A Neural Circuitry Basis for the Core Clinical Features of Schizophrenia

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Grand Needs and Challenges in Psychiatry

• Need: A diagnostic system based on an understanding of the underlying disease processes as opposed to syndromal diagnoses.
  – Challenge: The human brain is the most complex organ in the known universe.

• Need: Therapeutic interventions that target disease mechanisms as opposed to symptomatic treatments.
  – Challenge: Psychiatric illness impairs the most sophisticated functions of the human brain.

• Need: Effective delivery of therapeutic interventions in the real world as opposed to limited access, non-adherence and stigma.
  – Challenge: Psychiatric services and research remain markedly underfunded relative to the personal, medical and societal costs of psychiatric illnesses.
Potential for Grand Solutions in Psychiatry

- Understanding disease processes at the level of the affected neural circuits has the potential to provide...
  - An empirical substrate for diagnostic categories.
  - A rational basis for developing novel therapeutics.
  - An effective explanation to patients for the problem and the therapeutic solution.
Dissecting the Disease Process in Psychiatry

Pathogenesis → Pathophysicsology

Etiology Pathological Entity Clinical Syndrome

Prevention Treatment

The Clinical Heterogeneity of Schizophrenia

- Positive symptoms: Delusions, hallucinations, thought disorder
- Negative symptoms: Decreased motivation, diminished emotional expression
- Cognitive deficits: Impairments in attention, executive function, working memory
- Sensory abnormalities: “Gating” disturbances
- Sensorimotor abnormalities: Eye tracking disturbances
- Motor abnormalities: Posturing, impaired coordination
The Clinical Heterogeneity of Schizophrenia

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- **Motor abnormalities:** Posturing, impaired coordination

Thought Disorder: Consequence of Deficient Working Memory?

- **Loose Associations (Derailment)**
  - Speech in which one idea is followed by unrelated or only loosely connected ideas.

- **Working memory**
  - The transient maintenance of a limited amount of information in order to guide thought or behavior.

- The failure to maintain the context of thought or an overarching idea in order to guide thought/speech to the next logically connected thought/statement is manifest as loose associations.
Cognitive Deficits: A Core and Clinically Critical Feature of Schizophrenia

- Prevalent in schizophrenia
- Present in milder form in unaffected relatives
- Present and progressive before the onset of psychosis
- Persistent across the course of illness
- Predictor of long-term functional outcome
- Product of impaired cortical network oscillations

Impaired Prefrontal Gamma Oscillations during a Working Memory Task* in Patients with Schizophrenia

- Replicated in APD-naïve subjects
- Cho et al., PNAS, 2006
- Minzenberg et al., Neuropsychopharm 2010

* Preparing to Overcome Prepotency Task
Intracranial Prefrontal Gamma Band Power Increases with Working Memory Load in Humans

Howard et. al., Cereb Cortex 13:1369, 2003
Prefrontal gamma band power during the Sternberg WM task is lower in subjects with schizophrenia (Chen et al., Neuroimage Clin, 2014).

Dissecting the Disease Process in Schizophrenia

Pathogenesis
Pathological Entity
(Alterations in a Specific Prefrontal Cortical Circuit?)
Prevention
Treatment

Pathophysiology
(Lower Gamma Oscillations)
Clinical Syndrome
(Imaired Working Memory)
What alterations in dorsolateral prefrontal cortex (DLPFC) circuitry could contribute to weaker gamma oscillations and working memory impairments in schizophrenia?

Critical Issues in Interpreting Disease-Related Alterations: “The 5 C’s”

• Does any given finding represent...
  – An upstream cause?
  – A downstream detrimental consequence of a cause?
  – A compensatory response to a cause or consequence?
  – A comorbid factor that frequently accompanies the illness?
  – A confound due to experimental limitations?
Critical Issues in Interpreting Disease-Related Alterations: “The 5 C’s”

- Does any given finding represent...
  - An upstream cause?
  - A downstream detrimental consequence of a cause?
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  - A comorbid factor that frequently accompanies the illness?
  - A confound due to experimental limitations?

