Abstract #4342

Machine Learning-Enabled Digital Toxicology for Enhanced Discovery and Decisions

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ABSTRACT

Rapid advances in sensor technologies and computational capabilities, including artificial intelligence and machine learning-based approaches, provide a unique opportunity to enhance the value of animal studies. Complementing our usual biochemical, hematological, and histopathological assessments with continuous measures of behavior and physiology would provide a more dynamic, biologically-, and clinically-relevant characterization of potential drug safety liabilities. Non-invasive endpoints collected continuously and throughout their circadian cycle from animals in their home cage will provide novel insights but also require different approaches to data interpretation, decision-making, and study design. Rigorous validation processes applied to new capabilities will build confidence in the accuracy and relevance of the data generated by those capabilities.

As proof of concept, mice and rats were housed in individually-ventilated cages outfitted with computer vision cameras with infrared detection capabilities allowing continuous monitoring of group-housed animals throughout both light and dark cycles. Mice and rats were treated with a variety of test articles known to induce changes in activity (e.g. caffeine, chlorpromazine) and/or induce epileptic seizures (e.g. PTZ). Continuous, objective, and quantitative assessment of defined behaviors was done using machine learning-enabled algorithms applied in real time to raw computer vision video. These behaviors included activity, loss of righting reflex (LORR), and respiration.

Machine learning-defined digital measures applied to home cage computer video detected and quantitatively characterized dose-responsive changes in activity in mice and rats given single doses of caffeine or chlorpromazine. The onset and duration of effect was observationally and statistically identifiable in individual animals as a deviation from time-matched baseline activity. Chemically-induced seizures induced by pentylenetetrazole (PTZ) were also reliably detected with a digital measure for LORR.

The objective and quantitative measures revealed by digital sensors and ML-informed algorithms of animal behavior can significantly contribute to the primary aims of a toxicology study by detecting a test article-related effect as well as informing its exposure relationship, character, magnitude, duration, reversibility, adversity, and monitorability. A more dynamic and temporal characterization also allows a better integration with the plasma toxicokinetics of the test article providing insights into potential modes of action and likelihood of accommodation or progression.

STUDY DESIGNS

Mouse Neuromotor Proof of Concept

Cage	Treatment	N	Dose 1 time	Dose 2 time
C1	Vehicle	3	7:37 AM	4:49 PM
C2	Vehicle	3	7:40 AM	4:53 PM
C3	Vehicle	3	7:39 AM	4:51 PM
C12	Vehicle	1	7:41 AM	4:54 PM
C4	Caffeine 4 mg/kg	3	7:43 AM	4:47 PM
C5	Caffeine 4 mg/kg	3	7:45 AM	4:59 PM
C6	Caffeine 4 mg/kg	3	7:47 AM	5:01 PM
C7	Caffeine 4 mg/kg	3	7:48 AM	5:04 PM
C8	Caffeine 16 mg/kg	1	7:57 AM	5:13 PM
C9	Caffeine 16 mg/kg	3	8:00 AM	5:14 PM
C10	Caffeine 16 mg/kg	3	8:02 AM	5:17 PM
C11	Caffeine 16 mg/kg	3	8:04AM	5:22 PM
C13	Chlorpro 4 mg/kg	3	8:06 AM	5:22 PM
C14	Chlorpro 4 mg/kg	3	8:07 AM	5:24 PM
C15	Chlorpro 4 mg/kg	3	8:09 AM	5:26 PM
C16	Chlorpro 4 mg/kg	3	8:11 AM	5:28 PM
C17	Chlorpro 16 mg/kg	2	8:12 AM	5:29 PM
C18	Chlorpro 16 mg/kg	3	8:14 AM	5:31 PM
C19	Chlorpro 16 mg/kg	3	8:15 AM	5:33 PM
C20	Chlorpro 16 mg/kg	3	8:17 AM	5:35 PM

- Species/strain: 7 w.o. CD1 male mice
- Lab light cycle: 6 AM-6 PM
- **Dose 1**: approx. 1 hr. after light cycle
- 48 hr. washout
- **Dose 2**: approx. 1 hr. before dark cycle
- Cages in red excluded from analysis

