In vivo imaging of synapse formation on a growing dendritic arbor

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presented by

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Central question: How do dendritic structures organize during development?

Diagram removed for copyright reasons.
Eight stages of dendrite growth, with caption "Synapses Influence Dendrite Growth."

- Where and how are synapses formed during the growth of the dendrite?
- Are dendrite filopodia responsible for establishing synaptic contact and/or growth of the dendritic arbor?
- Most importantly what causes a dendritic growth to stabilize or retract?

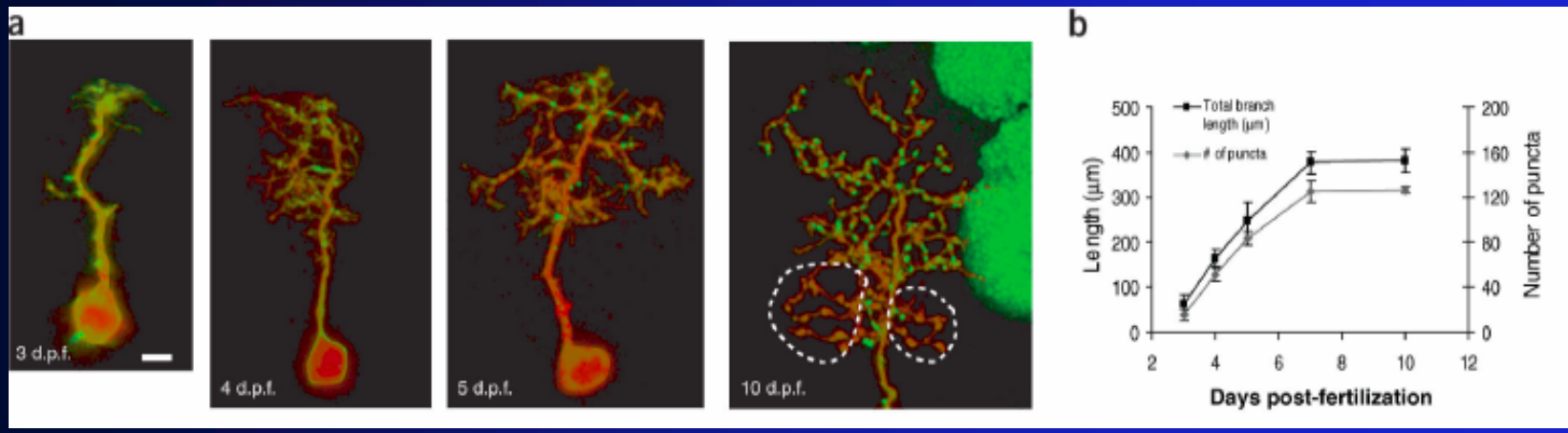
Methods

- Niell et al. used two-photon microscopy to monitor the formation of synapses and dendritic branch extension *in vivo* in zebrafish.
- They used PSD-95 tagged with GFP as well as DsRed to be able to visualize punctum formation and dendritic growth.
- This plasmid was injected into zebrafish embryos at 1-4 cell stage.

PSD-95 and excitatory synapses

- PSD-95 is a scaffolding protein which localizes to the post-synaptic density (PSD) in excitatory neurons.
- PSD-95 binds to glutamate receptors.
- Overexpression of PSD-95 enhances the formation of excitatory synapses with a corresponding decrease in inhibitory synapses.

