Development and Function of Neocortical Circuits: from Genes to Behavior

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Conserved circuit architecture across mammals
How cortical circuits are built?

**Neuronal diversity**
Genetic programs for neuron identity specification

**Connectivity**
Molecular mechanisms
Activity-dependent mechanisms

**Circuit activity**
Role of distinct neuron types in specific network functions
Neuronal diversity

Callosal projection neurons (CPN)

Intracortical commissural neurons

Subcerebral projection neurons (SCPN)

Corticothalamic projection neurons (CThPN)

Corticofugal neurons

Frontal cut

Longitudinal cut
Generation of neocortical projection neurons

Neuronal diversity

Adapted from Greig, Woodworth, Galazo et al., *Nat Rev Neurosci 2013*
Identification of genetic programs regulating neuronal diversity

Transcriptome comparison at multiple developmental stages

CThPN molecular program

(Galazo et al., Neuron 2016)
Corticothalamic identity specification

Transcriptome comparison at multiple developmental stages

**Representative Profile**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>GenBank ID</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> CThPN general Identity genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tle4</td>
<td>transcriptional regulator</td>
<td>NM_011600.2</td>
</tr>
<tr>
<td>Ges1</td>
<td>transcriptional regulator</td>
<td>NM_001145886</td>
</tr>
<tr>
<td>Itsn1</td>
<td>transcriptional regulator, axon guidance</td>
<td>NM_010587</td>
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<tr>
<td>Nrnb</td>
<td>transcriptional regulator</td>
<td>NM_001113210</td>
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<tr>
<td>Tbr1</td>
<td>transcriptional regulator</td>
<td>NM_009322.3</td>
</tr>
<tr>
<td>Cxcr5</td>
<td>transcriptional regulator, modulator of Wnt signaling</td>
<td>NM_133687.2</td>
</tr>
</tbody>
</table>

| **B** CThPN early development genes |                                            |                |
| Rad21         | cell division, transcriptional regulator   | NM_099009.4    |
| Rnpc2         | transcriptional regulator                   | NM_133242      |
| Rgs16         | regulator G protein signaling              | NM_011267      |
| Mllt1         | transcriptional regulator, chromatin remodeling | NM_022332.8  |
| Bcl2          | regulation of apoptosis                    | NM_177410      |
| Zmiz1         | transcriptional regulator, chromatin remodeling | NM_183208.3 |

| **C** CThPN intermediate development genes |                                            |                |
| Fog2          | transcriptional regulator, cell differentiation | NM_011766.5   |
| Cacna1e       | ion channel                                | NM_009782      |
| Cask          | synaptic scaffold, transcriptional regulator | NM_009806     |
| Gas7          | actin polymerization, neuron differentiation | NM_008088     |
| Nrf3          | transcriptional regulator                   | NM_010090      |
| Pcnx          | transmembrane protein, Notch signalling     | NM_018614      |
| Phr1          | ubiquitin ligase, axon guidance             | NM_207215      |
| Zmynd3        | transcriptional regulator, chromatin remodeling | NM_00119914.1 |

| **D** CThPN late development genes |                                            |                |
| Dmrt2         | transcriptional regulator, somite organization | NM_145831.3   |
| Klf9          | transcriptional regulator                   | NM_010638      |
| Nfya          | transcriptional regulator                   | NM_001110832   |
| Ror2          | transcriptional regulator, skeletal development | NM_013846     |
| Shb           | transcriptional regulator, cell differentiation | NM_001033306  |
| Smarcd3       | chromatin remodelling                       | NM_025891      |

| **E** Genes excluded from CThPN |                                            |                |
| Cav1           | signal transduction                        | NM_007818      |
| Gabrg1         | receptor                                   | NM_010252      |
| Neo1           | transcriptional regulator                   | NM_008684      |
| Rot3           | transcriptional regulator                   | NM_001043354   |
| Tmtc4          | transmembrane tetra-tripctopeptide containing repeat 4 | NM_028651 |
| Cdh10          | cell adhesion                              | NM_009685      |

Galazo, Emsley, Macklis, Neuron 2016
Tle4 regulates specification of Corticothalamic Identity

Adapted from Galazo, Sweetser, and Macklis. Submission to Nat Neurosci
Tle4 regulates specification of Corticothalamic Identity

Changes in Morphology

Reduced expression of CThPN-genes in Tle4 KO

Adapted from Galazo, Sweetser, and Macklis. Submission to Nat Neurosci
Tle4 regulates specification of Corticothalamic Identity

Upregulation of SCPN-genes in CThPN

Loss of CThPN identity

Adapted from Galazo, Sweetser, and Macklis. Submission to Nat Neurosci
Tle4 regulates specification of Corticothalamic Identity

Changes in Morphology

WT

Tle4 KO

CThPN → SCPN

Loss of CThPN identity

Acquired SCPN Identity

Changes in Molecular Identity

Increased Subcerebral Connectivity

Adapted from Galazo, Sweetser, and Macklis. Submission to Nat Neurosci
Tle4 regulates specification of Corticothalamic Identity

Tle4 → Specify CThPN identity

Repress SCPN identity

WT

Tle4 KO

CThPN → SCPN
Behavioral effects of Corticothalamic fate conversion
How Tle4 Specifies Corticothalamic Identity?

