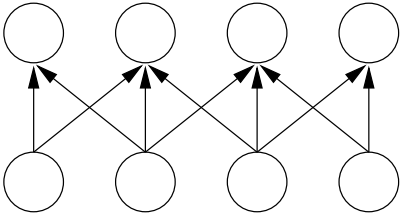
Extrinsic and intrinsic factors control the genesis of amacrine and cone cells in the rat retina

- Michael J. Belliveau and Constance L. Cepko (1999)
- Presented by Bo Morgan on March 7, 2004.

Introduction



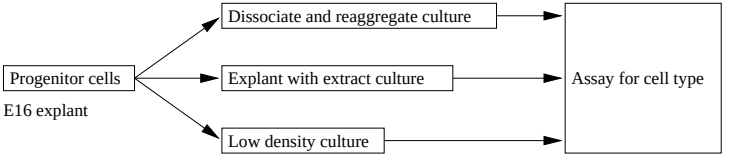
- **Do postmitotic cells influence progenitor cell fate decisions?**
- **Secreted factors shown to stimulate or inhibit rod cell development.**
- **Non-neuronal cells show feedback mechanisms from fated cells to the stem cell population.**

Why focus on the retina?

Diagram removed for copyright reasons.

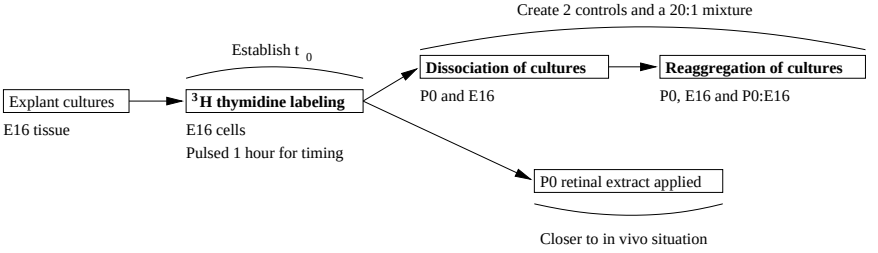
- **Evidence for particular order of production of cell types**
- **Evidence for particular portions of cell types**

How is prenatal retina progenitor cell fate affected by different surrounding cell populations?



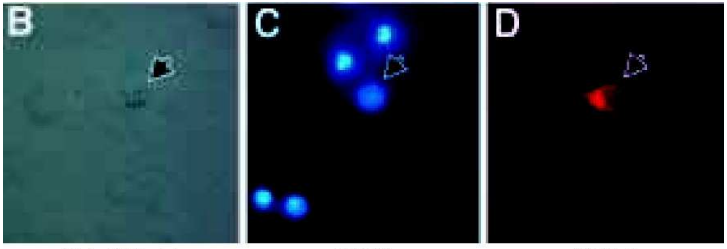
- **Which retinal cell types do the progenitor cells ultimately become?**
- **Three types of culture preparation methods:**
- **1. Dissociate and reaggregate with retinal postnatal cells**
- **2. Explant to culture with postnatal retinal extract**
- **3. Put progenitor cells into low density culture before mixing with postnatal cells.**

Methods Overview



- **$[^3H]$thymidine labeling of E16 cells to select progenitor population**
- **Dissociation and Reaggregation of cultures (E16, P0 and E16:P0)**
- **2 control solutions (E16 and P0) and 1 mixture solution (E16:P0) are prepared.**

$[^3H]$thymidine labeling of prenatal cells



Source: Belliveau, M. J., and C. L. Cepko. "Extrinsic and Intrinsic Factors Control the Genesis of Amacrine and Cone Cells in the Rat Retina." *Development* 126 (1999): 555-566.
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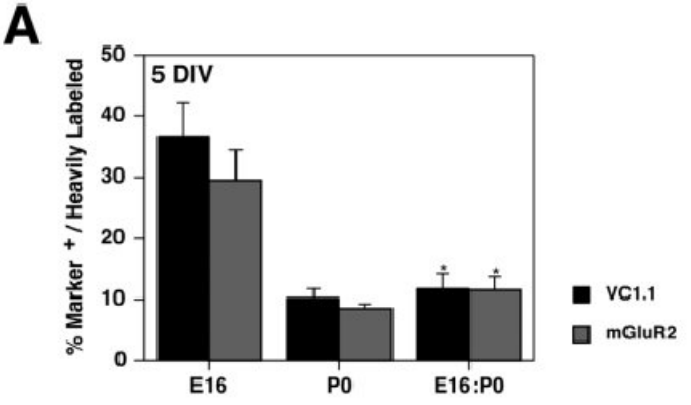
- **B. $[^3H]$thymidine-positive: cells dividing during $[^3H]$thymidine pulse**
- **C. DAPI-positive: cell nuclei**

