In ovo time-lapse analysis after dorsal neural tube ablation shows rerouting of chick hindbrain neural crest

Paul Kulesa, Marianne Bronner-Fraser and Scott Fraser
(2000)

Presented by Diandra Lucia

Background

- Neural crest cells emerge from neural tube and migrate away to form structures such as sensory ganglia, motor neurons, and others

- Guiding mechanism:
  - Signals from neural tube?
  - Signals from other emigrating neural crest cells?
  - Environmental signals?
  - Combination?
  - Are cells pre-specified to migrate to a specific location and become a particular structure? (Hox gene expression patterns)

Background

- Previous experiments suggest there are multiple guiding mechanisms
  - Different migratory paths/speeds, frequent path changes

- To examine importance of potential cues, normal migratory behavior had to be disrupted
  - Ablating subpopulations of premigratory hindbrain neural crest cells before stage 9 – when the regulative ability of neural tube declines - *resulted in normal facial structures*
    - neural tube is still developing; it regenerates and reforms neural crest

  - Ablating subpopulations of hindbrain neural crest cells after stage 9 still resulted in relatively normal embryos

    - Why?

Background

- Ablating after stage 9: neural crest cells rostral and/or caudal to ablation deviate from migratory paths to fill in for ablated tissue

- How/why does this happen?

Main Questions

- What are the cell trajectories and migration behaviors of non-ablated neural crest cells in ablated embryos?

- Where and when do the cell trajectory deviations take place?

Methods

- To answer these questions, they needed to be able to watch the activity of the neural tube and neural crest cells after ablation

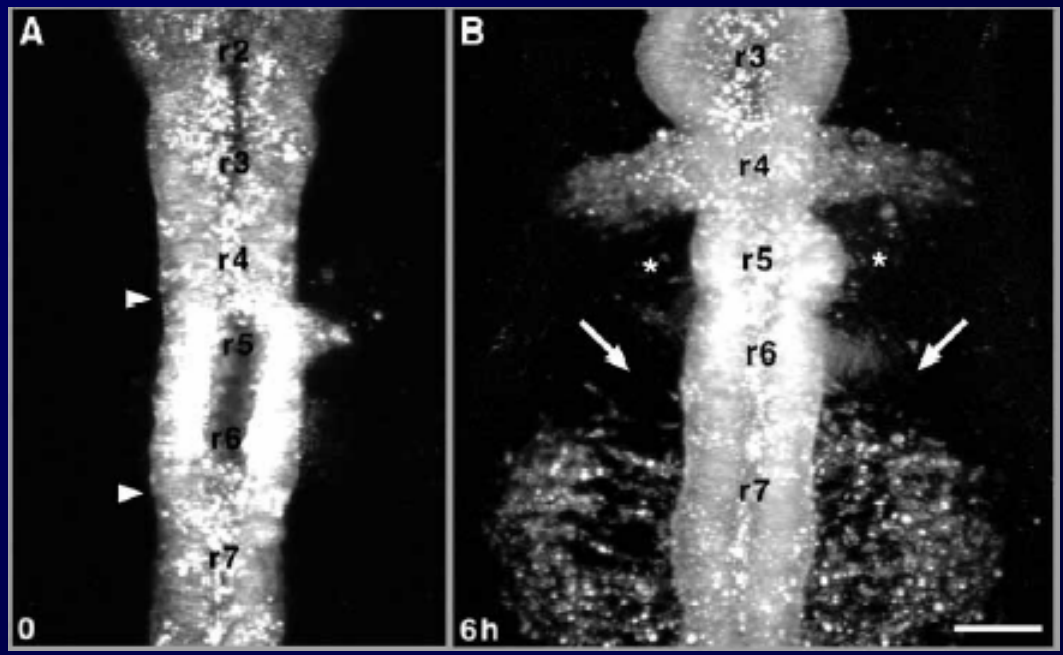
- They fluorescently labeled cells and used time-lapse recording and a computer program to track cell migration and see specific cell movements

- *In ovo* (*in vivo*) rather than *in vitro*, which was a limitation of previous experiments

