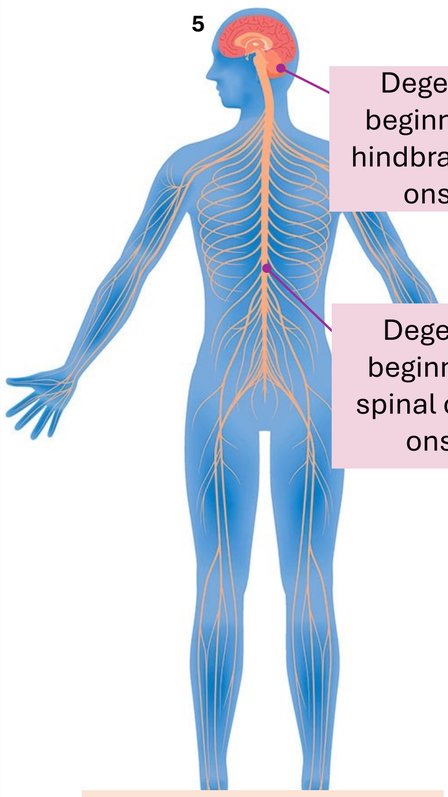
Modeling Bulbar-onset ALS using iPSC-derived Motor Neurons

Lauren Stoller[1], Nicole Teaney[2], Nisha Iyer[1]

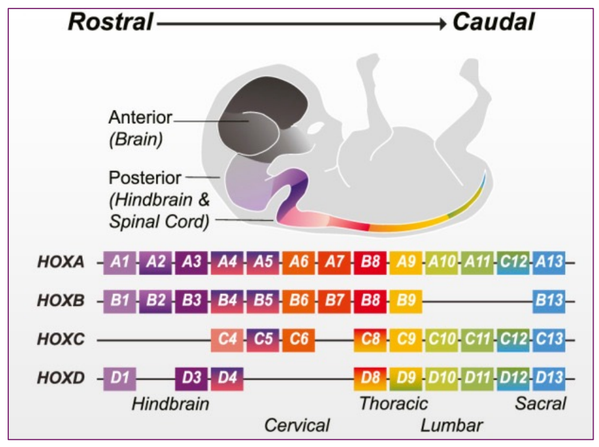
1. Department of Biomedical Engineering, Tufts University, Medford, MA; 2. Department of Neuroscience, Tufts Graduate School of Biomedical Sciences, Boston, MA

ALS presentation depends on location of onset

ALS is a fatal neurodegenerative disease caused by **progressive loss of motor neurons[1].** It is notoriously heterogeneous, with over 40 known associated genes and no singular cellular-level cause.



Bulbar-onset disease is known to be more aggressive[2-4]. **Why?**



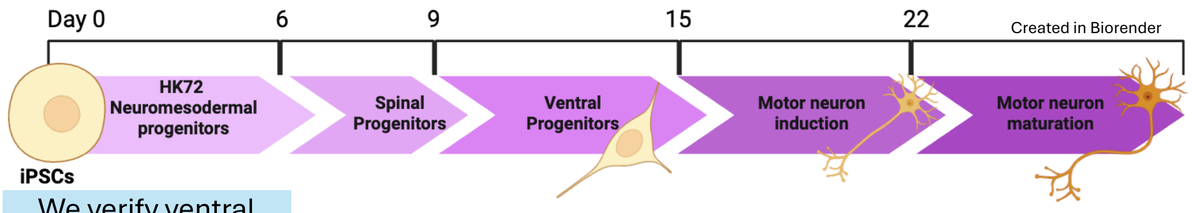
We use Hox patterning derived from developmental biology to create motor neurons specific to the hindbrain/bulbar region (HK72) from 3 lines of iPSCs:

- **Kolf2.1J:** healthy control line
- **FUS:** a mutation associated with bulbar-onset[1]
- **SOD1:** a mutation associated with spinal-onset[2]

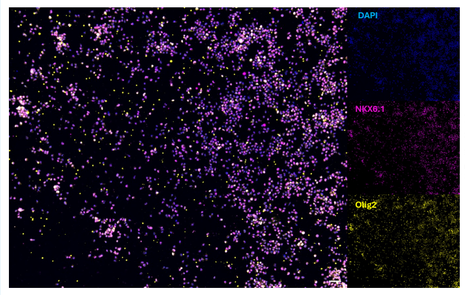
Key question: will we see different, more severe disease phenotypes in bulbar-region neurons with the bulbar-associated mutation?