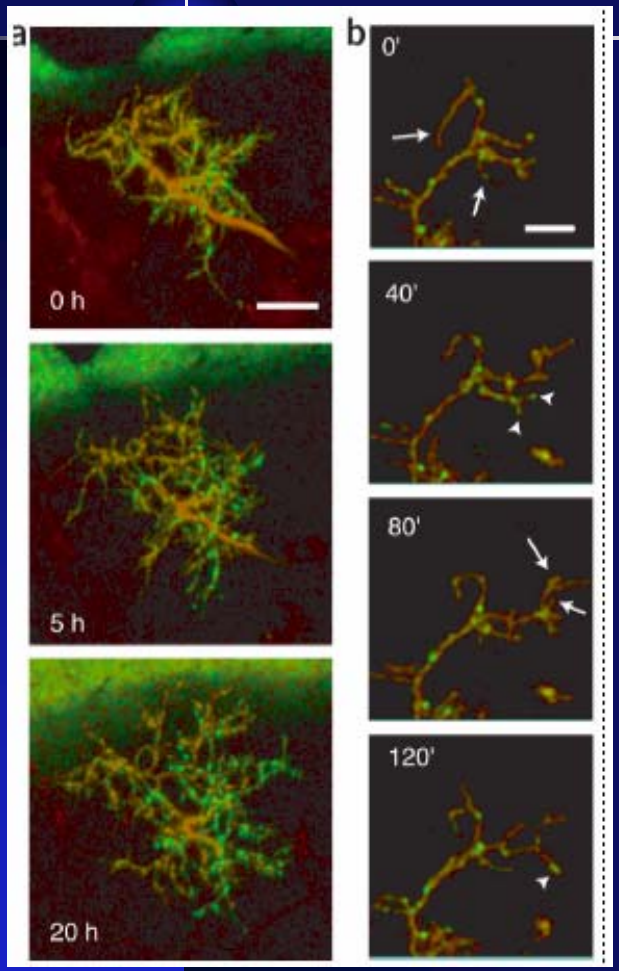
Punctum formation & dendritic growth are concurrent processes

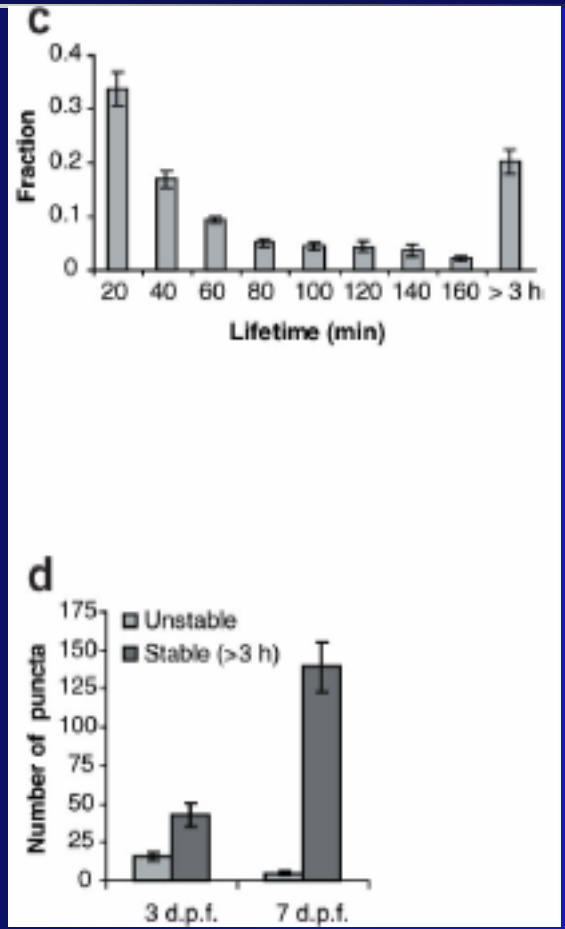


Source: Niell, C. M., M. P. Meyer, and S. J. Smith. "In Vivo Imaging of Synapse Formation on a Growing Dendritic Arbor." *Nature Neuroscience* 7 (2004): 254-260. Courtesy of the authors. Used with permission.

- Imaging of an individual tectal cell on consecutive days from 3 days post fertilization to 10 d.p.f.
- Arbor growth and PSD punctum formation in the zebrafish tectum are closely concurrent, with very little lag time between extension of a new dendritic process and formation of puncta upon it.

