446.21/EE1 - Vium Digital Vivarium™ Platform Enables Automated Disease and Drug Efficacy Assessment in an Experimental Autoimmune Encephalomyelitis Animal Model of Multiple Sclerosis

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Introduction

Current methods for screening and evaluating new therapeutics in rodent disease models are not only labor-intensive, but are also confronted by several challenges, including the ability to consistently produce unbiased and reproducible data. In Multiple Sclerosis (MS) pre-clinical research, one of the most commonly used rodent models is the Experimental Autoimmune Encephalomyelitis (EAE) model, where disease is induced by delivering an antigenic myelin-derived peptide[1,2]. EAE mouse models present with disease signs, including inflammation, demyelination, and motor dysfunction, which recapitulate human disease[1,2]. Researchers commonly score clinical signs of disease using a manual scoring system that rates disease severity at various stages[3]. Manual clinical scoring is not only labor-intensive, but may also be subjected to variability and possible side-effects produced by daily handling[4,5].

Vium has created a Digital Vivarium™ that uses an intelligent sensor and HD camera network along with computer vision, data algorithms, and cloud computing to improve *in vivo* research. The digital platform was used to identify behavioral and physiological signatures of disease in a mouse Myelin Oligodendrocyte Glycoprotein (MOG)-induced EAE model of MS. The Vium Digital Vivarium:

- Records continuous (24/7) metrics, such as motion and breathing rate
- Provides health endpoints with limited human interaction
- Enables efficacy screening of therapeutic compounds
- Allows real-time, remote data access over the internet to enable rapid decisions
- Retains data in auditable electronic and videographic records

Compared to conventional clinical scoring, we found that Vium's automated metrics were able to reproducibly detect MS disease, disease severity, and therapeutic efficacy of the standard of care (SOC) compound Fingolomid (FTY-720), a sphingosine-1-phosphate-receptor modulator.

Materials and Methods

Experiments were conducted in Vium's AAALAC-accredited Digital Vivarium™ in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by Vium's Institutional Animal Care and Use Committee.

Animals: 10-week old female C57BL/6 mice (Jackson Laboratory) were randomized into study groups, such that all groups had similar baseline body weights and motion profiles prior to induction. Mice were inoculated with subcutaneous injections of 50 µg/location (100 µg total) of MOG(1-125) or 100 µg/location (200 µg total) of MOG(35-55) in Complete Freund's Adjuvant (CFA) emulsion from a pre-loaded syringe (Hooke Laboratory). Inoculated mice were injected twice with 200 ng (400 ng total) of pertussis toxin (PTX): the first injection administered 1-6 hours post-MOG-CFA administration and the second injection 24 hours after the first PTX injection. Control (non-induced) mice were injected with saline.

Tracking and Measurement of MS: Post-induction, bodyweight and clinical status of mice were monitored. Similar to previously published literature[3], clinical signs of MS were assessed using a modified Disease Activity Index (DAI) scoring system, which is abbreviated as follows: 0=Normal; 0.5=Tail tip limp; 1=Limp tail; 1.5=Limp tail and hind leg inhibition; 2=Limp tail and weakness of hind legs OR obvious head tilt; 2.5=Limp tail and dragging of hind legs OR Strong head tilt; 3=Limp tail and complete paralysis of hind legs OR Severe head tilt; 3.5= Limp tail and complete paralysis of hind legs, inability to right, hind quarters are flat like a pancake; 4=Limp tail, complete hind leg and partial front leg paralysis; 4.5= Limp tail, complete hind leg and partial front leg paralysis, no movement around cage; 5=Severe paralysis.

Vium Smart Housing™: Cages are outfitted with sensors that stream data and environmental conditions 24/7. The Digital Vivarium recorded HD video, as well as collected, displayed, and analyzed Vium's validated motion and breathing rate metrics[6,7] continually in near real-time.

Treatment: After disease onset (DAI≥1), mice were orally treated daily with 10 mg/kg/day of FTY-720 or vehicle (saline).

Statistical analyses: One-way ANOVAs were used to assess changes in motion and breathing rate, while non-parametric tests (Mann-Whitney or Kruskal-Wallis) were used to assess DAI scores. Two-way ANOVAs with appropriate post-hoc tests were used to assess changes in clinical scores, motion, and breathing rate over time and between groups. A Pearson test was used for cross-correlation analysis, and regression analysis was used to plot the best-fit line. *P* values < 0.05 were considered significant.

