Strategy for Assaying Gene Expression in Human Brain Malformations

1) Obtain human tissue
- intraoperatively
-post-mortem

2) Obtain animal tissue

3) Fix in paraformaldehyde

4) Immunolabel

Generate cDNA from poly A mRNA
via *in situ* transcription from whole section

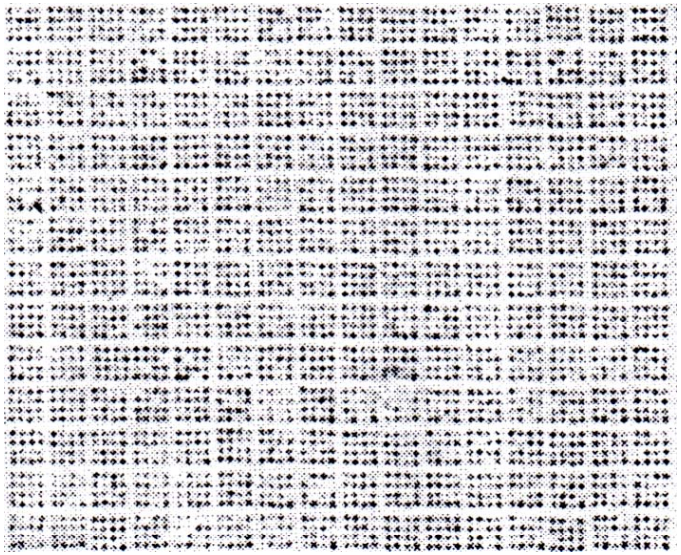
Amplify mRNA from cDNA, incorporate radiolabel

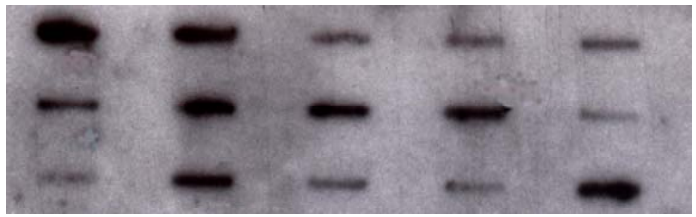
Probe cDNA array containing candidate genes

Analyze hybridization intensities

Microdissect single cells
Single cell mRNA amplification
Probe cDNA arrays

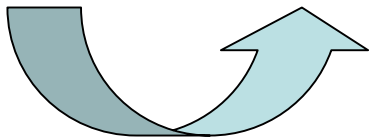
Crino et al., 1996

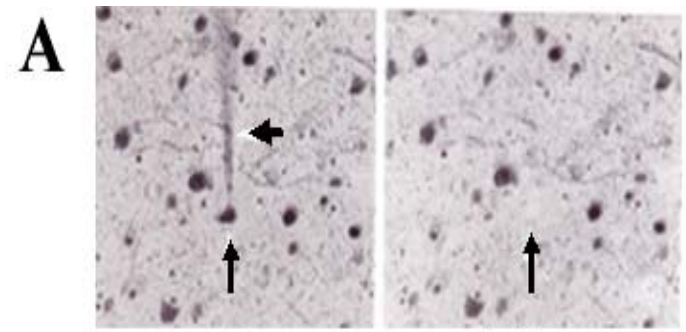




Tissue section
Small array

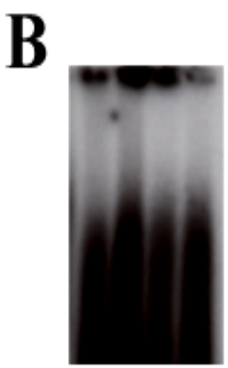
Tissue section
Large Array

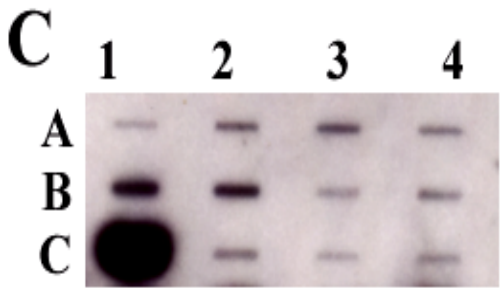




Hybridization intensity
Compare with “housekeeping” gene
Compare with internal standard
Compare to average of entire blot

