Discovery and development of digital biomarkers to inform home-cage neuro-behavioral assessments in the rodent

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DIGITAL IN VIVO ALLIANCE

ABSTRACT

Drug-induced neurobehavioral effects can lead to a host of unintended consequences that can be severe for patients leading to poor compliance or even death and development-limiting for sponsors. Accordingly, there are regulatory expectations to conduct a defined neurobehavioral assessment to support an Investigational New Drug Application (IND). These assessments are usually conducted as acute single dose studies in single-housed animals applying a protocol of mostly qualitative evaluations.

Advances in computer vision and machine learning (ML) offer an opportunity to re-invent the way neurobehavioral assessments are done in preclinical drug safety assessment. Continuous monitoring of animals in their home cage using computer vision enables the use of ML algorithms to derive 'digital biomarkers' that can provide continuous, quantitative, and objective reporting of 'taggable' behaviors in rodents. These digital approaches could be integrated into early dose-ranging toxicity studies, provide insights into repeat-dose effects and be more sensitive in detecting behavioral changes due to unintended neurological effects.

We collaboratively established a digital biomarker discovery and development pipeline to inform and support the development of a portfolio of digital biomarkers of rat behavior that will complement traditional neurobehavioral safety assessments. Our Digital Biomarker Development Plan included generation of 'discovery' video of untreated animals in home cage environment, identification of taggable behavioral features from those videos, and development of ML algorithms from the tagged data and experimental modulation of behavior to refine those models. A characterization and qualification strategy will ensure the analytical sensitivity and specificity of the resultant biomarkers as well as their toxicological relevance.

INTRODUCTION

Animal studies have supported the development of a growing portfolio of safe and effective medicines that are allowing patients to live longer and feel better. Those studies have been used across the drug discovery and development continuum from target validation; pharmacology modeling; characterization of absorption, distribution, metabolism and elimination; and even safety assessment. Though a broad set of biological endpoints are usually evaluated (particularly in the more standardized safety assessment studies), assessment of behavior is less frequent, more targeted, and often involves qualitative assessments.

Behavioral assessments are a primary endpoint in acute Central Nervous System (CNS) Safety Pharmacology Studies done to support a regulatory Investigational New Drug (IND) Application where the modified Irwin assessment is most common. Despite that assessment, patient-compliance or development-limiting CNS liabilities are occasionally revealed in human clinical trials. We hypothesized that earlier and more quantitative assessments of behavior using digital biomarkers in a home cage environment leveraging recent advances in automated computer vision sensors and machine learning (ML) might improve our ability to detect neurological liabilities at a time in development where we're better positioned to mitigate those liabilities without compromising the patent clock and with less resource.

> Digital biomarkers are characteristics or sets of characteristics, collected from digital health technologies, measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions.
>
> As defined by the U.S. Food & Drug Administration (Vasudevan et al., 2022)

The Digital In Vivo Alliance (DIVA) is a unique consortium of pharmaceutical leaders, innovative biotechs, and the public sector with a mission to collaboratively advance digital biomarkers for preclinical in vivo research through their development, validation, adoption, and regulatory acceptance. The Digital In Vivo Alliance collaboratively identifies biomarker development priorities with specific contexts of use across the drug discovery and development continuum. Members work collaboratively to share data, algorithms, and best practices in the discovery, development, application, and validation of digital biomarker to maximize the impact of in vivo research for therapeutic development and the advancement of our understanding of health and disease, supporting their adoption by industry, and developing plans to engage with regulators (e.g., US FDA) for acceptance in drug development.

We established a multi-disciplinary team of drug development scientists, basic researchers, and data scientists to define and develop digital biomarkers of locomotory activity as foundational capabilities in a portfolio of digital biomarkers that we believe will increase in sensitivity, specificity and biological resolution (e.g., gait analysis, social behaviors, etc.).