Summary of Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>Sex</td>
<td>47 M / 15 F</td>
<td>47 M / 15 F</td>
</tr>
<tr>
<td>Race</td>
<td>52 W / 10 B</td>
<td>46 W / 16 B</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.7 ± 13.8</td>
<td>47.7 ± 12.7</td>
</tr>
<tr>
<td>Postmortem Interval (hr)</td>
<td>18.8 ± 5.5</td>
<td>19.2 ± 8.5</td>
</tr>
<tr>
<td>Brain pH</td>
<td>6.7 ± 0.2</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>RNA Integrity Number</td>
<td>8.2 ± 0.6</td>
<td>8.1 ± 0.6</td>
</tr>
</tbody>
</table>

For brain pH, \( t_{122} = 2.6, p=0.01 \). For all others, \( t_{122} \leq 0.45, p \geq 0.65 \).
A Neural Circuitry Basis for the Core Clinical Features of Schizophrenia

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Circuitry of the Prefrontal Cortex

ACG = Anterior Cingulate    VM = Ventromedial Prefrontal Cortex

Cortical Layers

Pyramidal and GABA Neurons

White Matter
Pyramidal Neuron-Parvalbumin GABA Neuron Circuitry in DLPFC Layer 3 is Critical for both Gamma Oscillations and Working Memory

Critical Role of *Layer 3* Circuitry in Working Memory and Gamma Oscillations

- Persistent neuronal firing during the delay period of WM tasks arises from recurrent excitation among *layer 3* pyramidal cells in primate DLPFC (Goldman-Rakic, *Neuron* 1995; Wang et al., *Neuron* 2013).


- PV basket neurons are most numerous in *layer 3* of primate DLPFC (Conde et al., *J Comp Neurol* 1994).

- Gamma oscillations are generated in *layer 3* of primate association cortex (Buffalo et al., *PNAS* 2011).
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Pyramidal Neuron-Parvalbumin GABA Neuron Circuitry in DLPFC Layer 3 is Critical for both Gamma Oscillations and Working Memory

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Mechanisms of Neural Network Oscillations

PV Basket Neuron
Pyramidal Neurons

Asynchronous Firing

Gonzalez-Burgos and Lewis, Schizophrenia Bulletin, 2009
Is the “primary” problem in the excitatory drive to layer 3 pyramidal neurons?

Is Deficient Excitation Causal?

PV Basket Neuron

PV Basket Neuron

Pyramidal Neurons

Pyramidal Neurons

Gonzalez-Burgos and Lewis, Schiz Bull 2009

Normal

Impaired Synchrony

Neural Network Synchrony

Gonzalez-Burgos and Lewis, Schiz Bull 2009

PV Cell Terminal

GABA-A Receptor

PYR Cell Terminal

Glutamate Receptor

Excitatory Input

Feedback Inhibition

Recurrent Excitation
Dendritic Spines Receive the Excitatory Synapses to Pyramidal Neurons

Stephen Eggan

Comparison

Schizophrenia

Leisa Glantz
Lamina-Specific Reductions in Pyramidal Neuron Dendritic Spine Density in Schizophrenia

![Graph showing the change in spine density across different layers of pyramidal neurons in schizophrenia.]

- **Deep Layer 3 Pyramidal Neurons**
  - Change in schizophrenia: -23%, p = .003

- **Superficial 3**
  - Change in schizophrenia: -13%, NS

- **Deep 3**
  - Change in schizophrenia: +3%, NS

- **Layer 5**
  - Change in schizophrenia: +12%, NS

- **Layer 6**
  - Change in schizophrenia: +12%, NS

Glantz and Lewis, Arch Gen Psychiatry, 2000
Kolluri and Lewis, Am J Psychiatry, 2005

Potential Genetic Basis for a Primary Disturbance in Dendritic Spines/Excitatory Inputs to Pyramidal Neurons