Relative mRNA abundance

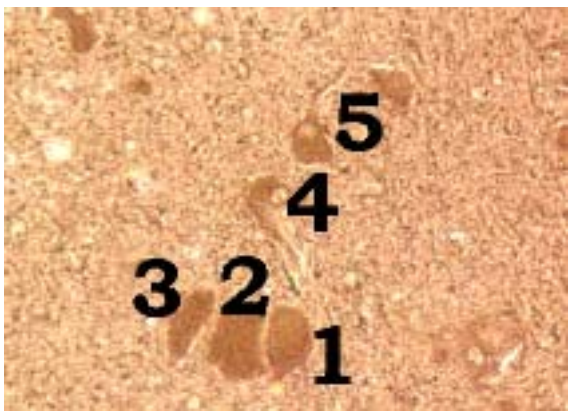


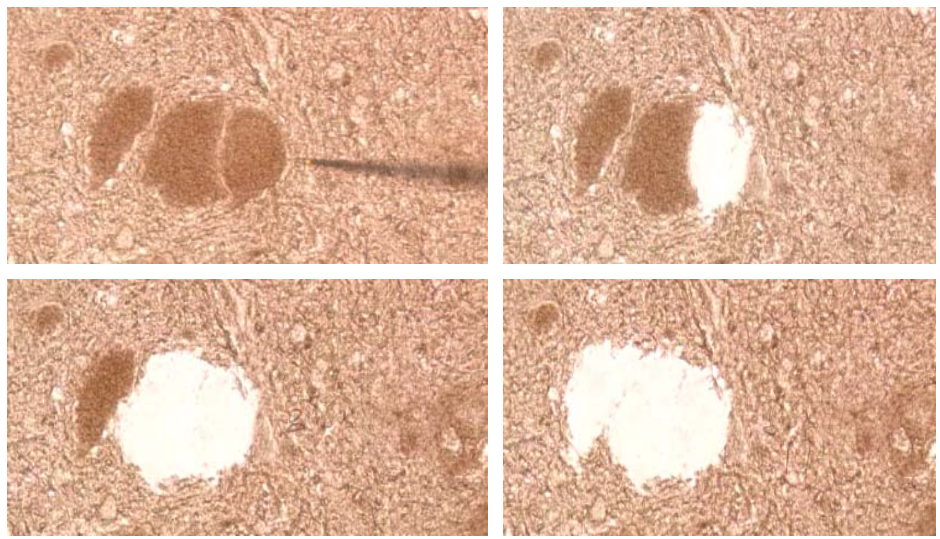


