability. Original data and detailed methods have been uploaded, for public access, to https://figshare.com/projects/Stentian_structural_plasticity_in_the_developing_visual_system/78012 (14).

ACKNOWLEDGMENTS. Funding sources are as follows: Natural Sciences and Engineering Research Council Canada Graduate Scholarship (T.N.R.), Sievers Award (M.M.), Jeanne-Timmins Costello and Molson awards (E.K.), and Canadian Institutes of Health Research Grant FDN-143238 (E.S.R.). We thank Martin Meyer for TeNT-Lc:EGFP, and Hollis Cline for pbGal4-VP16.