Machine Learning-Informed Digital Measures Improve the Sensitivity and Translational Relevance of In Vivo Neurodegenerative Disease Models

Amy E. Clipperton-Allen[1], Brian Berridge[2], Steve Ciciotte[1], Michael Ellis[1], Cat Lutz[1], Paige Martin[3], Timothy L. Robertson[4], Manuel E. Ruidíaz[4], Michael C. Saul[1], Natalie Bratcher-Petersen[2,4]

[1]The Jackson Laboratories, [2]Digital In Vivo Alliance, [3]Critical Path Institute, [4]TLR Ventures

BACKGROUND

- Machine learning-based **digital measures offer transformative insights** across various rare disease research domains.
- Digital measures hold untapped potential to significantly enrich neuromuscular disease research.
- We **compare digital *in vivo* measures to traditional endpoints** in mouse models of two neuromuscular diseases: **Friedreich's Ataxia** and **Amyotrophic Lateral Sclerosis**.
- These digital measures were developed by collaborators within the Digital In Vivo Alliance (DIVA) using **a novel home cage computer vision system** developed by The Jackson Laboratory (JAX).
- JAX Envision™ continuously **captures rodent behavior throughout both light and dark cycles**, enabling longitudinal studies **spanning weeks or months**.

METHODS

- Neuromuscular studies were conducted using two mouse strains and their relevant controls: B6.Cg-*Pvalb*$^{tm1(cre)Arbr}$ *Fxn*^{em2Lutzy} *Fxn*$^{em2.1Lutzy}$/J (**FXN**, JAX Strain #029721) for Friedreich's Ataxia and B6.Cg-Tg(SOD1*G93A)1Gur/J (**SOD1**, JAX Strain #004435) for Amyotrophic Lateral Sclerosis (ALS).
- Traditional measures, including rotarod performance, body weight, and neuroscore, were measured in both strains. In the **SOD1** model, additional assessments included CMAP/RNS and serum NfL, an ALS biomarker. For **SOD1** mice, traditional measures were performed at 6, 12, and 16 weeks, and for **FXN** mice at 9, 13, and 16 weeks.
- Digital measures were derived from videos of socially housed mice in home cages. Machine learning-based computer vision algorithms were applied to live video to compute continuous and longitudinal movement phenotypes (**Figure 1**).

Figure 1. JAX Envision™ collects video in the light (left) and dark (center) cycles and sends the results through a set of machine learning algorithms that are reported through a cloud-based user interface.

- Inferred sleep was computed using the criteria of Pack et al. (2007), which demonstrates that runs of inactivity of ≥40 seconds correlate with sleep.
- Activity breakpoints, when activity patterns change drastically, were computed with segmented line regression on circadian-detrended activity.

RESULTS: ALS (SOD1-G93A)

SOD1 Traditional Measures

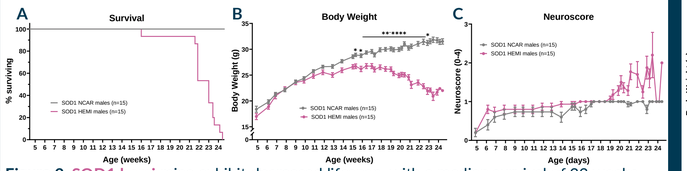

Figure 2. **SOD1 hemi** mice exhibit decreased lifespan, with a median survival of 23 weeks (**A**), and decreased body weight from 15 weeks (**B**). There were no significant differences in neuroscore, whereas **SOD1 hemi** mice received a neuroscore greater than 1 (**C**). There were no differences between **SOD1 hemi** mice and **NCAR** mice on rotarod performance at 6, 12, or 16 weeks (data not shown).

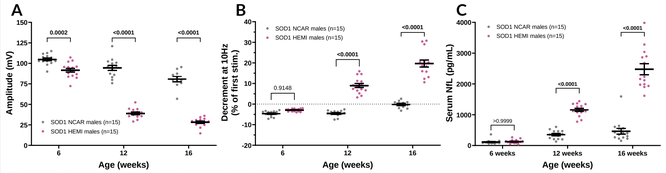

Figure 3. **SOD1 hemi** mice exhibit increasing deficits in compound muscle action potential (CMAP) with age, showing progressive muscle denervation and degeneration of motor neurons (**A**). **SOD1 hemi** mice also exhibit higher decreases in signal following repetitive nerve stimulation (RNS) at 12 and 16 weeks, indicating reduced transmission between muscle fibers and motor neurons through the neuromuscular junction (i.e., muscle recovery between stimuli is decreased; B). Serum NfL was also elevated in **SOD1 hemi** mice relative to **NCAR** mice at 12 and 16 weeks (**C**).

SOD1 Longitudinal Average Movement

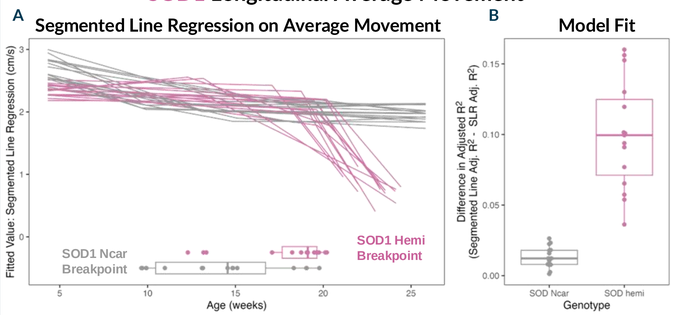

Figure 4. Average movement in **SOD1 hemi** mice drops near study endpoint and segmented line regressions have a median breakpoint of 19.1 weeks (**A**). **SOD1 hemi** segmented line model fits generally outperform straight line model fits relative to **NCAR** mice, strong evidence for change in slope (**SOD1 hemi** median adjusted R^2 increase: 0.0995; **B**).