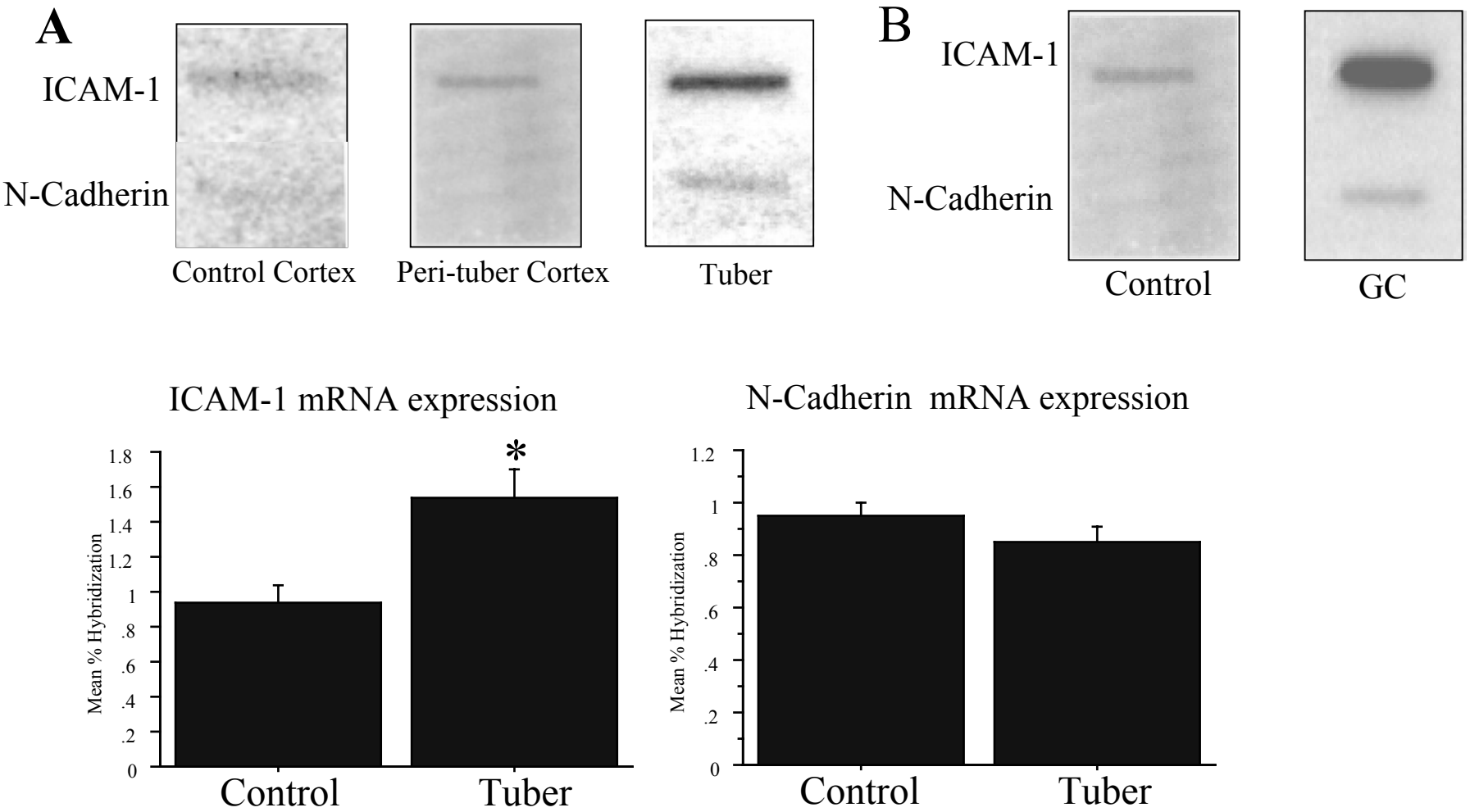
Why Single Cell mRNA Analysis?

