

# Current Hypotheses on the Etiology of Schizophrenia

Molecules, Neural Networks and Behavior

Roberto Fernández Galán, PhD  
Assistant Professor of Neurosciences  
Mt. Sinai Health Care Foundation Scholar  
Alfred P. Sloan Research Fellow

[rfgalan@case.edu](mailto:rfgalan@case.edu)

Phone: 216 368 0811

Room: Robbins, E-725

# Background Literature and Slides

- The *Schizophrenia Research Forum* online:

<http://www.schizophreniaforum.org>

- Lecture slides available at:

<http://www.case.edu/med/galanlab/teaching.html>

# Outline

- First Lecture
  - Introduction
    - What we know about Schizophrenia
  - Hypotheses on the etiology of the disease
    - Dopamine hypothesis
    - Glutamate (or NMDAR) hypothesis
    - GABA hypothesis
    - Genetic hypothesis
    - Epigenetic hypothesis
    - Synthesis of most of the above: A systems level perspective
- Second Lecture
  - Discussion of research papers

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# Basic Facts

- Schizophrenia has a heterogeneous presentation, with disorganized, **positive** (delusion, hallucinations), and **negative symptoms** (avolition, apathy, blunted affect, cognitive deficits) having different levels of prominence across time and across individuals.
- Schizophrenia is relatively common, affecting approximately 0.7% of the world's population.
- Prevalence is greater in men throughout most of adulthood, but is equal by the end of the risk period.

# Basic Facts

- Schizophrenia has a peak of **onset in young adulthood** and is rare before adolescence. Onset also interacts with sex, such that men are likely to become ill earlier in life than women.
- Liability to schizophrenia is **highly heritable** (~80%), and concordance between identical twins is almost 50%, suggesting a role for **environmental or stochastic influences as well**.
- All drugs with established anti-psychotic effects decrease dopamine neurotransmission, but anti-psychotic drugs are not effective for all schizophrenia symptoms.

# Etiological Facts

- Linkage studies (which identify regions of the genome where schizophrenia genes might be found) suggests a number of regions that show genome-wide significance (1q, 6p, 6q, 13q, 17p), with several other regions found across multiple studies.
- Association studies (which identify which form of a particular gene or part of a gene is associated with risk) suggest alleles in a number of promising genes confer risk, including RGS4, **DISC1**, DTNBP1, NRG1, DAOA and COMT.
- A number of early neurological insults and later life stressors confer additional risk for schizophrenia and other neurological disorders. Obstetrical complications and migrant status illustrate two of the largest effects known at this time.

# Pharmacological Facts

- While antipsychotics lead to some immediate improvement for many individuals, the time course varies widely and a minority of patients do not show a medication response until a month after beginning treatment.
- **Chronic exposure** to amphetamine, a dopamine agonist, can result in schizophrenia-like symptoms in some individuals.
- A **single exposure** to phencycline (PCP) and other NMDA receptor antagonists (such as ketamine) can result in schizophrenia-like symptoms in some individuals.

# Pathological Facts

- In post-mortem studies, pyramidal neurons in input layers of prefrontal cortex have a **reduced dendritic spine density**; whereas hippocampal neurons appear to be abnormally oriented with signs of arrested migration.
- Even in first-episode patients, the lateral and third ventricles are somewhat larger, whereas total **brain volume is slightly smaller**.
- Medial temporal lobe structures such as the hippocampus, and superior, temporal, and prefrontal cortices, as well as the thalamus **tend to be smaller** in patients with schizophrenia.

# Pathological Facts

- Overall grey matter and hippocampal volumes are also slightly smaller in healthy relatives of patients with schizophrenia.
- Functional abnormalities occur in a number of brain systems, including prefrontal and temporal cortices and subcortical structures.

# Behavioral Facts

- Cognitive tests are challenging for many, but not all, patients. The greatest deficits appear on tasks such as verbal memory, performance IQ, and short-term memory tasks.
- Longer duration of untreated psychosis is associated with a poorer treatment response.
- Patients have a 4.9% rate of suicide, which is far greater than the average risk in the United States.

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# Dopamine hypothesis

- The dopamine hypothesis is the oldest of the schizophrenia hypotheses. Although **clearly not sufficient** to explain the complexity of this disorder, it offers a direct relationship to some symptoms and their treatment.
- The first formulation of the dopamine hypothesis of schizophrenia proposed that hyperactivity of dopaminergic transmission was responsible for the disorder (Rossum, 1966). This was based on the early observations that **dopamine receptors are activated by psychostimulants**. Indeed, most antipsychotics bind to D<sub>2</sub> receptors.