Methods

- Cut a “window” into chick egg at 7-9 somite stage
- Injected DiI to visualize cells in the hindbrain neural tube just before the first somite pair
- Acrylic ring fixed into shell to serve as a window
- Microscope continually watched cell movements and this was recorded
- Made incisions in neural tube using a glass needle
  - Took out dorsal sections of tube in several different places, using rhombomeres as guideposts

r7 neural crest cells deviate rostrally in r5-r6 ablations



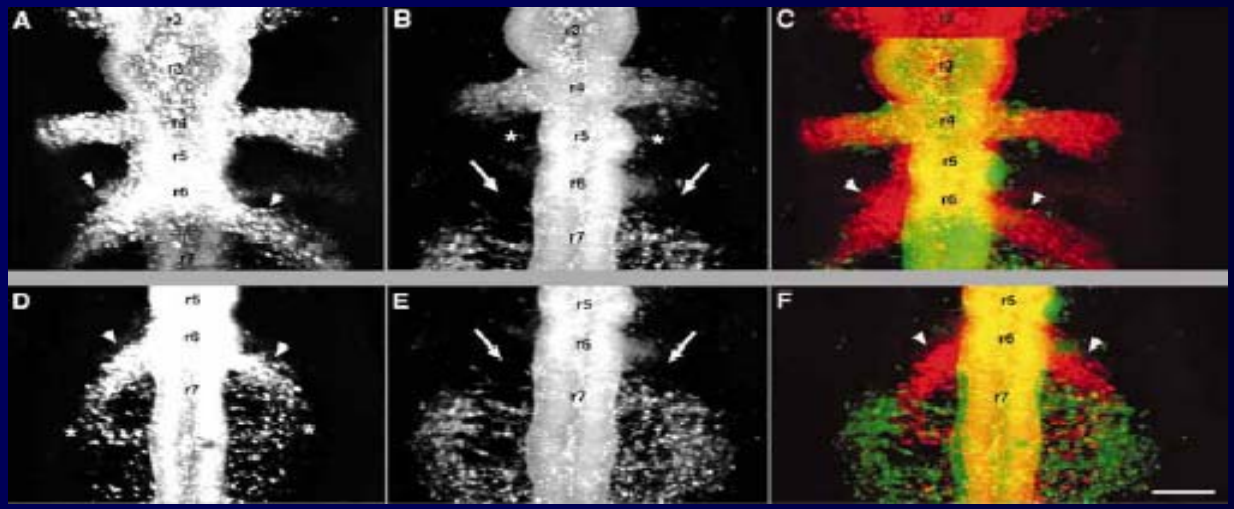
Source: Kulesa, P., M. Bronner - Fraser, and S. Fraser. "In Ovo Time- Lapse Analysis After Dorsal Neural Tube Ablation Shows Rerouting of Chick Hindbrain Neural Crest." *Development* 127 (2000): 2843 - 2852. Courtesy of The Company of Biologists. Used with permission.

Figure 1 – dorsal view

A. 2 hours after ablation – walls of n. tube started to close

B. 6 hours after – n. crest cells have emigrated from surrounding tissue to close up ablation.

r7 neural crest cells deviate rostrally in r5-r6 ablations



Source: Kulesa, P., M. Bronner - Fraser, and S. Fraser. "In Ovo Time-Lapse Analysis After Dorsal Neural Tube Ablation Shows Rerouting of Chick Hindbrain Neural Crest." *Development* 127 (2000): 2843 - 2852. Courtesy of The Company of Biologists. Used with permission.

Figure 2 – dorsal view

A. Normal embryo. Thick streams of n. crest cells migrate away from r4 and r6.

B. Ablated embryo. No n. crest cells migrate out from r6. Some n.c. cells migrate towards r5.

C. Overlapped image of normal (red) and ablated (green) embryos. No overlap of n.c. cells except near r4.

D. Normal. r7 cells spread out laterally in streams.

E. Ablated. No streams; lateral spread of r7 cells adjacent to n. tube.

F. Overlapped image. Little overlap of normal and ablated n.c. cell streams. r7 cells have migrated to beyond where typical interaction with r6 cells takes place.

