dizziness, headaches) persist in clinical trials and as post-marketing adverse effects. 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. 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. This poster will outline our digital biomarker discovery and development process, share representative examples of various contributions to that process, preliminary outputs and our validation/qualification strategy.

doi:10.1016/j.vascn.2025.107661

## 069

## Building a robust CNS screening strategy: Integrating in vivo and in vitro assays to better predict CNS risk

David A. Connor, Matthew Cato, XueJun Wu, Shannon Tringola, Eric I. Rossman, Ajeesh K. Cherian

*GSK, Collegeville, PA, USA*

Abstract

Attrition within clinical development due to central nervous system (CNS)-related adverse events continues to highlight the need to refine CNS screening strategies. The primary preclinical method to assess CNS risk is the Irwin/Functional Observation Battery (FOB). However, these tests are significantly limited by subjective assessment and temporal resolution. Automated neurobehavioral assessment, such as the Home Cage Analyzer (HCA), can overcome these issues. In addition, in vitro methods, such as microelectrode array (MEA), provide greater throughput and improved translation when using human iPSCs. Ideally, the combination of neurobehavioral and electrophysiological endpoints would converge to provide a clearer picture of CNS perturbance. Critically, a robust CNS screening strategy hinges upon understanding the limitations of such methods and identifying potential gaps in sensitivity between them. This study aimed to validate the Home Cage Analyzer (HCA) and test for convergence or difference in sensitivity between in vivo and in vitro screening using potent CNS active compounds from two distinct drug classes: psychostimulants and sedatives. For HCA experiments, eight male Wistar Han rats (Charles River) were implanted with temperature sensitive RFID transponders (Biomark USA) and pair housed in individually ventilated cages (Tecniplast). Rats were maintained under a 12-h light/dark cycle and habituated to the testing room and HCA rack for 1 week prior to initiation of dosing. Using a crossover Latin-square design, each rat received a single dose of d-amphetamine (0.25, 1, or 3 mg/kg), diazepam (0.5, 1.5, 5 mg/kg), or saline/vehicle. For MEA experiments, hiPSC-derived cortical neuron/astrocyte network (Fujifilm CDI) or rat cortex (E18.5; QBM Biosciences) was cultured in a Maestro Multi-electrode Array System (Axion BioSystems; 48-well plate). l-amphetamine in saline (0.12, 0.37, 1.11, 3.33, 10, 30 μM) or diazepam in DMSO (0.1, 0.3, 1, 3, 10 μM). As expected, we found a dose-dependent increase in activity after treatment with amphetamine in the HCA. Conversely, rats treated with diazepam showed decreased activity levels within the HCA. In line with neurobehavioral effects, diazepam caused a significant decrease in spiking and burst frequency in the MEA, resulting in a well-defined sedative phenotype. Surprisingly, amphetamine application in iPSCs exhibited no phenotypic response within the MEA, including no change in burst rate. Importantly, this lack of an effect in the MEA could be interpreted as a "clean" result, thus representing a missed hazard. In contrast, diazepam demonstrated consistent effects in the HCA and MEA. Together, these results indicate complementarity between in vitro MEA assay and in vivo automated HCA when screening sedatives while revealing a gap between these assays for amphetamine. Thus, a multi-modal approach is critical to a robust CNS screening strategy.

doi:10.1016/j.vascn.2025.107662

## 070

## Pharmacokinetics (PK) of psilocybin and psilocin in plasma following intravenous administration of psilocin and oral administration of psilocybin to male beagle dogs

Ines de Lannoy[a], Lilia Magomedova[a], Julia Izhakova[a], Christina de Rivera[a], James Lanthier[b], Jason Atkinson[b], Guy A. Higgins[a], Malik Slassi[b], Joseph A. Araujo[b]

[a]*Transpharmation Canada Ltd., Toronto, ON, Canada*
[b]*Mindset Pharma Inc., Toronto, ON, Canada*

