Natural history of seizures, spreading depolarizations and seizure-associated spreading depolarization in mouse models of epilepsy

Neela Codadu[1], Yunan Gao[1], Kevan Hashemi[2], Nicolas Mazarakis[3], Rob. C. Wykes[1,4].

[1] Department of Clinical & Experimental Epilepsy, UCL Queen Square Institute of Neurology, United Kingdom
[2] OpensourceInstruments, Waltham, MA 02452, USA
[3] Department of Brain Sciences, Imperial College London, United Kingdom.
[4] Centre for Nanotechnology in Medicine & Division of Neuroscience, University of Manchester, United Kingdom.

Introduction: Spreading depolarizations (SD) are pathological waves of neuronal and glial depolarization that slowly propagate through the brain. They have mostly been studied in the context of migraine with aura, stroke and traumatic brain injury.

In recent years it has become apparent that spreading depolarizations (SD) can occur in epileptic tissue; either independently or concurrently with seizures. Traditional electrophysiology approaches are poorly suited to detect SDs, and as such the impact of SDs to morbidity and mortality in epilepsy is an understudied area of research.

To investigate the natural history of SD's and seizure-associated SD's we performed long-term video-telemetry studies using multichannel, wireless, DC-coupled transmitters in models of temporal lobe epilepsy. We report the natural incidence rate of SD's , seizures, and seizure-associated SD's. Time-locked video provides the ability to describe the behavioral response to each of these events.

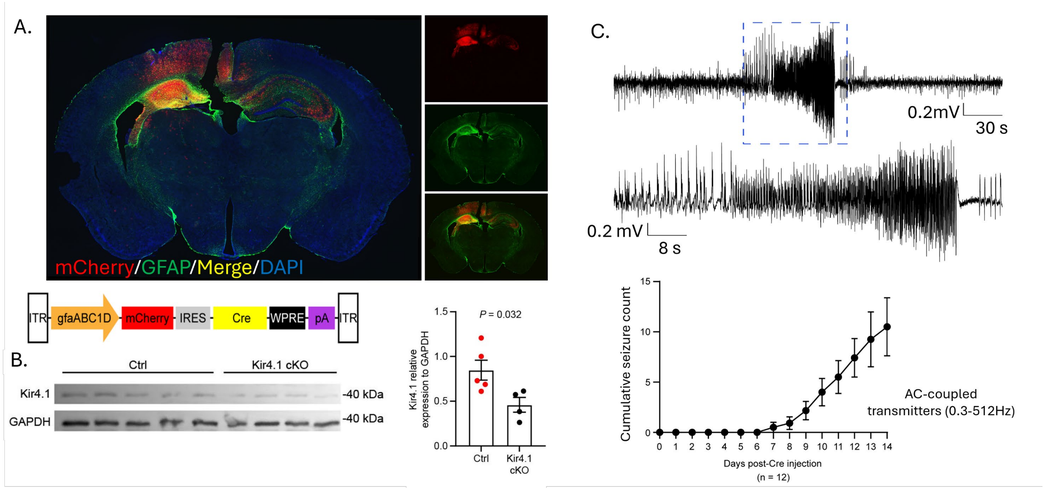
SD's and seizure-associated SD's are common events. We suggest that seizure-associated SD's play an important role in the severity of the post-ictal period. Further work in this area will help identify the contribution of seizure-associated SD's to post-ictal immobility, confusion and headache, in addition to studying their proposed involvement in mechanisms underlying SUDEP.

Knockout of the astrocyte potassium channels in adult hippocampus results in spontaneous seizures.

Astrocytes express Kir4.1 potassium channels, essential for potassium buffering and involved in glutamate uptake. Inadequate buffering of potassium and glutamate result in neuronal hyperexcitability. Reduced Kir4.1 expression has been reported in human and experimental epilepsies. To examine the impact of Kir4.1 loss to seizure and SD genesis we developed a novel model of temporal lobe epilepsy (TLE) based on focal conditional knock-out of astrocyte Kir4.1 channels within the hippocampus.