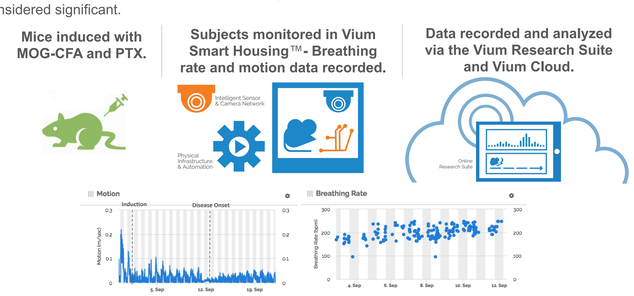

Figure 1: Depiction of study process and data flow featuring representative raw metrics from a MOG-induced mouse.

Results

Vium's motion and breathing rate metrics detect behavioral changes in the EAE-MOG model of MS.

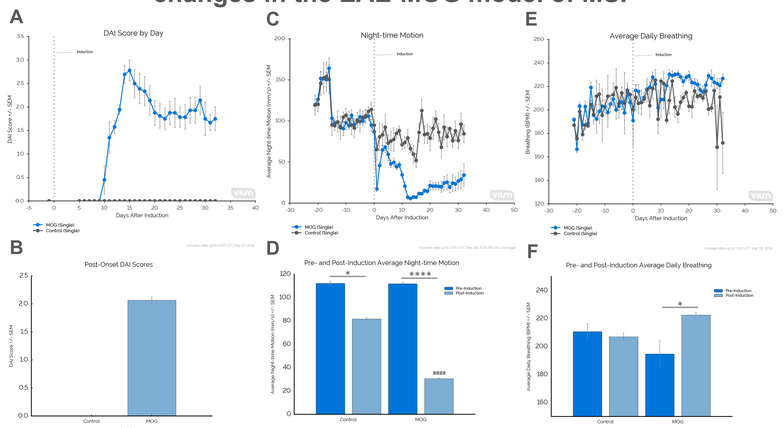

Figure 2: (A) Mice inoculated with the MOG(1-125) peptide showed MS disease signs when assessed by conventional DAI clinical scoring. (B) Mean DAI Score Post-disease onset (+/- SEM) for non-induced (Control) and MOG-induced (MOG) mice. (C) MOG mice showed a significant decrease in average night-time motion over the course of disease (*P<0.0001* by 2-way ANOVA). (D) Although Control mice showed slightly reduced motion levels post-induction (*P=0.02*), MOG mice showed a >50% reduction in mean night-time motion post- compared to pre-induction. (E) MOG mice also demonstrated a significant increase in breathing rate post-induction (*P=0.0004* by 2-way ANOVA). (F) Induced mice showed an increase in mean breathing rate post- compared to pre-induction. Dotted lines represent day of induction. *indicates significance (*P<0.05*) from Pre-induction, and # indicates significance (*P<0.05*) from Control. N=5-10/treatment group.

Vium's metrics detect disease severity and efficacy of SOC drug.

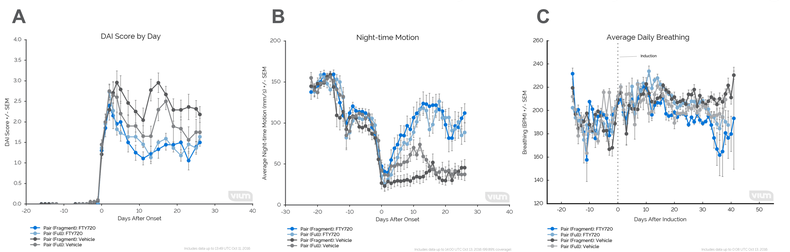

Figure 3: (A, B, C) Mice inoculated with the shorter MOG 35-55 peptide (Fragment) showed more severe clinical symptoms, motion loss, and breathing rate increase compared to mice inoculated with the longer MOG 1-125 peptide (Full) (*P<0.0001, P<0.0001,* and *P=0.0013* by 2-way ANOVA, respectively). (A, B) After disease onset (DAI ≥1), MOG mice treated with the SOC drug FTY-720 showed significant improvements in clinical scoring and motion levels. (C) MOG mice demonstrated increased breathing rate post-induction. FTY-720 treatment in mice inoculated with the Fragment MOG peptide attenuated this increase over the course of disease. For Figure C, dotted line represents day of induction. N=8-12/treatment group.