- **De novo mutations** are over-represented at loci encoding for glutamatergic post-synaptic proteins and proteins that regulate the actin filament dynamics essential for dendritic spine formation and maintenance. Fromer et al., *Nature* 506:179, 2014

- **Common alleles** associated with schizophrenia appear to be enriched for genes involved in glutamatergic neurotransmission. Ripke et al., *Nature* 511:421, 2014

- **Variants at the MHC locus** (complement component 4) associated with schizophrenia appear to regulate developmental pruning of dendritic spines. Sekar et al., *Nature*, 2016

- **These findings** provide a potential basis for a primary disturbance in dendritic spines in schizophrenia.
How is this apparent genetic liability moderated to create spine deficits predominantly on layer 3 pyramidal cells?

- Cdc42 is a RhoGTPase that regulates actin dynamics and spine number.
- Cdc42 mRNA levels are lower, and strongly correlated with spine deficits, in DLPFC layer 3 pyramidal neurons in schizophrenia (Hill et al. *Molecular Psychiatry*, 2006).
- Cdc42 effector protein 3 and 4 mRNA levels are upregulated in DLPFC layer 3 pyramidal cells from subjects with schizophrenia (Ide and Lewis, *Biol Psychiatry*, 2010; Datta et al. *Biol Psychiatry*, 2015).
- Together, lower Cdc42 and higher Cdc42EP3/4 could account for a cell type-specific dendritic spine deficit in layer 3 pyramidal cells.

### Altered CDC42 Signaling and Spine Deficits in Layer 3 Pyramidal Cells

<table>
<thead>
<tr>
<th>Healthy State</th>
<th>Molecular Alterations and Consequences in Schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ARHGDIA</strong></td>
<td><strong>ARHGDIA</strong></td>
</tr>
<tr>
<td>CDC42</td>
<td>CDC42</td>
</tr>
<tr>
<td>CDC42EP3</td>
<td>CDC42EP3</td>
</tr>
<tr>
<td>CDC42EP4</td>
<td>CDC42EP4</td>
</tr>
<tr>
<td>PAK1</td>
<td>PAK1</td>
</tr>
<tr>
<td>PAK2</td>
<td>PAK2</td>
</tr>
<tr>
<td>LIMK1</td>
<td>LIMK1</td>
</tr>
<tr>
<td>LIMK2</td>
<td>LIMK2</td>
</tr>
<tr>
<td>SEPT7</td>
<td>SEPT7</td>
</tr>
<tr>
<td>Cofilin</td>
<td>Cofilin</td>
</tr>
<tr>
<td>F-Actin</td>
<td>F-Actin</td>
</tr>
<tr>
<td>Spine Density</td>
<td>Spine Density</td>
</tr>
<tr>
<td>Note up- and down-regulation of mRNA levels.</td>
<td></td>
</tr>
</tbody>
</table>

Datta et al., *Biol Psychiatry* 2015
Dissecting the Disease Process in Schizophrenia: Hypothesis Building and Testing

- **Pathogenesis** (Cell Type-Specific Alterations in CDC42 Signaling)
- **Pathophysiology** (Lower Gamma Oscillations)
- **Etiology** (Genetic Liabilities in Actin Regulation)
- **Pathological Entity** (Fewer Dendritic Spines on Layer 3 Pyramidal Cells)
- **Clinical Syndrome** (Impaired Working Memory)

**Prevention**

**Treatment**

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**Postulates and Prediction**

- Schizophrenia is associated with chromosomal disturbances/genetic variants and gene expression alterations in the regulation of actin filament dynamics and hence in the capacity to form/maintain dendritic spines.

- Spine deficits are most prominent in layer 3 pyramidal cells due to altered levels of gene products selectively expressed in that cell type.

- Fewer spines and glutamatergic synapses reduce excitatory input to layer 3 pyramidal cells.