RESULTS: FRIEDREICH'S ATAXIA

FXN Traditional Measures

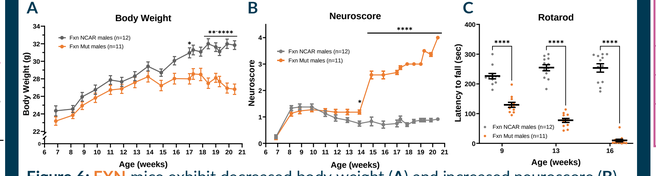

Figure 6: **FXN** mice exhibit decreased body weight (**A**) and increased neuroscore (**B**). Rotarod performance deficits were detected at 9, 13, and 16 weeks (**C**).

FXN Longitudinal Average Movement

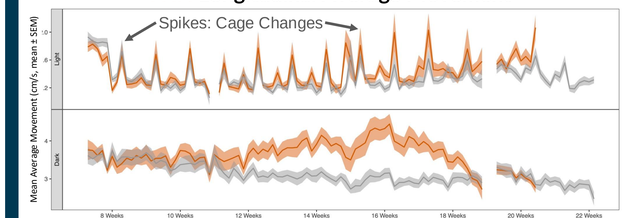

Figure 7: Longitudinal analysis of average movement demonstrates that **FXN** mice display increased movement in the dark period at approx. 12 weeks relative to their control **NCAR** mice. This phenotype reverses at approx. 16 weeks, and average movement declines in **FXN** mice until the study's endpoint criteria are reached.

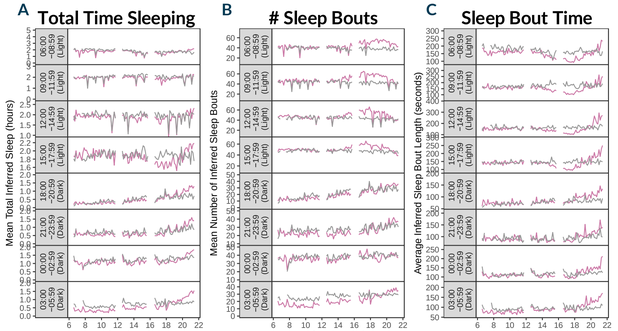

Figure 5. **SOD1 hemi** sleep disturbance relative to **NCAR** controls presents at 6 weeks toward end of dark period (**A**). **SOD1 hemi** mice progress to more sleep bouts in the dark period (**B**) that are shorter (**C**) during the light period after 14 weeks of age, reflecting fragmented sleep. Lines = mean, ribbons = ±SEM.

SUMMARY

Amyotrophic Lateral Sclerosis (ALS)

Readout	Preclinical Measures: Traditional: Measures	Preclinical Measures: Traditional: Age 1st Detected	Preclinical Measures: Digital: Measures	Preclinical Measures: Digital: Age 1st Detected	Clinical Measures
Activity & Coord.	Rotarod	Not by 16 WOA	Activity	7-8 WOA	Activity (Actigraphy) 6 min walk test
General Neuro.	Neuroscore	First score of 3+: 20 WOA	Combinatorial algorithms	*Digital Neuroscore*	ALS Functional Rating Scale-Revised (ALSFRS-R)
Sleep	Piezo sleep assay	N/A	Sleep/Wake time	7-8 WOA	Self-report Tracker (Fitbit) Sleep study

- Disrupted sleep patterns in **SOD1** mice:
  - Detected prior to traditional measures of clinical disease onset.
  - Sleep disruptions occur in patients with ALS.
- Identified unique breakpoint in **SOD1** mice (sudden decrease in average movement) correlated with a transition to end-stage disease phenotype:
  - Less subjective and less variability than traditional neuroscore → fewer mice for adequately powered studies.
  - Potential for continuous, non-invasive, longitudinal monitoring of disease progression → intervention at earlier timepoints, specific disease stages.

Friedreich's Ataxia

Readout	Preclinical Endpoints: Traditional: Measures	Preclinical Endpoints: Traditional: Age 1st Detected	Preclinical Endpoints: Digital: Measures	Preclinical Endpoints: Digital: Age 1st Detected	Clinical Measures
Activity & Coord.	Rotarod	9 WOA	Activity	Hyperactivity (dark phase): 12-19 WOA	Activity (Actigraphy) Timed walk tests 9-hole peg test
General Neuro.	Stargazing Neuroscore	14 WOA	Combinatorial algorithms	*Digital Neuroscore*	Friedreich Ataxia Rating Scale

- Identified new phases of disease progression in **FXN** mice:
  - Unique period of period of nocturnal hyperactivity in **FXN** mice from 12 weeks.
  - Decreased hyperactivity shortly before end-stage disease phenotype

CONCLUSIONS

The continuous, longitudinal monitoring provided by the **JAX Envision™** system has the potential to detect new phases of disease progression and allow for earlier detection of end-stage disease phenotypes without disturbing mice or requiring laborious manual scoring, leading to **improved data** and **smaller sample size** requirements than traditional measures.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the important contributions of a multidisciplinary team including Paige Martin, Steve Ciciotte, Michael Saul and the ML team at TLR Ventures.

DIGITAL IN VIVO ALLIANCE (DIVA)

The Digital In Vivo Alliance (DIVA) is a collaboration of pharmaceutical industry and academic scientists with a shared interested in the discovery, development, validation, and application of AI-enabled in vivo digital measures of animal behavior and physiology in their home cage environment. For more information, visit DIVA.bio.