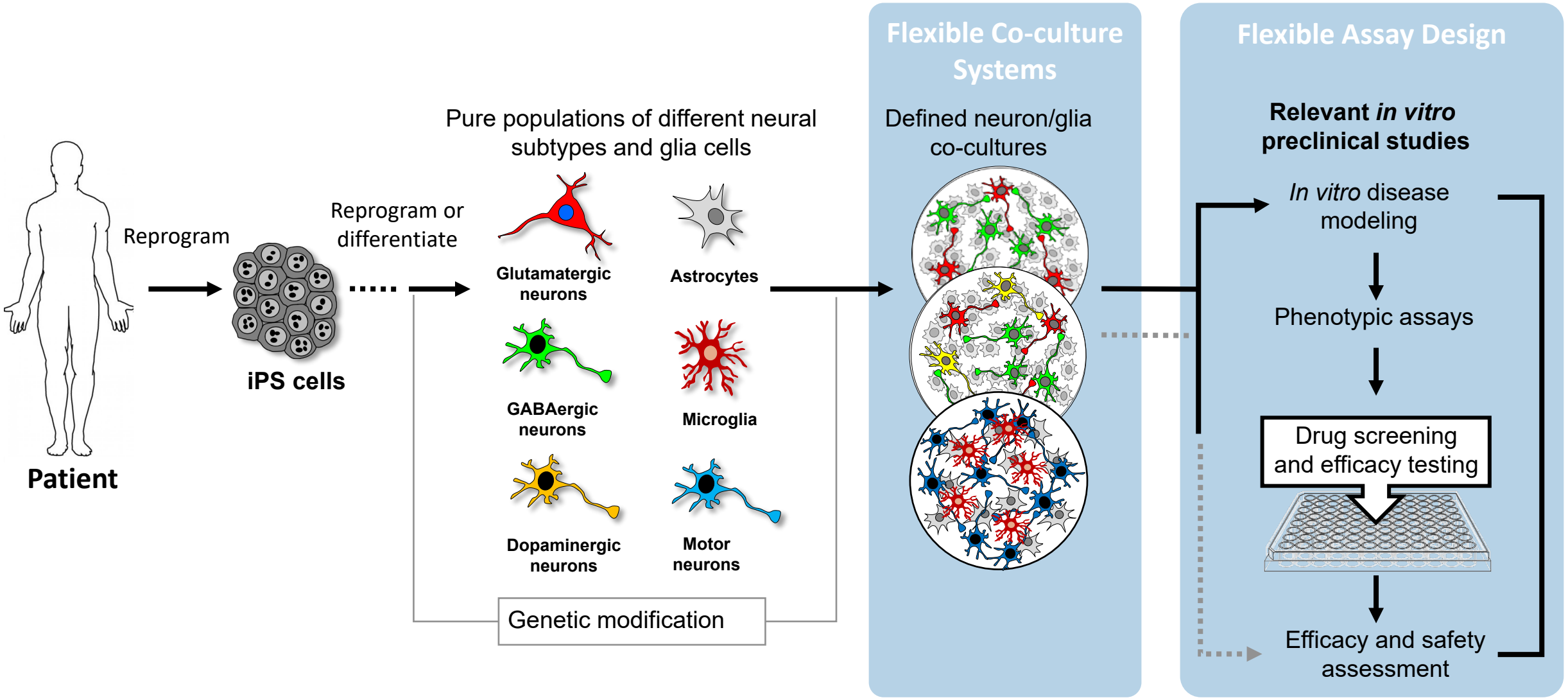
NeuCyte

Translatable Neuroscience

Technology Platform
and How We Plan to Apply It to KAND Disease Modeling

NeuCyte's Mission



➢ Advantages: **Human Biology**; short turn-around time, high reproducibility, high throughput, high flexibility, low cost

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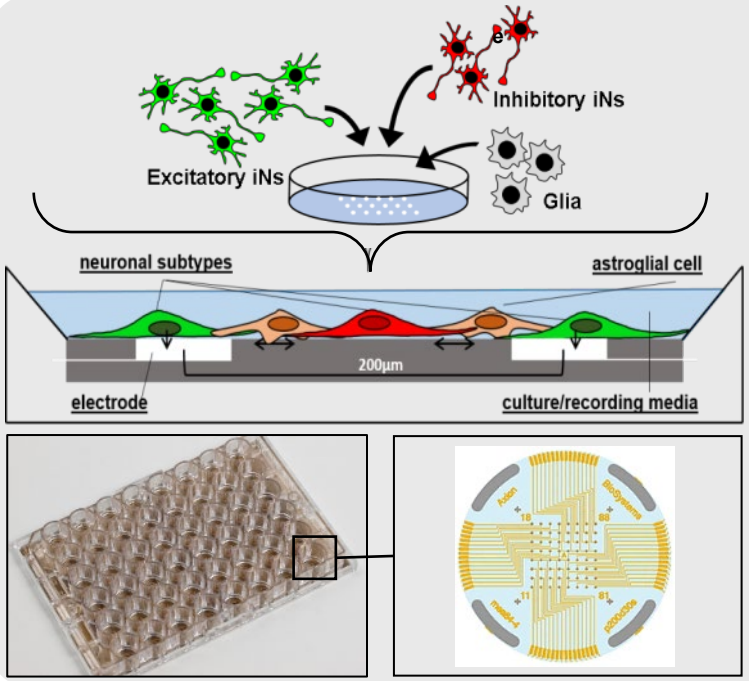
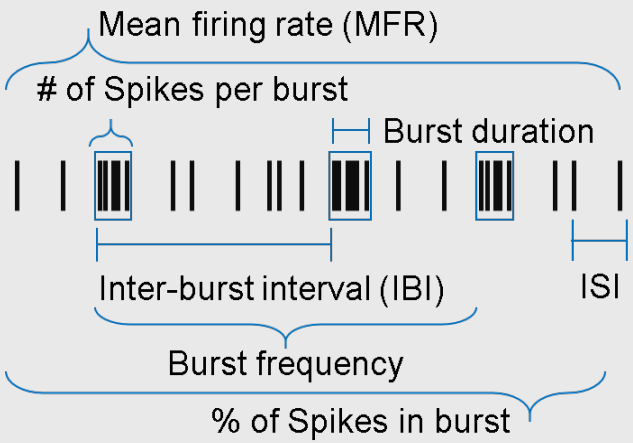
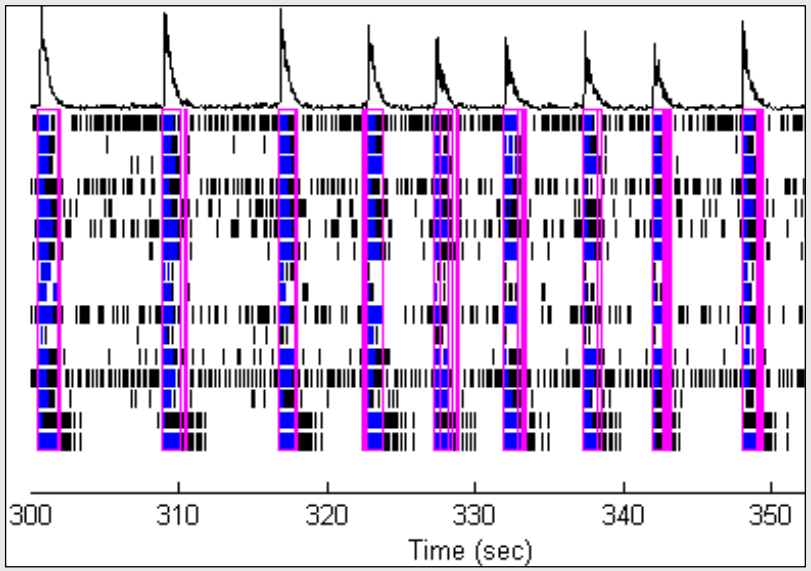
Phenotypic Screening: Medium to High Throughput Electrophysiology- Functional Readouts

Functional readouts of on multielectrode arrays (MEAs):

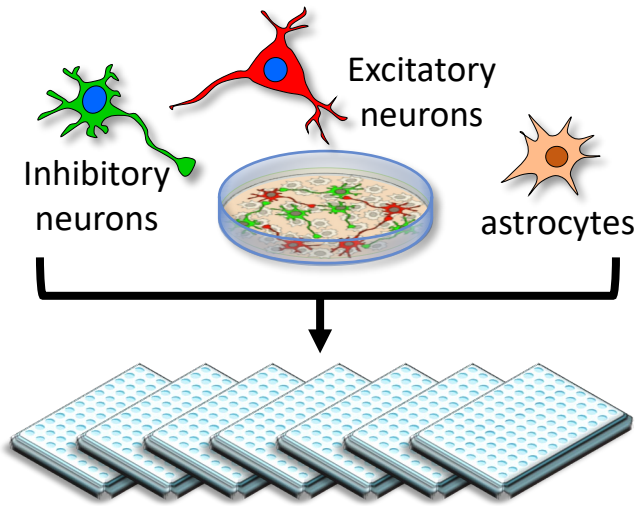
- Quantitative measurement of electrophysiological activity
- Functional integration of neuronal key features
- Flexible cell composition (enhance phenotypes)

