Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex

Anne J. Blaschke, Kristina Staley, and Jerold Chun, 1996

Presented by

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Programmed Cell Death

- Apoptosis
  - Fragments DNA
  - Nucleosomal ladders

- Found in post-mitotic cells
- Assumed not to occur in embryonic cortical development (specifically, in proliferative areas)

Experimental Goal

- Characterize the distribution and rates of PCD in the embryonic cerebral cortex (in mice) during embryonic days 10-18

In Situ End Labeling

- Identifies fragmented nuclear DNA in dying cells
  - Attaches labeled nucleotides to free DNA ends, which can then be visualized in tissue sections
- Past studies with ISEL
  - Identifies PCD specifically (Gavrieli et al, 1992)

ISEL+: Section incubated with mix containing terminal-deoxynucleotidal-transferase (TdT) and either a) digoxigenin-11-dUTP $\rightarrow$ incubated with blocking solution 1hr $\rightarrow$ incubated with anti-digoxigenin Fab fragments overnight; or b) [^{32}P]dCTP $\rightarrow$ incubated at 37^{o}C for 1hr $\rightarrow$ incubated at 65^{o}C for 2hr; $\rightarrow$ washed

In-Situ End Labeling +

- Does it identify cells undergoing PCD?
- Does it only identify these cells, or others as well?
- Does it do this in many areas of the nervous system?

The Effectiveness of Isel+ Labeling

- ISEL+ in the thymus
- ISEL+ in the retina
- ISEL+ marking in vitro apoptosis
- ISEL+ under heat inactivation
- Only PCD fragments?
  - RNase
  - DNase
  - Okazaki fragments

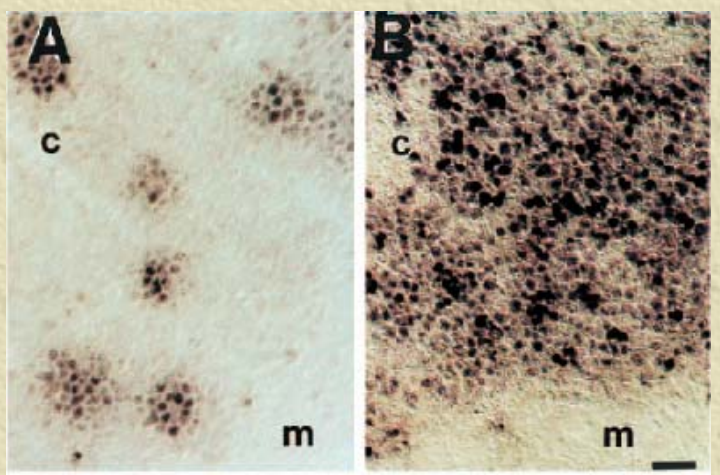
ISEL+ in the Thymus

- Dexamethasone is known to increase PCD amongst immature T-cells
  - ISEL+ detected this effect (compared with control)
  - Primarily in thymic cortex (97% death)
- DNA fragments undergoing apoptosis (nucleosomal ladders in LMPCR)

ISEL+ labeling of dying cells:

a) Normal thymus

b) Dexamethasone treated thymic section



Source: Blaschke, A. J., and K. Staley, et al. "Widespread Programmed Cell Death in Proliferative and Postmitotic Regions of The Fetal Cerebral Cortex." *Development 122*, no. 4 (1996): 1165-74. Courtesy of The Company of Biologists. Used with permission.

Thymus, cont.