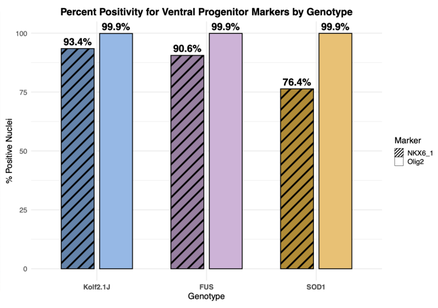
iPSC-derived motor neurons are derived by directed differentiation

Treating iPSCs with morphogens imitating embryonic development promotes motor neuron identity

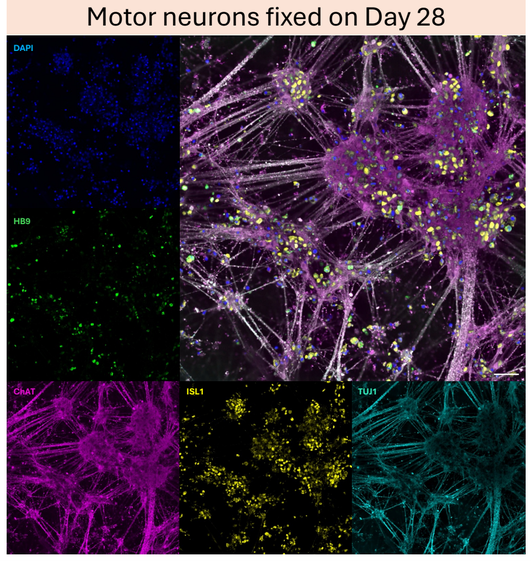


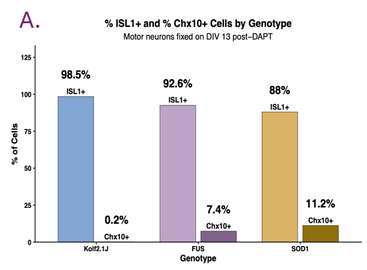
We verify ventral progenitor identity using immunofluorescent staining of ventral markers Olig2 and NKX6.1 to ensure correct patterning before maturation

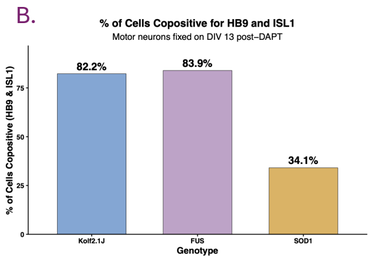




Motor neurons fixed on Day 28





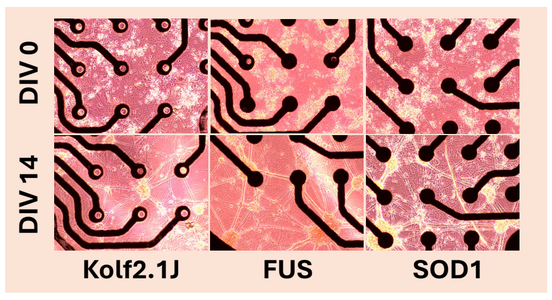


- Early motor neuron marker ISL1 and excitatory interneuron marker Chx10 are quantified to estimate culture purity (A)
- ISL1 and HB9, a marker for mature, post-mitotic motor neurons are quantified to estimate population maturity (B)

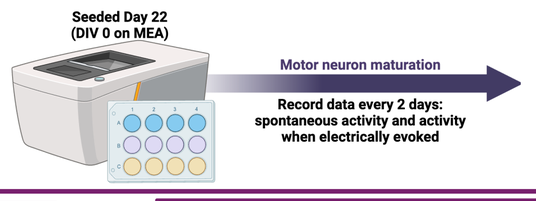
Region-specific iPSC-derived motor neurons allow for precise modeling and characterization of aggressive subtypes of ALS