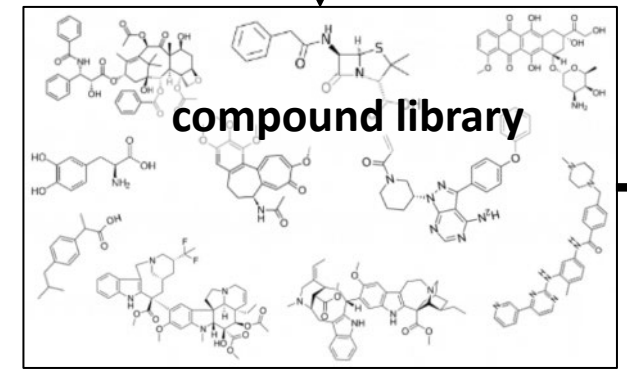




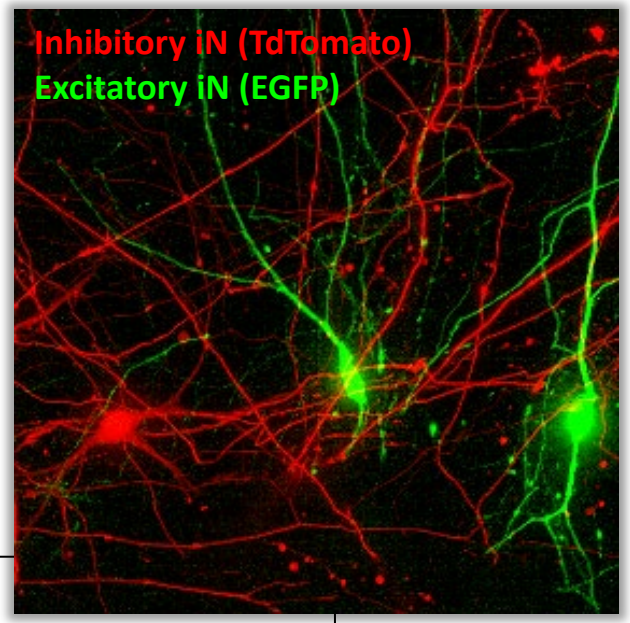


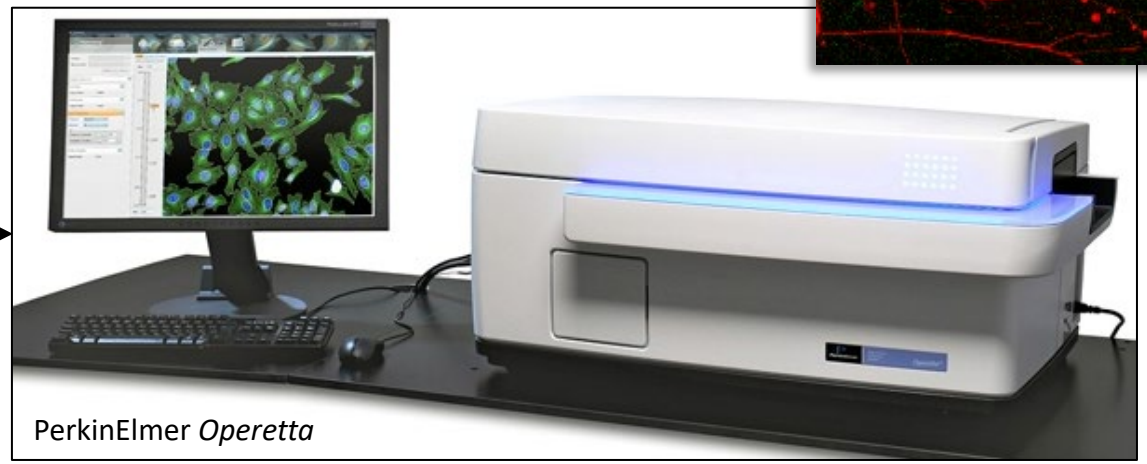
Phenotypical Screening: High-Content Imaging (HCI) - Morphological Endpoints






Sartorius *IncuCyte*




PerkinElmer *Operetta*

- Phenotypic drug screening based on neuronal morphology and network architecture

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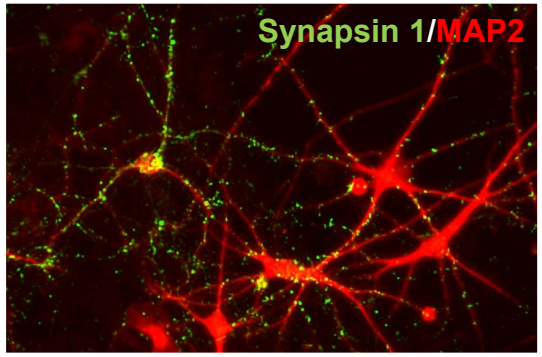
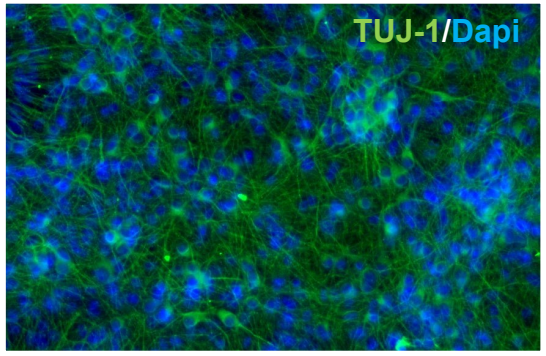
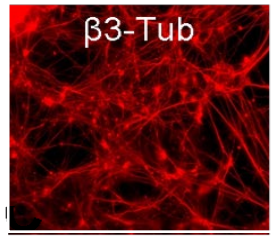
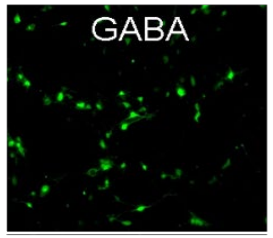
Characterization of the Co-Culture System

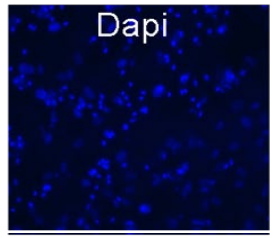
- Morphology and marker expression in human iN/glia co-cultures show mature neurons
- iNs exhibit intrinsic electrophysiology properties, synaptic function, circuit & network activity
- RNA-Seq show broad representation of neuronal signaling pathways