Consideration of Important Variables

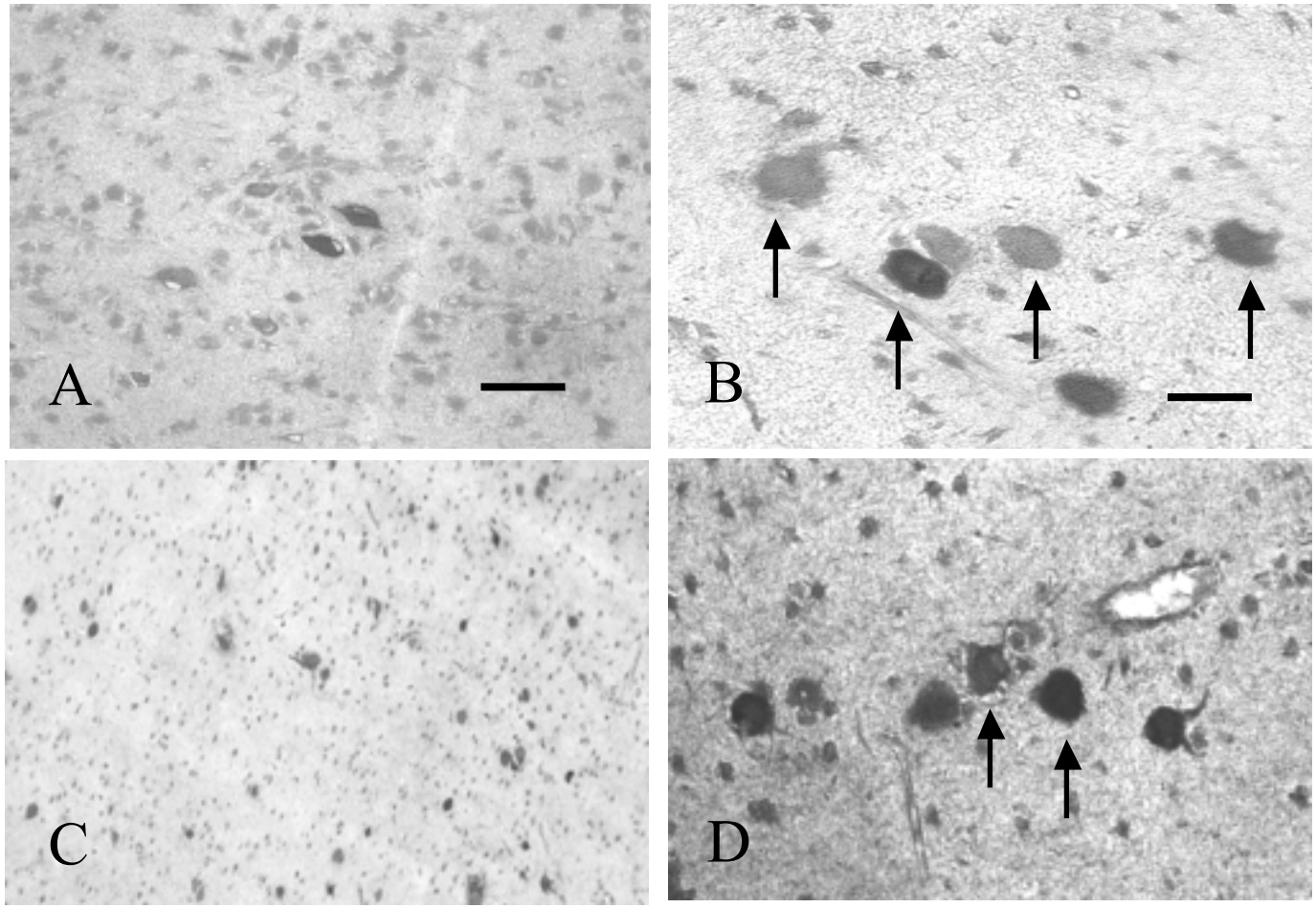
Neuronal Heterogeneity
Disease Heterogeneity
Analysis of Phenotypically Similar Cells
Selecting Disease Affected Neurons
Speed of Analysis







ICAM-1 mRNA expression in tuber and control cortex. **A**, Top, cDNA arrays showing increased hybridization of ICAM-1 mRNA in whole tuber sections compared with peri-tuberal and control cortex. Note similar N-cadherin hybridization levels in tuber and control cortex sections. Bottom, left, increased expression of ICAM-1 mRNA in tubers compared with control cortex. Right, similar expression levels of N-cadherin mRNA in tubers and control cortex. Graphs depict mean percent hybridization intensity of each mRNA$\pm$standard error (* $p<0.05$). **B**, ICAM-1 mRNA expression is increased in single ICAM-1 immunoreactive GC from tuber compared with single layer III pyramidal neuron (control) that did not exhibit ICAM-1 immunoreactivity. N-cadherin mRNA expression did not differ between these cell types.



Cellular expression of ICAM-1 in 4 representative tuber specimens. A,C note robust immunolabeling of tubers with ICAM-1. Scale bar, 300 microns. B,D higher magnification view of ICAM-1 immunolabeling in GCs (arrows). Smaller ICAM-1 labeled cells include DNs and rare astrocytes.