r7 neural crest cells deviate rostrally in r5-r6 ablations

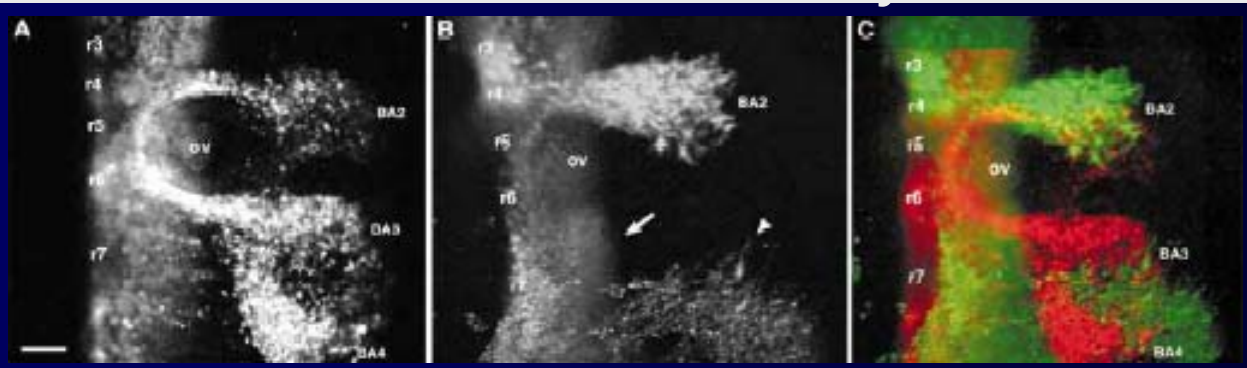


Figure 3 - right side view

A. Normal embryo. Second branchial arch stream of cells has migrated around otic vesicle and into branchial arch 2 (BA2). BA3 stream has split in 2, one stream going to BA3 and one to BA4.

B. Ablated. BA3 stream is gone. N.c. cells from r7 are starting to migrate rostrally. BA2 is normal.

C. Overlapped image. Shows how much the r7 cells have deviated towards ablated area.

Midbrain and r4 n.c. cells deviate to repopulate the regions adjacent to r1 and r3 in r1-r3 ablations