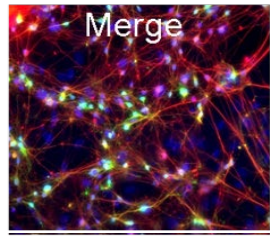


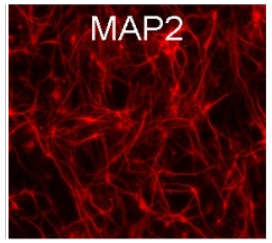


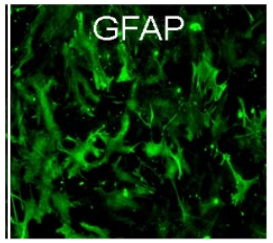


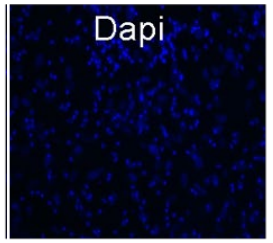


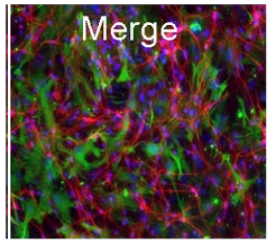


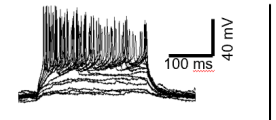


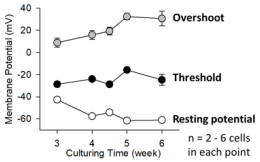


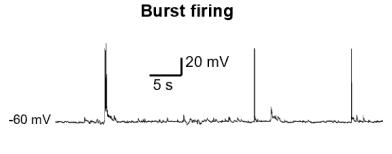


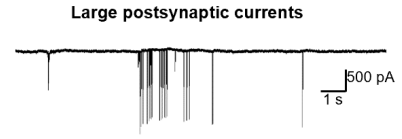


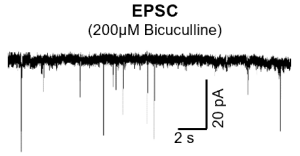


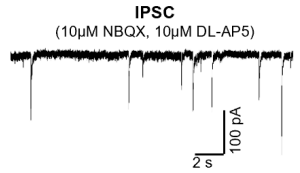


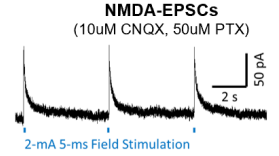


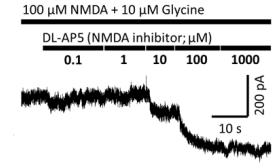


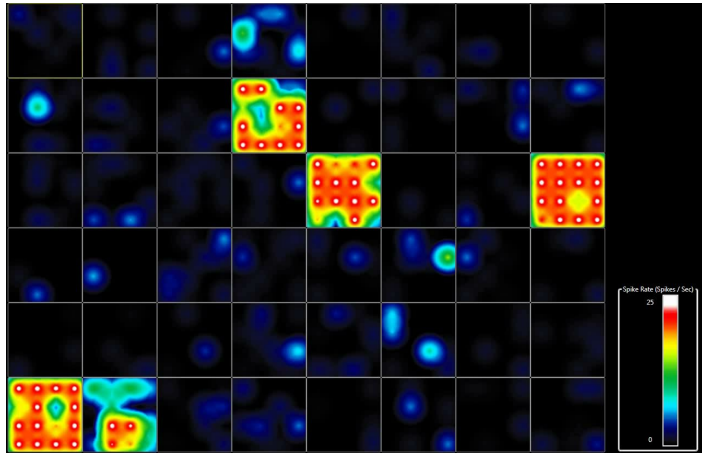


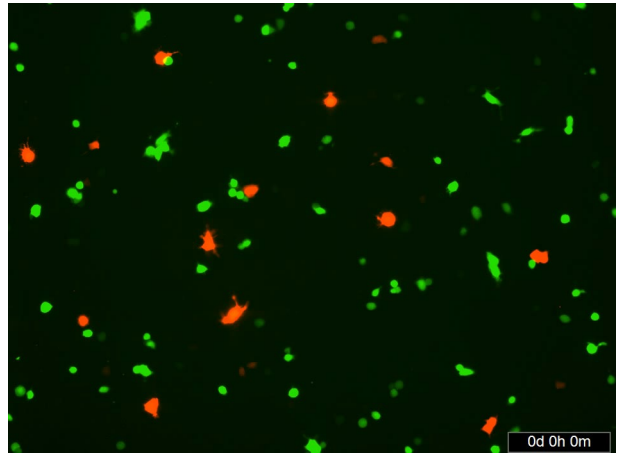




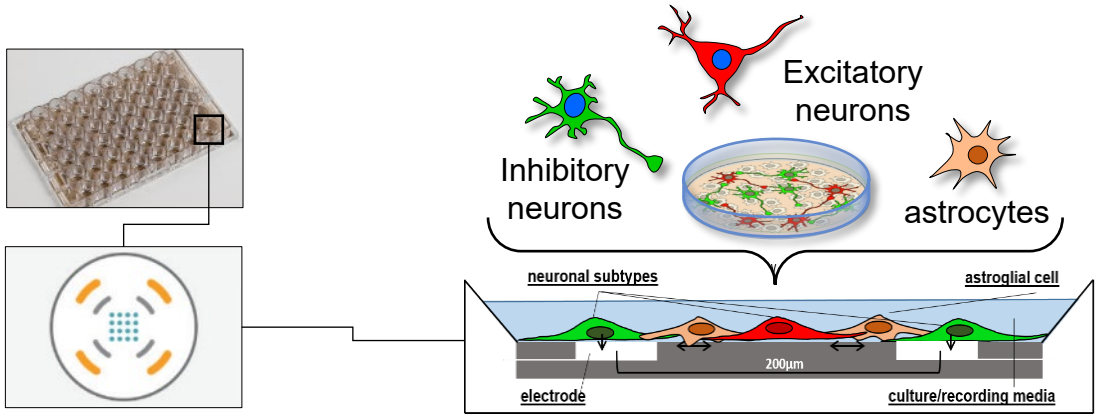


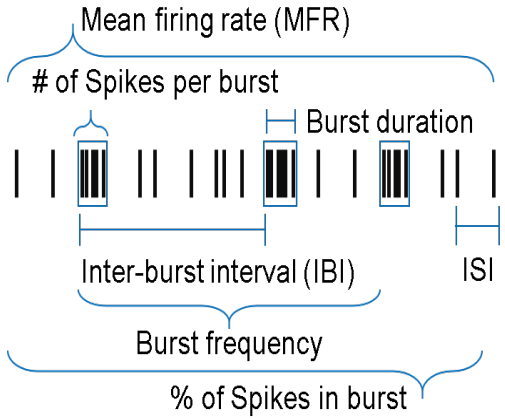




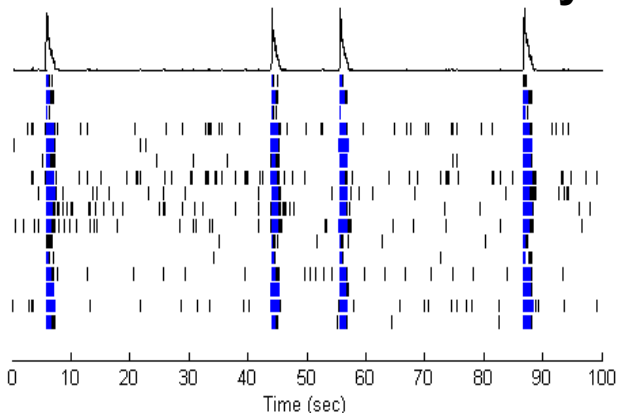


Reproducibility and Batch-to-Batch Consistency



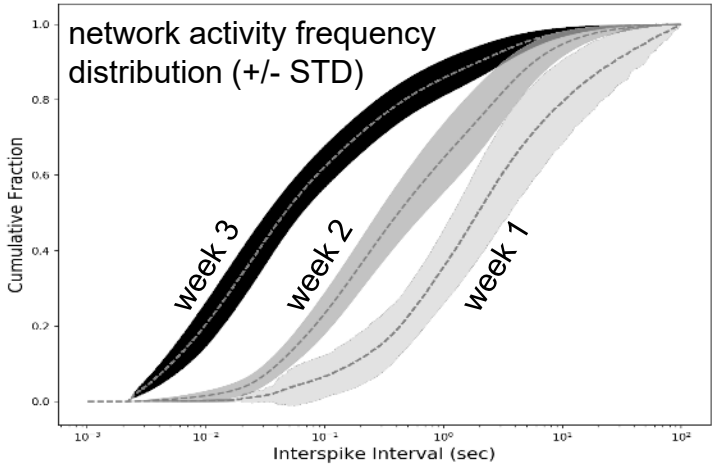


Intrinsic Network Activity

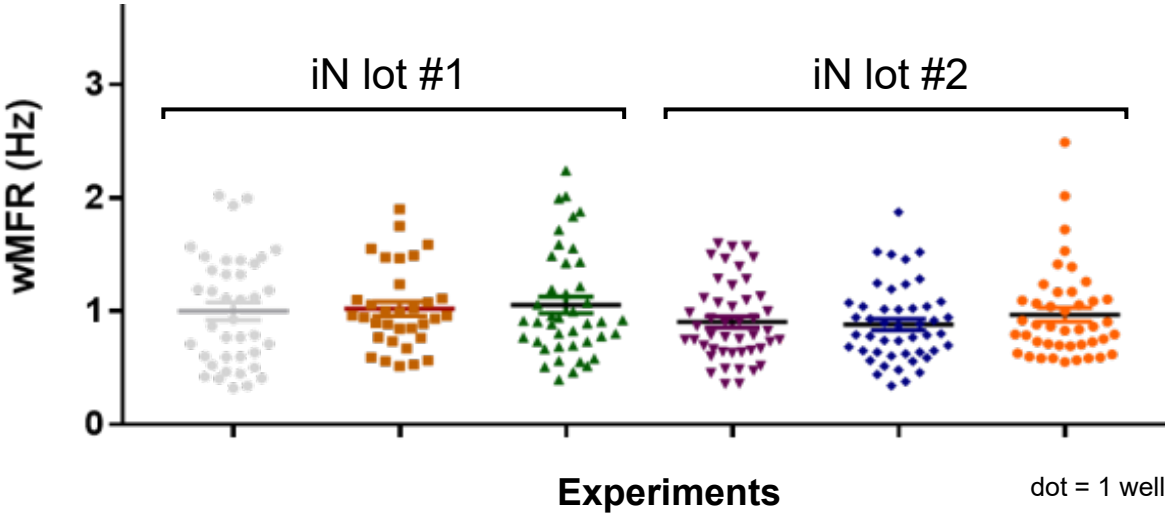


experiments
cell batches

Consistent Network Maturation



Robust screening endpoints



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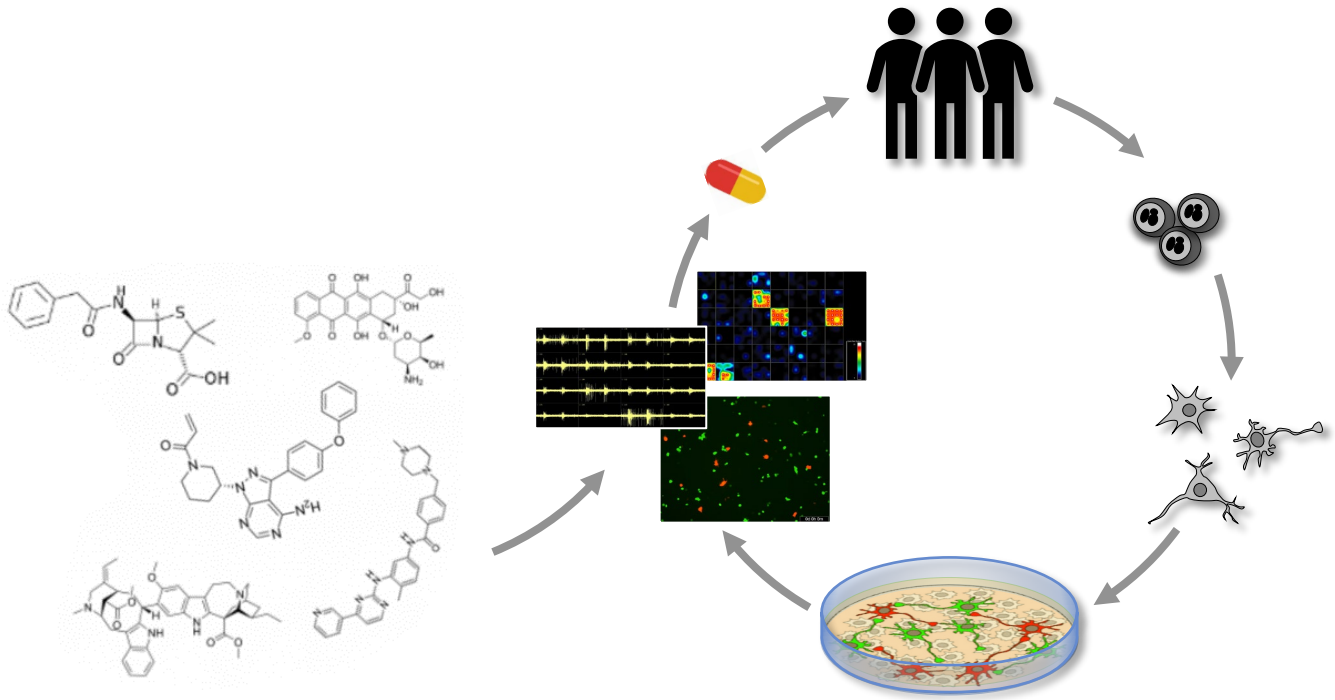
Human iPSC-Derived Neural Platforms for CNS Drug Discovery