# Dopamine hypothesis

- Given the predominant localization of dopaminergic terminals and D<sub>2</sub> receptors in subcortical regions such as the striatum and the nucleus accumbens, the classical dopamine hypothesis of schizophrenia originally focused on **subcortical regions**.
- More recently, functional imaging studies that these symptoms might arise from **altered prefrontal cortex function**.

# Dopamine hypothesis

- The dopamine hypothesis provides an explanation for the cause of positive symptoms.
- However, it does NOT provide a satisfactory explanation for the negative symptoms, for which the antipsychotics are not clinically effective.

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# Glutamate hypothesis

- The glutamate hypothesis of schizophrenia posits that the function of the N-methyl-D-aspartate (NMDA) receptor is compromised in this disease.
- NMDA receptors are a major subtype of glutamate receptors and mediate slow excitatory postsynaptic potentials (EPSPs). These slow EPSPs are considered critical for the proper expression of complex behaviors, such as associative learning, working memory, behavioral flexibility, and attention, many of which are impaired in schizophrenia.
- NMDA receptors also play an essential role in the development of neural pathways, including pruning of cortical connections during adolescence, making them a critical component of developmental processes whose malfunction may lead to schizophrenia.

# Glutamate hypothesis

- The idea of a glutamatergic abnormality in schizophrenia was first proposed by Kim, Kornhauber, and colleagues in 1980 based on their findings of low cerebrospinal fluid glutamate levels in patients with schizophrenia.
- This theory was not well received because: 1) these findings could not be replicated in subsequent studies and, 2) our limited knowledge of the glutamate system at the time suggested that disruptions in glutamate neurotransmission would result in overt toxicity and gross developmental abnormalities, something not seen in schizophrenia.
- In the last two decades, however, **basic and clinical evidence has been accumulating** to support the idea that aberrant NMDA receptor function subserves many aspects of molecular, cellular, and behavioral abnormalities associated with schizophrenia.

# Glutamate hypothesis

- Recreational use of a single low dose of an NMDA receptor antagonist such as phencyclidine (PCP) , ketamine or MK-801 produces "schizophrenia-like" symptoms **in healthy individuals** and profoundly exacerbates preexisting symptoms in patients with schizophrenia.
- The range of symptoms produced by these agents resembles **positive** (delusion and hallucination), **negative** (avolition, apathy, and blunted affect), and **cognitive** (deficits in attention, memory, and abstract reasoning) symptoms of schizophrenia, as well as disruptions in smooth-pursuit eye movements and prepulse inhibition of startle.
- Direct comparison of healthy volunteers receiving subanesthetic doses of ketamine and individuals with schizophrenia shows similar disruptions in working memory and thought disorder between the two groups, **suggesting that deficient activation of NMDA receptors may be a critical component of the cognitive deficits observed in schizophrenia.**

# Glutamate hypothesis

- Postmortem studies show changes in glutamate receptor binding, transcription, and subunit protein expression in the prefrontal cortex, thalamus, and hippocampus of subjects with schizophrenia.
- Recent imaging studies using a novel tracer for the NMDA receptor have reported **reduced NMDA receptor binding** in the hippocampus of medication-free patients. While this study remains to be replicated in a larger group of patients, it represents the first direct demonstration of NMDA receptor deficiency in schizophrenia.
- Glutamate neurons regulate the function of other neurons that have been strongly implicated in the pathophysiology of schizophrenia. These include GABA interneurons, whose morphology has been altered in schizophrenia, and dopamine neurons, which are the target of antipsychotic drugs.

# Glutamate hypothesis

- Two key pharmacological clues to the pathophysiology of schizophrenia—clinical efficacy of D2 receptor antagonist and increased probability of developing schizophrenia after cannabis use during adolescence—are consistent with deficient NMDA receptor function in schizophrenia.
- Cannabinoid CB1 receptor and D2 receptors are localized presynaptically on glutamate terminals and work to inhibit the release of glutamate.
- Cannabis, therefore, reduces glutamate release, in particular in corticostriatal regions, leading to **deficient activation of NMDA receptors**, whereas **reduced D2 receptor function** produces modest increases in glutamate release.

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# GABA hypothesis

- Tissue concentrations of the mRNA that encodes the 67 kiloDalton isoform of glutamic acid decarboxylase (GAD67), an enzyme that synthesizes GABA, are reduced in the DLPFC of individuals with schizophrenia.
- By contrast, mRNA and protein levels of GAD65, another enzyme that synthesizes GABA, are not altered in the DLPFC of people with schizophrenia, and the density of GAD65-immunoreactive axon terminals also remains unchanged.
- Interestingly, elimination of the *Gad65* gene in mice does not alter cortical levels of GABA, whereas reductions in GAD67 mRNA are associated with marked decreases in cortical GAD activity and GABA content.

