Brain cell diversity and the dynamic epigenome

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Illustration: Scott Nicoll
“There’s something very interesting about life,” Clark says, “which is that we do seem to be built of system upon system upon system. The smallest systems are the individual cells, which have an awful lot of their own little intelligence, if you like—they take care of themselves, they have their own things to do. Maybe there’s a great flexibility in being built out of all these little bits of stuff that have their own capacities to protect and organize themselves. I’ve become more and more open to the idea that some of the fundamental features of life really are important to understanding how our mind is possible. I didn’t use to think that. I used to think that you could start about halfway up and get everything you needed.”
Not all neurons are created equal:

Drawing of auditory cortical neurons, Ramón y Cajal (1899)

Fluorescently labeled neurons and glia, Livet, Sanes, and Lichtman (2007)

Each neuron has a unique:

1. Location (area, layer)
2. Connections (inputs, outputs)
3. Electrical and chemical responses
SAME BUT DIFFERENT

How epigenetics can blur the line between nature and nurture.

BY SIDDHARTHA MUKHERJEE

The author's mother (right) and her twin are a study in difference and identity.
How do cells acquire, maintain and adjust their diverse characters?
Brain development: A tightly orchestrated process

Timeline of major events in brain development. This diagram represents brain development beginning with neurulation, and proceeds through neuronal proliferation, neural migration, myelination, synaptogenesis, and apoptosis. Normal development of brain circuits for the Genesis of Neural Circuits.

Normal development of brain circuits. These markers increase approximately 50% of all neurons are eliminated during the periadolescence, followed by their pruning or competitive elimination. (For a comparison of ages and stages of rat treatment of developmental psychopathologies.

We also provide specific examples of neuropsychiatric disorders that are commonly seen by child psychiatrists and neurologists. These disturbances in neuronal migration can have profound neuro-functional consequences range from mental retardation to the extension of knowledge about prenatal brain development.

Evolutionary conservation of neurodevelopmental events, are both limited and inherently constrained by the extension of knowledge about prenatal brain development. Bystron et al, 2002; Huttenlocher and Dabholkar, 1997; Levitt, 2003). The remarkable evolutionary conservation of neurodevelopmental events and timing of synaptic production and elimination of the human receptors for dopamine GZ Tau and BS Peterson, 2000; 2002; 2008; 2009).

Dopamine D1 and D2 receptor expression is highest during the late stages of gestation, and postnatal human brain differed across different regions of the cortex. The density of synapses in the primary visual cortex. As neurons complete their migration, they extend axons and dendrites. Firing rates of nigrostriatal projections increase dramatically during the first weeks of life.

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Brain development: A tightly orchestrated process

Brain development: A tightly orchestrated process
Puzzle:
Different cells, same genome...

Drawing of pigeon cerebellar Purkinje and granule cells, Ramon y Cajal (1899)
Levels of organization in the genome

DNA wrapped around a histone particle
Levels of organization in the genome

- DNA: Isolated patches.
- The Nucleosome: Genes under active transcription.
- "Beads-on-a-String": Less active genes.
- The 30nm Fibre: Further levels of organization.
Levels of organization in the genome

30nm Fibre

Active Chromosome

During interphase.

The Metaphase Chromosome

During cell division.

Add further scaffold proteins.
Modifications to DNA are epigenetic “punctuation marks”

Epigenetics:
Punctuation. Is. Key.

STOP CLUBBING BABY SEALS!!

STOP CLUBBING, BABY SEALS!
The genome as a computational network

ENCODE: Encyclopedia of DNA elements
Cytosine DNA Methylation

- Covalent modification of genomic cytosine (mC)
- Key roles in imprinting, X-inactivation, transcription repression, cancer
- Stable and heritable
- Yet, reversible and potentially activity-dependent
- *Rett syndrome*: An autism-spectrum disorder caused by Methyl-C Binding Protein (MECP2) loss of function

**CG**: Highly methylated in all cell types

**non-CG (CH)**: Generally unmethylated after differentiation
Epigenetic regulation in insects

Queen and female worker bees

Royal jelly
Epimutation:
Two forms of the toadflax plant with identical genotype but different inherited DNA methylation patterns.
Mammals: You are what you eat (so eat your vitamins!)

