Intraneuronal mechanism of C4A mediation of synapses without microglia

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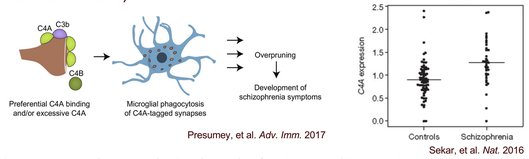
Background

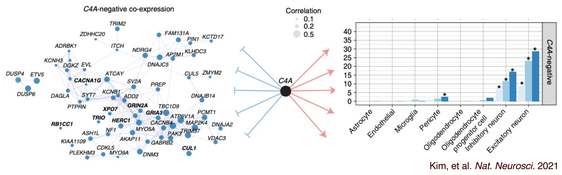
Schizophrenia (SZ) is a chronic illness with unclear pathophysiology and no disease-modifying treatments (McCutcheon, et al. *JAMA Psych.* 2020). Converging evidence points to the synapse as the primary site of pathology in SZ (Trubetskoy, et al. *Nature.* 2022). Neuroimaging and postmortem studies find a decrease in synaptic density in the brains of individuals with SZ (Osimo, et al. *Mol. Psych.* 2019; Onwordi, et al. *Nat. Comm.* 2020). Decreased synaptic density correlates with cognitive impairments (Yoon, et al. *Psych Res.* 2023). However, many details are unclear, such as whether synaptic density reductions occur in all or selectively in particular synaptic subtypes. Similarly, the mechanism that leads to lower synaptic density is unclear. An influential hypothesis explaining the synaptic deficit is the *excessive synaptic pruning hypothesis* which posits that SZ arises due to excessive elimination of synapses by microglia during development, perhaps through upregulation of complement C4A gene expression in the context of high-risk C4 copy number variation (CNV) (Yilmaz, et al. *Nat. Neurosci.* 2020; Sekar, et al. *Nature* 2016).



Presumey, et al. *Adv. Imm.* 2017

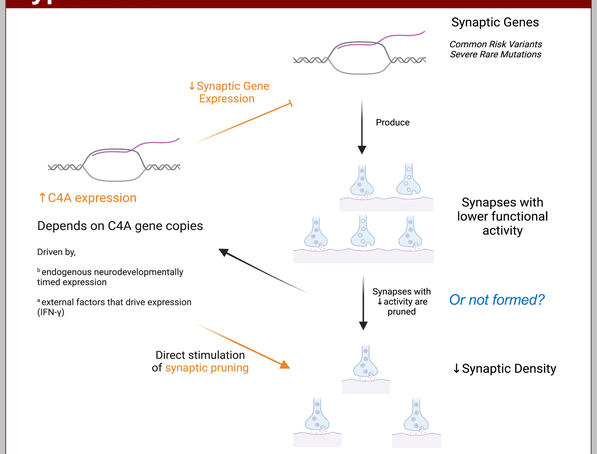
Sekar, et al. *Nat.* 2016

However, a large clinical trial of minocycline, a known microglial inhibitor, in patients with early schizophrenia was negative (Deakin, et al. *Lancet Psych.* 2018), which suggests another mechanism may be involved. Evidence from clinical postmortem studies demonstrates that a set of synaptic genes are downregulated when C4A is upregulated, particularly in excitatory cortical neurons (Kim, et al. *Nat. Neurosci.* 2021). This data suggests a transcriptional or intraneuronal mechanism could be contributing to low synaptic density in the context of high C4A gene expression.



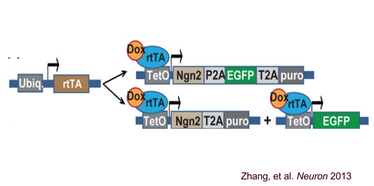
Kim, et al. *Nat. Neurosci.* 2021

Hypothesis

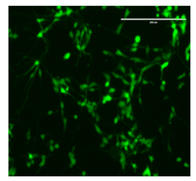


We hypothesize that increased C4A gene expression downregulates synaptic gene expression in glutaminergic neurons resulting in decreased synaptic density.