- Human neurophysiology, morphology & neural network properties
  - Early access to human-relevant data
- Flexible cellular co-culture systems based on induced neuron (iN) technology
  - Short turnaround time
  - High reproducibility
  - High throughput
  - Low cost
- Flexible assay design
  - Phenotyping
  - Drug screening
  - Lead optimization
  - Neurotoxicity



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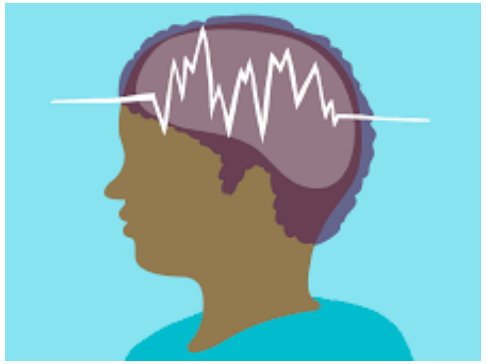
Human Neuron-Based Seizure Models for Discovery of Novel Anti-Epileptic Drugs

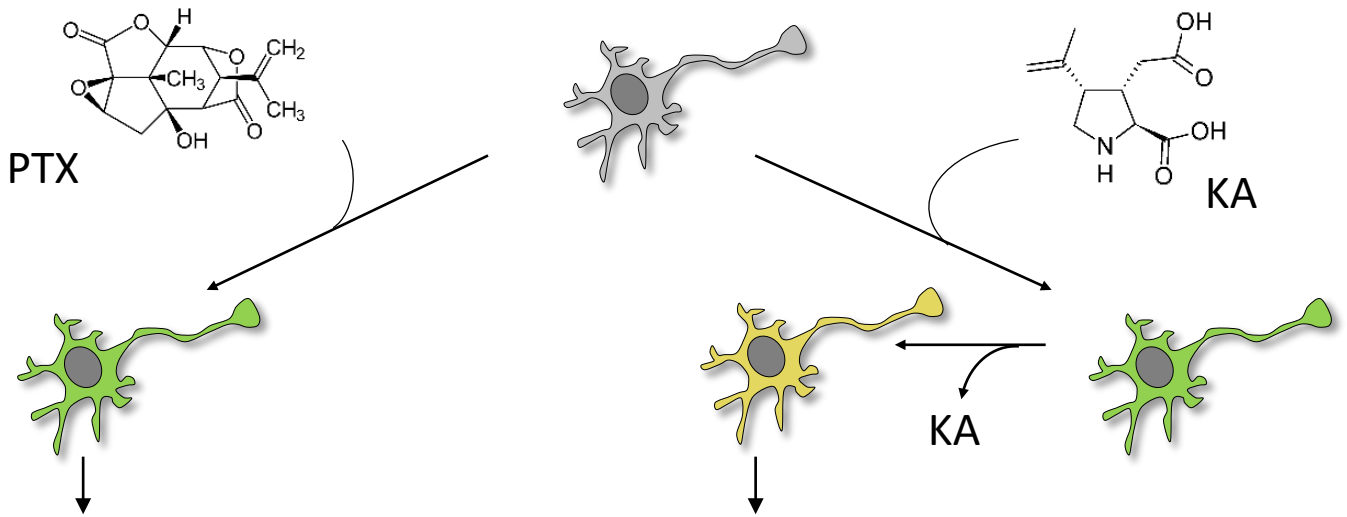


Epilepsy – *In Vitro* Seizure Models for Drug Screening

Chemically induced seizure models

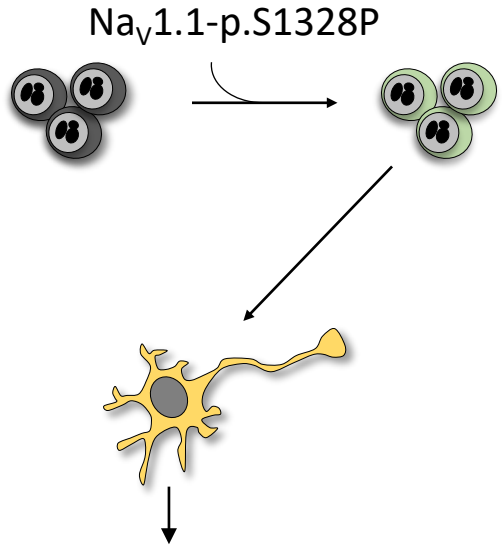
provoked concurrent

provoked sequential



Genetic models

spontaneous



High-Throughput Screening, Drug Testing, Lead Optimization, Neurotoxicity

Picrotoxin (PTX)	Kainic acid (KA)	Dravet syndrome
acute	acute/chronic	chronic
temporary hyperactive state	permanent hyperactive state	inherent (hyperactive) disease state
limitation of pathway interference	all pathways available	specific disease mechanism

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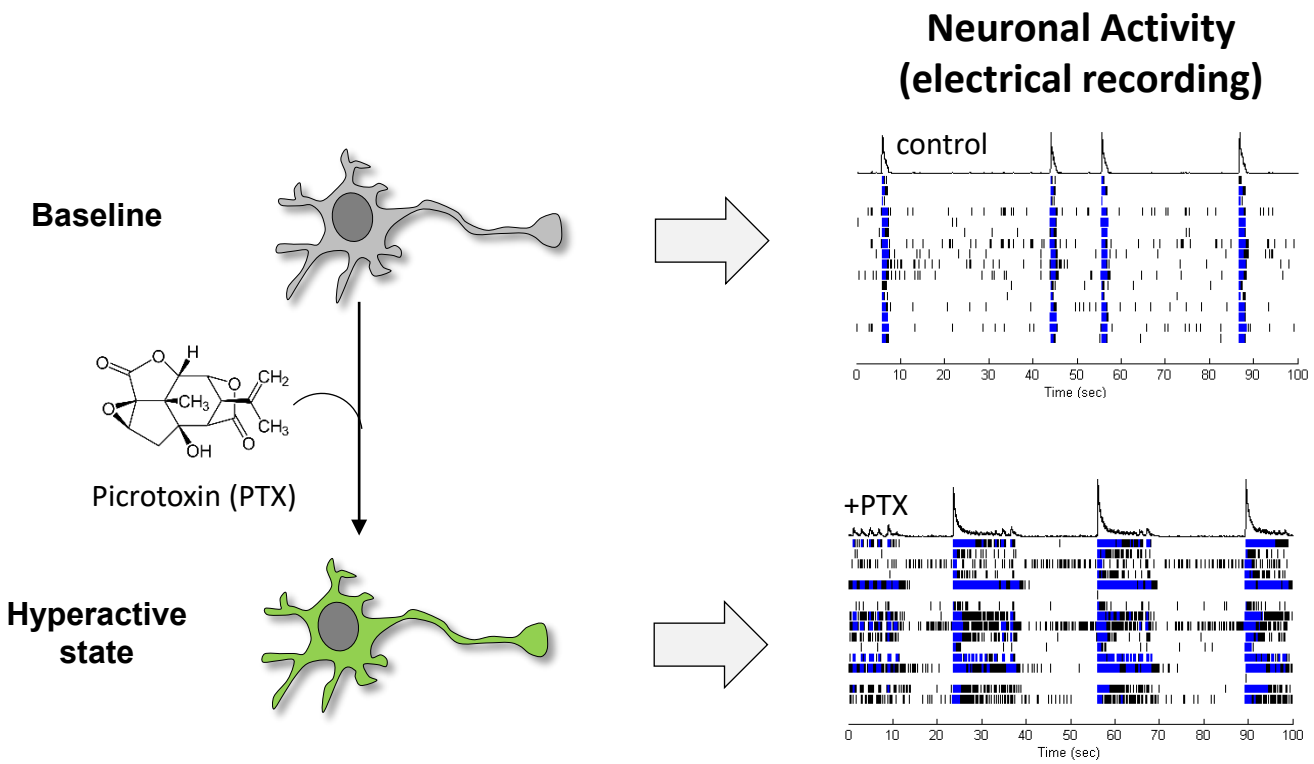
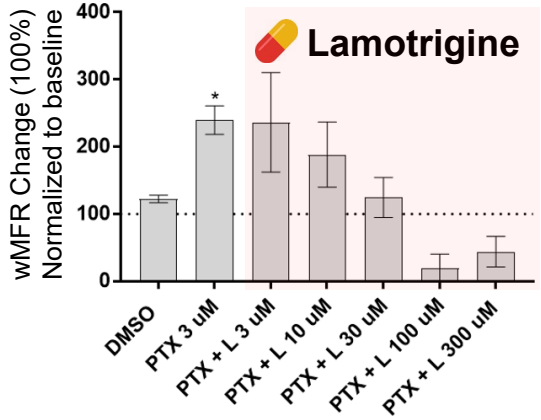
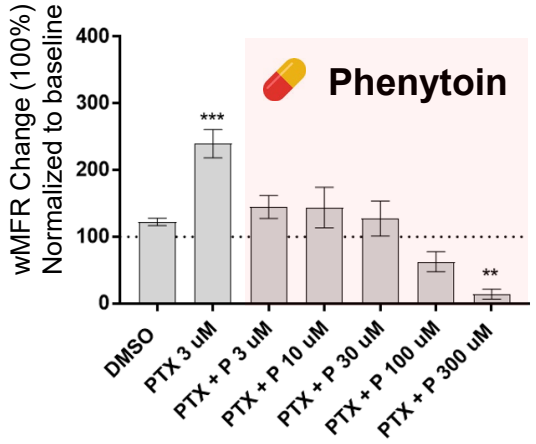
Epilepsy: First Human Cell Based Seizure Model

