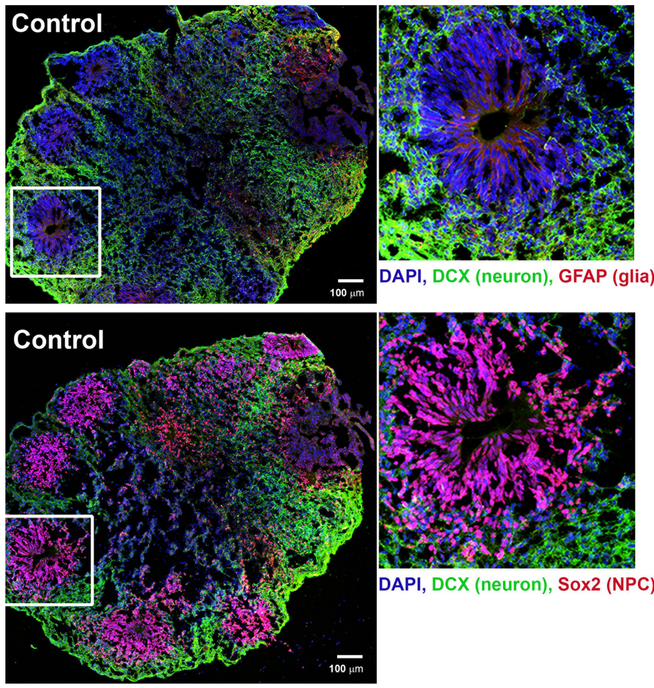
3-D Cerebral Organoids Model the Development of Autism Pathology

Pranaya Pakala, Colin Johnson, Storm Davis, Haroon Dar, Adrienne Orbita, Karen Litwa, PhD.
Brody School of Medicine at East Carolina University
Department of Anatomy and Cell Biology

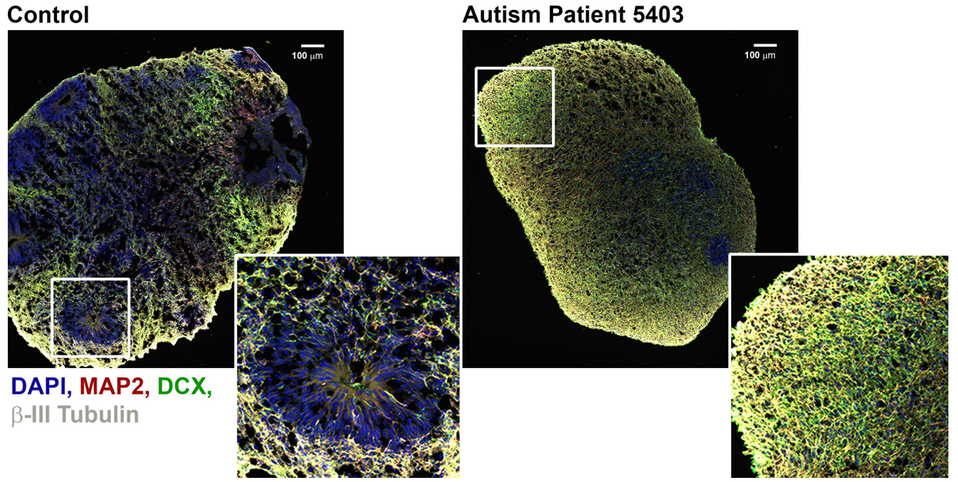
Abstract

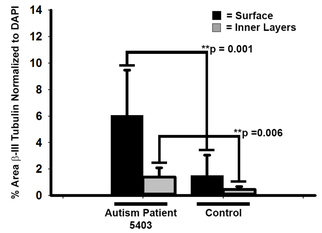
Autism is a genetically complex neurodevelopmental disorder in which patients exhibit social deficits in both verbal and non-verbal forms of communication and display restricted and repetitive behaviors. Emerging evidence suggests that altered neural connectivity, particularly at the level of synaptic connections, contributes to disease pathology. In idiopathic autism cases, post-mortem patient brain samples exhibit increased numbers of excitatory synaptic connections in cortical brain regions that govern social behavior (PMID: 21346746). However, the use of post-mortem brain samples prevents researchers from capturing the development of this altered brain circuitry. Thus, we set out to develop a physiologically relevant model of idiopathic Autism that recapitulates defective neuronal circuitry at the level of both neurite and synapse formation. We began by reprogramming Autism patient fibroblasts into human induced pluripotent stem cells (hIPSCs), which we subsequently differentiated into 3-D cerebral organoids ('mini-brains') using a low-attachment protocol (PMID: 26005811). Similar to the *in vivo* cerebral cortex, these 'mini-brains' contain diverse brain cells, including neural progenitor cells, both excitatory and inhibitory neurons, and supporting glial cells. Additionally, brain ventricles develop. However, in Autism hIPSC-derived mini-brains, we observe dramatic differences in 'mini-brain' organization. In neurotypic controls, neurons develop around brain ventricles and their neurites associate with one another to form a patterned organization within the subventricular zone. By contrast, Autism-derived 'mini-brains' have negligible ventricle formation and their neurites form a disorganized meshwork throughout the organoid. Furthermore, Autism-derived 'mini-brains' exhibit increased levels of excitatory synapse formation. Thus, we describe a model that recapitulates the development of altered brain circuitry associated with idiopathic Autism. Importantly, this model will enable us and other researchers to dissect out the molecular mechanisms contributing to Autism pathology and to test whether specific pharmacologic intervention can rescue altered neurite and synapse formation associated with Autism.