- Prediction: DLPFC layer 3 pyramidal cells are hypoactive in schizophrenia with less drive for mitochondrial energy production.
Does deficient energy production occur as a consequence of fewer dendritic spines on layer 3 pyramidal neurons?

Gene Expression Profiling Supports Lower Activity of DLPFC Layer 3 Pyramidal Cells in Schizophrenia

<table>
<thead>
<tr>
<th>LMD of Nissl-stained Pyramidal Cell in Human DLPFC</th>
<th>Altered Gene Expression is Enriched in Layer 3 Pyramidal Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gray Matter</td>
</tr>
<tr>
<td>Gene</td>
<td>% Δ</td>
</tr>
<tr>
<td>COX7A1</td>
<td>-9.4</td>
</tr>
<tr>
<td>UQCRQ</td>
<td>-2.3</td>
</tr>
</tbody>
</table>

Lower Expression of Genes Regulating Energy Production in Layer 3 Pyramidal Cells

<table>
<thead>
<tr>
<th>Mitochondrial Gene Pathways</th>
<th>Q-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactome Electron Transport Chain</td>
<td>&lt;10⁻⁷</td>
</tr>
<tr>
<td>Kegg Parkinsons Disease</td>
<td>&lt;10⁻⁷</td>
</tr>
<tr>
<td>Reactome Glucose Regulation of Insulin Secretion</td>
<td>&lt;10⁻⁵</td>
</tr>
<tr>
<td>Kegg Oxidative Phosphorylation</td>
<td>&lt;10⁻⁵</td>
</tr>
</tbody>
</table>

N = 36 matched subject pairs

Arion et al., Molecular Psychiatry 2015
In vivo findings support lower DLPFC network activity during working memory in subjects with schizophrenia

- “Although altered patterns of activation are occasionally observed in samples of patients with schizophrenia, meta-analyses of working memory in schizophrenia have converged on hypoactivation of the dorsolateral prefrontal cortex as the most common finding.”

Can a “causal” deficit in dendritic spines lead to the “consequence” of psychosis in schizophrenia?

- Cognitive deficits, including those that depend on DLPFC circuitry, emerge before the onset of psychosis (Reichenberg et al., *Am J Psychiatry* 167:160, 2010).

- DLPFC activation during cognitive tasks is inversely related to measures of striatal dopaminergic function in schizophrenia (Meyer-Lindenberg et al., *Nat Neurosci* 5:267, 2002).

- Psychosis is associated with excessive dopamine release in the associative striatum (Howes et al., *Arch Gen Psychiatry* 69:776, 2012).

- In mice, deletion of the actin-related protein-2/3 (ARP2/3) complex produces cortical spine deficits, elevated striatal dopamine neurotransmission and antipsychotic-responsive hyperlocomotion (Kim et al., *Nat Neurosci* 18:883, 2015).

- Is the ARP2/3 complex signaling pathway altered in DLPFC layer 3 pyramidal neurons in schizophrenia?
A Neural Circuitry Basis for the Core Clinical Features of Schizophrenia

David Lewis, MD

Sunday, September 17, 2017

ARP2/3 Complex Signaling Pathway

Healthy

Datta et al., Am J Psychiatry 2016

Lower Expression in Schizophrenia of ARP2/3 Complex Components in Layer 3 Pyramidal Cells

ACTR2
-10.9%
Paired: F_{1,34}=5.8, p=0.021
Unpaired: F_{1,70}=11.4, p=0.001

ACTR3
-15.2%
Paired: F_{1,34}=11.4, p=0.002
Unpaired: F_{1,70}=16.9, p<0.001

ARPC3
-15.9%
Paired: F_{1,35}=12.9, p=0.001
Unpaired: F_{1,70}=9.3, p=0.003

Expression of 6 of 7 ARP2/3 subunits is lower.