# GABA hypothesis

- In individuals with schizophrenia, most GABA neurons in layer 3-5 (but not 6) of the prefrontal cortex express normal levels of GAD67mRNA. However, ~25–30% of GABA neurons do not express this transcript at a detectable level.
- In the same cohort of individuals, expression of the GABA membrane transporter 1 (GAT1), a protein responsible for reuptake of released GABA into nerve terminals, was also decreased with almost identical cellular patterns.
- Therefore, in schizophrenia, both the synthesis and reuptake of GABA seem to be greatly reduced in a subset of inhibitory neurons, at least in the prefrontal cortex.

# GABA hypothesis

- In the prefrontal cortex of individuals with schizophrenia, the density of pyramidal-neuron axon initial segments that are immunoreactive for the GABA $\alpha$ 2 subunit is increased by >100% compared with control subjects.
- This seems to reflect higher levels of  $\alpha$ 2 subunits at the axon initial segment rather than an increase in the density of pyramidal neurons or of their axon initial segments.
- Since the  $\alpha$ 2 subunit endows the GABAergic synapses with slower kinetics, it seems that **the postsynaptic neuron is trying to compensate for the lack of released neurotransmitter**, by increasing the time window over which chloride ions flow through the synapse.

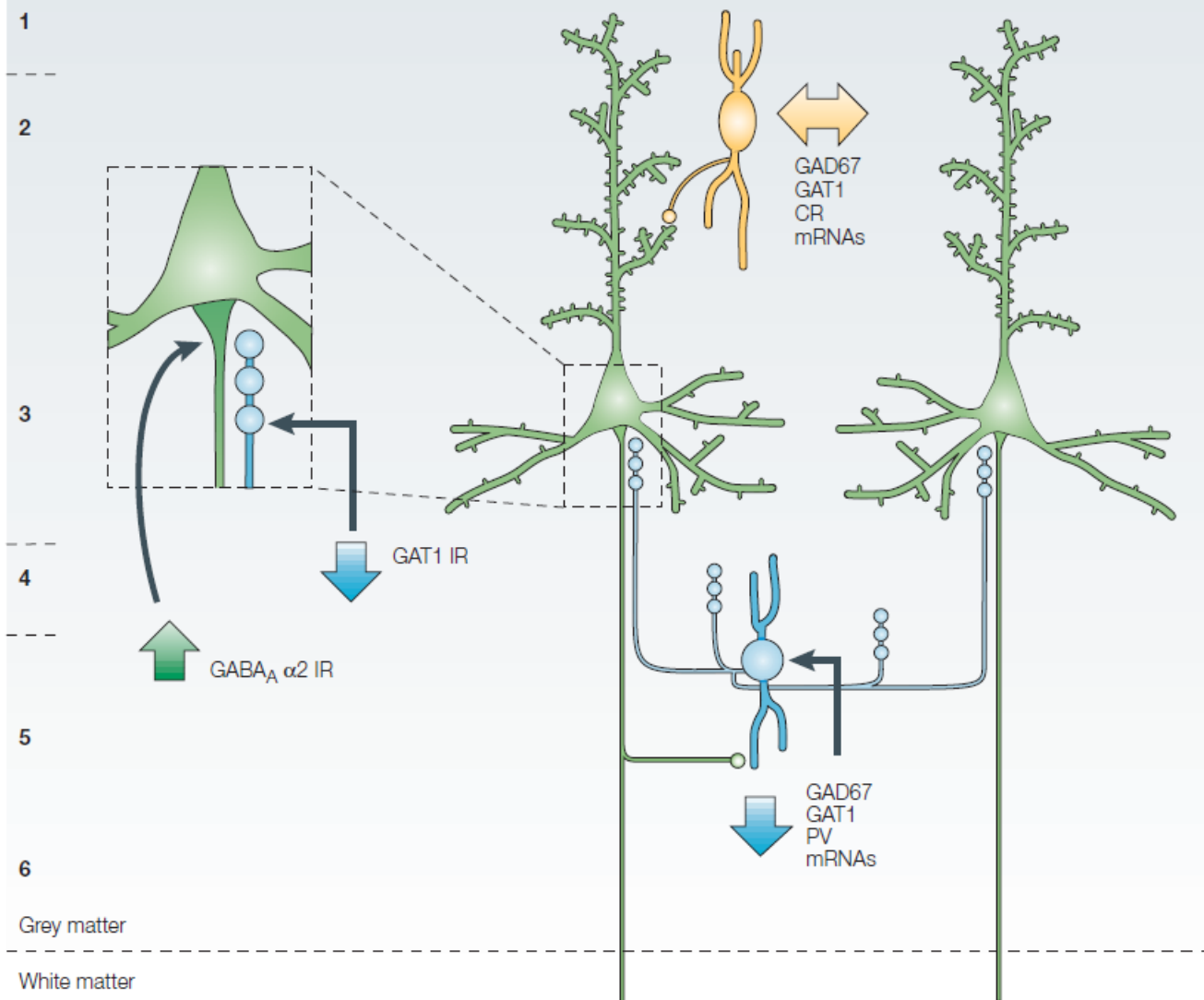


Figure 6 | **Schematic summary of alterations in GABA circuitry in the dorsolateral prefrontal cortex of individuals with schizophrenia.** Reduced levels of gene expression in chandelier neurons (blue) are associated with a decrease in immunoreactivity (IR) for GABA (γ-aminobutyric acid) transporter 1 (GAT1) in the axon cartridges of these neurons and an upregulation of GABA<sub>A</sub> (GABA type A) receptor α2 subunit immunoreactivity in the postsynaptic axon initial segment of pyramidal neurons (green). Gene expression in calretinin (CR)-expressing subpopulations of GABA neurons does not seem to be altered (yellow). GAD67, 67 kD isoform of glutamic acid decarboxylase; PV, parvalbumin; 1–6, layers of dorsolateral prefrontal cortex.