METHODS

Digital Biomarker Discovery and Development Life Cycle

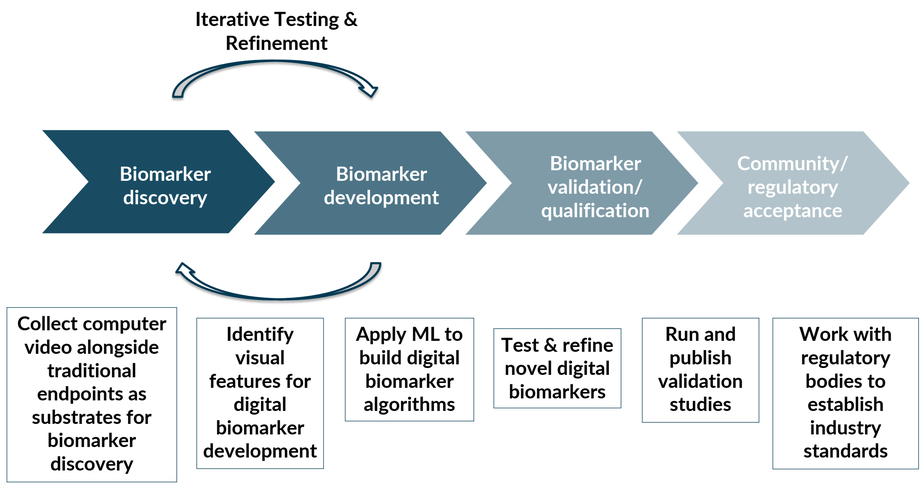

Figure 1. Digital biomarker discovery and design is supported by a multi-disciplinary collaboration that defines a relevant biomarker, identifies 'taggable features', designs studies that represent the biological activity of interest, and uses machine learning-based computational approaches to develop digital biomarker algorithms.

Digital Data Supply Chain and Biomarker Development Process

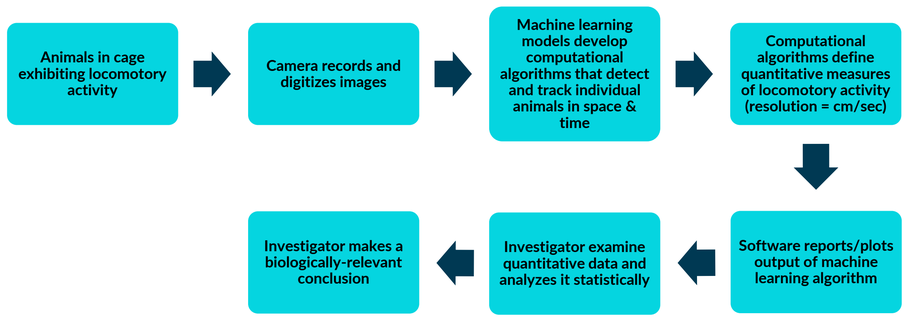

Figure 2. A digital data supply chain defines the path of raw data through the development process to the eventual application by end-users ensuring that the development process results in a relevant and useful product.

Enabling Sensor Technology

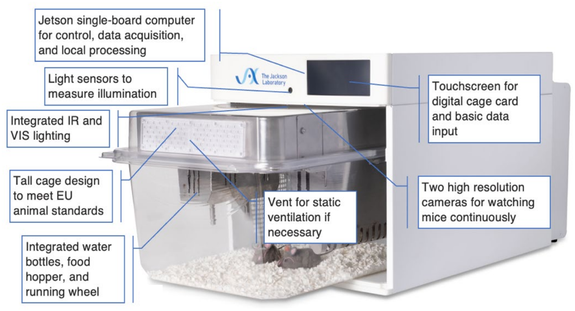

Figure3. Digital 'smart' cages incorporate computer vision biosensors into home cages that fit existing infrastructure within the vivarium allowing seamless integration into existing workflows. Continuous raw video is streamed to a secure cloud for algorithm development and analysis.

ML Development Schema & Taggable Features

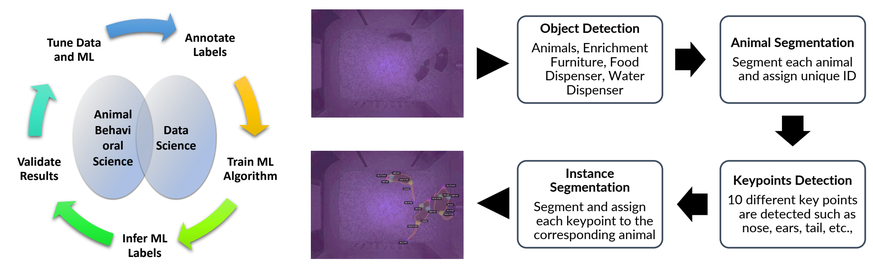

Figure 4. ML Development Schema (left). ML workflow to extract taggable features (right)