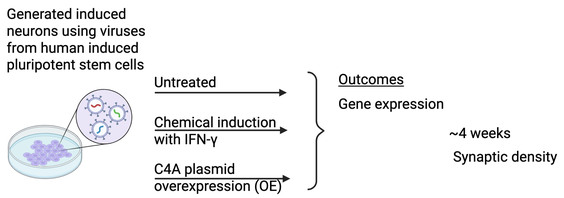
Methods: Glutaminergic model neurons from human induced pluripotent stem (iPS) cells



Zhang, et al. *Neuron* 2013

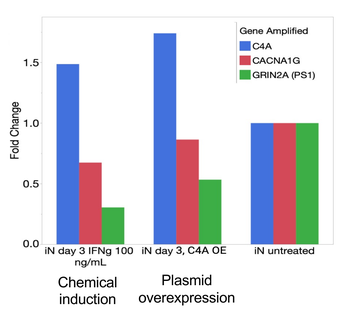


Cortical excitatory neuronal like cells

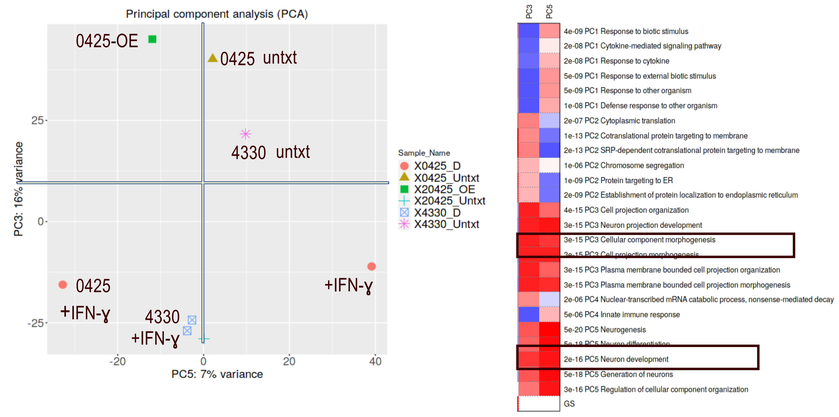


Left diagram shows the Tet-inducible promoter that expresses Ngn2 to induce human iPS cells towards glutaminergic cortical neuron-like cell (iN). **Middle** A picture of the iN cells, also expressing GFP ~4 days after viral infection. **Right** Experimental paradigm. After induction of control iPS cells into iNs, C4A gene expression is upregulated by either chemical induction (IFN-ɣ) or plasmid overexpression (origene). Outcomes include gene expression at different time points and synaptic density.

Results: Synaptic gene expression is downregulated with the upregulation of C4A gene expression

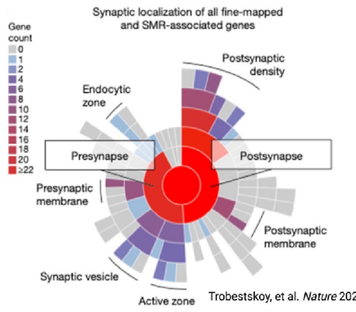


Quantitative PCR of isolated total RNA from treated iNs (normalized by GAPDH). Induction of C4A expression either by plasmid overexpression or chemical stimulation (using IFN-γ, a known C4 inducer) resulted in downregulated mRNA expression of GRIN2A and CACNA1 in induced neurons (iNs).



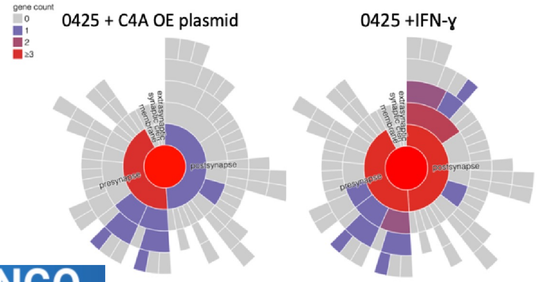
Bulk RNA-sequencing of treated iNs. Principal Component Analysis (nf-core) shows interesting separations along PCA3 (y-axis) and PCA5 (x-axis). Treated and untreated iNs separate along PCA5, where the treated cells are downregulated in genes involved in "Neurogenesis, Neuron differentiation and Neuron development." Plasmid overexpression vs. chemical induction separate along PCA3: where the treated cells are downregulated in genes involved in "Neuronal projection, and morphogenesis."