Lewis et al. (2005),  
 Nat. Rev. Neurosci.  
 6: 312-324.

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# Genetic hypothesis

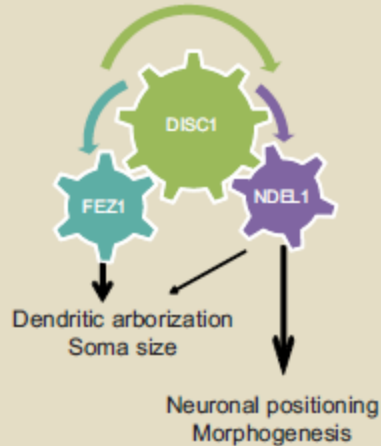
- The gene *DISC1* (Disrupted In Schizophrenia - 1) was identified in the late 60's in a large Scottish family that segregates with schizophrenia and other mental disorders.
- *DISC1* is a scaffold protein that has a multifaceted impact on neuronal development.
- Exogenous manipulation of *DISC1* in mice results in a spectrum of neuronal abnormalities, depending on the timing and locus of the perturbation.

# Genetic hypothesis

- During embryonic cortical development, knockdown of DISC1 at E13 accelerated cell cycle exit and neuronal differentiation whereas at E14.5 leads to inhibition of neuronal migration and disorganized dendritic arbors.
- During adult neurogenesis, suppression of DISC1 also leads to decrease proliferation of neural progenitors and an array of neuro-developmental defects in newborn dentate granule cells, including hypertrophy, misplacement, impaired axonal targeting, and accelerated dendritic growth and synaptogenesis.

# Genetic hypothesis

**Table 1. A Model of Interaction between DISC1 with FEZ1 or NDEL1 in Regulating Distinct Aspects of Newborn Neuron Development in the Adult Mouse Hippocampus**



shRNA	Neuronal positioning	Soma hypertrophy	Ectopic dendrites	Enhanced dendritic outgrowth
Control	0	0	0	0
DISC1#1	+++	+++	+++	+++
FEZ1	0	+++	0	++
NDEL1	++	+	++	+
DISC1#3	0	0	0	0
FEZ1+DISC1#3	0	+++	0	++++
FEZ1+NDEL1	++	+++	++	+++
DISC1#3+NDEL1	++	+++	+++	+++

Shown on the left is a schematic diagram of the model on signaling mechanisms of DISC1 in regulating new neuron development during adult hippocampal neurogenesis. Shown on the right is a summary table of effects of different genetic manipulations that lead to defects in neuronal positioning, soma hypertrophy, ectopic dendrites, and enhanced dendritic outgrowth of newborn neurons in the adult dentate gyrus at 14 dpi ("0" represents normal and the severity of phenotypes is represented by the numbers of "+").