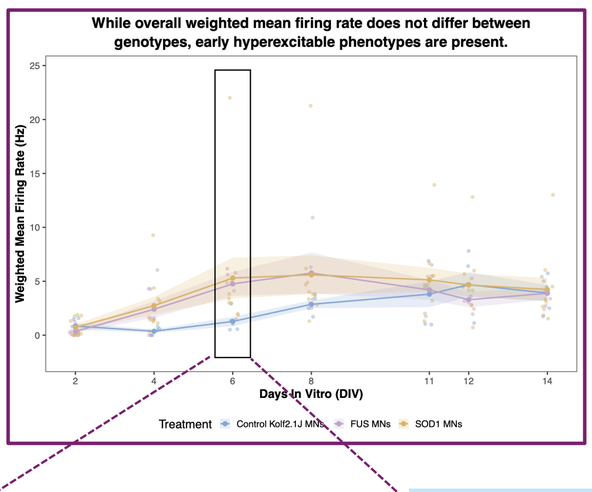
See detailed methods here:

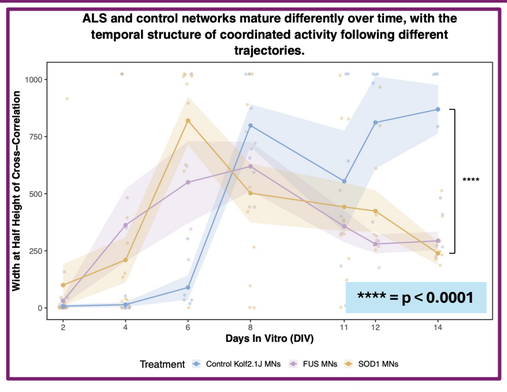
ALS motor neurons display early spontaneous hyperactivity phenotype

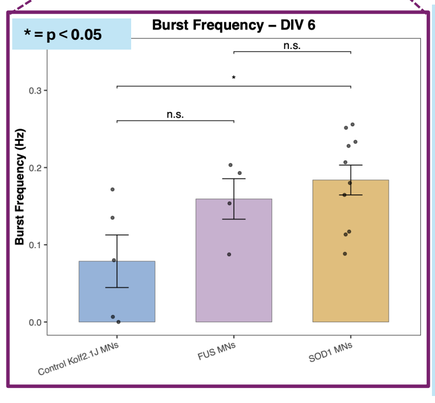


Following 7 days of neuronal induction, motor neurons were seeded into multielectrode arrays to mature and begin electrophysiological measurements.



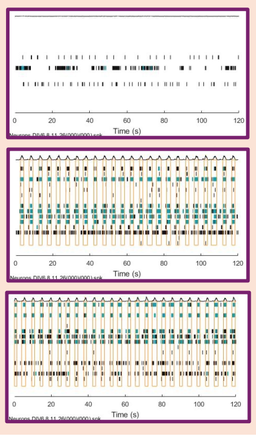






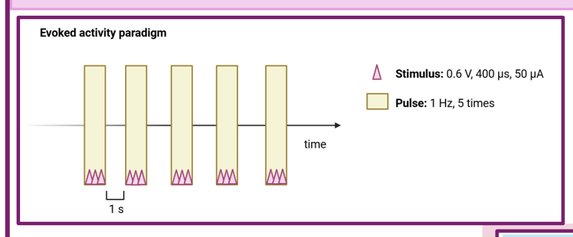
ALS neurons experience more <u>bursts</u> per second compared to control, but similar total spikes per second