- **Wild type**
  - SCPN → CThPN
  - Tle4
    - Specify SCPN identity
    - Repress CThPN identity

- **Fezf2 KO**
  - SCPN → CThPN
  - Fezf2
    - Specify SCPN identity

- **Tle4 KO**
  - CThPN → SCPN
  - Fezf2
    - Specify SCPN identity
How Tle4 Specifies Corticothalamic Identity?

**Wild type**

- **CThPN**
  - **Fezf2** repression
    - **Fezf2 locus**
    - Repression of SCPN program
  - **Tle4** specification
    - Specifying CThPN identity

**Tle4 KO**

- **CThPN** → **SCPN**
  - **Fezf2** activation
    - **Fezf2 locus**
    - Activation of SCPN program
  - **Tle4** repression
    - Specifying SCPN identity

Adapted from Galazo, Sweetser, and Macklis. Submission to *Nat Neurosci*.
Genetic programs driving neuronal identity differentiation

CPN identity program

Clim1
Lmo4
EphB1
Ctip2
Fezf2
Cited2
Satb2
Cux1

CThPN identity program

Sox5
Tbr1
Tle4
Fog2
Ctip1
Coup.tf1
Genetic programs driving neuronal identity differentiation

Autism Associated genes (SFARI human genetic database)
How stable is neuronal identity over time?

Tle4

Tle4 KO

Tle4 deletion postnatally

Tle4 OFF
Tle4 is necessary to maintain CThPN identity stable

Adapted from Galazo, Sweetser, and Macklis. Submission to Nat Neurosci
Postnatal reprogramming of Corticothalamic identity

Adapted from Galazo, Sweetser, and Macklis. Submission to Nat Neurosci
Connectivity

Molecular mechanisms
Activity-dependent mechanisms

Early development

Molecular mechanisms
Target recognition

Activity-dependent

Late development

Map refinement

Guidance cues

Target Innervation

Intra-target cues

Activity-dependent pruning
Connectivity  Molecular mechanisms

A. CThPN general identity genes

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<td>transcriptional regulator</td>
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<td>ltnl</td>
<td>transcriptional regulator, axon guidance</td>
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<td>Tbr1</td>
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<td>Cacna5</td>
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B. CThPN early development genes

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<td>Rad21</td>
<td>cell division, transcriptional regulator</td>
<td>NM_099009.4</td>
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<td>Rnpcl</td>
<td>transcriptional regulator</td>
<td>NM_133242</td>
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<td>Rps16l</td>
<td>regulator G protein signaling</td>
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<td>Mll1</td>
<td>transcriptional regulator, chromatin remodeling</td>
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<td>Bcl2</td>
<td>regulation of apoptosis</td>
<td>NM_177410</td>
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<td>Zn14a1</td>
<td>transcriptional regulator, chromatin remodeling</td>
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C. CThPN intermediate development genes

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<tr>
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<tbody>
<tr>
<td>Fog2</td>
<td>transcriptional regulator, cell differentiation</td>
<td>NM_011766.5</td>
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<td>Ca2a1</td>
<td>ion channel</td>
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<td>Cask</td>
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D. CThPN late development genes

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<tr>
<td>Dmr2</td>
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<td>Klf4</td>
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<td>Smarcd3</td>
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E. Genes excluded from CThPN

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<tr>
<td>Cav1</td>
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<td>Gdab7</td>
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<td>Cdh10</td>
<td>cell adhesion</td>
<td>NM_005860</td>
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Galazo, Emsley, Macklis, Neuron 2016
Connectivity Molecular mechanisms

WT

Somatosensory Cortex Motor Cortex

FOG2 cKO

Somatosensory Cortex Motor Cortex

Abnormal MOTOR connectivity

Motor Cortex

Somatosensory Cortex

WT Fog2 cKO

WT Fog2 cKO

Adapted from Galazo et al., Neuron 2016
• Fog2 de novo mutations linked to Autism risk (Hashimoto et al., 2016)