D. VC1.1-positive: amacrine cells

Prenatal amacrine progenitor cells are affected by postnatal retina cells



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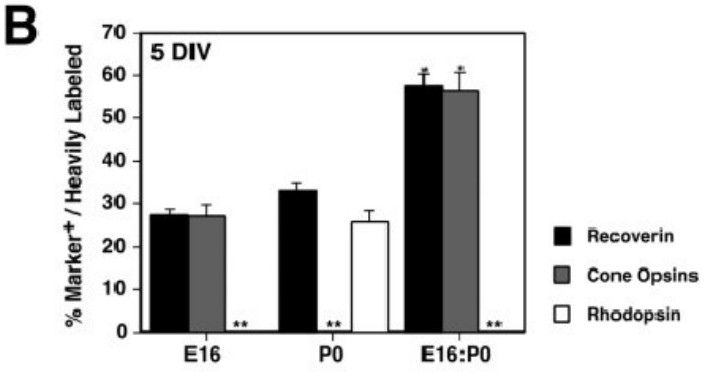
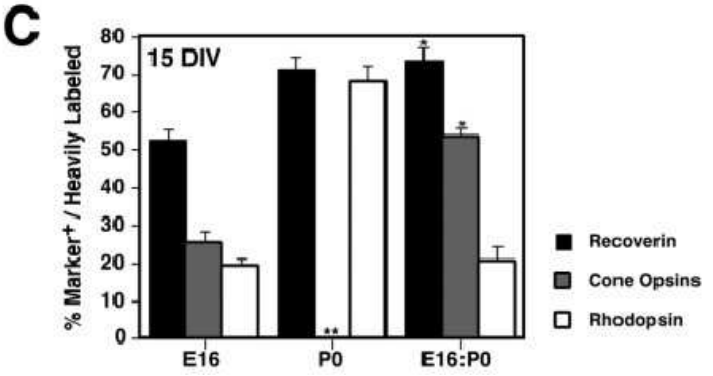
- **Ratio of prenatal amacrine progenitor cells conforms to postnatal progenitor amacrine cell ratio.**
- **VC1.1, mGluR2: amacrine cell indicators**

Prenatal photoreceptor progenitor cells are affected by postnatal retina cells



- Percentage of labeled progenitors with photoreceptor fate rises.
- Photoreceptor fate intrisically already established as cone.
- Perhaps some of these cones would have become amacrine cells in the absence of postnatal influence.

10 days later



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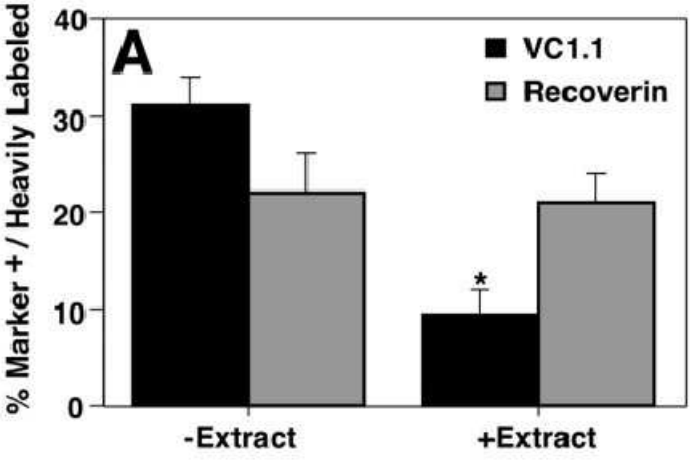
- % photoreceptors extrinsically signaled to increase
- % rods not affected
- % cones extrinsically signaled to double

Closer to in vivo



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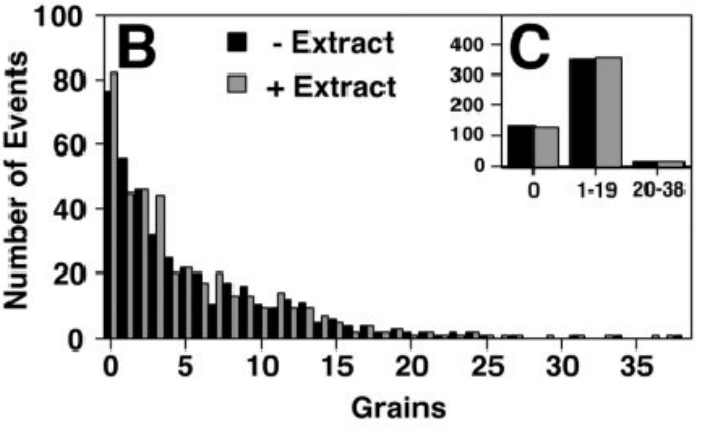
- **P0 extract applied to E16 explant tissue.**
- **No dissociation or reaggregation performed.**