Long-term imaging of arbor growth and puncta formation





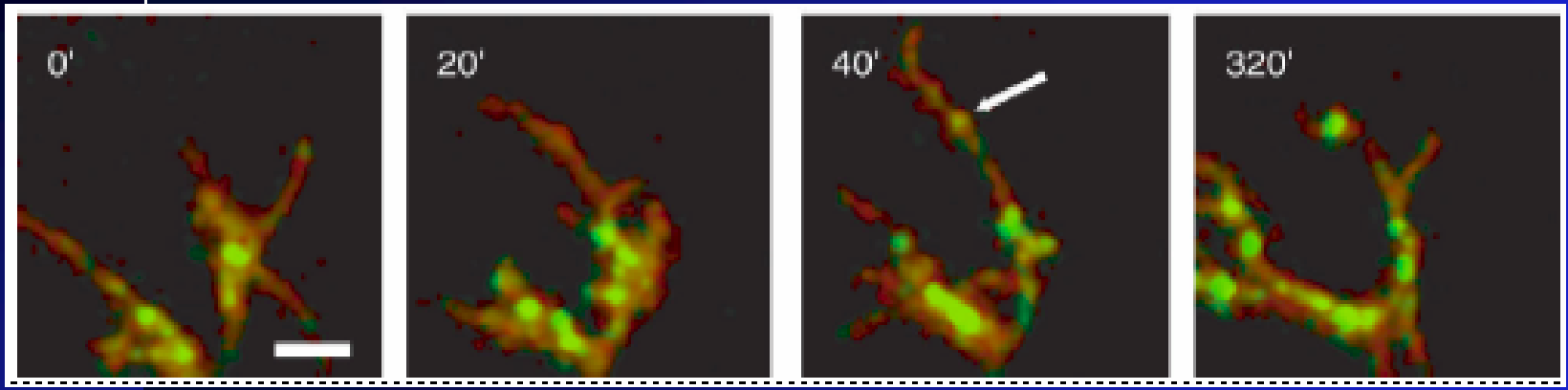
a) Series of still images starting at 3 d.p.f.

b) Series of images at 40 min intervals indicating transient filopodia and puncta

c) Puncta between 3-4 d.p.f. persist for less than 3h.

d) As dendritic arbor matures stable puncta increase.

Punctum-centric stabilization of arbors



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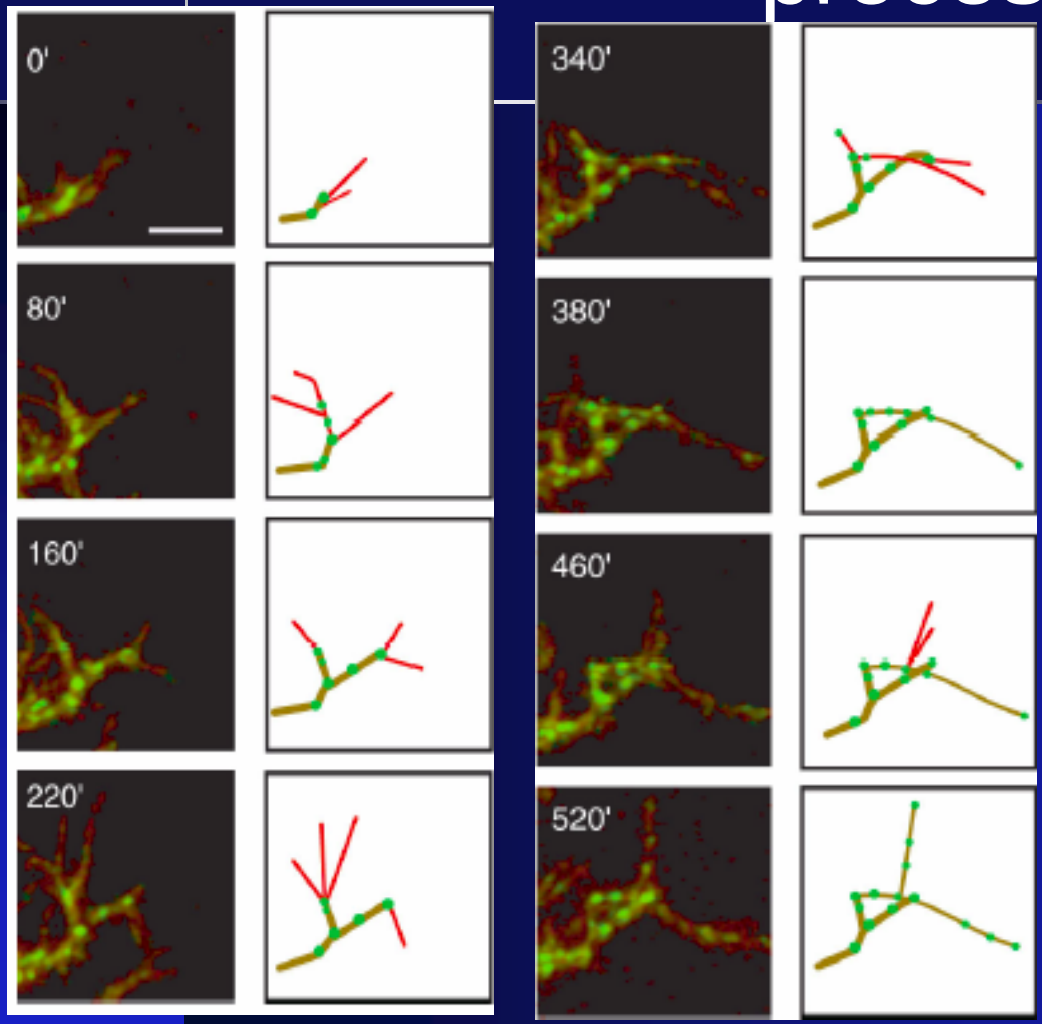
- A series of images reveals a typical mode of dendrite growth and puncta formation.
- 20’ a filopodia extends
- 40’ a punctum starts forming on it
- 320’ the punctum increases in intensity and the filopodia retracts.