Datta et al., Am J Psychiatry 2016
Lower Expression in Schizophrenia of Nucleation Promotion Factors Regulating the ARP2/3 Complex

<table>
<thead>
<tr>
<th>Gene</th>
<th>Log2 Microarray Signal in Healthy Comparison Subject</th>
<th>Log2 Microarray Signal in Schizophrenia Subject</th>
<th>Paired</th>
<th>Unpaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTTN</td>
<td>-16.4%</td>
<td>Paired: $F_{1,35}=4.1$, $p=0.052$</td>
<td>Unpaired: $F_{1,34}=3.9$, $p=0.053$</td>
<td></td>
</tr>
<tr>
<td>WASL</td>
<td>-10.1%</td>
<td>Paired: $F_{1,35}=7.4$, $p=0.010$</td>
<td>Unpaired: $F_{1,35}=7.7$, $p=0.007$</td>
<td></td>
</tr>
<tr>
<td>CYFIP1</td>
<td>-14.3%</td>
<td>Paired: $F_{1,35}=3.1$, $p=0.088$</td>
<td>Unpaired: $F_{1,34}=4.7$, $p=0.033$</td>
<td></td>
</tr>
</tbody>
</table>

Expression of 3 of 4 NPF transcripts is lower.

Datta et al., *Am J Psychiatry* 2016

Deficient ARP2/3 Complex Signaling and Dendritic Spine Deficits in Layer 3 Pyramidal Cells in Schizophrenia

Expression alterations not attributable to antipsychotic medications or other comorbid factors.

Datta et al., *Am J Psychiatry* 2016
Can a “primary” deficit in dendritic spines account for psychosis in schizophrenia?

- Cognitive deficits, including those that depend on DLPFC circuitry, emerge before the onset of psychosis (Reichenberg et al., *Am J Psychiatry* 167:160, 2010).

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- In mice, deletion of the actin-related protein-2/3 (ARP2/3) complex produces cortical spine deficits, elevated striatal dopamine neurotransmission and antipsychotic-responsive hyperlocomotion (Kim et al., *Nat Neurosci* 18:883, 2015).

- The ARP2/3 complex signaling pathway is downregulated in DLPFC layer 3 pyramidal neurons in schizophrenia (Datta et al., *Am J Psychiatry*, 2016).

- Interpretation: Spine deficits in the DLPFC (and resulting cognitive dysfunction) are upstream of subcortical hyperdopaminergia (and resulting psychosis) in schizophrenia.

When during development do the cortical spine deficits arise in schizophrenia?
Developmental Hypotheses of Lower Excitatory Synapse and Dendritic Spine Densities in Schizophrenia

Number of Cortical Excitatory Synapses and Spines

- Normal Development
- Late: excessive spine pruning
- Early: deficit in spinogenesis
- Possible Paths to Psychosis
- Psychosis Threshold

Adapted from McGlashan and Hoffman (2000)

Evidence of Cognitive Deficits in Schizophrenia Prior to the Onset of Spine Pruning

- Healthy (N=517)
- Schizophrenia (N=31)
- Persistent depression (N=185)
- Mild cognitive impairment (N=120)

Scaled Score on Digit Symbol Coding Subtest
Scaled Score on Similarities Subtest

Age at Assessment (Years)

Meier et. al., Am J Psychiatry, 2014

(Taps processing speed, attention and working memory)
(Taps verbal concept formation and reasoning)
Is the timing of developmental shifts in expression of molecular regulators of spines consistent with the idea that spine deficits in DLFPC layer 3 pyramidal cells arise prior to the onset of psychosis?

Most Molecular Regulators of Spine Density Altered in Schizophrenia Exhibit Early Postnatal Shifts in Expression

Dienel et al., *Neurobiology of Disease*, 2017
Accounting for Layer 3 Pyramidal-Parvalbumin Cell Circuit Dysfunction in Schizophrenia

- In DLPFC layer 3, the “cause” is a deficit in the number of pyramidal neuron dendritic spines resulting in lower excitatory drive to layer 3 pyramidal neurons.
- As a consequence, net neural activity is reduced in DLPFC layer 3 circuitry.
- Prediction: Homeostatic synaptic plasticity mechanisms produce multiple, pre- and post-synaptic “compensations” in PV basket cell inhibition of layer 3 pyramidal neurons, all of which reduce feedback inhibition.