Modeling Neuronal Connectivity



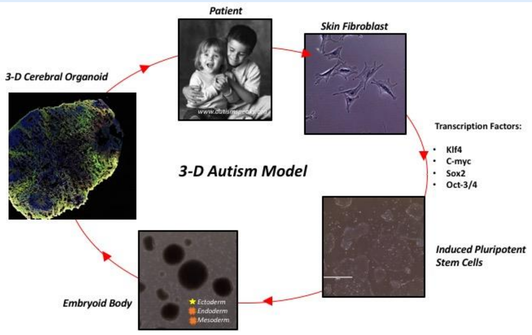
'Mini-Brains' Recapitulate Cerebral Development. Neurotypic control 'mini-brains' (~90 days old) differentiate into neurons (doublecortin, DCX, green) and supporting glial cells (GFAP, red, top) and neural progenitor cells (Sox2, red, bottom). Enlarged brain ventricles are shown to the right. The neural progenitor cells localize to proliferative regions of the ventricular zone, which are surrounded by neuronal differentiation in the subventricular zone. We are currently analyzing whether differentiation into specific brain cell types is altered in idiopathic Autism using this 'mini-brain' model.



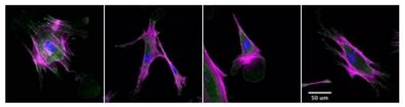


In the control brain, neurons develop around ventricular zones, where they associate with one another, as seen by increased intensity of neurites similar to neuronal tracks that form *in vivo*. Whereas the autistic model shows a disorganized meshwork of neuronal connections with no clear pattern or centralized association. Comparing % Area of the axonal marker, β-III Tubulin, between the two models (left), we see a much larger axonal area occupied by the autistic model, indicative of this lack of centralized associations or patterned neuronal connections.

Background

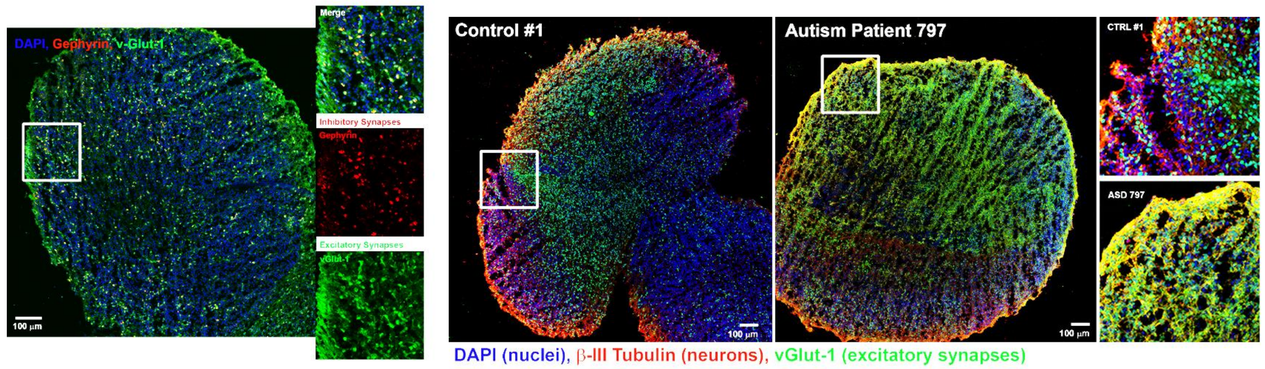


Patients of Interests



Fibroblast:	Control	797	5278	5403
Gender	Male	Male	Female	Male
Ethnicity		Caucasian	Caucasian	Caucasian
Social Interaction (10)	n/a	24	22	30
Communication & Language (Verbal/Non-Verbal: 8/7)	n/a	20/13	21/11	n/a/14
Restricted & Repetitive Behaviors (3)	n/a	6	5	5

Modeling Synaptic Connectivity



'Mini-Brains' Capture Increased Excitatory Circuits Associated with Autism. Left: Mini-Brains form both excitatory (vesicular glutamate transporter, vGlut-1, green) and inhibitory (gephyrin, red) synaptic connections. **Right:** Using approximately 90-day old min-brains, we stained for developing neural networks with the axonal marker Beta III Tubulin, and the excitatory synaptic marker, vesicular glutamate transporter 1 (vGlut-1). The autism patients (representative patient 797 shown) exhibit increased levels of excitatory synapses (green) normalized to the nuclear DAPI stain.

Conclusions and Future Directions

1. Conclusions:
   a. 3-D cerebral organoids ('mini-brains') closely approximate brain development by forming both inhibitory and excitatory neural circuits together with proliferative niches of neural progenitor cells and supporting glial cells.
   b. These 'mini-brains' also capture the development of idiopathic Autism pathology, namely disrupted brain circuitry at the level of both neurite organization and increased excitatory synapse formation.
2. Future Directions
   a. Does altered neurite formation disrupt cortical layering in Autism?
   b. Does altered actomyosin regulation underlie disorganized neurite formation and elevated excitatory synapse formation?
   c. Is cell type differentiation altered in 3-D cortical organoids? For example, are NPC and glial populations increased in Autism?