- Similar findings in adult small intestine and embryonic limb bud
- Conclusion: ISEL+ detects normal apoptosis in thymus and other tissues, and is sensitive to relative increase in apoptosis after dexamethasone treatment

ISEL+ in the Retina

- 66% of ganglion cells from birth eliminated by the first week (Crespo et al, 1985); similar among amacrine cells during second week (Horsburgh and Sefton, 1987)
- ISEL+ found similar magnitude and time course

Decreasing ganglion cell death

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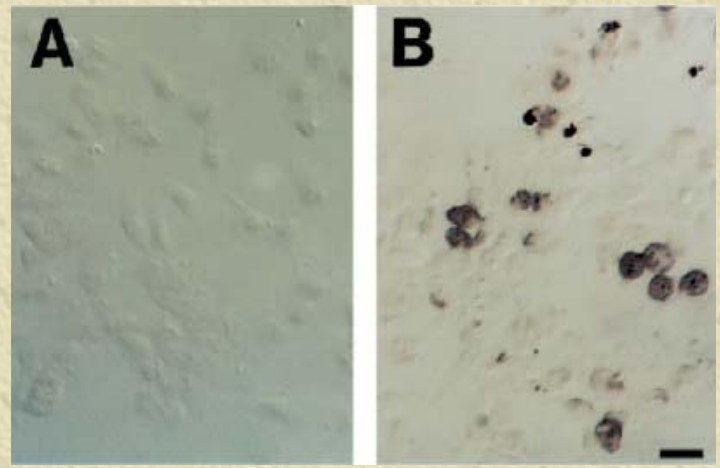
UV-Induced Apoptosis

- Undifferentiated P19 cells grown as monolayer on coverslips
- ISEL+ found rare or no labeled cells on control slides
- ISEL+ found many labeled cells in cultures exposed to UV light

ISEL+ labeling
without UV light (A),
and with UV light (B)



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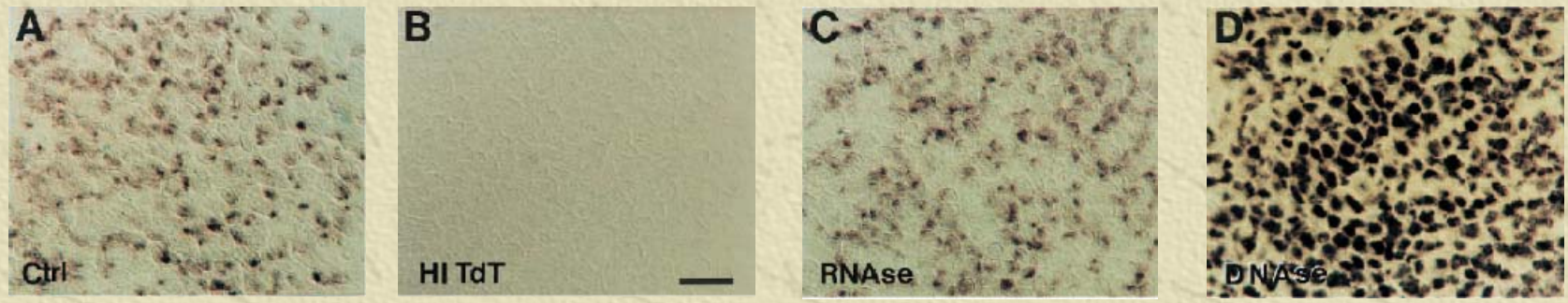
ISEL+ Validation

- Heat inactivation prevented labeling
- RNase produced no change
- DNase allowed labeling of nearly all cells
- Conclusion: ISEL+ labels DNA but not RNA through TdT activity (not histological artifact)

From E16 mouse ventricular zone.