Nutrients supporting healthy methylation:
- Folic acid
- B-vitamins
- SAM (S-adenosyl methionine)

Especially important for pregnant mothers/infants
Maternal care induces life-long changes in DNA methylation and stress resilience in offspring

- Low quality maternal care
  - Stress behavior of offspring ↑
  - Glucocorticoid receptor (GR) expression in brain ↓
  - GR promoter is hypermethylated in hippocampus in low-quality group [Weaver, …, Szyf, Meaney (2004)]

- High quality maternal care
  - Stress behavior of offspring ↓
  - Glucocorticoid receptor (GR) expression in brain ↑
  - Maternal Care ↓
  - Offspring DNA methylation ↓
  - Offspring GR expression ↓
  - Offspring stress behavior ↓
Testing the cognitive role of epigenetic modifications requires genome-scale, base-resolution neuronal epigenome profiling.
Studying gene networks: Shotgun sequencing
Shotgun bisulfite sequencing measures the DNA methylation landscape

Genomic DNA → Random fragmentation → Bisulfite conversion of unmethylated C → Converted → Protected → Deep sequencing → Computational analysis/statistics

\[ c(\tau) = \sum_i m_i m_{i+\tau} \]

R. Lister and J. Ecker, Genome Research (2009)
Two DNA sequence contexts for methylation

**CG:**
Highly methylated in all cell types

**non-CG (CH):**
Generally unmethylated after differentiation
A surprise: Substantial non-CG methylation in neurons


See also: Xie et al., Cell (2012); Zeng et al., Am. J. Hum. Gen. (2012)
How does methylation accumulate during brain development?

Timeline of major events in brain development. This diagram represents brain development beginning with neurulation, and proceeding through neuronal migration, synaptogenesis, pruning, myelination, and apoptosis.

The neuro-ontogenic process in humans begins at gestation, and proceeds through the formation of the primary brain vesicles, which develop into the forebrain, midbrain, and hindbrain.

During the embryonic period, monoamine neurons are detectable by embryonic ages 2.2. Postnatal brain development continues throughout life, with periods of vulnerability that may affect brain development.

Normal development of brain circuits integrates established and emerging knowledge of development and treatment of developmental psychopathologies.

Neuropsychopharmacology

Figure 1 (Jessell and Sanes, 2000; Rash and Grove, 2006b; Rhinn et al., 1993; Marin-Padilla, 1988). In this review, we will illustrate how knowledge of brain development can be applied to pediatric neurology.

We also provide specific examples of neuropsychiatric disorders that are associated with abnormalities in brain development.

DeFelipe et al. (1997); Huttenlocher et al., (1997)

mCH

Non-CG methylation accumulates throughout childhood and adolescence

Human

Mouse

Non-CG methylation accumulates throughout childhood and adolescence
Non-CG methylation increases during years 0-16, coinciding with synaptogenesis and pruning

*DeFelipe et al. (1997); Huttenlocher et al., (1997)
Methylation patterns are strongly conserved between individuals

Conservation suggests there could be a biological function

However, there is no causal evidence yet (stay tuned)
Does DNA methylation contribute to brain cell diversity?

Drawing of auditory cortical neurons, Ramón y Cajal (1899)

Fluorescently labeled neurons and glia, Livet, Sanes, and Lichtman (2007)
Cell types have unique DNA methylation fingerprints

Transcription factor MEF2C:
- Implicated in neurogenesis and cortical development
- Hypermethylated (i.e., repressed) in glia
mCH is a characteristic feature of neurons, not astrocytes.
Non-CG DNA methylation is a specific feature of mature neurons.
Identifying gene methylation patterns is a “Big Data” challenge

Lister*, Mukamel*, et al. (2013)
Unbiased clustering of methylation profiles identifies distinct gene sets

Principal component (PC) analysis of genome-wide methylation patterns
Unbiased clustering of methylation profiles identifies distinct gene sets.
Sub-types of neurons: Excitatory and inhibitory cells create balance
Excitatory and inhibitory neurons: Natives and immigrants

Excitatory cells radiate upward within cortex

Inhibitory cells migrate to the cortex
What is the DNA methylation landscape in major neuron cell types?