ALS lines exhibit more network bursts than controls at DIV 6

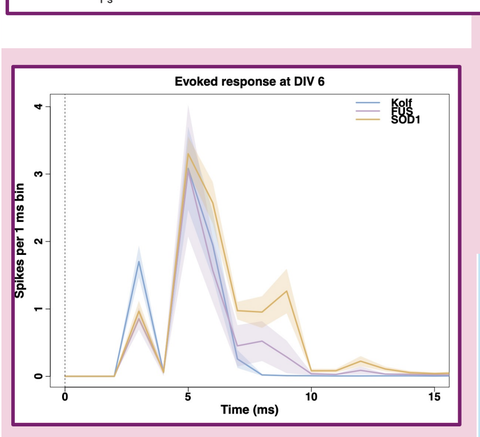


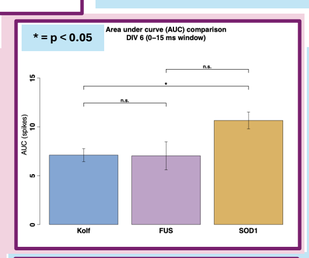
ALS neurons exhibited a narrower width at half height of cross-correlation by DIV 14 compared to controls, indicating that coordinated activity occurred within a shorter temporal window and suggesting altered temporal organization of network dynamics during maturation.

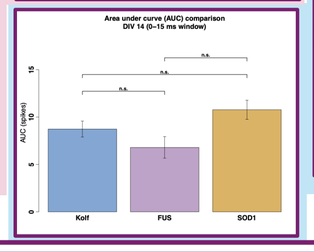
SOD1 tends to have a prolonged and less coordinated response in response to evoked stimulation

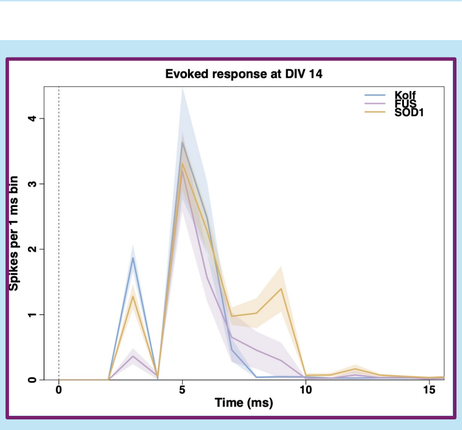


This stimulation paradigm, repeated 10x per recording day, delivers a precise electrical input at one point within the neural network. We can then examine how response propagates throughout the network post-stimulus.









Applications and Future Directions

- Establishing a parallel caudal motor neuron model to determine how genotype interacts with spinal cord region to influence disease vulnerability
- More assays to capture disease phenotypes (metabolism)
- Culturing MNs in 3D (mono-culture and co-culture)

Together, these steps move toward a more physiologically relevant *in-vitro* disease model for investigating cellular pathology and evaluating potential therapeutics.

References

1. Hu, Y. *et al.* Pathological mechanisms of amyotrophic lateral sclerosis. *Neural Regen Res* **19**, 1036–1044 (2023).
2. Zoccolella, S. *et al.* Analysis of survival and prognostic factors in amyotrophic lateral sclerosis: a population based study. *J Neurol Neurosurg Psychiatry* **79**, 33–37 (2008).
3. Shellikeri, S. *et al.* The Neuropathological Signature of Bulbar-onset ALS: A Systematic Review. *Neurosci Biobehav Rev* **75**, 378–392 (2017).
4. Requardt, M. V., Görlich, D., Grehl, T. & Boentert, M. Clinical Determinants of Disease Progression in Amyotrophic Lateral Sclerosis—A Retrospective Cohort Study. *J Clin Med* **10**, 1623 (2021).
5. Central Nervous System: brain and spinal cord. https://qbi.uq.edu.au/brain/brain-anatomy/central-nervous-system-brain-and-spinal-cord (2017).
6. Iyer, N. R. & Ashton, R. S. Bioengineering the human spinal cord. *Front Cell Dev Biol* **10**, 942742 (2022).

Acknowledgements

I would like to thank the Iyer Lab for their continued support and encouragement throughout the research process. I would especially like to thank my mentor, Nicole Teaney; this work would not be possible without her.

Thank you to the Tufts Laidlaw team, especially Andrew Singleton, for their support and project funding.