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

Rat Neuromotor Proof of Concept

Cage	Treatment	N	Dose 1 time	Dose 2 time
C1	Vehicle	2	6:58 AM	4:47 PM
C2	Vehicle	2	6:59 AM	4:49 PM
C3	Vehicle	2	7:00 AM	4:50 PM
C4	Vehicle	2	7:01 AM	4:51PM
C5	Caffeine 4 mg/kg	2	7:04 AM	4:54PM
C6	Caffeine 4 mg/kg	2	7:05 AM	4:56 PM
C7	Caffeine 4 mg/kg	2	7:07 AM	4:58 PM
C8	Caffeine 4 mg/kg	2	7:09 AM	4:59 PM
C9	Caffeine 24 mg/kg	2	7:12 AM	5:02 PM
C10	Caffeine 24 mg/kg	2	7:14 AM	5:04 PM
C11	Caffeine 24 mg/kg	2	7:16 AM	5:05 PM
C12	Caffeine 24 mg/kg	2	7:18 AM	5:07 PM
C13	Chlorpro 4 mg/kg	2	7:20 AM	5:10 PM
C14	Chlorpro 4 mg/kg	2	7:22 AM	5:11 PM
C15	Chlorpro 4 mg/kg	2	7:24 AM	5:13 PM
C16	Chlorpro 4 mg/kg	2	7:25 AM	5:15 PM
C17	Chlorpro 16 mg/kg	2	7:28 AM	5:18 PM
C18	Chlorpro 16 mg/kg	2	7:30 AM	5:20 PM
C19	Chlorpro 16 mg/kg	2	7:32 AM	5:21 PM
C20	Chlorpro 16 mg/kg	2	7:34 AM	5:24 PM

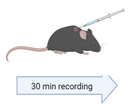
- Species/strain: 6 w.o. SD male rat
- Lab light cycle: 6 AM-6 PM
- **Dose 1**: approx. 1 hr. after light cycle
- 48 hr. washout
- **Dose 2**: approx. 1 hr. before dark cycle
- Cages in red excluded from analysis

Treatment	# Cages
Vehicle	4
Caffeine 4 mg/kg	4
Caffeine 24 mg/kg	4
Chlorpro 4 mg/kg	4
Chlorpro 16 mg/kg	4

Figure 1. 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 an activity digital biomarker would reveal expected changes in activity behavior.

Mouse Seizure Proof of Concept

S.C. Pentylenetetrazole (PTZ) injection (80 mg/kg)



Manual Annotation of Loss of Righting Reflex (LORR)

Manual Selection of Labeled Frames

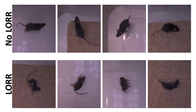


Figure 2. Inducible PTZ seizure assay in WT C57BL6/J mice recorded behavior in mice in digital cages for 30 mins.

METHODS

Enabling Sensor Technology

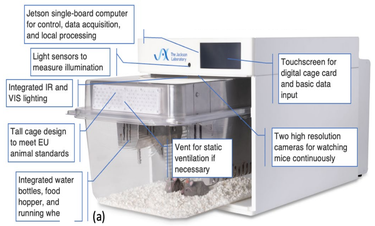


Figure 3. Digital 'smart' cage prototypes incorporate computer vision biosensors into home cages that fit existing infrastructure within the vivarium allowing seamless integration into existing workflows. Continuous raw video is streamed to a secure cloud for algorithm development and analysis.

Individualized animal behavior data enabled by specialized ear tags

Digital Biomarker Development Life Cycle

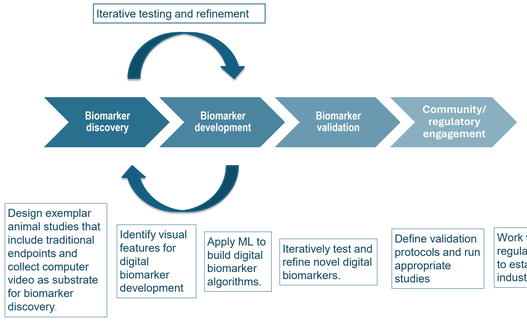
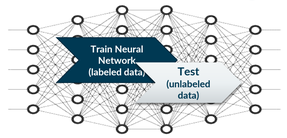


Figure 4. Digital biomarker discovery and development are supported by a multi-disciplinary collaboration that defines a relevant biomarker, identifies 'taggable features', designs studies that represent those features, and uses machine learning-based approaches to develop digital biomarker algorithms. The quantitative outputs of those algorithms are validated for their analytical rigor and clinical relevance.

ML-defined Digital Biomarker Algorithms



Neural Network

Pros:
- Preserves information from original data
- No need to manually define heuristics

Cons:
- Not explainable

Figure 5. Neural networks are 'trained' with labeled video frames demonstrating the behavior or characteristic of interest. The model is then tested and cross-validated against a set of 'testing' data. As cases are discovered where the model does not perform well, additional labeled video frames representing the new cases can be added to the training set and used to augment the model. Iterative cycles of training and testing improve the sensitivity and specificity of the algorithm.