No cell death or proliferation



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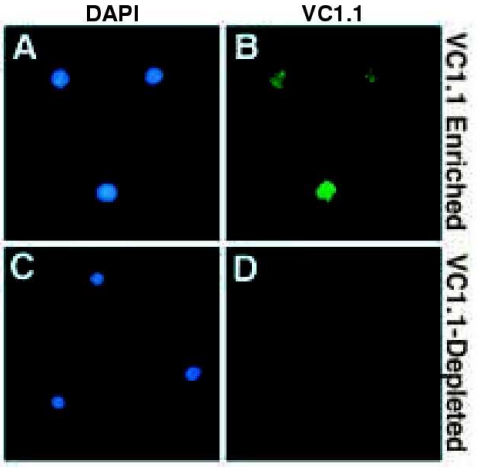
- **Same results with and without extract.**
- **Cell death → lower grey curve**
- **Cell proliferation → leftward grey curve.**

FACS machine



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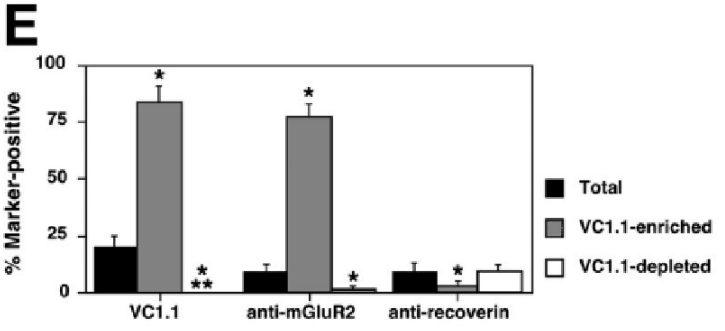
- **Flourescence -activated cell sorter**
- **Brightest and dimmest 12% collected**

FACS machine test



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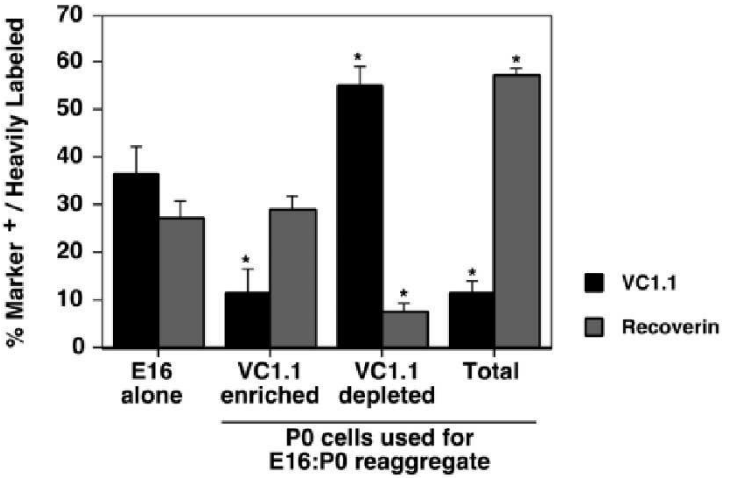
- **Amacrine cells are in VC1.1-enriched and not in VC1.1-depleted cultures.**

Negative feedback between amacrine cells



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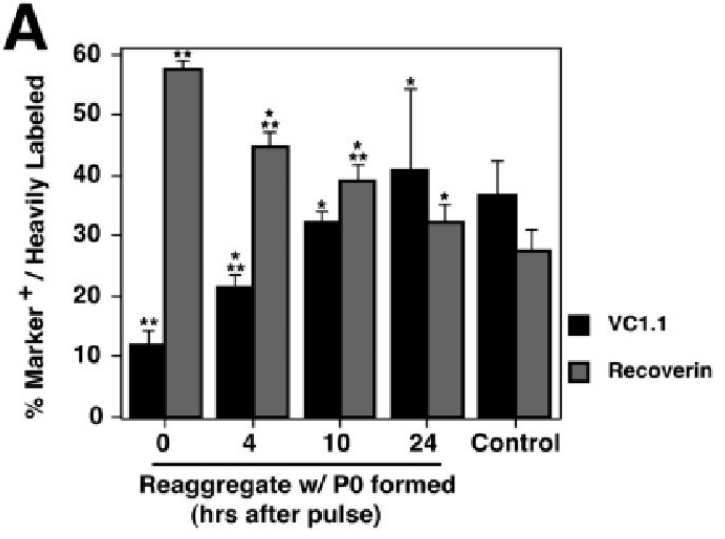
- **P0 amacrine cells inhibit E16 progenitors from becoming amacrine cells.**