Are changes at PV basket cell inputs consistent with a compensatory downregulation of feedback inhibition of layer 3 pyramidal neurons?
1. Less GABA and lower presynaptic strength
   (Curley et al., *Am J Psychiatry*, 2012)

2. Fewer receptors and lower postsynaptic strength
   (Glausier and Lewis, *Neuropsychopharm*, 2012)

3. Less hyperpolarization
   (Arion and Lewis, *Arch Gen Psychiatry*, 2011)

4. Greater suppression of GABA release
   (Volk et al., *Cerebral Cortex*, 2012)

Do these findings represent four different "causal" pathologies, each of which leads to lower PV basket cell inhibition of pyramidal cells and reduced gamma band power in schizophrenia?

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**Prefrontal Gamma Band Power**

Feedback Inhibition

Recurrent Excitation

**Healthy: Normal E/I Balance**

**Schizophrenia: "Re-Set" E/I Balance**

Lewis et al., *TINS* 2012
How are PV basket neurons regulated to reduce feedback inhibition of layer 3 pyramidal neurons?

Potential Mechanisms for Down-regulating Activity of PV Neurons: NARP

- Neuronal activity-regulated pentraxin 2 (NARP) is expressed by pyramidal cells in response to neuronal activity.
- NARP is secreted from presynaptic axon terminals at glutamatergic synapses onto PV neurons.
- NARP clusters GluR4-containing AMPARs that generate the fast EPSCs in PV neurons required for gamma oscillations.
### Potential Mechanisms for Down-regulating Activity of PV Neurons: NARP

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- NARP is secreted at presynaptic axon terminals in glutamatergic synapses onto PV neurons.

- NARP clusters GluR4-containing AMPARs that generate the fast EPSCs in PV neurons required for gamma oscillations.

- **Prediction:**
  - Lower activity in layer 3 pyramidal neurons leads to less NARP expression.
  - Less NARP expression leads to weaker excitatory inputs to PV neurons resulting in a proportional activity-dependent down-regulation of GAD67 expression.

### Lower levels of NARP mRNA predict lower levels of GAD67 mRNA in subjects with schizophrenia.

![Graph showing normalized NARP mRNA level in schizophrenia subject vs. comparison subject](image)

![Graph showing normalized GAD67 mRNA level](image)

- **Pair analysis:**
  - Normalized NARP mRNA level: $F_{1,55} = 25.8, p < 0.001$
  - Normalized GAD67 mRNA level: $F_{1,114} = 21.0, p < 0.001$

- **Scatter plots:**
  - NARP mRNA level: $r = 0.55, p < 0.001$
  - GAD67 mRNA level: $r = -0.05, p = 0.70$

*Kimoto et al., JAMA Psychiatry 2015*
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The Disease Process in Schizophrenia: Current Model

- **Pathogenesis**
  - (Cell Type-Specific Alterations in CDC42 Signaling)

- **Pathophysicsology**
  - (Lower Gamma Oscillations)

- **Etiology**
  - (Genetic Liabilities in Actin Regulation)

- **Pathological Entity**
  - (Fewer Dendritic Spines on Layer 3 Pyramidal Cells)

- **Clinical Syndrome**
  - (Cognitive Impairments and Subsequent Psychosis)

- **Prevention**
- **Treatment**
Acknowledgments

- Dominique Arion, Holly Bazmi, Ray Cho, John Corradi, Dibs Datta, Sam Dienel, John Enwright, Lisa Glanz, Jill Glausier, Masa Ide, George Tseng, Allan Sampson, David Volk

- Support from NIMH, Bristol-Meyer Squibb, Pfizer, UPMC

- The many family members who generously gave consent for brain tissue donation from their deceased loved ones and who patiently participated in our diagnostic interviews.