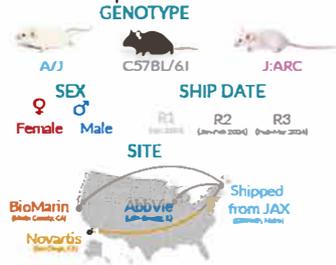


INTRODUCTION

- The **replication crisis** challenges all aspects of preclinical research.
- Factors that complicate cross-site replication are **interlaboratory variation and noise**, perhaps from short-duration studies.
- Digital *in vivo* measures show great promise in enhancing study replicability, potentially through **long-duration studies**.
- Variation in digital *in vivo* studies must be measured and mastered.

METHODS

- Study design based on a classic study interlaboratory variation study (Crabbe et al., 1999), was run over 3 replicates at 3 sites.



- 9-week replicability study: 21 days per cage, 3 mice per cage, collected **73,504 hours** of individual mouse behavior.
- Advanced machine learning from a beta version of the **JAX Envision™** platform used to generate the outcome measure of cage activity (average speed in cm/s per mouse).

EXPERIMENT TIMELINE (in Envision™ App)

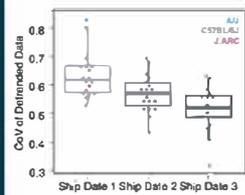


EARTAG PLACEMENT (enables individual animal tracking)

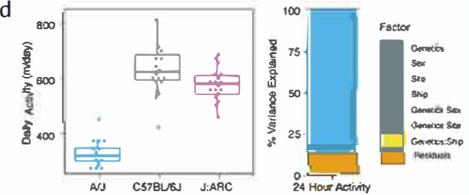
- Analysis used linear modeling:

$$\text{activity} = \beta_0 + \beta_1 \cdot \text{site} + \beta_2 \cdot \text{ship} + \beta_3 \cdot \text{genotype} + \beta_4 \cdot \text{sex} + \beta_5 \cdot (\text{genotype} \times \text{sex}) + \beta_6 \cdot (\text{site} \times \text{genotype}) + \beta_7 \cdot (\text{ship} \times \text{genotype}) + \epsilon$$
- Hourly aggregated data down-sampled at different durations.

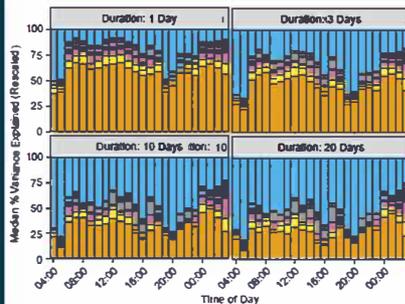
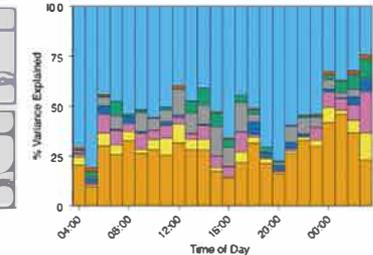
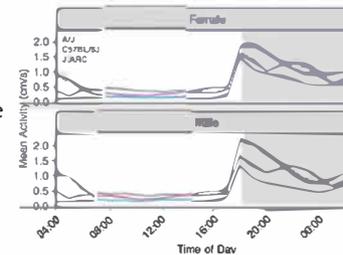
RESULTS



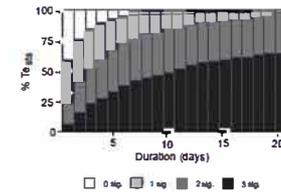
- Statistically significant differences were observed in hour-to-hour variation due to ship date (**left**). Earlier ship dates had higher coefficients of variation.
- Despite this, long-duration digital phenotyping elicits a highly replicable genotype effect in averaged 24-hour aggregates (**right**).



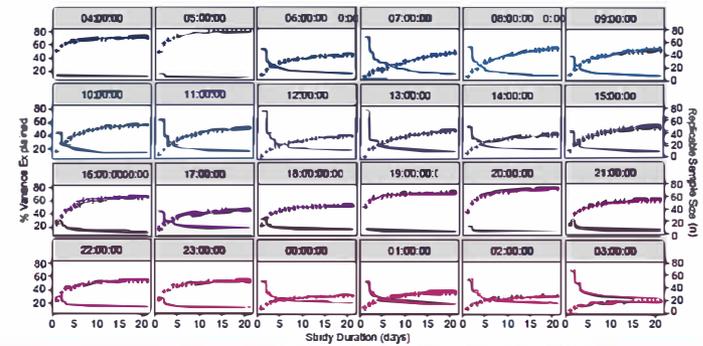
- Hour-to-hour aggregates showed genotype effects varying over the day (**near right**, mean \pm SEM).
- Resulting dataset demonstrates strong genotype effects during most hours.
- Residuals and technical terms were high during typical working hours (**far right**, 08:00-16:00).



- Down-sampled dataset indicated that **noise decreased with longer duration** (**left**, see residuals in orange).
- Increasing duration resulted in greater replication of statistically significant effects across sites (**right**, dark gray = replicated findings across three sites).



- Increasing study duration strongly increased effect size within the first 10 days of the study regardless of the hour of the day sampled (**right**, + signs).
- This resulted in **decreases in sample size required for replicable results** for each hour (**right**, stair step lines).
- Replicable sample size was lowest during the dark cycle.



CONCLUSIONS

- Digital *in vivo* studies **enhance experimental replicability**.
- In contrast to the usual short duration of behavioral experiments, **long-duration sampling in the home cage increases replicability**.
- The **time of day** is an important factor determining experimental replication.
- By sampling animals for longer, it is possible to **decrease the sample size** needed for a replicable experiment.

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The Digital In Vivo Alliance (DIVA) is a collaboration of pharmaceutical industry and academic scientists with a shared interest in the validation and application of AI-enabled *in vivo* digital measures. For more information, visit DIVA.bio.