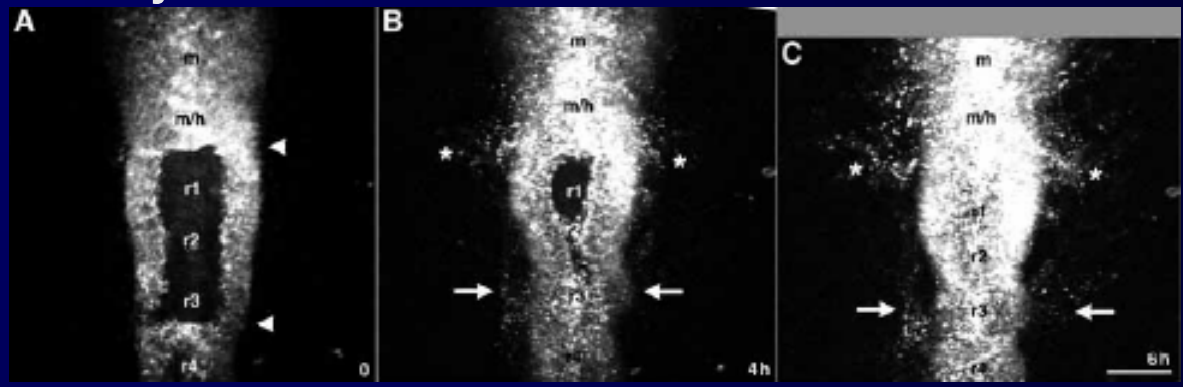


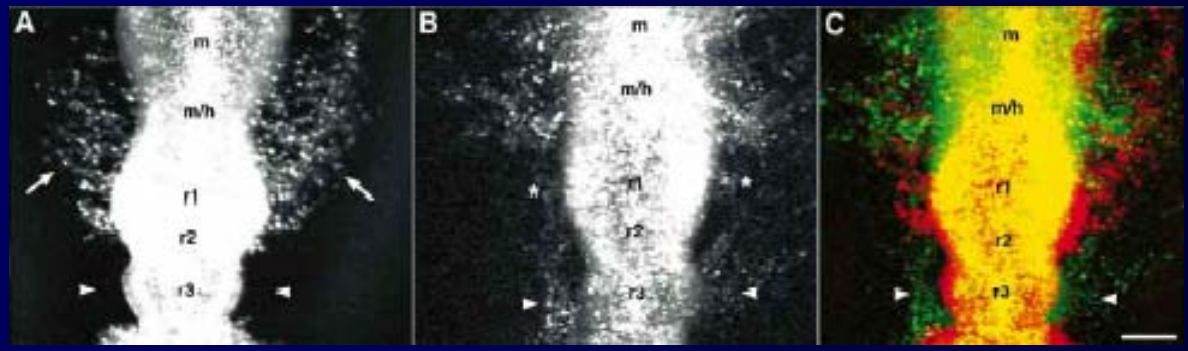
Figure 4 - dorsal view

A. 2 hours after ablation.

B. 4 hours after ablation; tube has already almost closed on the ablated site. Notice the n.c. cells surrounding the area, especially at the midbrain/hindbrain boundary, r1 and r3.

C. 6 hours – tube is mostly closed.

Midbrain and r4 n.c. cells deviate to repopulate the regions adjacent to r1 and r3 in r1-r3 ablations



Source: Kulesa, P., M. Bronner - Fraser, and S. Fraser. "In Ovo Time-Lapse Analysis After Dorsal Neural Tube Ablation Shows Rerouting of Chick Hindbrain Neural Crest." *Development* 127 (2000): 2843 - 2852. Courtesy of The Company of Biologists. Used with permission.

Figure 5 - dorsal view

A. Normal embryo. Notice caudolateral pattern of n.c. cell migration from midbrain and midbrain/hindbrain boundary to r2. Cell-free zone around r3.

B. Ablated. Cells surround r3. Patchy distribution of cells around midbrain and m/hbrain boundary. Cells fill in region around r1.

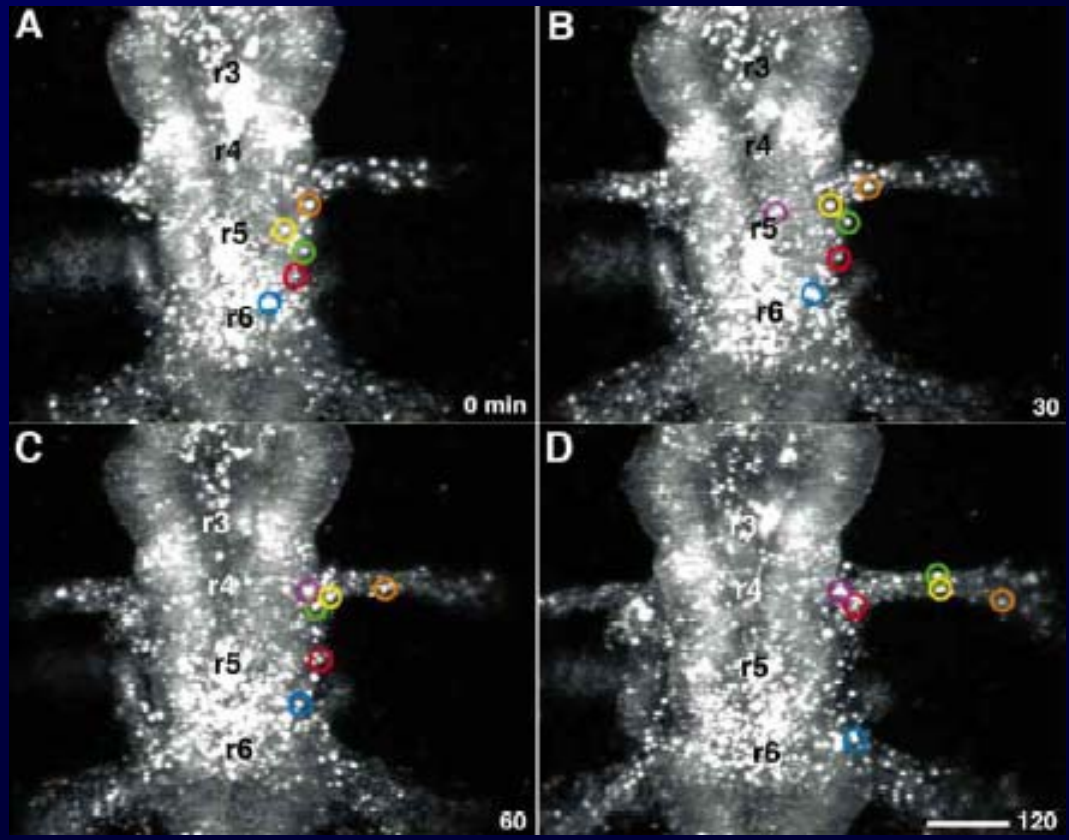
C. Overlap (normal = red, ablated = green). Only red cells around r1-r2, only green around r3.