Schizophrenia GWAS hits



Trobestskoy, et al. *Nature* 2022

iPS-derived model neurons (iNs)



SYNGO DEFINING THE SYNAPSE

Plasmid overexpression

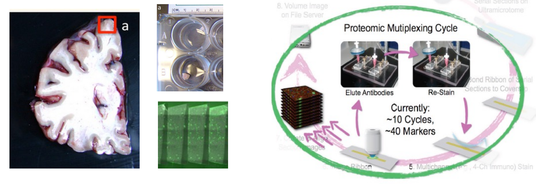
Chemical induction

Differential gene expression (DEG) analyses of RNA-seq of the untreated iNs compared to those treated with IFN-γ or transfected with C4A overexpressing plasmid overlap with GWAS-loci in Syngo (annotated database of synaptic genes, Koopman, et al. *Neuron* 2019). More specifically, C4A protein overexpression downregulates a fraction of the DEGs compared to IFN-γ, suggesting that the C4A protein (or mRNA) may be exerting an effect on specific portions of synaptic architecture.

Summary and Ongoing Work

- Increasing C4A gene expression downregulates synaptic gene expression (not just synaptic pruning)
  - Q: Due to C4A gene alone or it is part of transcriptional network? (C4A gene knockdowns)
  - Q: do these transcriptional changes translate to changes in synaptic density changes? (Synaptic density changes)
    - In model neuron (iN) system
    - Clinical postmortem tissue: determining the correlation between high C4A gene expression and measured synaptic density.

Array Tomography to study synapses

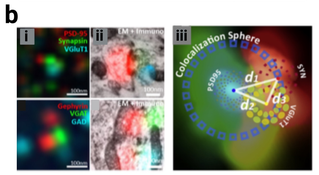


Outline of AT methodologies. Left Fixed tissue is sectioned in a planar array, stained and imaged. **Right** The AT proteomic imaging cycle involves multiple rounds of immunostaining, imaging, and antibody elution and allows collection of immunofluorescence data from up to 40 antibody channels at full resolution.

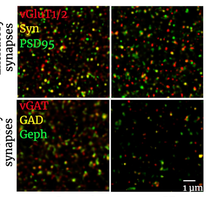
a

Clinical Brain Diagnosis	Age	Sex	Race	PMI	Manner of death
None	89	Female	White	5.67	Natural
SZ	76	Male	Asian	17.25	Natural

b



c



	Control	SZ
SD_{Ex}	0.69 syn per µm³	0.44 syn per µm³
SD_{In}	0.26 syn per µm³	0.06 syn per µm³
Ratio	2.7	7.3

Example of Array Tomography Imaging of clinical tissue from the NIH NeuroBioBank (a) Sample donor demographic information, PMI and manner of death. **(b) i-** Excitatory synapses: vesicular glutamate transporters 1 and 2 (vGlut1/2), synapsin (Syn) and postsynaptic density protein 95 (PSD95). Inhibitory synapses: vesicular GABA transporter (vGAT), glutamic acid decarboxylase (GAD1/2) and gephyrin (Geph). **ii-** Confirmation of synaptic structure of the same anatomical location using EM. **iii-** Synapses are quantified using Image Analysis Suite, an algorithm that includes deconvolution of fluorescent puncta and computational localization of defined synaptic proteins within a defined centroid. **(c)** Sample images of synaptic protein labeling in human postmortem tissue from control and SZ donor (DLPFC L3). Quantification of excitatory and inhibitory synaptic density.

Acknowledgements

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