Human and Non-Human Primate Developmental Gene Expression Data Analysis

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A Transcriptional Atlas of Mammalian Brain Development

Allen Mouse Brain Atlas
- Comprehensive ISH for 20,000 unique transcripts
- Digital annotated reference atlas

Allen Developing Mouse Brain Atlas
- 7 developmental stages, comprehensive ISH for ~2000 genes
- Stage-specific annotated reference atlases

Allen Human Brain Atlas
- Adult human brain gene expression atlas
- Dense microarray analysis (~1000 brain regions)
- MRI, DTI imaging
- Targeted ISH analysis

NIH Blueprint Non-Human Primate (NHP) Atlas
- Neurodevelopmental gene expression atlas
- Targeted ISH analysis
- MRI, histological reference series
- High anatomical resolution microarray analysis

BrainSpan Atlas of the Developing Human Brain
- Targeted developmental transcriptome analysis
- Digital annotated MRI, DTI, histological ref. atlases
- Targeted ISH analysis
Methodological approaches to neuroanatomically comprehensive, genome-wide transcriptional analysis of the brain

Mouse brain ISH profiling

- Sagittal Series
- Subdivide slab into 2x3 blocks

Human/NHP brain microarray profiling

- Whole brain
- MRI
- 1cm coronal slabs

- Cryosectioning
- Macrodissection
- Laser Microdissection

- Microarray Analysis

- Human/NHP brain microarray profiling
- Mouse brain ISH profiling

- Laser Microdissection
- Macrodissection
Balancing anatomical specificity with anatomical, developmental and transcriptome coverage

BrainSpan RNA-seq
- Expert targeted (16) structure dissection
- Comprehensive developmental coverage
- Full sequence-level transcriptome coverage

BrainSpan microarray
- Expert laser microdissection
- Fetal timepoints only
- Comprehensive (300) structural coverage
- Gene-level transcriptome coverage

Allen Human Brain Atlas
- Expert structure dissection in cortex, striatum, cerebellum for comprehensive spatial coverage
- Expert laser microdissection for subcortical nuclei
- Gene-level transcriptome coverage: ~1000 samples/brain

NHP atlas
- Expert laser microdissection
- Targeted structural (~60) coverage
- Postnatal development (prenatal coming soon)
- Gene-level transcriptome coverage
The NIH Blueprint NHP Atlas: A neurodevelopmental atlas of gene expression in non-human primate brain

Goal: Provide research tools and primary data to the neuroscience community through construction of a searchable, digital gene expression atlas of the rhesus macaque brain spanning postnatal brain development.

Postnatal Development

• Microarray-based transcriptional analysis
  o Medial prefrontal, visual cortex, hippocampus, striatum, amygdala
  o Macro- and laser microdissection of fine substructures
  o N=3 male per stage

• Cellular resolution in situ hybridization
  o Uniform sampling across same 5 structures
  o N=3 male per stage
  o 46 genes/structure

• MRI, Nissl-based anatomical reference

http://www.blueprintnhpatlas.org
NIH Blueprint NHP Atlas
Postnatal brain development and experimental design

Target structures:
Primary Visual Cortex (V1)
Medial Prefrontal Cortex
Hippocampus
Ventral striatum
Amygdala

Similar prenatal profiling in progress over the next 2 years


http://www.blueprintnhpatlas.org
Molecular signatures of brain region and developmental stage in rhesus macaque macro-structure microarray data

Largest developmental gene signatures are associated with early developmental stages

Rui Luo, Gescwind lab, UCLA
Primary features of spatiotemporal patterning in postnatal development

Genetic similarities seem to mirror ontogenetic/phylogenetic relationships

Genetic signatures associated with structure and age. Earliest ages are most distinct.

Different brain regions have both common and unique age-related signatures

Weighted gene co-expression network analysis (WGCNA)
Molecular/cellular architecture of primate hippocampus
(What we would like to characterize with transcriptional profiling)
Fine anatomical transcriptional profiling across macaque postnatal development

Sampling paradigm:

- Hippocampal subfields
- Amygdalar subnuclei
- Striatal divisions
- Cortical layers (1,2,3,4,5,6, WM)
  - V1, V2
  - Anterior cingulate
  - Orbitofrontal cortex
  - Gyrus rectus
  - Dorsolateral prefrontal
High anatomical resolution quantitative transcriptional profiles from birth to adulthood

NIH Blueprint NHP Atlas

Parvalbumin (PVALB)

0 Months
Newborn

3 Months
Infant

12 Months
Juvenile

48 Months
Young Adult

Increase over postnatal development

Stratum Oriens

Stratum Oriens
Transcriptional profiling of specific hippocampal subfields/cell types

Hippocampal Formation

- CA2sp
- CA3sp
- CA4
- DGgcl
- DGpo
- DGsgz
- CA1sr
- CA1sp
- CA1so
- Sub
- Stratum Oriens (CA1so)
- Stratum Radiatum (CA1sr)
- Stratum Pyramidale (CA1sp)
- CA2
- Stratum Pyramidale (CA2sp)
- CA3
- Stratum Pyramidale (CA3sp)
- CA4
- Dentate gyrus
- Polymorph layer (DGpo)
- Granule cell layer (DGgcl)
- Subgranular zone (DGsgz)
- Subiculum (S)

*3 biological replicates per time point
Transcriptional similarities reflect constituent cell types, samples with similar cell types appear to become increasingly similar as they mature.
WGCNA identifies gene modules associated with specific hippocampal subfields, cell types and developmental stages.
Characterizing the neurogenic niche of the subgranular zone of the dentate gyrus where neurogenesis persists into adulthood.
Transcriptional dynamics involved in developmental and adult dentate gyrus granule cell neurogenesis
WGCNA identifies gene networks enriched in the neurogenic subgranular niche with different temporal profiles
Molecular signatures of postnatal neurogenesis in the hippocampal dentate gyrus

Neurogenesis-related module eigengene pattern matches developmental decline in hippocampal neurogenesis (# of dividing cells)

ISH validates enrichment in SGZ and developmental downregulation
Transcriptional architecture of the primate neocortex
Microarray analysis of specific neocortical layers and areas in adult rhesus macaque

Bernard et al. (2012) Neuron
Cortical layers have highly specific gene expression patterns that can be identified by microarrays and confirmed with other methods.