Neighboring crest cells fill in the second branchial arch stream in r3-r4 ablation

Figure 6

N.c. cells from r5 are circled to make it easier to follow their individual migration paths.

Normally, circled cells would be part of the r6 exiting stream (branchial arch 3), but most join the r4/r5 exiting stream (branchial arch 2).



Source: Kulesa, P., M. Bronner - Fraser, and S. Fraser. "In Ovo Time-Lapse Analysis After Dorsal Neural Tube Ablation Shows Rerouting of Chick Hindbrain Neural Crest." *Development* 127 (2000): 2843 - 2852. Courtesy of The Company of Biologists. Used with permission.

N.c. cell trajectories become less ordered in ablated embryos

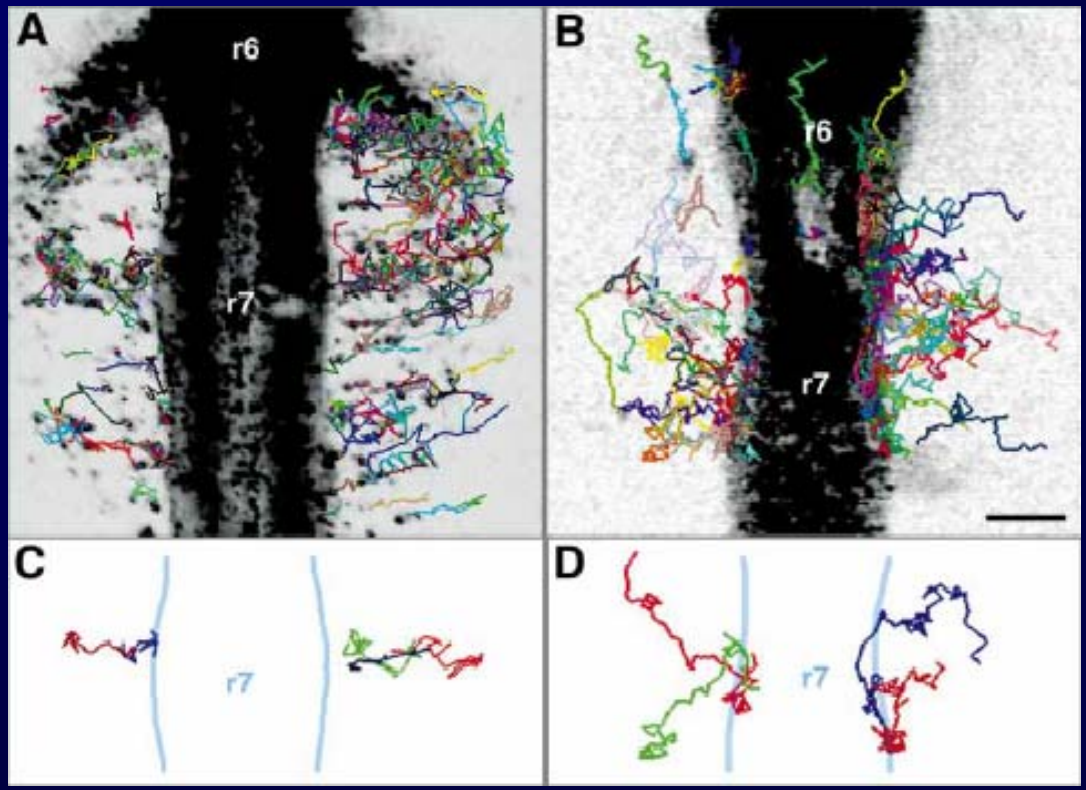