(A) Control; (B) Heat inactivated; (C) RNase; (D) DNase



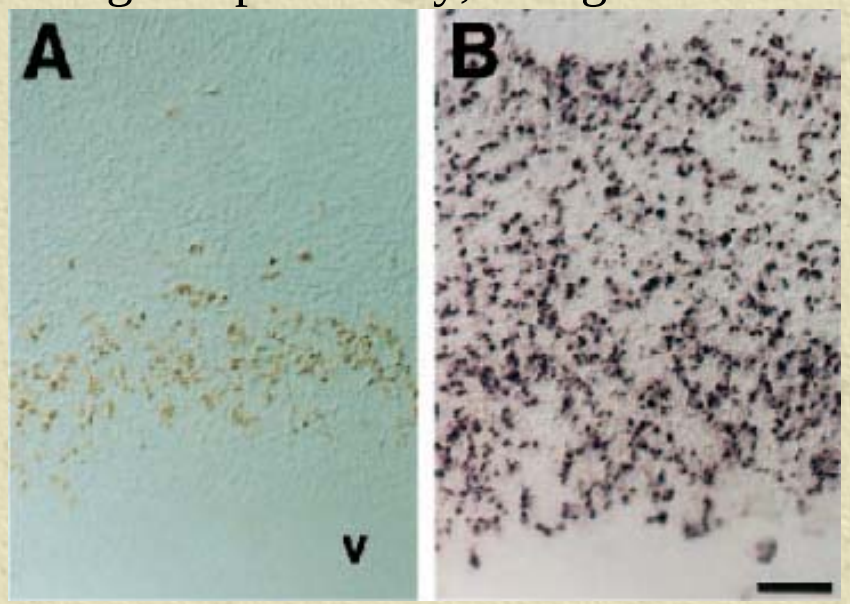
ISEL+ Validation, cont.

- Is ISEL+ labeling Okazaki fragments?
- Labeling with BrdU and thymidine compared with ISEL+ for cells of developing cortex:
  - ISEL+ does not label the same region specifically, though there is overlap

    A) BrdU and thymidine;

    B) ISEL+

    (immediately adjacent sections)



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Summary

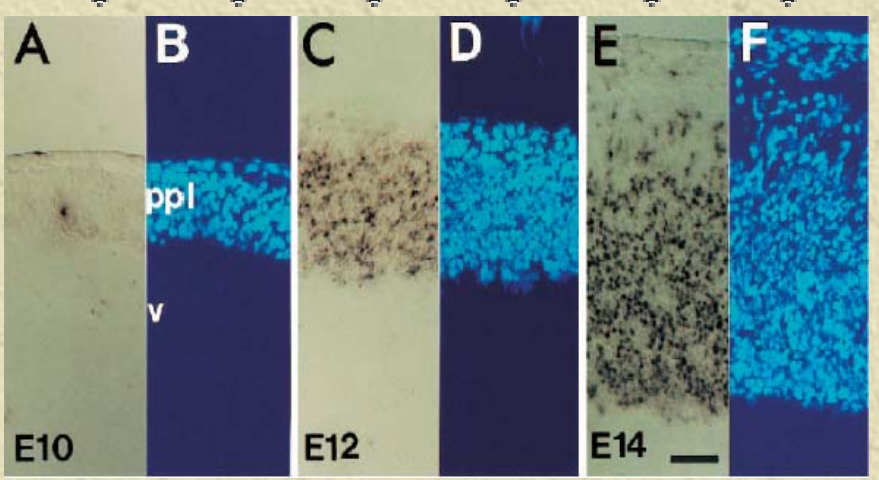
- ISEL+ recognizes PCD in 3 different systems
- Labeling all appropriate DNA
- Not labeling RNA
- Not simply labeling Okazaki fragments

- ISEL+ seems to be a good method for labeling PCD in embryonic cortical development

ISEL+ in Embryonic Cortex

- Location and extent of PCD across E10-18, into adult cortex (of mice)
  - In proliferative zones, or just post-mitotic zones?