- A Chemically Induced Seizure Model based on Picrotoxin (PTX)
  - Quantitative network activity phenotype
    - ✓ Mixed excitatory/inhibitory iN co-cultures
    - ✓ Potent $GABA_A$ antagonist (PTX) induce tonic-clonic seizures
    - ✓ Rescue seizurogenic phenotype by AED dosing







➢Dose-dependent response of the PTX seizure model to standard AEDs

Epilepsy: Concordance Between *In Vitro* PTX Model Efficacy and Clinical Efficacy

Compound	Clinical status	PTX Model efficacy	Target	Target expression in iNs
(-)-Cannabidiol	Approved AED	Yes	unclear / possible GABAa	Yes
Carbamazepine	Approved AED	Yes	Nav	Yes
Cenobamate	Approved AED	Yes	Nav, presynaptic GABA release	Yes
Ethosuximide	Approved AED	Yes	T-Cav	Low
Everolimus	Approved for TSC-associated POS	Yes	mTOR inhibitor	Yes
Ezogabine	Approved AED	Yes	Kv opener	Yes
Lacosamide	Approved AED	Yes	Nav blocker	Yes
Lamotrigine	Approved AED	Yes	Nav	Yes
Levetiracetam#	Approved AED	Yes	SV2 / unclear	Yes
Perampanel	Approved AED	Yes	AMPA-R non-comp antagonist	Yes
Phenytoin	Approved AED	Yes	Nav (hydantoin class)	Yes
Rufinamide	Approved AED	Yes	Nav / unclear	Yes
Stiripentol*	Approved for Dravet (add-on)	weak w PTX model	GABAa / KATP / unclear	Yes / Low
Topiramate	Approved AED	No (up to 100uM)	unclear/Nav/Cav/CA/GABAa/AMPAr	Yes
Valproate	Approved AED	Yes	unclear/Nav/GABA_level/HDAC	Yes
Buspirone hydrochloride	Approved for anxiety/Phase II	Yes	5HT1A agonist	No (novel target?)
(S)-Carisbamate	Failed in Phase III**/new phase II	Yes	unclear/Nav/Cav/NMDA-r	Yes
Mexiletine hydrochloride	Approved for arrhythmia	Yes	Nav	Yes
Safinamide	Approved for PD	Yes	MAO-B / Nav / Cav	Yes
Licarbazepine	Extra AED suggested by KOL	Yes	Nav	Yes
Mephenytoin	Extra AED suggested by KOL	Yes	Nav (hydantoin class) / unclear	Yes
Methsuximide	Extra AED suggested by KOL	Yes	T-type Cav	Low
Acetazolamide Sodium	Extra AED suggested by KOL	No (up to 100)	carbonic anhydrase inhibitor / unclear	Yes / Low on some subtypes
Sulthiame	Failed to obtain approval in U.S.	No (up to 100)	carbonic anhydrase inhibitor / unclear	Yes / Low on some subtypes
VRT-043198	Failed to obtain approval in U.S.	No	ICE/caspase-1,4/IL1b/	Yes / Low on some subtypes

\# Not effective in acute seizure models (MES) and chemically induced seizure (PTZ), but active in models of acquired and genetic epilepsy, in particular in kindling models

* Interacts with barbiturate site / interferes with PTX site

** Withdrawn by JNJ after FDA reviewed clinical data. Phase III for the adjunctive treatment of partial onset seizures (JNJ); initiated a new phase II trial for Lennox-Gastaut Syndrome (SK)

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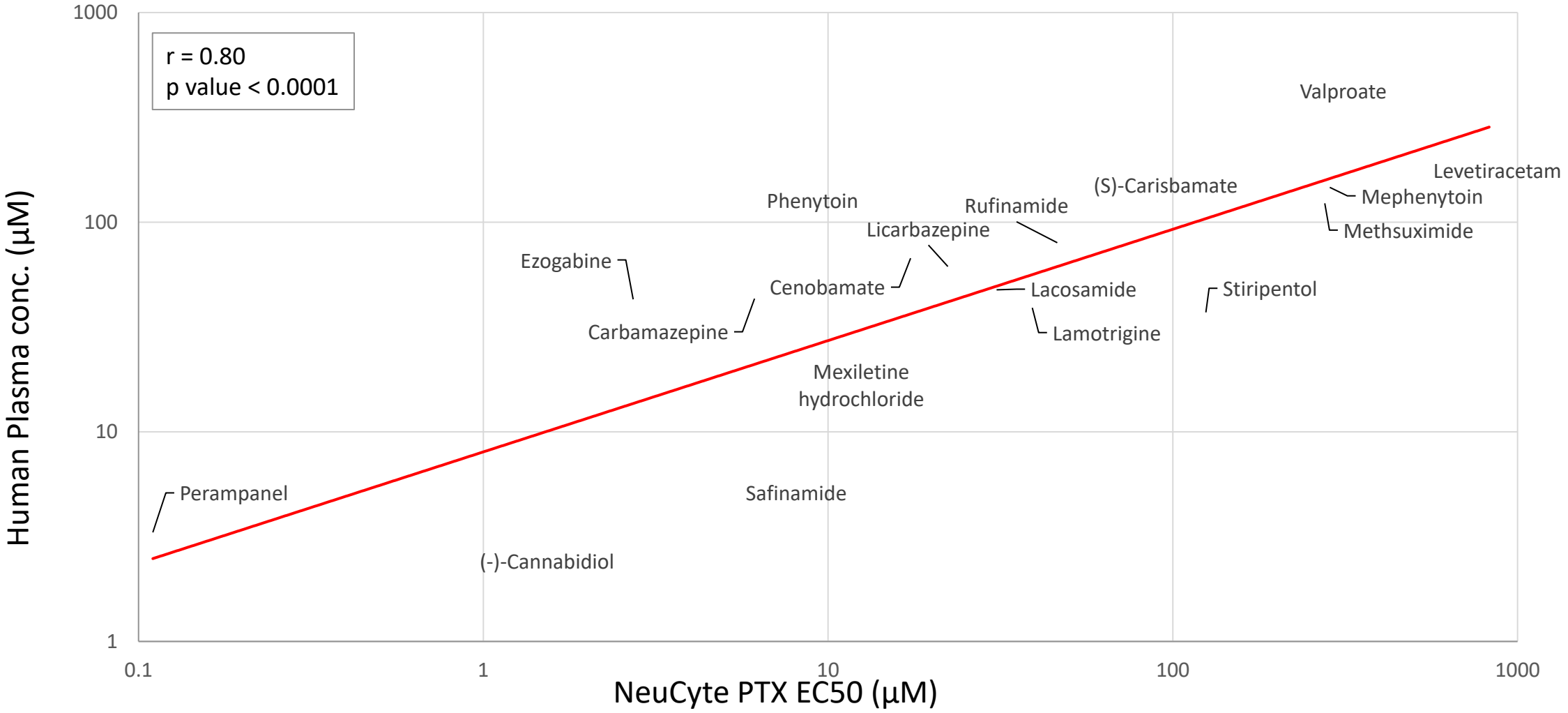
Efficacies of 18 AEDs on PTX Human *In Vitro* Seizure Model: High Correlation with Clinical Data



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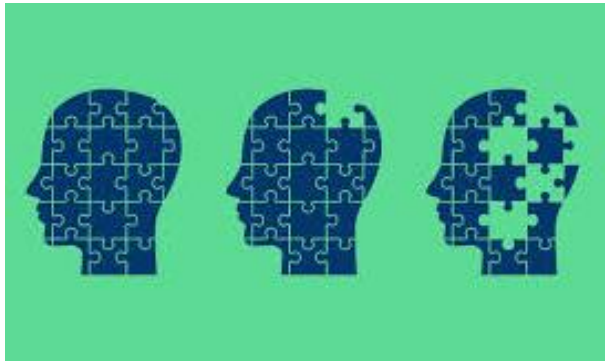
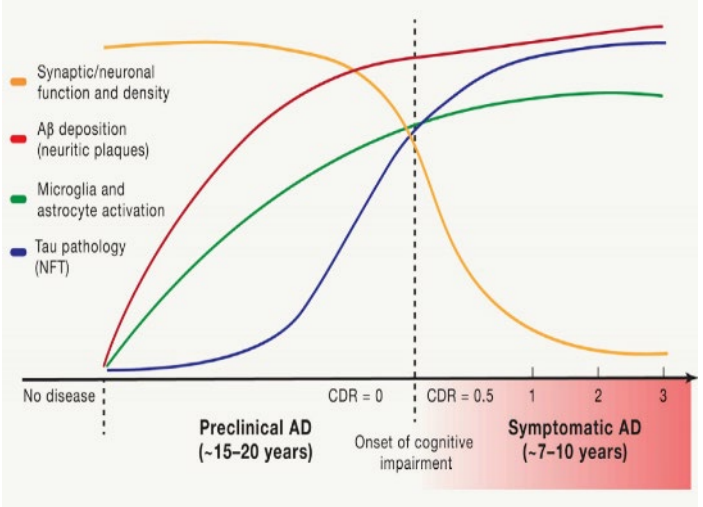
Neurodegeneration & Neuroinflammation Drug Discovery Programs