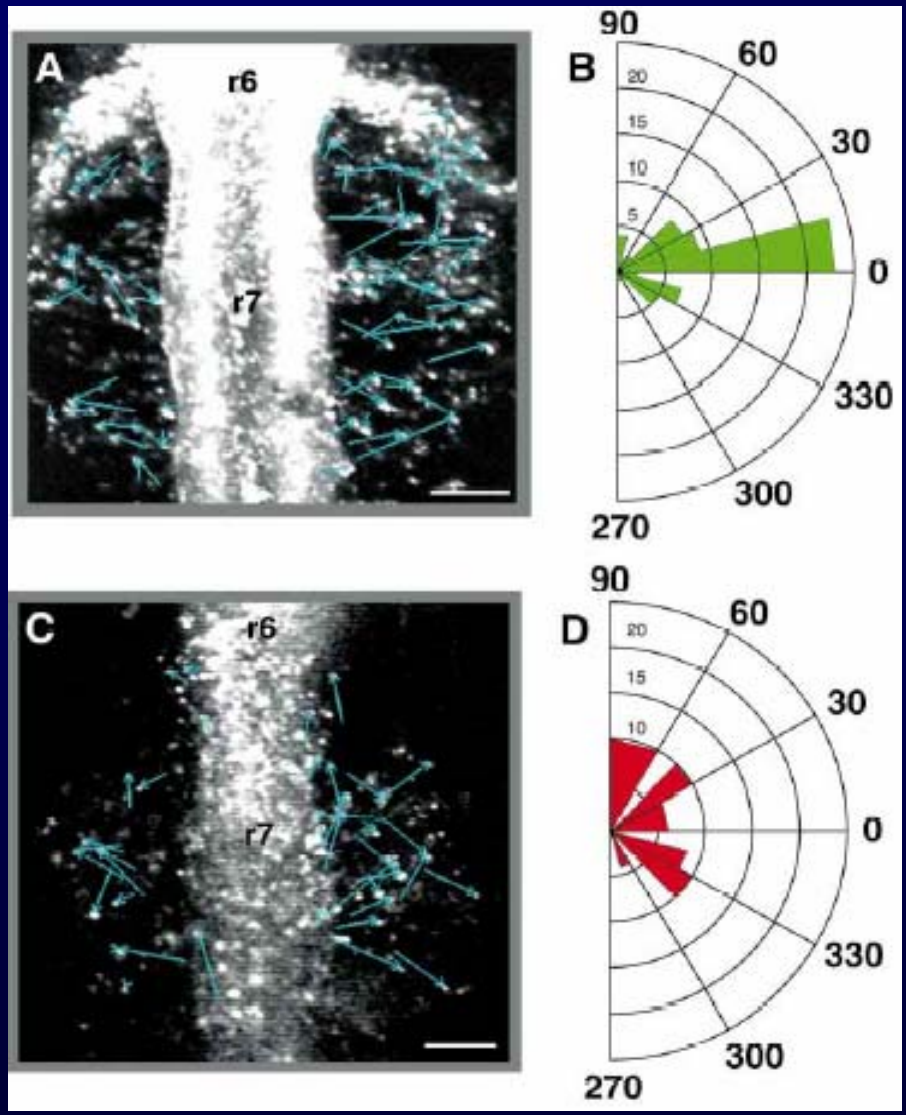
Figure 7 - dorsal view; inverted colors

A. Normal. Lateral trajectories from r6 and r7. Where r6 and r7 interact, trajectories turn, indicating direction changes towards each other.

B. r5-r6 ablated. Trajectories suggest chaotic migratory behavior of r7 cells. Some r7 cells migrate towards r5.

C. Typical cell trajectories in normal and ablated embryos.

N.c. cell trajectories become less ordered in ablated embryos



Source: Kulesa, P., M. Bronner - Fraser, and S. Fraser. "In Ovo Time-Lapse Analysis After Dorsal Neural Tube Ablation Shows Rerouting of Chick Hindbrain Neural Crest." *Development* 127 (2000): 2843 - 2852. Courtesy of The Company of Biologists. Used with permission.