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# Epigenetic hypothesis

- Recent thinking suggests that the origins of schizophrenia may not lie strictly in DNA sequence variations. Instead, these may be coupled with epigenetic dysfunctions as the key etiopathogenic factors.
- The proposed mechanism, which is a modification of the GABAergic hypothesis, states that aberrant gene expression of GAD1, GAD67, GAT1, NMDA receptors NR2, and others observed in GABAergic neurons of schizophrenic patients is a consequence of promoter hypermethylation mediated by the overexpression of DNA methyltransferase I.

# Epigenetic hypothesis

- Methylation and demethylation may act as neuronal gene expression switches.
- Some researchers argue that methylation patterns may be reversible and also could be intimately linked to histone deacetylation.
- In fact, the histone deacetylase inhibitor valproic acid, which is commonly prescribed to psychotic patients as a mood stabilizer, is thought to act in part by increasing DNA demethylation.

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# A systems-level synthesis attempt

- In modern accounts of schizophrenia, researchers have proposed that the disorder arises from a **failure to integrate the activity of local and distributed neural circuits**.
- Recently there has been a considerable amount of interest in the role that gamma-band EEG oscillations might play in the cognitive abnormalities that characterize schizophrenia.
- Gamma oscillations represent the synchronization of neural firing and are thought to mediate representation and selection of information in the brain, and thus may be an important mechanism that mediates processes such as perception, selective attention, and working memory.
- Consistent with the notion that neuronal synchronization may be impaired in schizophrenia, **a growing number of studies have reported abnormal gamma oscillations associated with sensory stimulation, perception, and target-detection tasks in subjects with schizophrenia**.

# A systems-level synthesis attempt

- In animals, **NMDA receptor blockade has been found to reduce the number of parvalbumin-immunoreactive neurons in the entorhinal cortex**. This reduction is accompanied by robust disruption in gamma rhythms.
- These effects may be mediated primarily by the NR2A subunit. In a recent study, in a primary neuronal culture system, Kinney and colleagues have found that **NR2A is particularly enriched in PV-containing GABA cells at both the transcript and protein levels, when compared to pyramidal cells**.
- **NR2A, but not NR2B selective antagonists, down-regulate parvalbumin mRNA and protein expression and expression of GAD67 mRNA in parvalbumin-containing cells**. In light of these findings, it is indeed very interesting that, in schizophrenia, GAD67 mRNA expression has been found to be selectively reduced in PV-containing neurons.

# A systems-level synthesis attempt

- These findings lead to the following hypothesis:

Hypofunction of NMDA receptors on GABAergic interneurons, especially those that contain parvalbumin, disrupts the synchronization of neural circuits in the gamma frequency (40-100 Hz) by altering inhibitory control of pyramidal cell networks.

# A systems-level synthesis attempt

- The normal interaction between pyramidal cells and parvalbumin-positive interneurons is thought to be a “push-pull” mechanism that generates oscillations of neuronal activity in the cortex.
- This push-pull mechanism seems to be defective in schizophrenia.