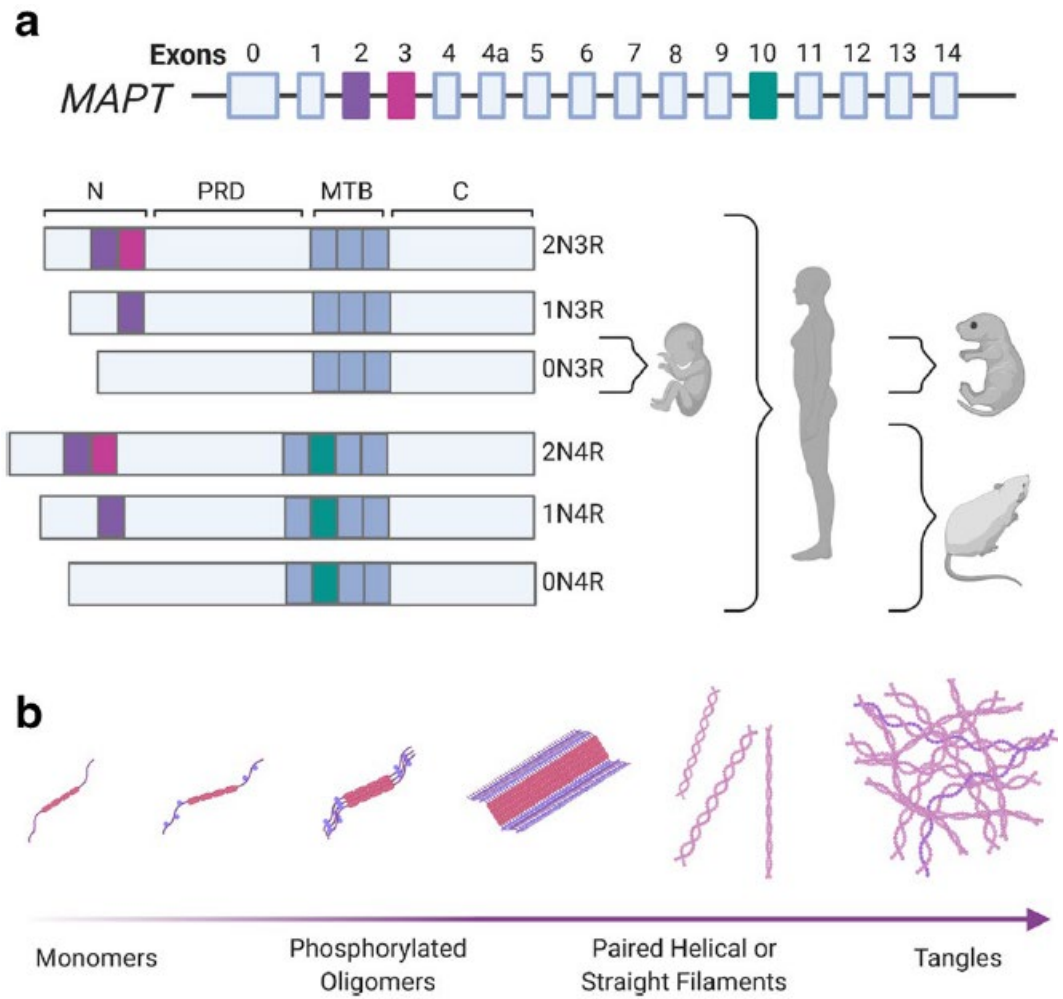


Why Starting with Tauopathy

- Tauopathies are linked to neuronal death and development of AD
- Six tau isoforms in human brain with variant 3R or 4R C-terminal repeats
  - Only the shortest 3R isoform in fetal brain
  - MAPT mutations found in AD affected by tau
  - Different 3R:4R isoform ratio by altering exon 10 splicing
- Mice only express isoforms with 4R in adult brain

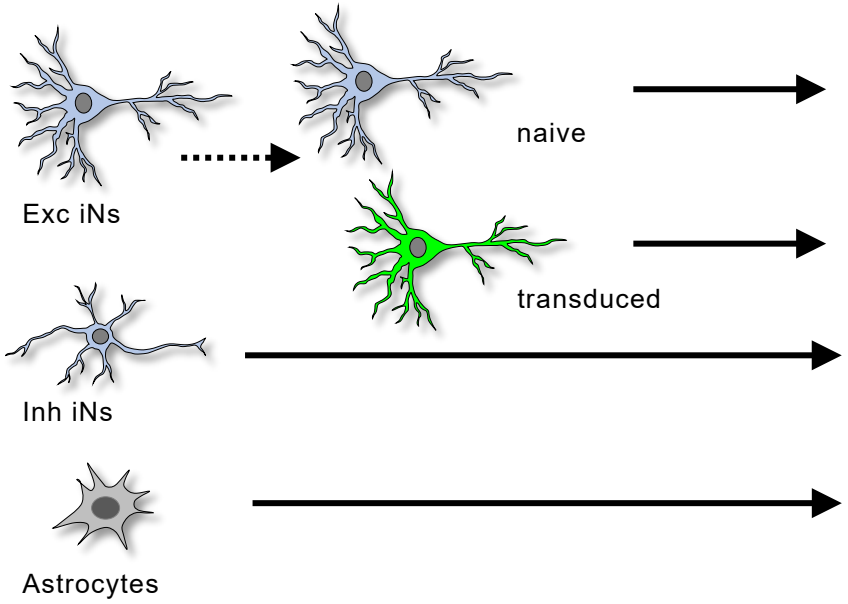


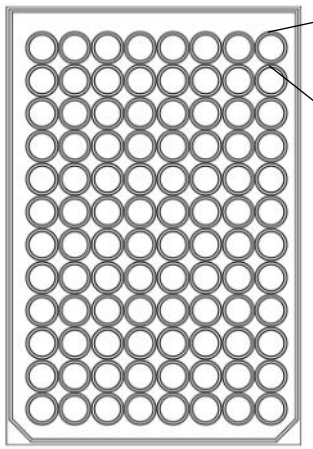
Long and Holtzman *Cell 2019*



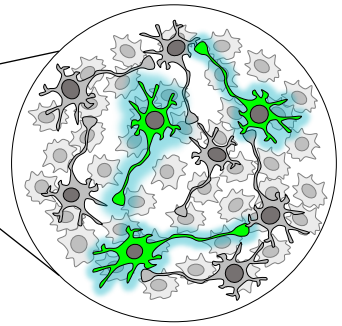
Kent et al., *Acta Neuropathologica 2020*

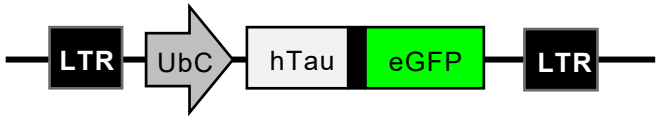
AD Tauopathy Pilot Program





Optical 96-well plates





3 of 6 Tau splicing isoforms are unique to human

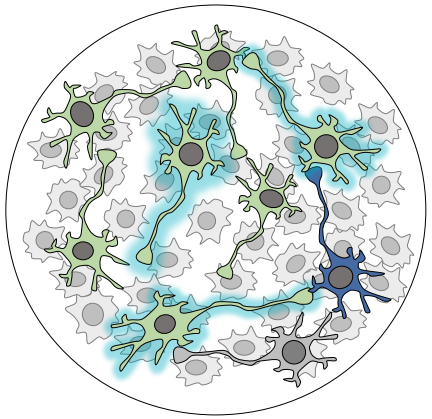
- Compounds
- Antibodies
- ASOs/AAVs
- shRNA KD screen
- CRISPR/Cas9 screen

Neural network activity

- wMFR
- Network burst frequency
- synchrony
- "training performance" by repeated stimulation

High-content imaging

- Tau pathologies
  - P-Tau
  - PHF
  - Misconformed Tau
- Neuronal morphology (dystrophic dendrites)
- Neuronal cell loss



- Quantification of pathological tau spreading
- Assessment of neuronal health/ function, and synaptic function

Usenovic et al., *Journal of Neuroscience 2015*

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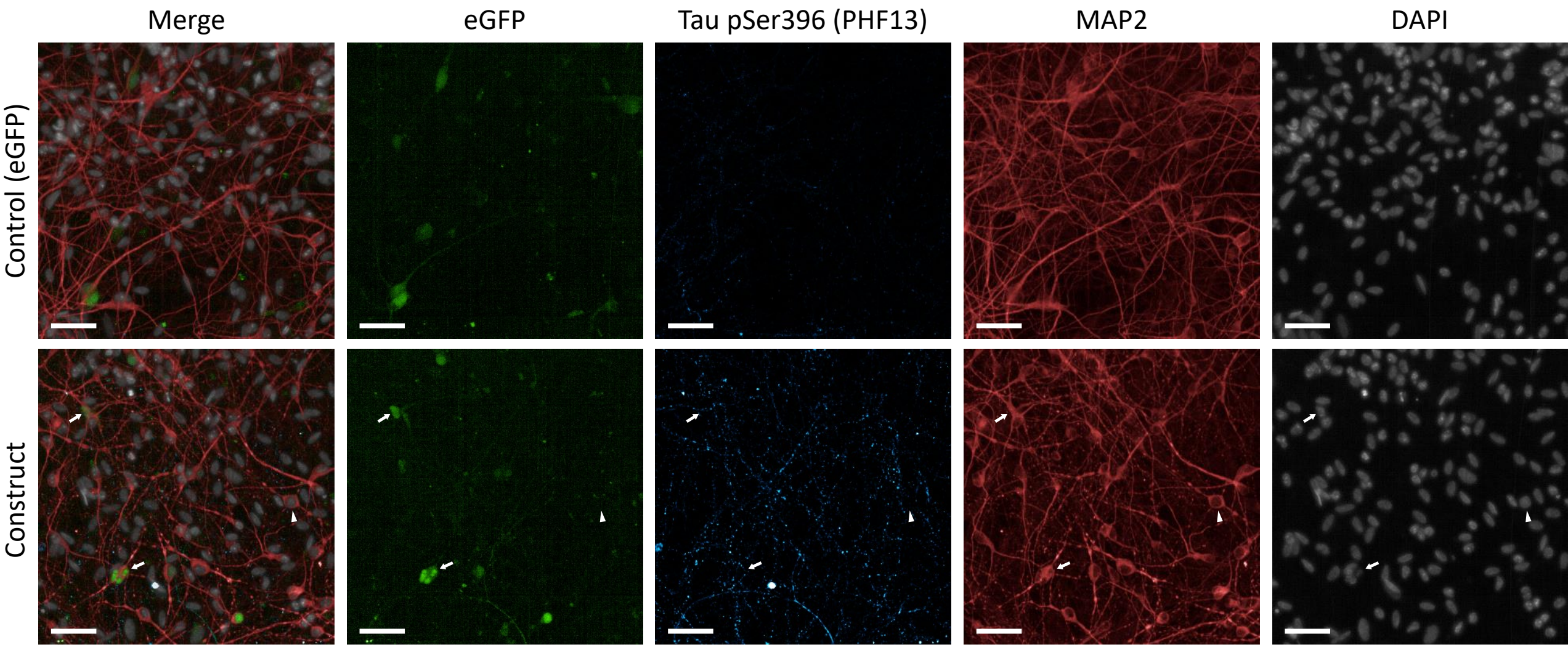
Preliminary Data: Tau Spreading – Aggregation in Naïve iNs