Classification of inhibitory interneuron cell types
Cluster analysis of 411 single cells + 14 bulk methylomes

Spearman correlation of gene-body mCH
Data dimensionality

- A dataset with \( p \) “features” (e.g. genes) and \( n \) “observations” (e.g. cells)

- If both \( p \) and \( n \) are large (>1,000), it becomes difficult to visualize, analyze and interpret the data
Dimensionality reduction by Principal Components Analysis (PCA)

- Principal components analysis (PCA) projects high-dimensional data onto a smaller number of “most interesting” dimensions
Example: Projection of 3D global geography onto 2D maps
tSNE (t-Stochastic Neighbor Embedding)
Visualizing cells in a high-dimensional space
Linear and non-linear dimensional reduction

Principal components analysis

$t$-Distributed stochastic neighbor embedding
(van der Maaten, Hinton 2008)
tSNE visualization of single human neurons

Hierarchical clustering of human neuronal clusters

- hL2/3
- hDL-3
- hDL-1
- hDL-2
- hL4
- hL5-1
- hL5-2
- hL5-3
- hL5-4
- hL6-2
- hL6-3
- hL6-1
- hVip-1
- hVip-2
- hNdnf
- hNos
- hPv-1
- hSst-1
- hSst-3
- hSst-2
- hPv-2
Example: Adgra3 is a novel marker of PV+ interneurons.

Fig. S11. Double ISH experiments validate novel markers predicted by mCH.

(A-B) Relative mCH level (mCH Z-score) of Sulf1 and Tle4. The z-score is defined as the mCH value minus its mean over all cells, divided by the standard deviation across cells.

(C-D) Double in situ RNA hybridization results using probes for Sulf1 and Tle4 in mouse FC. (C) and (D) show two coronal sections both in mouse FC with (C) located more rostral than (D).

(E-F) Relative mCH level (mCH Z-score) of Adgra3 and Pvalb.

(G) Double in situ RNA hybridization results using probes for Adgra3 and Pvalb.
How many cell types are there? 

**Lumpers vs. Splitters**

It is good to have hair-splitters & lumpers

*(Darwin, 1857)*

Splitters make very small units – their critics say that if they can tell two animals apart, they place them in different genera ... and if they cannot tell them apart, they place them in different species. ...

Lumpers make large units – their critics say that if a carnivore is neither a dog nor a bear, they call it a cat

*(George Simpson, 1945)*
Determining cell types through the integration of multi-modal datasets

Yamawaki (2014) eLIFE

Brain adapted from Allen Brain Atlas
Determining cell types through the integration of multi-modal datasets

Cell type defined by multiple modalities

- Directly link transcription and epigenetic regulation in the same cell type
- Provide cross-modal validation of cell types predicted from one data modality
- Provides foundation for functional cell types

Yamawaki (2014) eLIFE
Brain adapted from Allen Brain Atlas
Multi-omics data integration requires imputation.
For each cell in modality A, find K neighbors in modality B
This requires a linking assumption, e.g. low gene body mCH corresponds with high mRNA expression
Use neighbors to impute missing information for A
Integrated cluster analysis

Multimodal projection
- 5,352 snmC cells
- 11,878 snATAC cells
- 6,083 snRNA cells

Gene visualization for Tshz2
- chr: 169,630,000-169,643,000
- Tshz2

clusters

Normalized gene body mCH (scale reversed)
Normalize ATAC counts
mRNA expression (log TPM)
Exploring cell type specific DNA methylation patterns with interactive data visualizations (http://brainome.org)
FOXP2: A “language gene”?  

- Mutations in FOXP2 linked to language disorders (verbal dyspraxia)  
- Also linked with vocalization in songbirds
FOXP2 (associated with language) is expressed in different cortical layers in mouse and human frontal cortex.
~60M years of evolution led to greater divergence in excitatory compared with inhibitory neuron epigenomes.
Complex biological networks: Genes and Brains

Brains (neural networks)

C. Elegans connectome (1986)

Genomes (gene networks)


- Encode and store innate information
- Encode and store learned information
- Transmit information
- Enable complex, recurrent interactions
Take-home message

The methylation status at ~1 billion cytosines in the genome is potentially an information-rich, stable yet flexible substrate for information storage/processing.
Open questions:

- Functional role of mCH?
- Differences between 100s of neuronal subtypes?
- Impact of experience/learning?
- Disruption in neuropsychiatric disorders?
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