Beyond seizure control: Profiling Levetiracetam's effect on epileptogenesis and comorbid behaviours in kindled mice

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INTRODUCTION

Corneal kindling provides a controlled platform to study epileptogenesis and its associated behavioural changes. Using this model, we evaluated whether the antiseizure drug levetiracetam not only suppresses seizures but also modulates behavioral domains relevant to epilepsy comorbidities. By profiling locomotor, risk-related, and self-care behaviors in chronically kindled mice, this work demonstrates how preclinical assays can be extended beyond seizure endpoints to characterize drug effects on comorbidity-related phenotypes, highlighting the translational value of this platform for antiepileptic drug discovery and screening.

METHODS

Corneal Kindling Training

Male C57BL/6 mice (20–30 g) underwent corneal kindling with twice-daily stimulations (AM and PM, ≥6 h apart) using 2 mA, 3 s, 60 Hz pulses delivered via corneal electrodes. Stimulations continued until animals reached the fully kindled state.

Treatment with Levetiracetam began once average racine score reached ~3. A subset of animals began levetiracetam (LEV, 60 mg/kg, i.p.) treatment, administered 1 h before each stimulation, and continued throughout the remaining kindling sessions. Non-kindled controls (UNK-VEH) received electrode placement without current delivery.

Behavioural Assessments

Locomotor Activity (LMA): 60-min open-field test quantifying total distance, ambulatory episodes, and rearing.

Canopy Test: Evaluates anxiety, risk-related exploration and disinhibition; time and distance in open vs. covered zones plus manual scoring of stretch-attends and over the edge assessments.

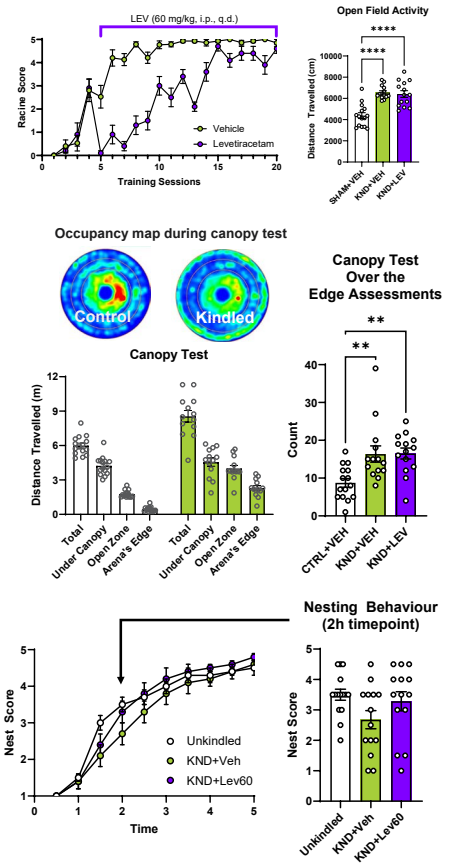
Nest Building: Five-hour ethological assay of self-care and motivation; nest quality scored every 30 min on a 5-point scale (Deacon RM, 2006).

Acoustic Startle: Startle amplitude recorded in response to loud acoustic pulses; average response across trials as measure of sensorimotor reactivity.

Running Wheel Activity: Animals were placed in an arena with a running wheel for a 20h session. Wheel rotations were recorded continuously.

MoSeq (Motion Sequencing): Depth-camera-based, unsupervised behavioural profiling used to decompose activity into discrete "syllables" representing recurring motor motifs (Gschwind et al., 2023). Each mouse's activity was recorded for 30 min and analyzed using a machine-learning algorithm (MoSeq) to generate the behavioural fingerprints.

RESULTS



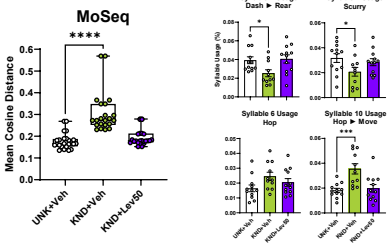
Kindled mice were more active than controls (p<0.001) and showed increased risk-taking in the canopy test, with elevated "over-the-edge" assessments (p<0.01). Levetiracetam (60 mg/kg) did not affect these behaviors. Nest-building was impaired in kindled animals but partially restored by levetiracetam, suggesting recovery of goal-directed behavior.

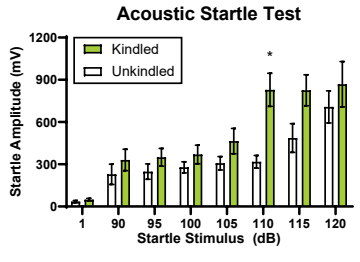
RESULTS - Motion Sequencing (MoSeq)

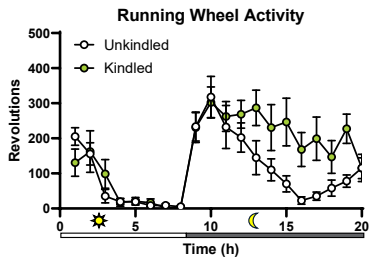
Unsupervised motion sequencing analysis revealed changes to behavioural syntax across groups. **Unkindled mice** primarily expressed low-numbered syllables (1–40), common, well-established behaviours reflecting efficient and adaptive movement patterns.

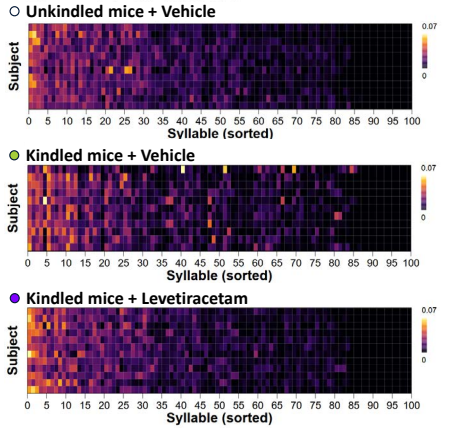
Vehicle + Kindled mice shifted toward higher syllables (≈60–85), representing atypical actions emerging during epileptogenesis. Cosine analyses shows deviation from normal behavioural syntax (p<0.001).

Kindled + Levetiracetam restored use of common motifs. Cosine-distance analysis showed chronic levetiracetam treatment restored mean cosine distance back to typical behaviour.









CONCLUSIONS

- Corneal kindling produced a consistent hyperactive and disinhibited phenotype, evident in open-field and canopy tests.
- Levetiracetam (60 mg/kg) did not reduce hyperactivity but partially restored goal-directed behaviour (nest building).
- MoSeq revealed a shift toward atypical, less utilized behavioural syllables in kindled mice; levetiracetam normalized this pattern.
- Running-wheel recordings showed preserved circadian rhythm but excessive nocturnal activity, suggesting elevated arousal rather than rhythm disruption.
- These findings establish corneal kindling as a translational platform to assess both seizure control and comorbidity-relevant behavioural outcomes of antiepileptic drugs.

REFERENCES

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