Scale bar = 50 µm

Arrowhead – Naïve iNs
Arrow – Transduced iNs

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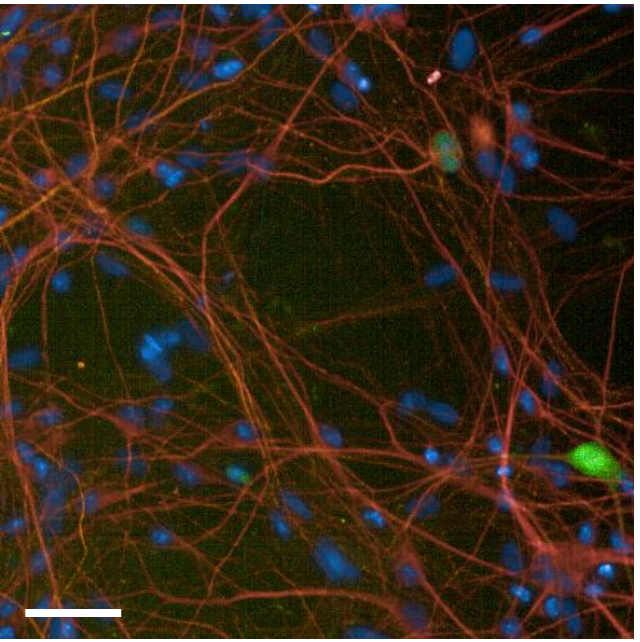
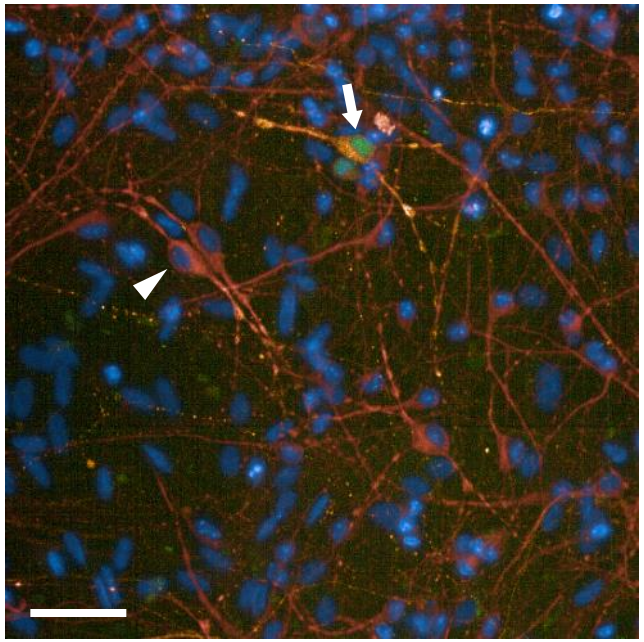
Preliminary Data: Tauopathy-Associated Morphologies in Naïve iNs

Control (eGFP)



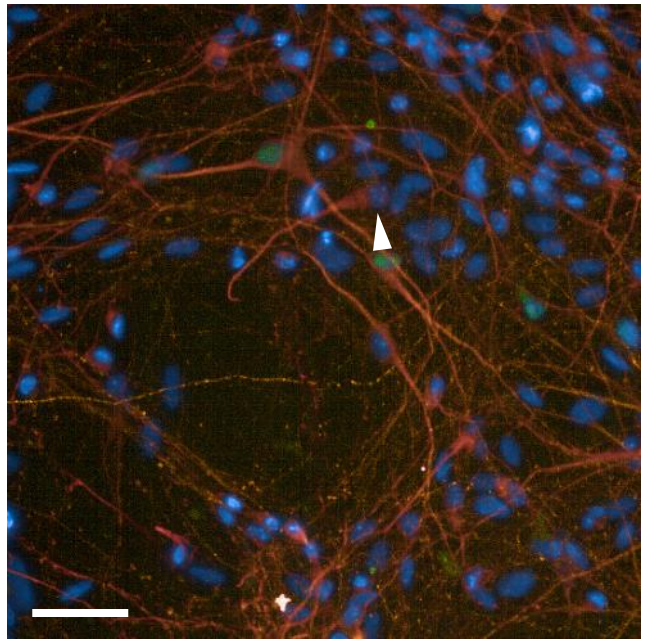
Scale bar = 50 µm

Construct



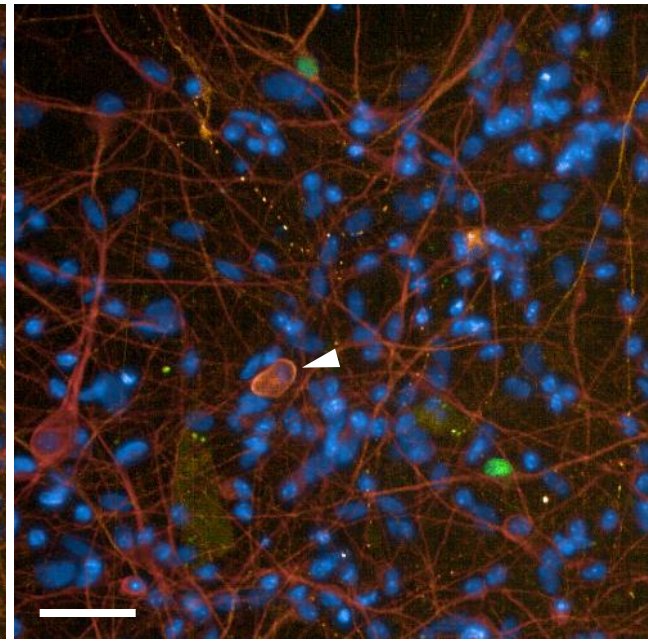
Varicosity

Construct



Shortened Neurites

Construct



Cell Death

Arrowhead – Naïve iNs
Arrow – Transduced iNs

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KIF1A Project Plan

Project Goals

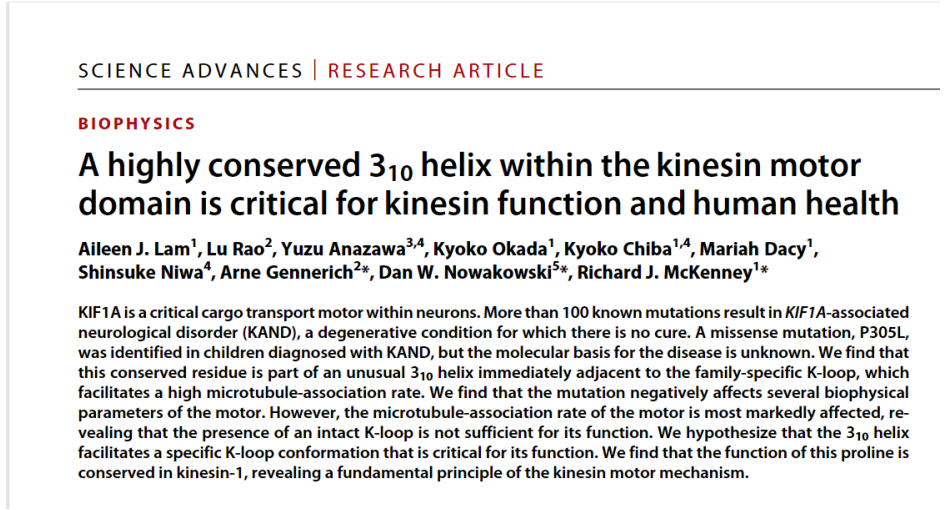
- Characterize KIF1A mutant iPSC lines and derived neurons
- Build in vitro disease model for screening drug candidates for the KAND community

Proposed Modules

- Module I: iPSC and neuron characterization of select variants
  - Characterize and qualify Coriell iPSCs
  - Produce induced neurons from qualified iPSCs
  - Identify most relevant phenotypes and develop assays on the induced neurons
  - Target validation based on drug candidates to be screened
- Module II: In vitro modeling and compound screening
  - Finalize assay development
  - Compound screening

Variant Selection

- P305L
  - High MAF
  - Mutation in critical protein domain
  - Symptoms severe
    - Seizure
    - Developmental delay
  - Molecular mechanism being reported
- E253K
  - High MAF
  - Mutation in critical protein domain
  - Symptoms severe
    - Seizure
    - Developmental delay
  - Molecular mechanism being reported