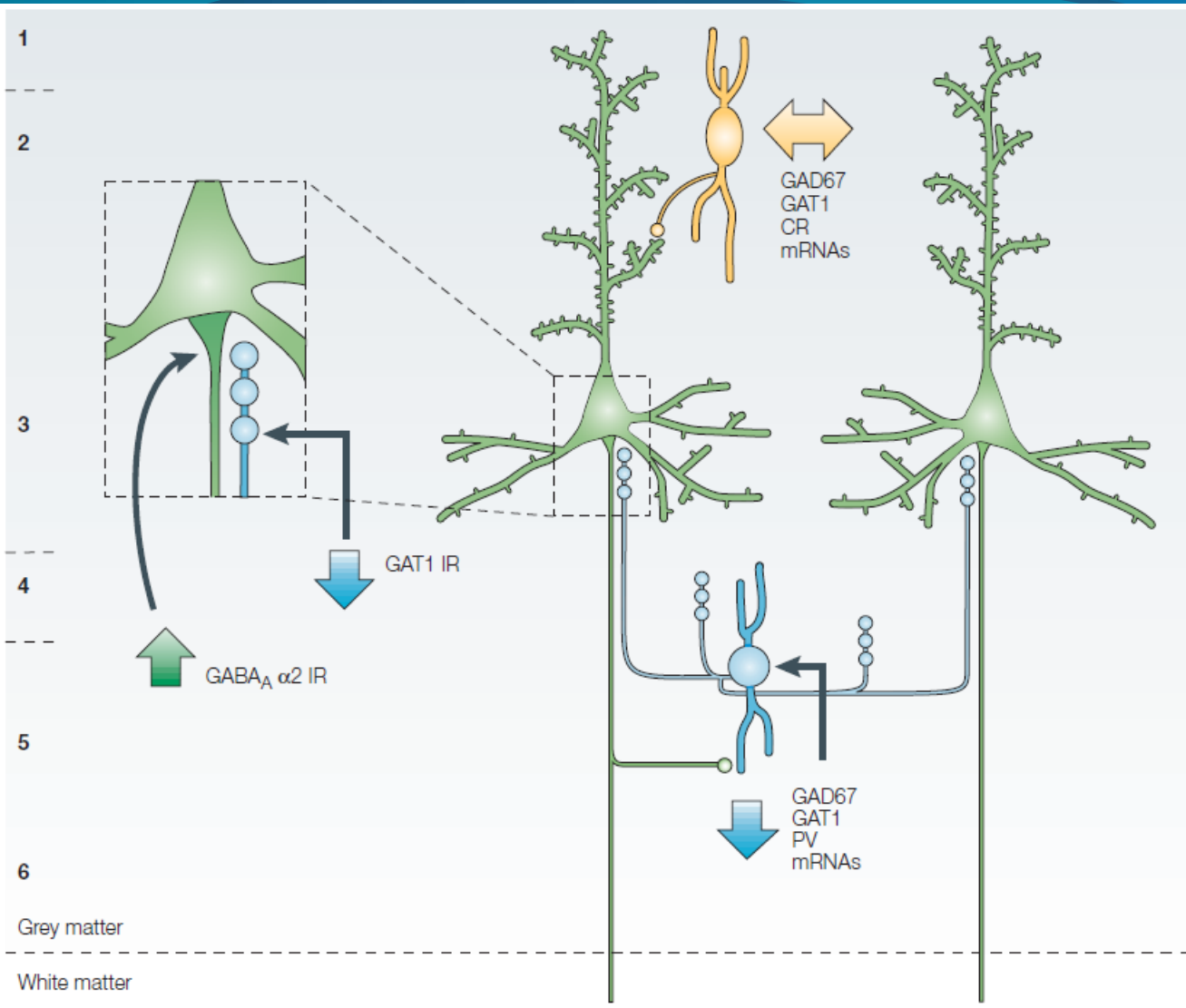
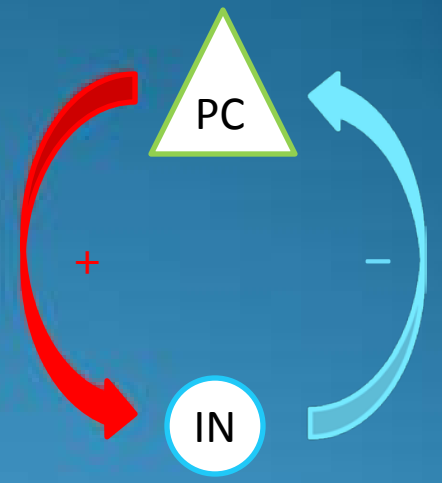


Figure 6 | **Schematic summary of alterations in GABA circuitry in the dorsolateral prefrontal cortex of individuals with schizophrenia.** Reduced levels of gene expression in chandelier neurons (blue) are associated with a decrease in immunoreactivity (IR) for GABA ( $\gamma$ -aminobutyric acid) transporter 1 (GAT1) in the axon carriages of these neurons and an upregulation of  $GABA_A$  receptor  $\alpha 2$  subunit immunoreactivity in the postsynaptic axon initial segment of pyramidal neurons (green). Gene expression in calretinin (CR)-expressing subpopulations of GABA neurons does not seem to be altered (yellow). GAD67, 67 kD isoform of glutamic acid decarboxylase; PV, parvalbumin; 1–6, layers of dorsolateral prefrontal cortex.