ISEL+ in Embryonic Cortex



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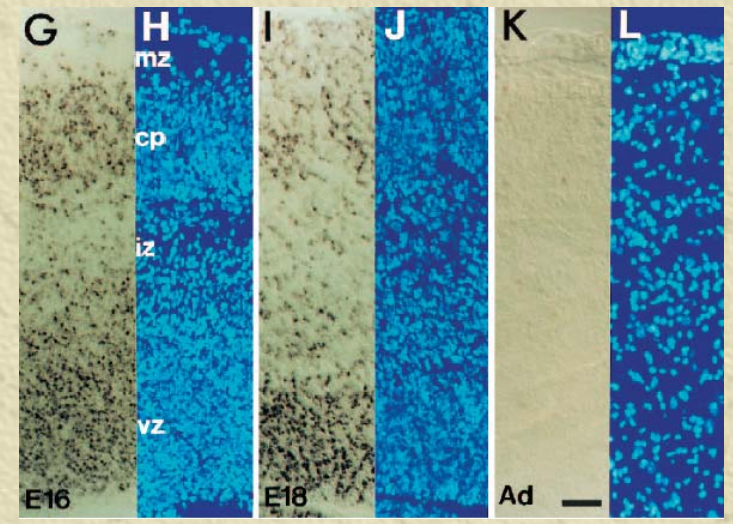
- A,C,E,G,I,K: ISEL+ staining of embryonic mouse cortex
- B,D,F,H,J,L (DAPI staining):
  - Shows labeling is in nucleus
  - Also allows information about percentage

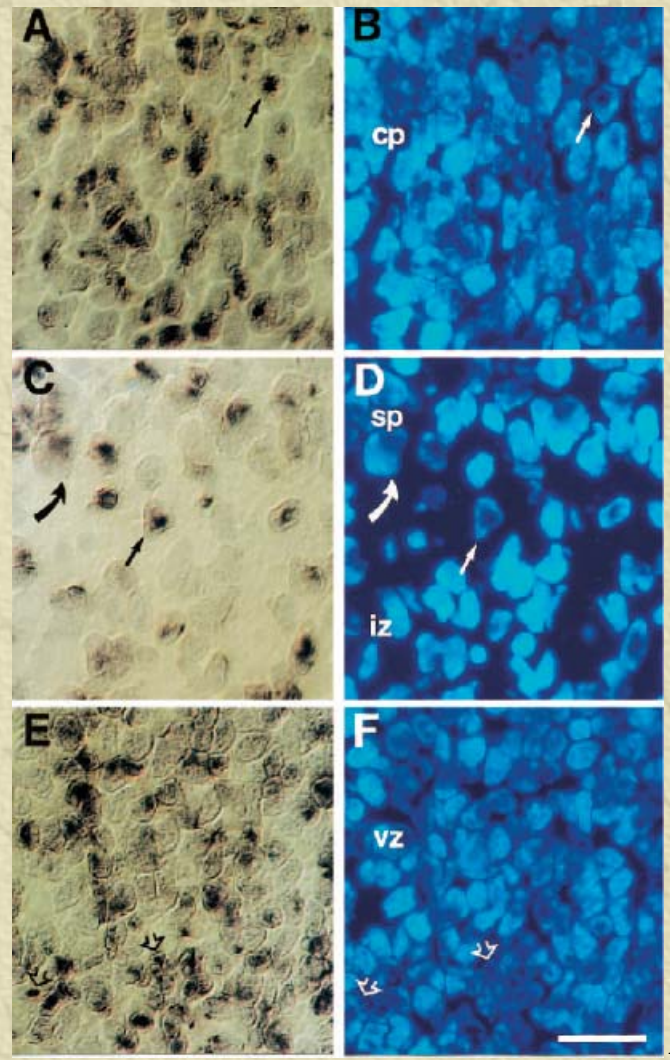


PCD Distribution

Cortical Plate (postmitotic)

Subplate, Intermediate Zone (postmitotic)

Ventricular Zone (proliferative)



All at E16

- Some post-mitotic regions
- Mostly, however, in proliferative zones
- Degree of PCD in embryonic cortex consistent with PCD documented in other parts of nervous system
- Results from LMPCR of DNA in accord with ISEL+