RESULTS- Mouse Neuromotor

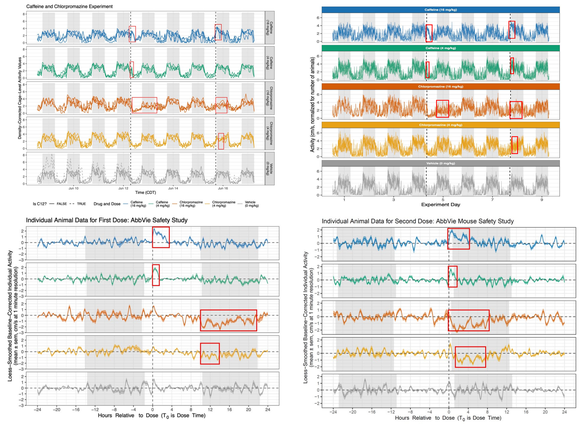


Figure 6. Male CD1 mice were treated with single oral doses of either caffeine or chlorpromazine at 2 different dose levels and at 2 different times in their daily circadian cycle. Continuous monitoring with computer vision and quantitation of activity revealed an expected circadian cycle of activity with the mice significantly more active during the dark hours but also changes in activity level at both the cage and individual animal level induced by treatment with either caffeine (increased activity) or chlorpromazine (decreased activity). Variability in the duration of those responses was dose-related. The ability to monitor during the dark cycle and even expose the animals to drug during the dark cycle affected their response (e.g. note the delayed decrease in activity in response to chlorpromazine in mice dosed at the beginning of the light cycle. Analysis of time normalized data increased the sensitivity for detecting a change.

RESULTS- Rat Neuromotor

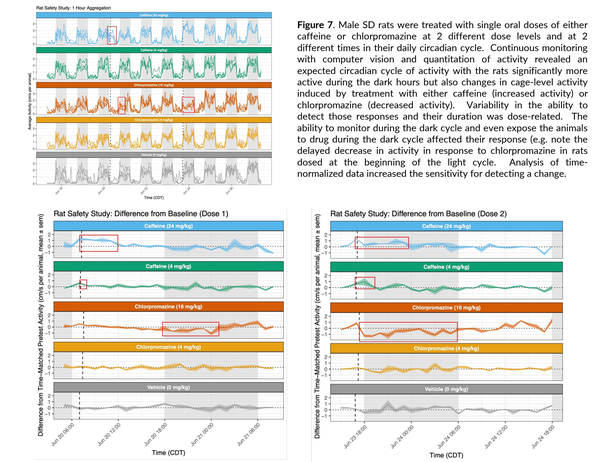
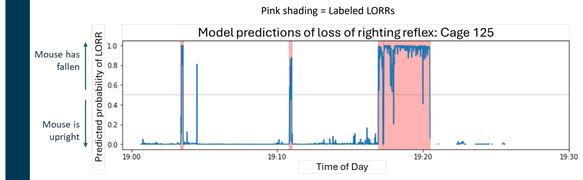


Figure 7. Male SD rats were treated with single oral doses of either caffeine or chlorpromazine at 2 different dose levels and at 2 different times in their daily circadian cycle. Continuous monitoring with computer vision and quantitation of activity revealed an expected circadian cycle of activity with the rats significantly more active during the dark hours but also changes in cage-level activity induced by treatment with either caffeine (increased activity) or chlorpromazine (decreased activity). Variability in the ability to detect those responses and their duration was dose-related. The ability to monitor during the dark cycle and even expose the animals to drug during the dark cycle affected their response (e.g. note the delayed decrease in activity in response to chlorpromazine in rats dosed at the beginning of the light cycle. Analysis of time-normalized data increased the sensitivity for detecting a change.

RESULTS- Mouse Seizure



	Predicted non-LORR	Predicted LORR
True non-LORR	188	9
True LORR	7	172

Model trained on PTZ-data (tested on PTZ data)

96% of true LORR samples are identified by the model
5% of LORR detections are false positives

Figure 8. Video of acute seizures induced in male C57/BL6/J mice with a single injection of pentylenetetrazole (PTZ) was used to trained an ML algorithm to detect loss-of-righting reflex (LORR) as a stereotypical 'seizure' behavior. Testing of the algorithm with video from the same study but not used to train the algorithm revealed good specificity. The algorithm will be evaluated and refined further using video data from more traditional models of epilepsy like the SCN1a model of juvenile onset heritable epilepsy.

CONCLUSIONS/SUMMARY

- Sensor-based and AI-enabled digital measures of behavior and physiology from mice and rats in their home cage environment are an opportunity to increase the informativeness, objectivity, and translational relevance of our non-clinical toxicology studies.
- The continuous, quantitative and objective data provided by these approaches will reveal novel xenobiotic effects and enable a more accurate characterization of their temporal onset, duration, magnitude, progression or reversibility, and relationship to systemic exposure kinetics.
- The data represented here provide an important proof-of-concept that will require further refinement of these core measures to improve their informativeness and generalizability. They also provide a basis for a rapidly growing portfolio of digital measures that will complement and contextualize our traditional measures.
- The usefulness of these measures is highly dependent on our confidence in their analytical and clinical validity. Accordingly, the DIVA team is developing a framework of verification and validation that will support the application and acceptance of these novel measures.
- **Conflict of Interest-** The authors report no financial conflicts of interest.
- Presenting author can be contacted at brberridge@b2pathologysolutions.com

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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. The DIVA members presenting this work gratefully acknowledge the efforts of members from AbbVie and Biomarin who generated the proof-of-concept work represented here. For more information, visit DIVA.bio.