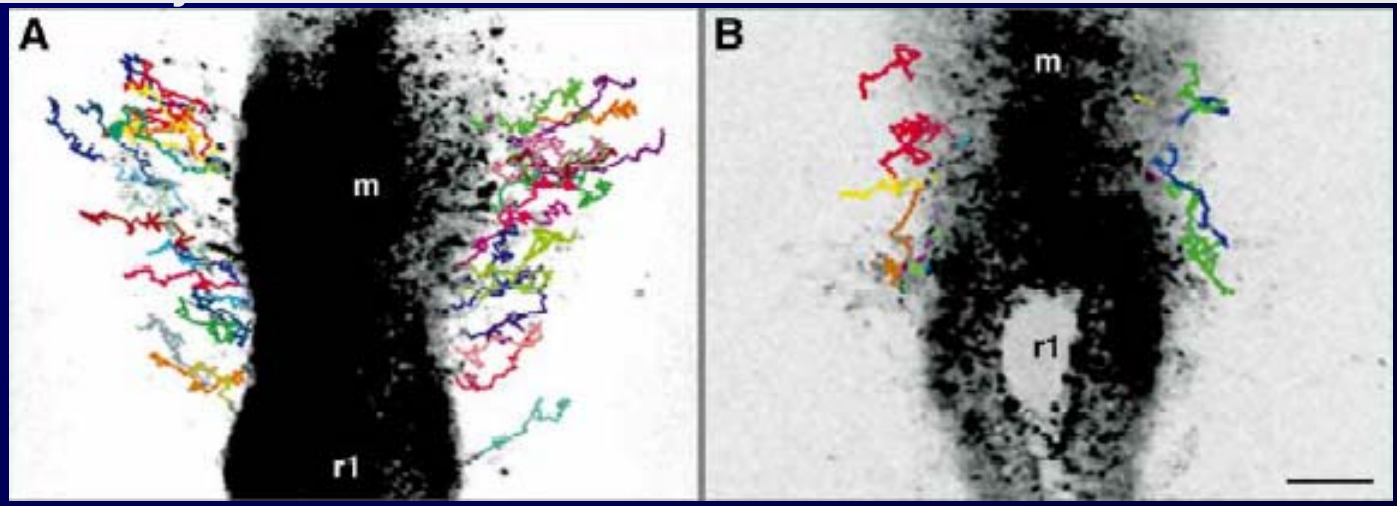
Quantification of cell trajectories

Figure 8

A,B. Normal embryo. Most cell trajectories are pretty lateral.

C,D. Ablated. Larger range of cell trajectories angling away from lateral direction.

N.c. cell trajectories become less ordered in ablated embryos



Source: Kulesa, P., M. Bronner - Fraser, and S. Fraser. "In Ovo Time-Lapse Analysis After Dorsal Neural Tube Ablation Shows Rerouting of Chick Hindbrain Neural Crest." *Development* 127 (2000): 2843 - 2852. Courtesy of The Company of Biologists. Used with permission.

Figure 9

A. Normal embryo with n.c. cell trajectories traced (migrating from the midbrain to r1 towards BA1 stream).
B. r1-r3 ablated embryo. Cell trajectories point caudolaterally, backwards away from first branchial arch streams.

