Transpharmation
Science that translates into results

Behaviour and biomarkers in mouse corneal kindling as a model for epilepsy, neuroinflammation and gliosis

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Guy A. Higgins, Leo B. Silenieks, Daniel Feng, Matt Brown, Lauren Stam, Róisín Winters, Will Bastedo, Martin Sutton, Anne Oakley, Julie Vintuks, Meera Navaratnam, Manuela Mitsogiannis, Jack Prenderville

INTRODUCTION

Corneal kindling represents a robust model for testing novel treatments for epilepsy and its associated comorbidities[1]. This study aimed to characterize the corneal kindling process in two mouse strains, C57BL/6J (C57) and CD-1 in addition to evaluating the comparative efficacy of three reference antiepileptic drugs, namely Carbamazepine (CBZ), Sodium Valproate (VPA) and Levetiracetam (LEV). We also explored behavioural phenotypes emergent from the corneal kindling process. Underlying neuroinflammatory and gliosis markers were measured in the temporal lobe. Our data highlights the utility of this model in studying epilepsy and associated comorbidities.

METHODS

Corneal Kindling: C57BL/6 and CD-1 mice received a twice daily mild electroshock (2mA, 3s duration, 60Hz) via corneal electrodes until consistent kindled state (stage 3-5 seizures, Racine scale). Daily stimulation for maintenance between tests.

Pharmacology: Reference anti-epileptic drugs (CBZ - 10, 30, 60mg/kg, I.P., 1h p.t.t. and LEV - 10, 30, 60, 100, 150mg/kg, I.P., 1h p.t.t. and VPA - 100, 300, 600mg/kg, I.P., 1h p.t.t.) were tested using a repeated measures design. On test days, the AM stimulation score is used as the baseline control and PM stimulation is preceded by the respective drug injection.

Elevated Plus Maze: Animals freely explore the maze for 5 mins.

Canopy Test: Circular elevated test arena consisting of a smaller centered sheltered canopy and allowed to freely explore for 5 mins.

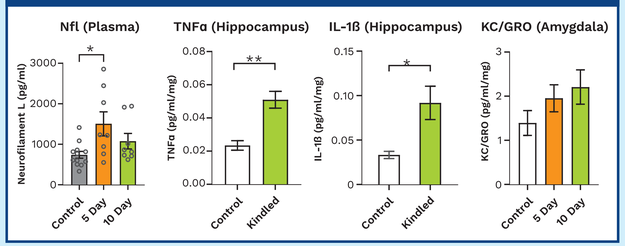
Forced Swim Test: Mice are placed into glass cylinder filled with water (20-25°C) for 6 mins. Latency (seconds) to immobility and duration of immobility during the last 4 mins. Is recorded.

Fear Conditioning: Mice are placed in a chamber with a cue light, 2.9-kHz tone, and shock floor. In the conditioning session, they receive a foot shock (0.5mA) after a 20-sec light-tone stimulus at 3 intervals. The test session replicates the stimulus without the shocks, recording freezing behaviors. The extinction phase over three days evaluates the diminishing fear response using the same test conditions.

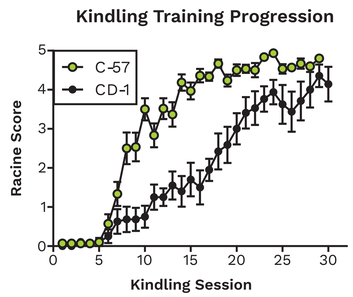
Immunohistochemistry: Immunostaining (Millipore #AB5541 1:5000) Images acquired using a brightfield microscope and immunostaining quantified with ImageJ.

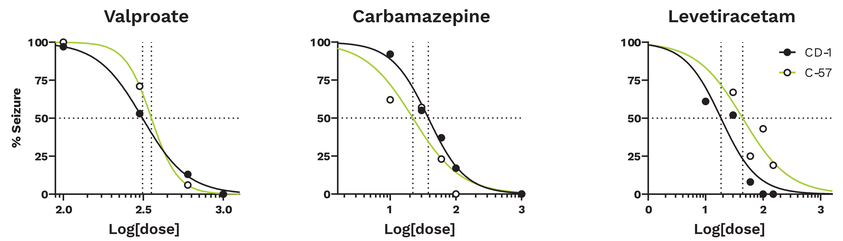
Biomarkers: Brain and plasma biomarkers were quantified using MESO QuickPlex SQ 120 custom panels (Mouse Pro-inflammatory Panel (Cat. No. K152A0H-2) and Neurofilament Light Chain (Cat. No. F217X3)).