• Hand stereotypies, delayed or no speech, seizures, mild to severe intellectual disability associated to human Fog2 deletions (Thierry et al., 2013; Tan et al., 2012; Wat et al., 2011)
Activity

Connectivity
Activity-dependent mechanisms

Connectivity

Cux1 → Kv 1.3 → Excitability → Contralateral Projection

Cux1 shRNA

Cux1
Reduced expression
Increased Excitability
Contralateral Projection

WT

Cux1

Adapted from Rodriguez-Tornos, …, Galazo et al., Neuron 2016
Connectivity
Activity-dependent mechanisms

Cux1 → Kv 1.3 → Excitability → Contralateral Projection

Reduced expression:
Cux1

Increased Excitability → Contralateral Projection

Rescued expression:
Cux1

Normalized Excitability → Rescue Contralateral Projection

Adapted from Rodriguez-Tornos, ..., Galazo et al., Neuron 2016
Connectivity
Activity-dependent mechanisms

Adapted from Rodriguez-Tornos, …, Galazo et al., Neuron 2016
Connectivity

Activity
Diversity of neurons
Genetic programs for neuron identity specification

Connectivity
Molecular mechanisms
Activity-dependent mechanisms

Circuit Function
Circuit Function: Sleep mediated memory consolidation

Synchronization necessary for memory consolidation

Slow Oscillation

Ripples

Corticothalamic-Driver

Corticothalamic-Modulator
Genetic access to Corticothalamic populations for optogenetic manipulation and recording

Corticothalamic-Modulator  Corticothalamic-Driver

Galazo, Varela, Wilson, ongoing
Electrophysiology in behaving animals

Extracellular recording

Filter 1 - 500Hz

Filter 300Hz – 6 KHz

Local Field Potential

Spikes

500ms

1ms

Galazo, Varela, Wilson, ongoing
Recording neural activity in freely moving animals

By Fabian Klossterman
Corticothalamic function in memory consolidation

CThPN- Modulator
CThPN- Driver

PFC
HC
Thal

Cortical Activity

Hippocampus Activity

Ripple

Ripple Onset

Galazo, Varela, Wilson, ongoing
Diversity of activities correlated with HC-Ripples

CThPN- Modulator
CThPN- Driver

PFC
HC
Thal

Cortical Activity

Hippocampus Activity

Ripple Onset

Galazo, Varela, Wilson, ongoing
Memory consolidation paradigm

- Memory Encoding (Training)
- Post-training Sleep
- Memory Retrieval

Optogenetic manipulation

CThPN-Modulator
- Inhibition

CThPN-Driver
- Stimulation

PFC-HC Synchronization
(slow oscillation-ripples)
Future goals and questions

Genetic programs → Neuron types → Connectivity → Activity

Genetic programs
Neuron types
Connectivity
Activity
Future goals and questions

1. How is neuronal identity maintained during circuit development?

2. How do early activity regulate neuronal differentiation and circuit formation?

3. How different neuronal populations contribute to early cortical activity patterns

4. Can early network activity reveal developmental problems in cortical circuits
Future goals and questions

1. How is neuronal identity maintained during circuit development?

   • Are mechanisms stabilizing identity permanently active in neocortical neurons?

   • What are the key molecular targets to stabilize connectivity during circuit development?

   • How is identity differentiation and stability influenced by the activity and other external factors?
Future goals and questions

2. How do early activity patterns regulate neuronal differentiation and circuit formation?

- Can developmental oscillations modulate genetic programs of differentiation?
- What molecular targets are regulated by activity-driven feedback mechanisms?

Simultaneous recordings with tetrodes in multiple areas

Adapted recording device for neonates

Ultra-sound guided implantation

Galazo, ongoing
Future goals and questions

1. How is neuronal identity maintained throughout development?

2. How do early activity regulate neuronal differentiation and circuit formation?

3. How different neuronal populations contribute to early cortical activity patterns

4. Can early network activity reveal developmental problems in cortical circuits
Jeff Macklis (Harvard)
Luciano Custo Greig (Harvard)
Ryann Fame (Harvard)
Erica Gornstein (Harvard)
Chloe Greppi (Brandeis)
Ryan Humphries (Harvard)
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Mollie Woodworth (Harvard)
Jessica MacDonald (Syracuse)
Kadir Ozkan (Harvard)
Hari Padmanabhan (Harvard)
Vibhu Sahni (Harvard)

Marta Nieto Lopez (CNB, Spain)
David Sweetser (Mass. General Hospital)
Xi Cheng (Mass. General Hospital)

Matt Wilson (MIT)
Carmen Varela (MIT)
Pedro Feliciano (MIT)
Francisco Flores (MIT)
Hector Penagos (MIT)
Jon Newman (MIT)

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