Abstract

Psychedelic drugs such as psilocybin (PSY) are becoming widely recognized for their therapeutic potential to treat psychiatric conditions, including depression and addictive disorders. PSY is rapidly metabolized to psilocin (PSI) which underlies its primary pharmacological properties. Despite PSY being evaluated in Phase 2/3 clinical trials, relatively little preclinical data for PSY exists. The present study evaluates the PK of PSY and PSI following a single 0.3 mg/kg intravenous (i.v.) dose of PSI and a single oral (p.o.) dose of 1 mg/kg PSY to groups of three male Beagle dogs. The concentration of PSY and/or PSI was determined in serially collected blood plasma samples using a qualified LC-MS/MS method. Following i.v. dosing of PSI, plasma concentration profiles exhibited a biphasic decay. The estimated half-life ($t_{1/2}$) of psilocin averaged 2.15 h. The systemic clearance was 1520 mL/h/kg and steady-state volume of distribution was 3190 mL/kg. Following p.o. dosing of PSY, PSY plasma concentrations were either below the lower level of quantitation (<1 ng/mL) or not measurable (no peak). Maximum plasma concentrations of PSI (mean $C_{max} = 111$ ng/mL) were attained at 1 h post-dose. The estimated apparent half-life of PSI was similar (2.46 h) to that following i.v. PSI administration. The p.o. bioavailability of PSI calculated based on the assumption that PSY is completely metabolized to PSI was 64.8 %. A few clinical signs were observed following dosing and appeared to be due to the pharmacology of PSI (5HT-2A receptor agonist). The dogs were examined at 30 min (i.v. dosing) and 60 min (p.o. dosing) post-dose. All dogs exhibited abnormal mydriasis and had no spontaneous blink, and thus, eye lubricant was regularly applied to prevent corneal ulceration. One of the 3 dogs administered PSI i.v. was vocal and anxious but settled down when comforted by staff. No other major clinical signs were observed. All dogs appeared normal when offered food at ~6 h post-dose. The current study demonstrates that PSY is rapidly metabolized to PSI in the dog and that the half life of PSI is similar following both i.v. administration of PSI and oral administration of PSY. Moreover, at

the doses used in the current study, behavioral effects were linked to the anticipated pharmacological effects of the PSI and no dose-limiting effects were observed.

doi:10.1016/j.vascn.2025.107663

## 071
## Establishment of functional observational battery test in large animals (dogs and non-human primates)

Xiaoyue Liang, Jufeng Wang, Bin He

*Pharmaron, Beijing, Beijing, China*

Abstract

This study is to establish the functional observational battery (FOB) tests in large animals (dogs and non-human primates) at our facility, which provided the safety pharmacology evaluation of the test substance on the central nervous system in non-rodent species. For dog FOB study, 16 beagle dogs were assigned to four groups (saline, morphine, ethanol, and methanol). Neurological functions (FOB) were evaluated prior to dose initiation, 0.5 h and 24 h post-dose. For non-human primates FOB, six cynomolgus monkeys were used. All animals received the same treatments, including giving saline and 5.6 mg/kg ketamine *via* intramuscular injection (i.m.) on Day 1 and Day 3, and 6.4 mg/kg amphetamine sulphate *via* oral administration on Day 7, Day 10 and Day 22, respectively. FOB observation was evaluated in cynomolgus monkeys prior to dose initiation, 1 h and 24 h post-dose with saline (i.m.) and ketamine, and at 2, 24 and 48 h post-dose with amphetamine sulphate. Our results were 1) For the FOB study in beagle dogs, treatment of morphine changed the posture, produced abnormal gait, affected the cranial nerves (decreased olfactory response) and vestibulocochlear function (loss gag reflex, no response in hypoglossal assessment), and decreased postural reaction; treatment of ethanol produced abnormal gait, decreased postural reaction and motor activity; treatment of methanol produced salivation, emesis, abnormal excreta, abnormal gait, and decreased postural reaction. 2) For the FOB study in non-human primates, abnormal findings are listed as follows: Treatment with ketamine produced low arousal, abnormal posture (sitting), decreased locomotor activity, abnormal response to food (unable to chew/swallow), slow respiration, ataxia, dysmetria, absent vocalization and proprioception; Treatment with amphetamine sulphate produced high arousal, increased locomotor activity positive Babinski reflex with dose-response. In conclusion, the above results showed that dog and monkey were ideal animal models to evaluate drug effect to neurological function.