Neural interactions that putatively lead to gamma oscillations



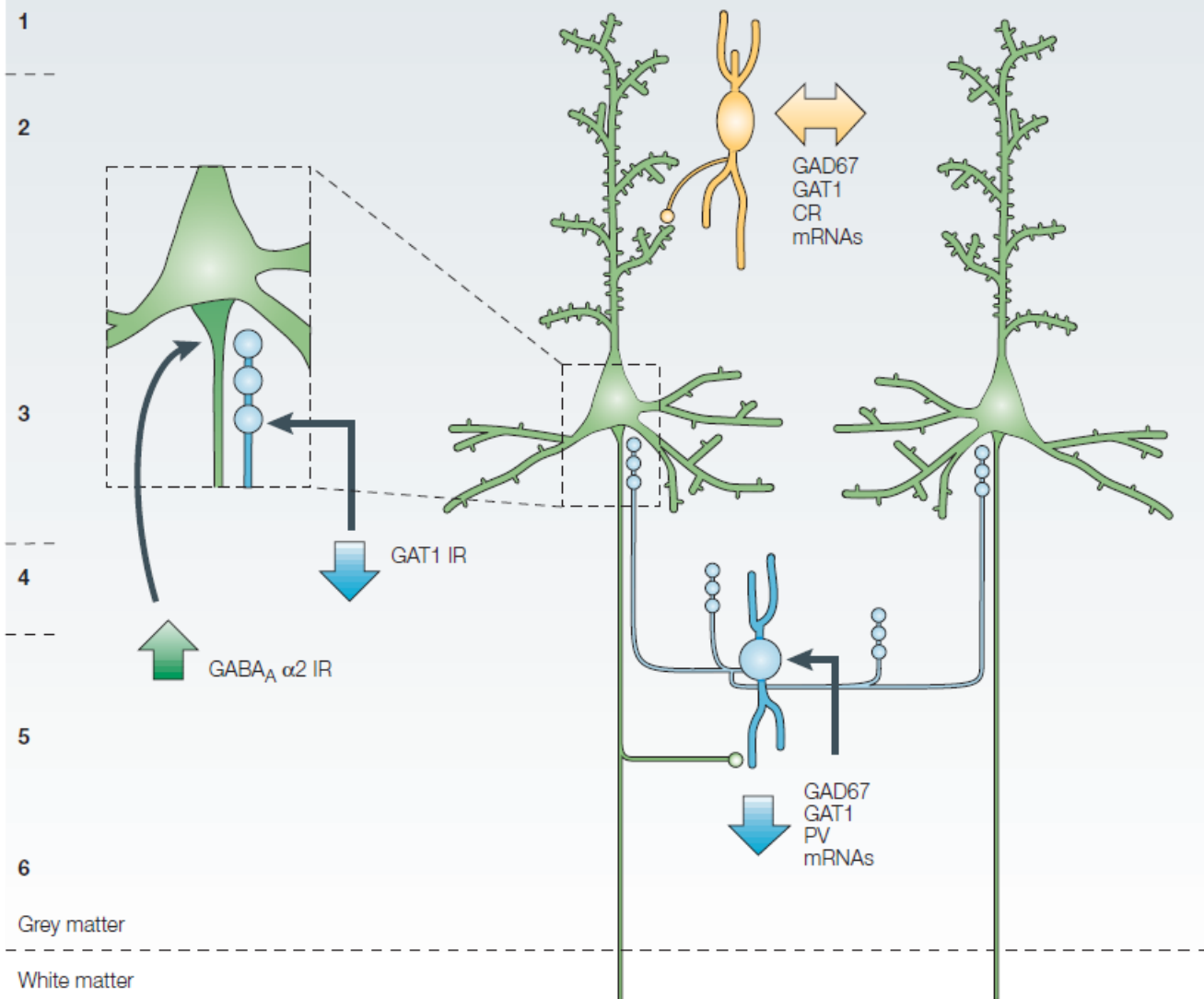
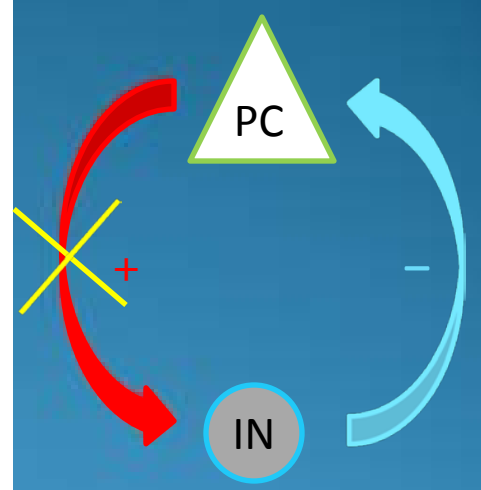
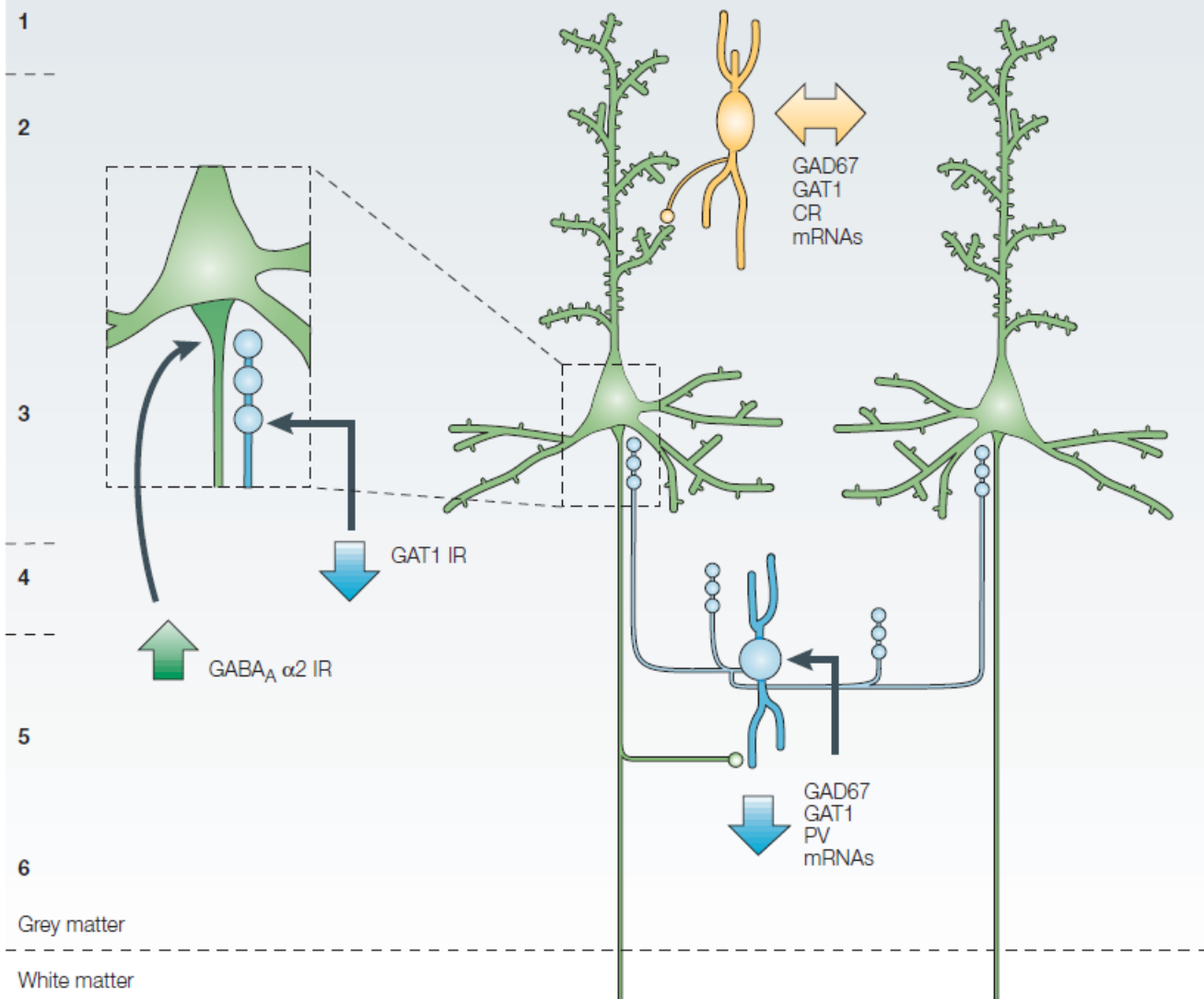