Neurobehavioral Proof-of-Concept Study Design

Cage.name	Treatment	MouseNO.EoS	Dose 1_Time	Dose 2_Time
C1	Vehicle	3	6/12/23 7:37 AM	6/15/23 4:49 PM
C2	Vehicle	3	6/12/23 7:40 AM	6/15/23 4:53 PM
C3	Vehicle	3	6/12/23 7:39 AM	6/15/23 4:51 PM
C12	Vehicle	1	6/12/23 7:41 AM	6/15/23 4:54 PM
C4	Caffeine4mpk	3	6/12/23 7:43 AM	6/15/23 4:47 PM
C5	Caffeine4mpk	3	6/12/23 7:45 AM	6/15/23 4:59 PM
C6	Caffeine4mpk	3	6/12/23 7:47 AM	6/15/23 5:01 PM
C7	Caffeine4mpk	3	6/12/23 7:48 AM	6/15/23 5:04 PM
C8	Caffeine16mpk	1	6/12/23 7:57 AM	6/15/23 5:13 PM
C9	Caffeine16mpk	3	6/12/23 8:00 AM	6/15/23 5:14 PM
C10	Caffeine16mpk	3	6/12/23 8:02 AM	6/15/23 5:17 PM
C11	Caffeine16mpk	3	6/12/23 8:04 AM	6/15/23 5:22 PM
C13	Chlorpro4mpk	3	6/12/23 8:06 AM	6/15/23 5:22 PM
C14	Chlorpro4mpk	3	6/12/23 8:07 AM	6/15/23 5:24 PM
C15	Chlorpro4mpk	3	6/12/23 8:09 AM	6/15/23 5:26 PM
C16	Chlorpro4mpk	3	6/12/23 8:11 AM	6/15/23 5:28 PM
C17	Chlorpro16mpk	2	6/12/23 8:12 AM	6/15/23 5:29 PM
C18	Chlorpro16mpk	3	6/12/23 8:14 AM	6/15/23 5:31 PM
C19	Chlorpro16mpk	3	6/12/23 8:15 AM	6/15/23 5:33 PM
C20	Chlorpro16mpk	3	6/12/23 8:17 AM	6/15/23 5:35 PM

- Species/strain: 7-week-old, CD1 male mice
- Lab light cycle: 6AM-6PM
- **Dose 1**: approx. 1 hour after light cycle on June 12
- **Dose 2**: approx. 1 hour before dark cycle on June 15
- Cages in red excluded from analysis

Treatment	# of Cages (N)
Vehicle	3
Caffeine4mpk	4
Caffeine16mpk	3
Chlorpro4mpk	4
Chlorpro16mpk	3

Th	F	Sa	Su	M	Tu	W	Th	F	Sa
6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17

Figure 5. Short, single dose studies with well-characterized neuroactive compounds (caffeine, chlorpromazine) were done in male CD1 mice and Sprague Dawley rats to determine if continuous monitoring with a locomotory activity digital biomarker would reveal expected changes in activity behavior but also reveal changes that wouldn't be detected in usual cage side observations. The data represented here is from CD1 mice. A locomotory activity digital biomarker is in development for the rat.

RESULTS

Exploring approaches to data visualization and analysis

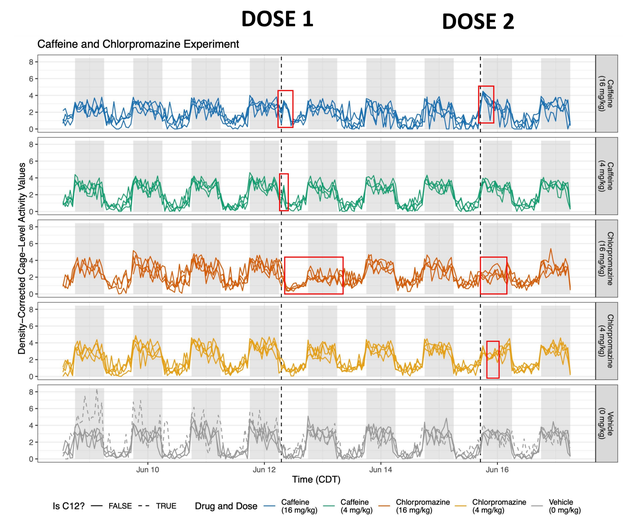

Figure 6. Graphing of the raw activity data at the cage level over the course of the study revealed a regular pattern of circadian variability with expected increases in activity during the dark cycle and decreased activity during the light period. It also revealed dose dependent changes in activity associated with dosing of either caffeine as a neurostimulant or chlorpromazine as a neurodepressant.

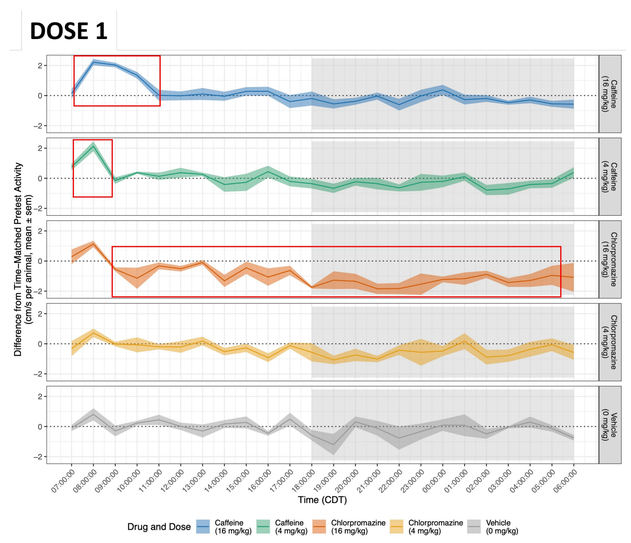

Figure 7. Normalizing the data to time-matched pre-dose levels smooths the within cycle and circadian variability and increases the sensitivity for visualizing the magnitude and duration of the effects.