Table 1: Summary statistics of post-disease onset measurements with SOC drug FTY-720

Measurements	Fragment MOG (35-55)-Vehicle	Fragment MOG (35-55)-FTY-720	Full MOG (1-125)-Vehicle	Full MOG (1-125)-FTY-720
Mean Post-Disease Onset DAI Score	2.38 ± 0.20	1.56 ± 0.11*	2.00 ± 0.19	1.64 ± 0.09
Mean Change in Night-time Motion	0.45 ± 0.09	0.90 ± 0.08***	0.49 ± 0.07	0.81 ± 0.04*
Mean Change in Breathing Rate	1.12 ± 0.02	0.99 ± 0.03**	1.05 ± 0.01	1.11 ± 0.03

*indicates *P<0.05* between FTY-720 and vehicle groups of each MOG peptide. Data presented are Mean ± SEM. Change in Night-time motion = Mean Night-time Motion Post-disease onset / Pre-disease onset; Change in Breathing Rate = Mean Breathing Rate Post-disease onset / Pre-induction.

Results

Vium's motion metric tracks changes in MS clinical scoring.

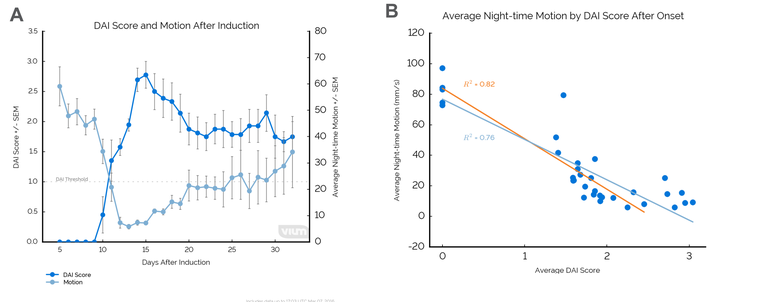

Figure 4: (A) Vium's night-time motion (light blue) tracks with DAI scoring (dark blue). Data shown is from a representative experiment with MOG(1-125)-induced mice (N=10). Dotted line indicates DAI threshold for onset (DAI ≥1). Error bars ± SEM. (B) There is a significant negative correlation between the Average DAI Score and Average Night-time motion metric (R=-0.8695, *P<0.0001*). Shown are control and MOG(1-125)-induced mice from three independent experiments, as well as best-fit line and corresponding R^2. Orange line indicates best-fit for DAI scores between 0 and 2.5, while blue line indicates best-fit for DAI scores between 0 and 3.5. N=33 mice.

Table 2: Summary statistics of disease course from three independent experiments using MOG (1-125)

	Controls	Expt. 1	Expt. 2	Expt. 3
Motion				
Incidence (%)	0 of 5	10 of 10	10 of 10	8 of 9
Onset (days after induction)	N/A	11.20 ± 1.03	10.70 ± 0.82	12.1 ± 0.82
Peak Severity (days after induction)	N/A	11.7 ± 0.95	11.2 ± 0.79	12.6 ± 0.92
DAI Score				
Incidence(%)	N/A	10 of 10	10 of 10	9 of 9*
Onset (days after induction)	N/A	11.30 ± 0.95	10.60 ± 0.52	12.78 ± 0.83
Peak Severity (days after induction)	N/A	13.6 ± 1.71	12.4 ± 1.07	14.7 ± 1.0

*One animal reached DAI ≥1, but was asymptomatic later on. Data presented are Averages ± SD.
Incidence for Motion = # of animals with >50% motion loss; Peak Severity for Motion = day of largest motion loss.

Conclusion

In a MOG-induced EAE rodent model of MS, the Vium Digital Vivarium™ and Vium's metrics can be used to:

- Track MS disease progression comparably to conventional clinical scoring
- Evaluate compound therapeutic efficacy
- Identify disease onset and severity
- Increase the efficient use of resources in drug discovery studies

Our results demonstrate how a low-touch, high-tech platform can pave new ways for more rapid and reproducible disease evaluation and high-throughput drug discovery for MS. This platform has broad applications for other autoimmune diseases, including Lupus, as well as neurological diseases, such as Parkinson's, Huntington's, and Amyotrophic Lateral Sclerosis (ALS).

References

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[2] Denic A, Johnson AJ, Bieber AJ, Warrington AE, Rodriguez M, Pirko I. The relevance of animals models in multiple sclerosis research. Pathophysiology. 2011; 18(1):21-9.
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[6] Vium Activity Metric White Spec
[7] Vium Breathing Rate Metric White Spec