Dendritic stabilization is an iterative process



Previous theories proposed that synapses moved along a filopodium or were pulled into the dendritic shaft.

Here we see that the formation of punctum converts a filapodium into a stable branch.

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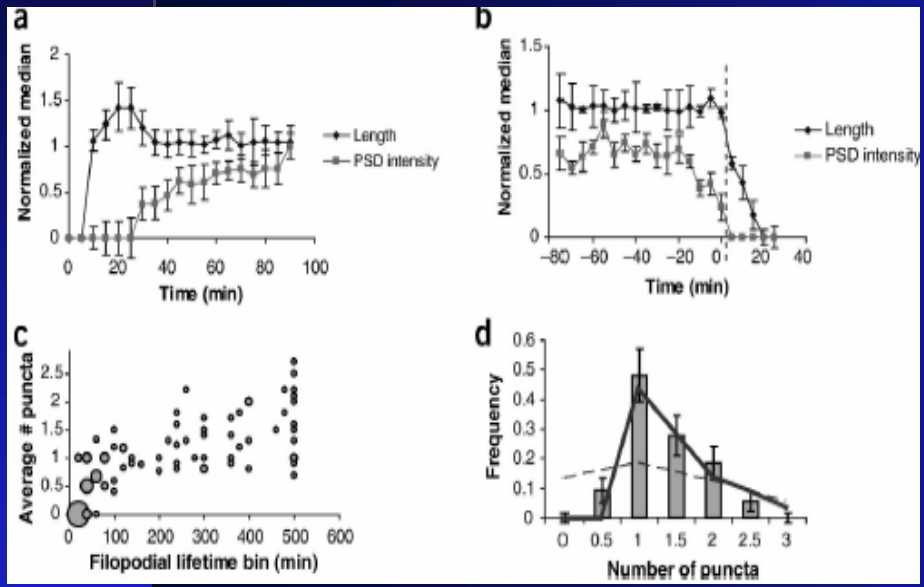
Is filopodia stabilization independent or dependent on synapse formation?



a) Filopodia retract back to the location of the stable punctum. (15 events from 4 cells, normalized with respect to the punctum and median-avg'd)