Layer-specific gene expression signatures

Validation of laminar patterns by ISH
Molecular relationships between cortical layers most strongly reflect spatial proximity

Adjacent layers are most similar to one another.

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Fueling Discovery
Molecular relationships between cortical regions reflect proximity

Adjacent areas are most similar to one another
Transcriptional similarity mirrors cortical topography in adult human neocortex
Differential V1 gene signatures conserved in human and non-human primate, not in mice

- Human SYT6
- Mouse Syt6
- Same layer, no V1 enrichment in mouse
- Different cell population in mouse
- Same layer, no V1 enrichment in mouse

Human SYT6
Mouse Syt6
BrainSpan Atlas of the Developing Human Brain

Data access & visualization

Data analysis, mining & API access

Transcriptional profiling

Gene expression at cellular resolution (ISH)

Anatomy Reference Framework

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UCLA: Dan Geschwind, Giovanni Coppola, Steve Horvath

Allen Institute

Harvard/MGH: Bruce Fischl

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Allen Institute

BrainSpan

ATLAS OF THE DEVELOPING HUMAN BRAIN
**High Resolution Prenatal Brain Component**

**Reference Atlases**

- **Stage III:** 15pcw
- **Stage IV:** 21pcw (Public in November 2011)

**In Situ Hybridization**

- **Stage III:** 15pcw
- **Stage IV:** 21pcw

**Fine structure LCM/microarray analysis**

- **Stage III:** 15pcw, 16pcw
- **Stage IV:** N=2 21pcw (first brain public in November 2011)

Maximizing precious resources: 6 fetal specimens will yield ~1200 microarrays, 2 reference atlases and 6400 ISH slides

~1200 arrays total scope
Neocortical development

Inside-out generation of neurons destined for successive cortical layers

Transient layers during early prenatal development

Figure 1 | The Boulder Committee’s 1970 schematic model of human neocortical development, and a proposed revision. A | The Boulder Committee’s original summary diagram of neocortical development. B | Our revised version. Comparison of these two illustrations summarizes our redefinition of the sequence of events and the formation of transient compartments, including the preplate (PP) and the intermediate and subplate zones (IZ and SP). The panels in part B correspond to the following approximate ages (for the lateral part of the dorsal telencephalon): a: embryonic day (E) 30; b: E31–E32; c: E45; d: E55; e: gestational week 14. CP, cortical plate; I & IZ, intermediate zone; M & MZ, marginal zone; S & SVZ, subventricular zone; (SG), subpial granular layer (part of the MZ); V & VZ, ventricular zone. Part A reproduced, with permission, from REF. 4 © (1970) Wiley.
Prenatal neocortex laser microdissection laminar analysis

Sampling Strategy

- SG: Subpial granular zone
- MZ: Marginal zone
- CPo: Cortical plate, outer
- CPI: Cortical plate, inner
- SP: Subplate zone
- IZ: Intermediate zone
- SZo: Subventricular zone, outer
- SZi: Subventricular zone, inner
- VZ: Ventricular zone

Clustering of cortex samples

Color Key

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ATLAS OF THE DEVELOPING HUMAN BRAIN
LCM microarray profiles accurately capture anatomical specificity

Reelin expression in Cajal-Retzius cells

RELN
Lamina-specific gene expression in 21pcw neocortex

Zic family member 1 (odd-paired homolog, Drosophila)

ZIC1

Calbindin 2

CALB2

LIM domain only 4

LMO4

Forkhead box P1

FOXP1

ZIC1 ISH

CALB2 ISH

LMO4 ISH

FOXP1 ISH
Lamina-specific gene expression in 21pcw neocortex

- Subventricular Zone
- Ventricular Zone
- Ganglionic Eminences

Gene expression:
- Paired box 6 (PAX6)
- Eomesodermin (TBR2) (EOMES)
- Vimentin (VIM)
- Glial fibrillary acidic protein (GFAP)

Images:
- PAX6 ISH
- EOMES ISH
- VIM ISH
- GFAP ISH
Laminar expression mirrors developmental process

Subpial Granular Zone-Enriched
- Ectoderm development
- Epithelium development
- Blood vessel development

Marginal Zone-Enriched
- Regulation of transcription

Outer Cortical Plate-Enriched
- Cellular protein metabolic process
- Protein polymerization
- Phospholipid metabolic process

Inner Cortical Plate-Enriched
- Neuron projection development
- Axonogenesis

Subplate Zone-Enriched
- Regulation of synaptic transmission
- Synaptic vesicle transport
- Negative regulation of cell migration

Subventricular Zone-Enriched
- DNA replication
- Mitosis
- Cell cycle checkpoint

Subpial granular zone
Marginal zone
Cortical plate, outer
Cortical plate, inner
Subplate zone
Intermediate zone
Subventricular zone, outer
Subventricular zone, inner
Ventricular zone
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www.alleninstitute.org or www.brain-map.org
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NIH Blueprint NHP Atlas

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BrainSpan Atlas of the Developing Human Brain

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