RESULTS: Tissue Biomarkers



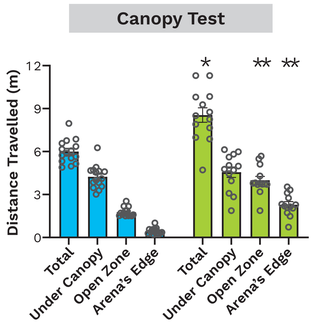
RESULTS: Epileptogenesis and Pharmacology

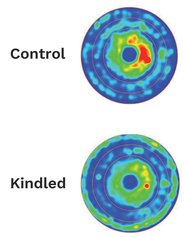


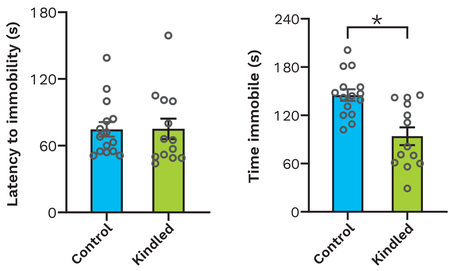


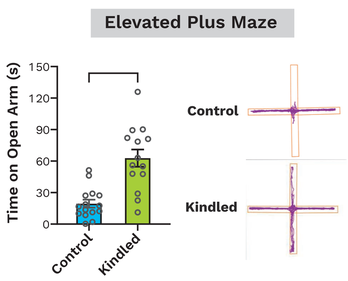
ED_{50}				
	C-57	356 mg/kg	22 mg/kg	44 mg/kg
	CD-1	313 mg/kg	38 mg/kg	19 mg/kg

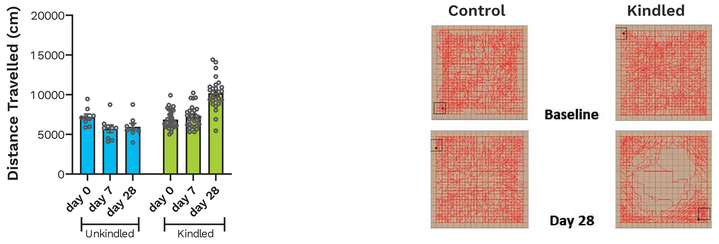
RESULTS: Behavioural Tests

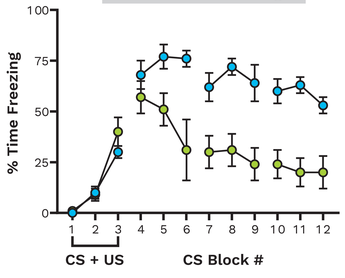


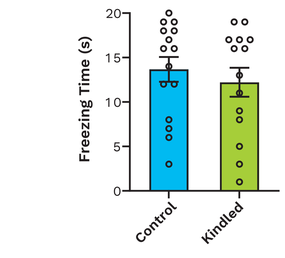


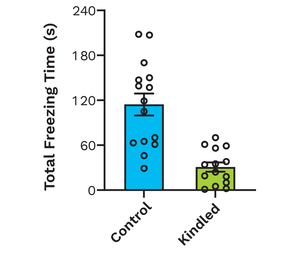




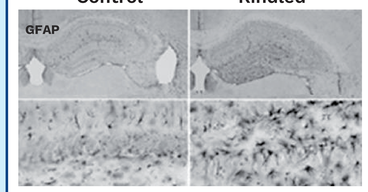


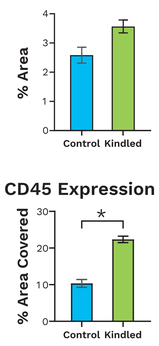


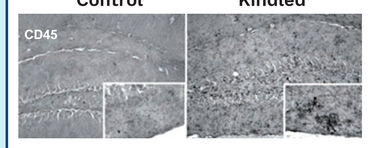




RESULTS: Immunohistochemistry







CONCLUSIONS

- C-57 mice reached stable kindling stage 5 seizures 2x as quickly as CD-1 mice, with less attrition (5% vs 35%).
- Clinical AEDs (VPA, CBZ, LEV) demonstrated comparable efficacy against evoked seizures in both mouse strains.
- Kindling causes hyperactivity and decreased risk assessment behaviors across several behavioral assays.
- Both groups displayed similar freezing responses in the 1st probe trial, implying retention of the CS/US association. During extinction, kindled mice demonstrated hastened extinction learning.
- Increased hippocampal GFAP and CD45 expression is consistent with astrocyte and microglial activation.
- Increase in proinflammatory cytokines (IL-1ß and TNFα) indicate neuroinflammation within temporal lobe structures including hippocampus and amygdala.
- Corneal kindling is a promising approach to explore neuroinflammation, gliosis, and neurobehavioural deficits characteristic of some clinical epilepsies[2]. (hyperactivity, impaired risk assessment, altered emotional regulation, impulsivity, attention deficits).

REFERENCES

1 Matagne, A, & Klitgaard, H. (1998). Validation of corneally kindled mice: a sensitive screening model for partial epilepsy in man. Epilepsy research, 31(1), 59-71.

2 Williams, AE, Giust, JM, Kronenberger, WG, & Dunn, DW. (2016). Epilepsy and attention-deficit hyperactivity disorder: links, risks, and challenges. Neuropsychiatric disease and treatment, 287-296.