b) Retraction of a filopodia is preceded by disassembly of the punctum

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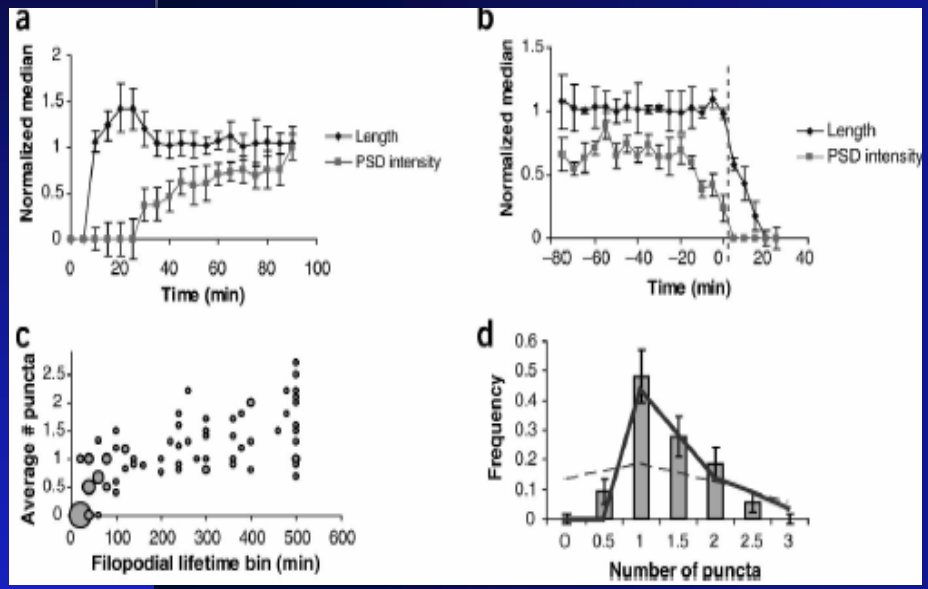
Is filopodia stabilization independent or dependent on synapse formation?(cont'd)



c) Avg # puncta vs terminal filopodial lifetime.

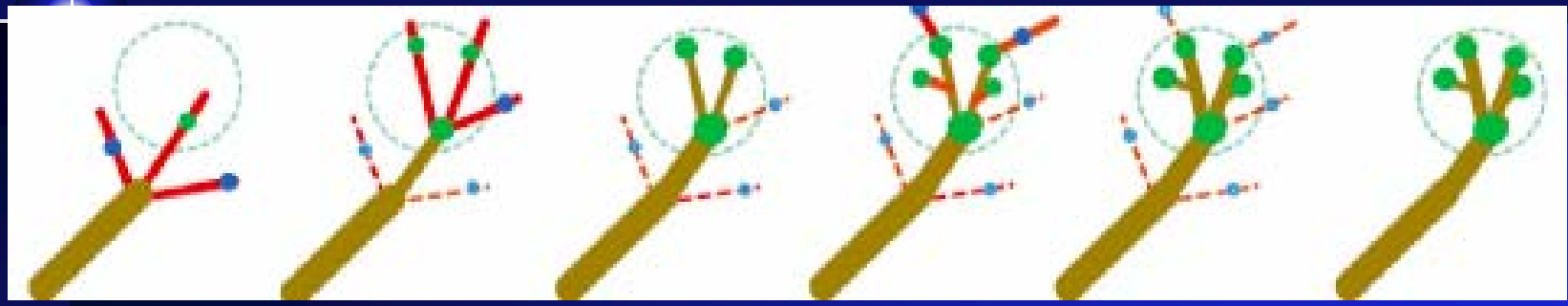
No terminal filopodia persisting longer than one hour bears less than one punctum.

If filopodia could stabilize independent of synapse formation, there would exist some processes without any punctum

d) A histogram of the # of puncta appearing on processes that lasted for more than one hour.

If the formation of a puncta were random, there might be some cases of filopodia w/o any puncta.

A model for synaptotropic guidance of dendrite growth



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- New filopodia (red) extend from a dendritic branch.
- Those that make proper synaptic contacts (green) are stabilized as new branches.
- Those that don’t make synaptic contacts (blue dots) are eliminated.
- This iterative process result in arborization within a field of appropriate synaptic connections.

Conclusions

- Punctum formation and dendritic stabilization are closely related processes.
- The presence of a synapse stabilizes the dendritic branch.
- Disassembly of a synapse leads to branch retraction.

Questions

- How are synapses maintained?
- Are there presynaptic signalling molecules?
- Puncta are not spines, how much can we infer from this model?
- Does overexpression of PSD-95 affect the shape of dendritic arbors by shifting the ratio of excitatory to inhibitory synapses?