doi:10.1016/j.vascn.2025.107664

## 072
## An iPSC-derived neurotoxicity screening platform demonstrates compound toxicity on calcium activity, synapses, viability and cell proliferation

Md Fayad Hasan, Kara L. Gordon, Christine G. Rines, Nicole Suarez, Ameena Pascua, Alyson Smith, Patrick McDonough, Jeff Price

*Vala Sciences, San Diego, CA, USA*

Abstract

Neurotoxicity is a leading cause of drug failures preclinically and clinically. Development of predictive *in-vitro* systems for CNS safety testing that are predictive, high throughput, and reduce the use of animals in testing are desirable. In this study, we sought to use human induced pluripotent stem cells (hiPSC) to develop and validate three platforms for toxicity testing. The platforms include neural progenitor cells (NPCs), two-dimensional, and three-dimensional cultures of mature neurons, astrocytes and microglia. We tested a range of compounds such as chemotherapy agents, channel effectors, environmental toxicants, and HIV anti-retroviral drugs (ARV) to assess outcomes such as cell viability, proliferation, calcium imaging, and synapses. We developed assays for NPCs or mixed cultures in 384-well plates. Cultures were treated with compounds for acute (min-hours) or chronic time points (days). For live cell assays, nuclei were stained with a nuclear dye for single cell identification and viability measurements. Click-iT EdU kits were used to assess cell proliferation and fluorescent calcium dyes for imaging calcium transients. Fixed endpoint labeling was performed with antibodies detecting neurites, pre- and post-synaptic markers for neurite measurements and synapse count. High speed imaging and fixed sample imaging was performed on Vala Science's Kinetic Image Cytometer (KIC IC200) and single cell analysis was performed on Vala's CyteSeer Image Analysis software. We observed a decrease in NPC viability and cell proliferation after ARV treatments. Calcium activity was reduced in neuron co-cultures after treatment with ARVs and carboplatin. 4-aminopyridine increased both transients and synchronization of neuron culture models. ARVs and lead reduced neuronal synaptic puncta. In conclusion, we have demonstrated the ability to quantify toxic effects of clinically relevant compounds in these three hiPSC-derived platforms that are relevant for studying neurotoxicity in neurodevelopment and in developed CNS systems. We plan to develop more assays for assessing toxicity in these platforms such as mitochondrial and ER toxicity.

doi:10.1016/j.vascn.2025.107665

## 073
## Multiplexed cell-based assays for evaluating the structure and function of excitable cells

Kjell Steps, Austin Passaro, Daniel Millard, Denise Sullivan, Ben Streeter, Stacie Chvatal

*Axion BioSystems, Atlanta, GA, USA*

Abstract

The flexibility and accessibility of induced pluripotent stem cell technology has allowed complex human biology to be reproduced *in vitro* at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of *in vitro* models of neuron and cardiomyocyte electrophysiology to be used in screening applications in drug discovery and safety. Furthermore, advanced cell preparations, such as organoids, are under investigation with aims toward establishing mature human phenotypes *in vitro*. For the development and validation of relevant *in vitro* neuronal and cardiac models, it is critical to evaluate the structure and function of neuronal synapses and networks, as well as cardiomyocyte viability, electrophysiology, and contractility. The objective of this work is to develop and validate a multiplexed structure-function assay as an efficient approach for evaluating neuronal and cardiomyocyte models *in vitro*. A planar grid of microelectrodes embedded in the substrate of each well interfaces with cultured cellular networks to continuously monitor both electrophysiological function and structural viability. The electrodes detect the raw electrical activity from the cells to identify changes in function, while structural effects, such as morphological changes and cell viability, are detected as changes in impedance at the cell-