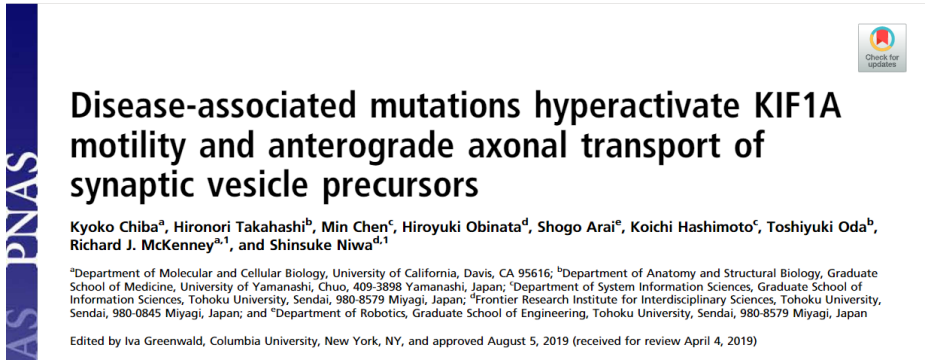
SCIENCE ADVANCES | RESEARCH ARTICLE

BIOPHYSICS

A highly conserved 3_{10} helix within the kinesin motor domain is critical for kinesin function and human health

Aileen J. Lam[1], Lu Rao[2], Yuzu Anazawa[3,4], Kyoko Okada[1], Kyoko Chiba[1,4], Mariah Dacy[1], Shinsuke Niwa[4], Arne Gennerich[2]*, Dan W. Nowakowski[5]*, Richard J. McKenney[1]*

KIF1A is a critical cargo transport motor within neurons. More than 100 known mutations result in *KIF1A*-associated neurological disorder (KAND), a degenerative condition for which there is no cure. A missense mutation, P305L, was identified in children diagnosed with KAND, but the molecular basis for the disease is unknown. We find that this conserved residue is part of an unusual 3_{10} helix immediately adjacent to the family-specific K-loop, which facilitates a high microtubule-association rate. We find that the mutation negatively affects several biophysical parameters of the motor. However, the microtubule-association rate of the motor is most markedly affected, revealing that the presence of an intact K-loop is not sufficient for its function. We hypothesize that the 3_{10} helix facilitates a specific K-loop conformation that is critical for its function. We find that the function of this proline is conserved in kinesin-1, revealing a fundamental principle of the kinesin motor mechanism.



PNAS

Disease-associated mutations hyperactivate KIF1A motility and anterograde axonal transport of synaptic vesicle precursors

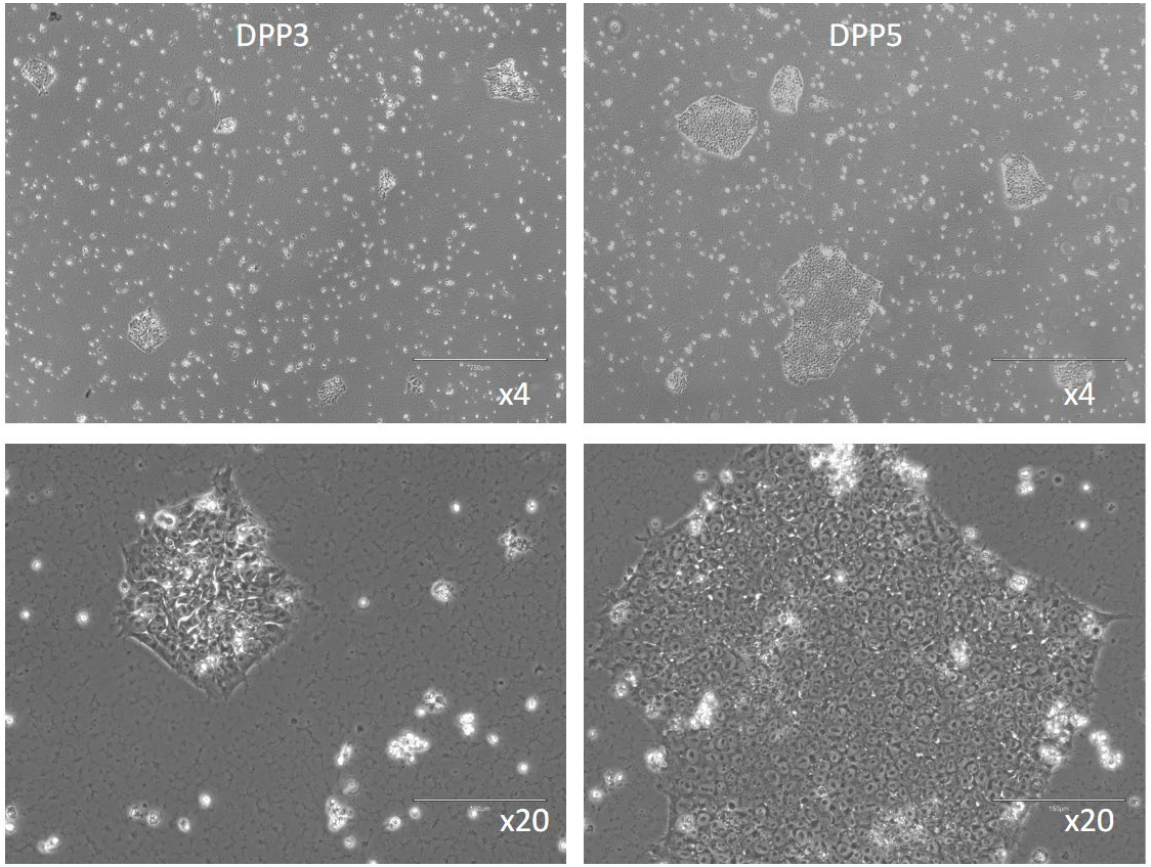
Kyoko Chiba[a], Hironori Takahashi[b], Min Chen[c], Hiroyuki Obinata[d], Shogo Arai[e], Koichi Hashimoto[c], Toshiyuki Oda[b], Richard J. McKenney[a,1], and Shinsuke Niwa[d,1]

[a]Department of Molecular and Cellular Biology, University of California, Davis, CA 95616; [b]Department of Anatomy and Structural Biology, Graduate School of Medicine, University of Yamanashi, Chuo, 409-3898 Yamanashi, Japan; [c]Department of System Information Sciences, Graduate School of Information Sciences, Tohoku University, Sendai, 980-8579 Miyagi, Japan; [d]Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, Sendai, 980-0845 Miyagi, Japan; and [e]Department of Robotics, Graduate School of Engineering, Tohoku University, Sendai, 980-8579 Miyagi, Japan

Edited by Iva Greenwald, Columbia University, New York, NY, and approved August 5, 2019 (received for review April 4, 2019)

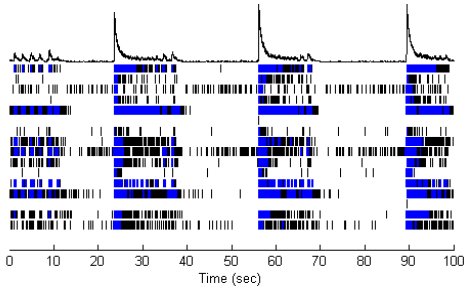
Characterize and qualify Coriell iPSCs through standard protocol

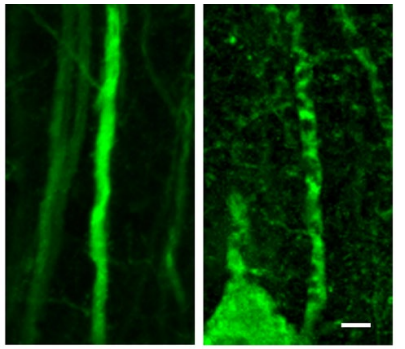
- Ensure purity of cell population
- Sterility and mycoplasma testing
- Genomics QC
  - Karyotyping
  - To do in parallel with iN production
    - SNV and CNV surveillance: Whole exome sequencing
      - We are in the process of establishing a pipeline to use WES for CNV detection
- Expansion and banking
  - We will bank 30-40 vials in our own facility
    - We will transfer half or more to KIF1A ORG at the right time



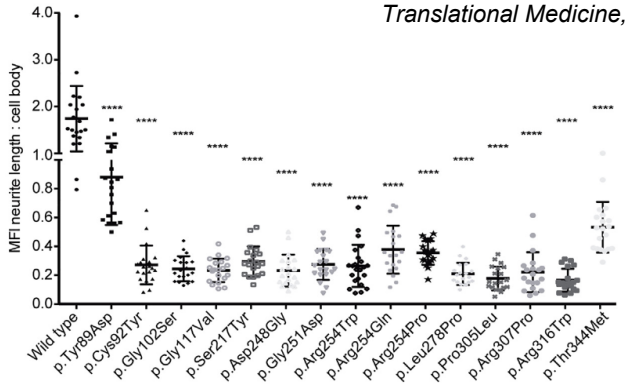
Identify phenotypes and develop assays on the induced neurons

- Phenotypes to look into (current thinking)
  - Seizure-like hyper excitability measured by MEA (microelectrode array)
  - Structural changes detected by HCI (high content imaging)
    - Dendrite morphology/Spine density
    - MFI neurite length/cell body ratio
    - Synapse count





Genc et. al, Clinical and Translational Medicine, 2021



Boyle et. al, Human Genetics and Genomics Advances, 2021

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"KIF1A.ORG, together with its community of collaborators, have been working hard to identify, utilize and develop therapeutic candidates for KAND," said Dr. Dominique Lessard, Chief Scientific Officer, KIF1A.ORG. *"This research and development community urgently needs in vitro models that resembles human biology and can be used to quickly screen potential treatments with a translatable value on an industrial level. We believe NeuCyte is one of the partners that can help us achieve this objective."*

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Thank you!

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