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NMDAR dysfunction alters the PC drive to the IN





...which in turn alters the feed-back from the IN onto the PC

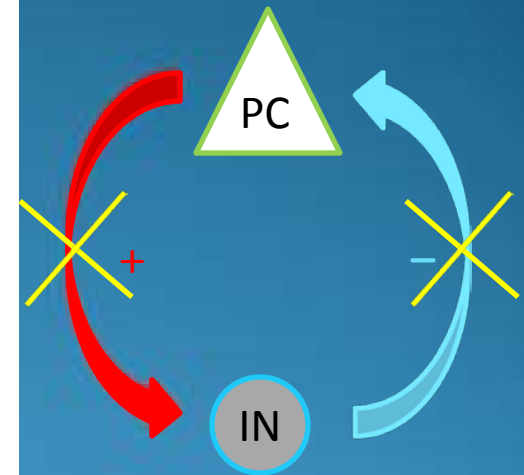


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# Papers for discussion

- M. M. Behrens et al. (2007). Ketamine-Induced Loss of Phenotype of Fast-Spiking Interneurons Is Mediated by NADPH-Oxidase. **Science**, 318: 1645-1647.
- S.B. Powell et al. (in press). Behavioral and neurochemical consequences of cortical oxidative stress on parvalbumin-interneuron maturation in rodent models of schizophrenia. **Neuropharmacology**.