N.c. cell trajectories become less ordered in ablated embryos

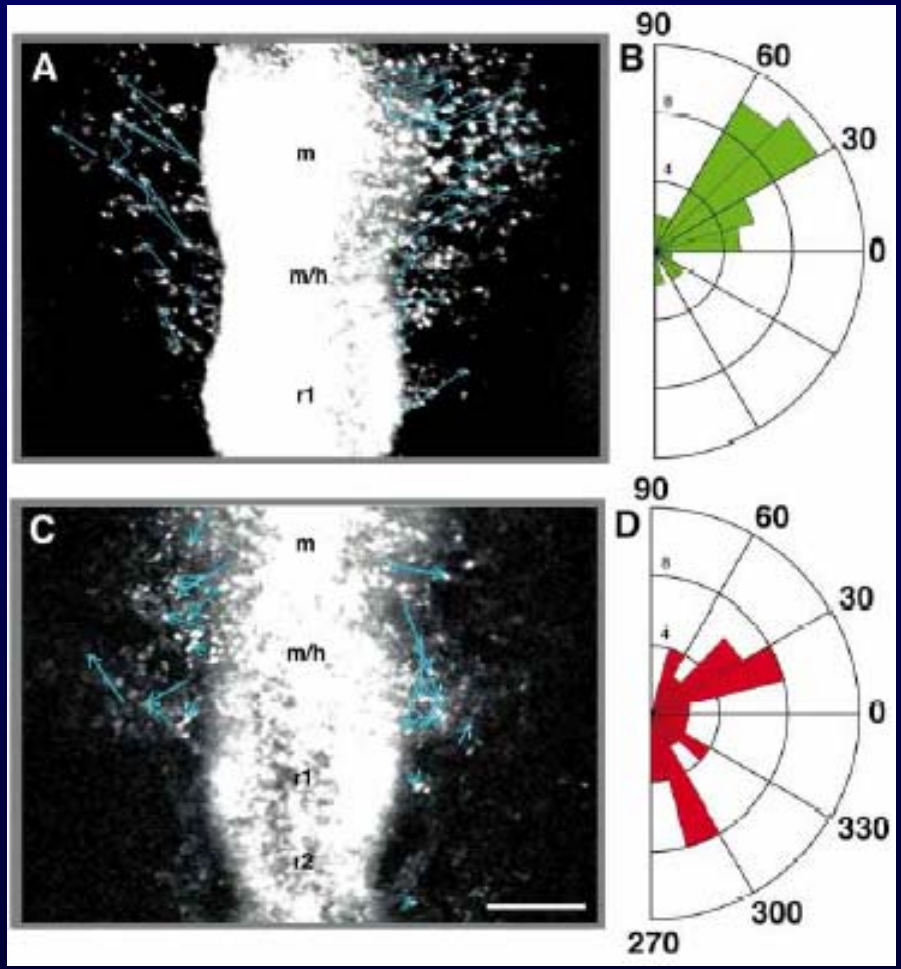


Figure 10

A,B. Normal embryo with cell trajectory vectors marked. Most of them point rostrolaterally.

C,D. Histogram confirms that most of them have a +0-60 degree angle up from the lateral direction.

Source: Kulesa, P., M. Bronner - Fraser, and S. Fraser. "In Ovo Time-Lapse Analysis After Dorsal Neural Tube Ablation Shows Rerouting of Chick Hindbrain Neural Crest." *Development* 127 (2000): 2843 - 2852. Courtesy of The Company of Biologists. Used with permission.

Summary of Observations

Background knowledge recap:

- Cranial neural crest cells are able to regulate and repopulate ablated areas of tissue in the neural tube and branchial arches
- After stage 9, non-ablated neural crest cells “fill in” ablated portion
- Neural crest cells change their migratory paths in order to do so

This experiment found:

- Which cells deviate from their normal paths, where, and when
  - *Local* populations of cells
- Areas of cell-cell interactions are the areas with most re-routing

Implications of Observations

"Neural crest cells change their migratory paths"

- This wouldn't happen if cells were pre-specified to go to a certain location and become a certain structure
- This wouldn't happen if cell-environment interactions were the deciding factor, either

"*Local* populations of cells" deviate to fill in for missing cells

- Suggests that entire neural crest is still flexible

- The fact that cells *nearby* the ablations are the ones responding indicates that there must be some cell-cell or cell-environment interaction

Implications of Observations

"Areas of cell-cell interactions are areas with most re-routing"

- Suggests that cell-cell interactions play an important role in determining migratory routes

- Absence of interaction with cells or environment can cause changes in cells' migratory direction; suggests that cell-cell and/or cell-environment interactions play a role

- Timing and position of route changes suggests that cells shape their routes based on their interactions with other cells

Further Observations

- Ablation causes a change in the *nature* of cell-cell interactions:
  - In normal embryos, cells usually migrate in chains; closely together and very organized.
  - In ablated embryos, cells often migrate individually and more chaotically.
- What does this imply about how the cells communicate with each other? What processes may have been disrupted by ablation? No answer is evident from this experiment.

Future Research

- More experimentation is needed to determine more specifically where migratory cues are coming from
  - More ablation studies
  - Chemical pathway blocking
  - Gene manipulation

Questions