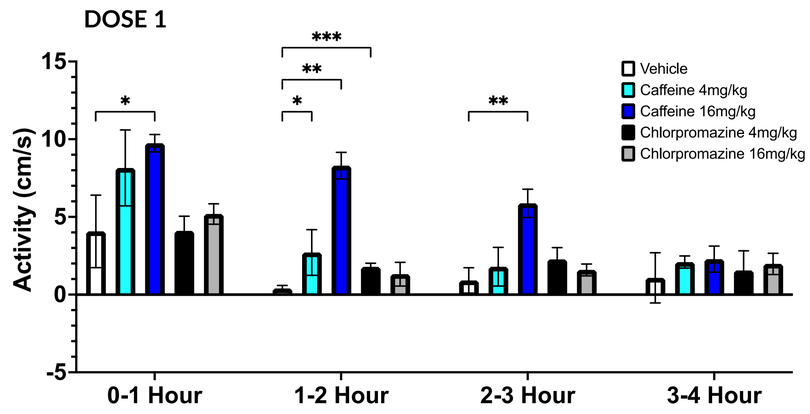

Figure 8. Statistical analysis of the home cage-level raw activity data revealed agent and dose-dependent statistically-significant changes in activity. This data focuses on the initial 4-hour period when internal exposures were expected to be highest.

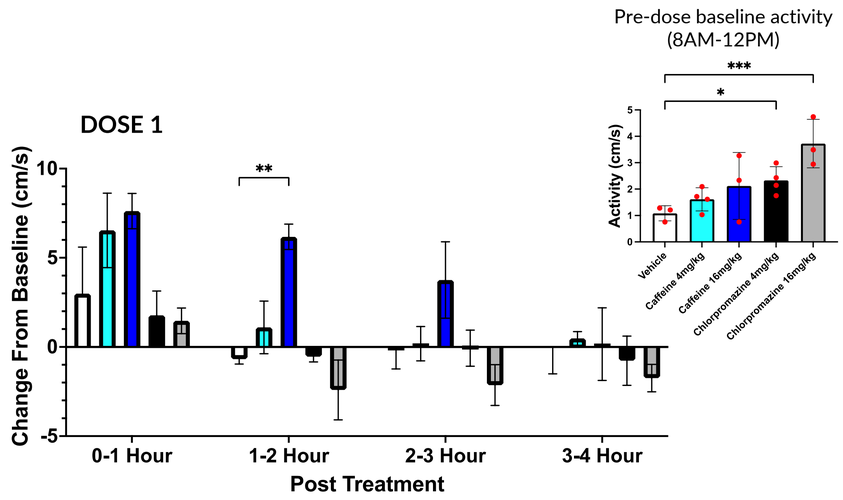

Figure 9. Statistical analysis of pre-dose baseline data revealed significant variability in that baseline data that was reflected in and undermined treatment-related effects.

CONCLUSIONS

Digital biomarker discovery and development requires a multidisciplinary partnership between disease biologists, animal scientists and data scientists. The biologists define the biological events to be 'biomarked', their contexts of use and analytical approaches. Animal scientists provide guidance on vivarium management, animal cage environment and husbandry practices that influence how the sensor technology is incorporated into the home cage environment. Data scientists apply machine learning (ML) approaches to develop algorithms that translate raw sensor (e.g., video) data to quantitative measures. Statisticians and analytical data scientists design approaches to evaluate continuous data to inform relevant decisions.

- Single dose challenges with either caffeine or chlorpromazine were expected to increase or decrease locomotory activity for short periods of time in mice and rats. It was not known whether dosing at the beginning of the light cycle (the usual practice in drug safety assessment) for either compound would have a different effect than dosing at the beginning of the dark cycle which is not usual due to logistical complexities (e.g., animal care staff/study scientists working through the night hours).
- The ML-defined locomotory activity digital biomarker was able to detect the expected effects at the cage level in mice (rat data pending) and characterize their temporal pathogenesis with high sensitivity during both the light and dark cycles.
- There was a difference in the effects when dosing occurred at the beginning of the light cycle vs. the dark cycle. For example, there was a much more prolonged depressant effect of a high dose of chlorpromazine when dosed during the light cycle rather than the dark.
- Baseline normalization of the locomotory activity data increases the sensitivity for detecting treatment-related effects but that sensitivity is undermined by baseline variability. It will be useful in the future to randomize animals to treatment groups using baseline locomotory activity assessments during the acclimation period.
- Digital biomarkers applied to animals in a home cage environment will provide a unique opportunity to detect treatment effects earlier in development and likely redefine our understanding of the pathogenesis of those effects with more continuous monitoring.

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