Sensitive time period



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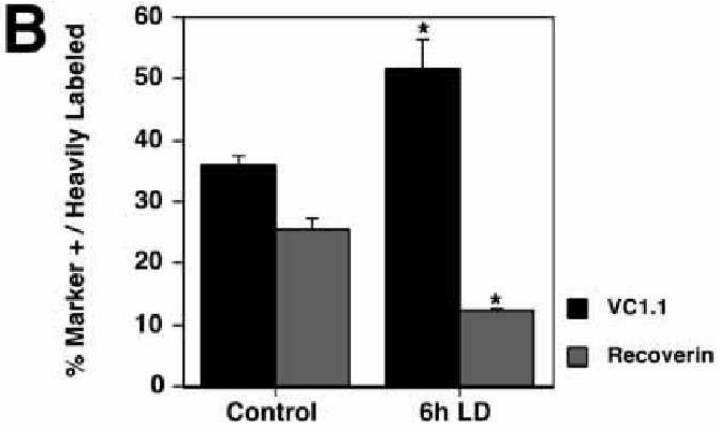
- **E16 progenitor cells are affected by extrinsic signaling for only about 4-10 hours.**

Without amacrine negative feedback



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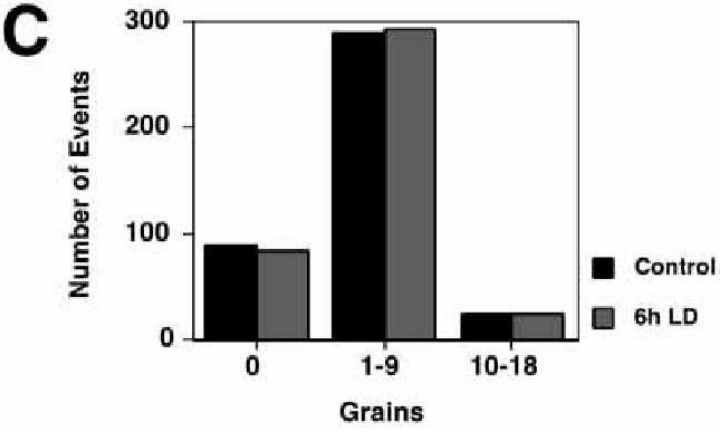
- **Low density culture prevents extrinsic signaling.**
- **Amacrine cells % rises without extrinsic signaling from other cells.**

No cell death or proliferation



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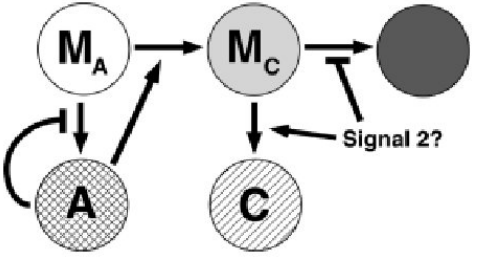
- **Graphs are roughly equal.**
- **Low density culture did not cause proliferation or cell death.**

Hypothesized Model



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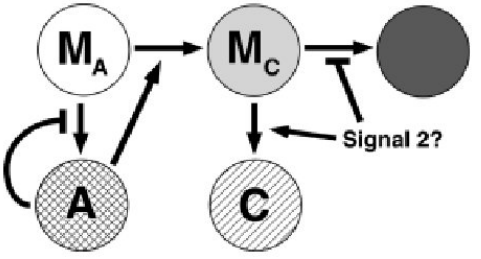
- **Extrinsic regulation of intrinsic fate biases**
- M_A**: Intrinsically biased to become an amacrine cell**
- M_C**: Intrinsically biased to become a cone cell.**
- **This could be a process which regulates the production of each retinal cell type.**

Observations

- **Postnatal rat retina provides an extrinsic signal that inhibits the production of amacrine cells from prenatal progenitors.**
- **Postnatal rat retina provides an extrinsic signal that affects the production of cone cells.**
- **Intrinsic biases of prenatal cells limit the effects of extrinsic signaling from postnatal cells.**

Future Research



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- **Do postnatal amacrine cells directly signal amacrine biased progenitor cells to switch to a cone bias state?**
- **Is there a second signal that promotes a state transition from M_C, cone bias state, to M_R, rod bias state?**
- **If so, what gives this signal?**

Questions