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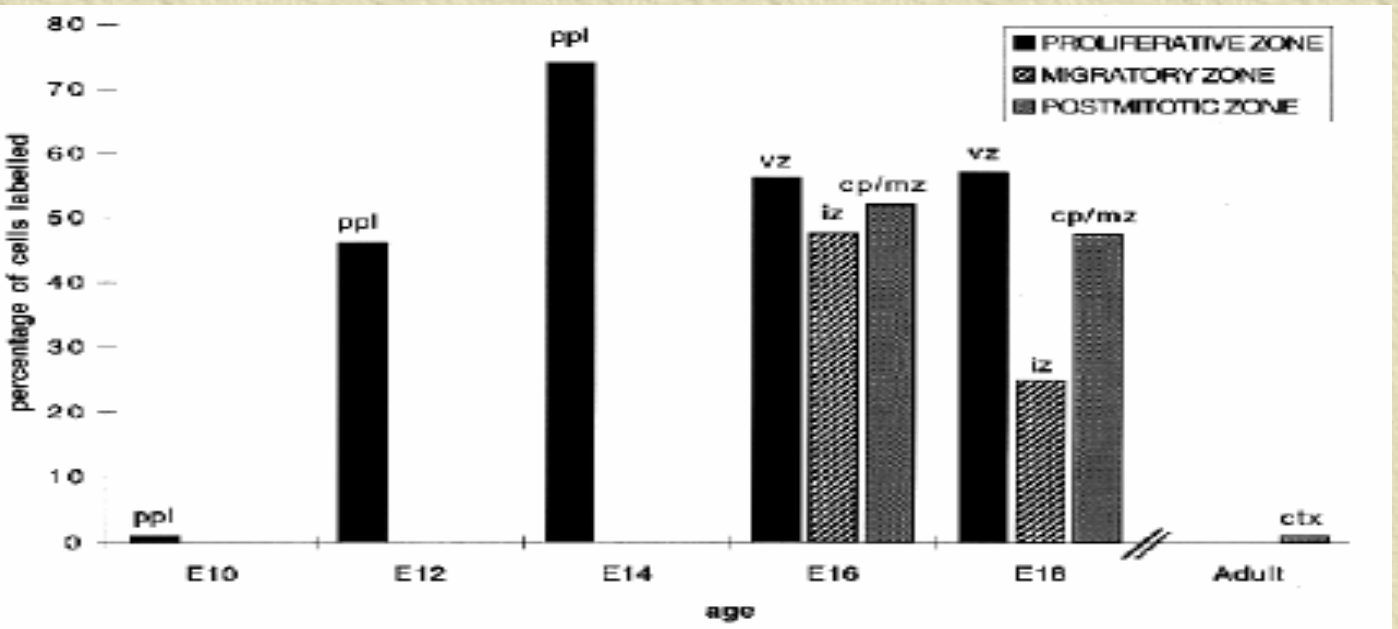
Quantitative Distribution

- Few dying cells at E10 or in adult cortex (<1% of cells)
- Death range of 25-70% (ave. 50%) from E12-E18



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Conclusions

- PCD is a major factor in embryonic cortical development
  - An average of 50% of cells undergo PCD from E12-E18 (peak at E14)
  - Most of the PCD in proliferative zones
- ISEL+ is a sensitive and effective method of labeling PCD

Implications

- If there is so much cell death, but overall cell numbers increase, there must be a dramatic rate of cell production
  - Single blast cell estimated to give rise to 250+ neurons (Caviness et al, 1995)
  - Such production, plus PCD, may explain actual numbers found
- Reassessing past studies
  - Ex: retroviral lineage estimates of size, distribution and composition of cortical clones
    - Many clonal types may have died from PCD
    - PCD may account for discrepancies between in vitro and in vivo (larger number of cells in vitro)

Speculation

- PCD in proliferative zones
  - PCD in postmitotic regions explained by matching of neurons to targets
  - Matching is not a useful explanation in proliferative zones
  - What are the mechanism(s) in proliferative zones? Different selectivities for death?
- Possible characterization of PCD
  - Peak at E14 allows selection of first cortical neurons, with correct phenotypes, which are then a template for later generated cells
  - PCD in cortex similar to PCD in thymus, where this selection does take place

Issues

- Okazaki fragments
- Rate of cell removal
  - No real-time visualization
  - Estimates from other studies, but they were not as sensitive as ISEL+