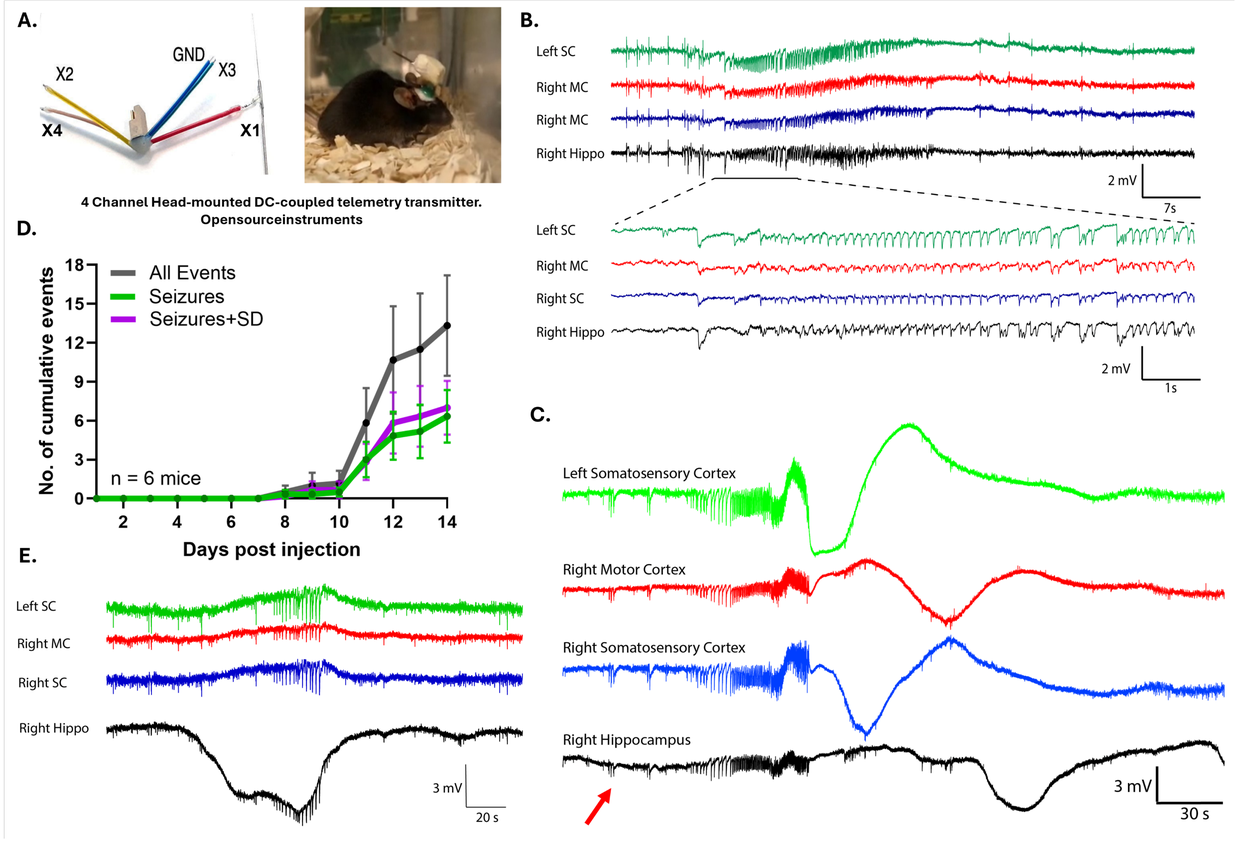
Viral vector-delivered cre-recombinase was used to knock out Kir4.1 expression in the hippocampus of adult Kir4.1-flox mice (Kir4.1-cKO). Continuous AC and DC-coupled wireless video-telemetry was used to monitor spontaneous seizures and SDs.

In Kir4.1-cKO mice, spontaneous epileptiform events emerged ~1-week post-viral injection.



A. Viral vectors with an astrocyte specific promotor were used to knockdown expression of Kir4.1 unilaterally in the hippocampus (with some reduction in the overlaying cortex). **B**. This results in ~50% reduction of hippocampal Kir4.1 protein assessed by Western Blotting. **C**. Spontaneous seizures emerge ~ 1 week post injection of virus.

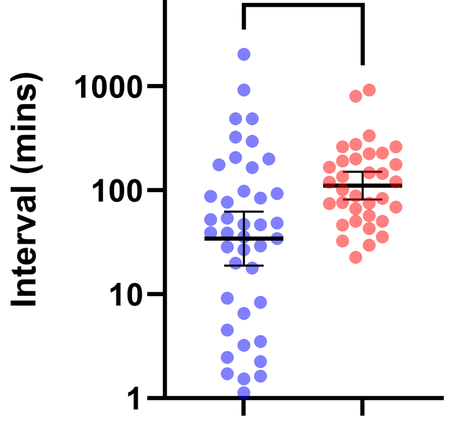
DC-coupled recordings detect seizures, seizures with an associated spreading depolarisation and focal hippocampal depolarisations.



A. 4 channel head-mounted DC-coupled wireless telemetry device from opensourceinstruments. 3 cortical leads and 1 depth electrode to allow recording from the hippocampus. **B**. Spontaneous generalised seizure without SD. **C.** Spontaneous generalised seizure with SD. SD was only observed when seizures generalised and was always first detected in a cortical region – this varied and could even be first originating in the contralateral cortex. Note red arrow indicates a slow pre-seizure DC shift in the hippocampal lead, the presumed seizure focus. **D**. ~50% of spontaneous seizures were associated with an SD. **E.** Focal hippocampal depolarisations were also detected. These tended to trigger global large amplitude spikes.

Time-locked video analysis indicates that seizures associated with an SD tended to have a more severe behavioural response than seizures alone. Additionally, post-ictal immobility was often greatly increased in duration following seizures associated with SD. This suggests that SD impacts on the severity of the post-ictal period.

Do Spreading Depolarisations 'reset' the brain and increase the inter-seizure interval?.



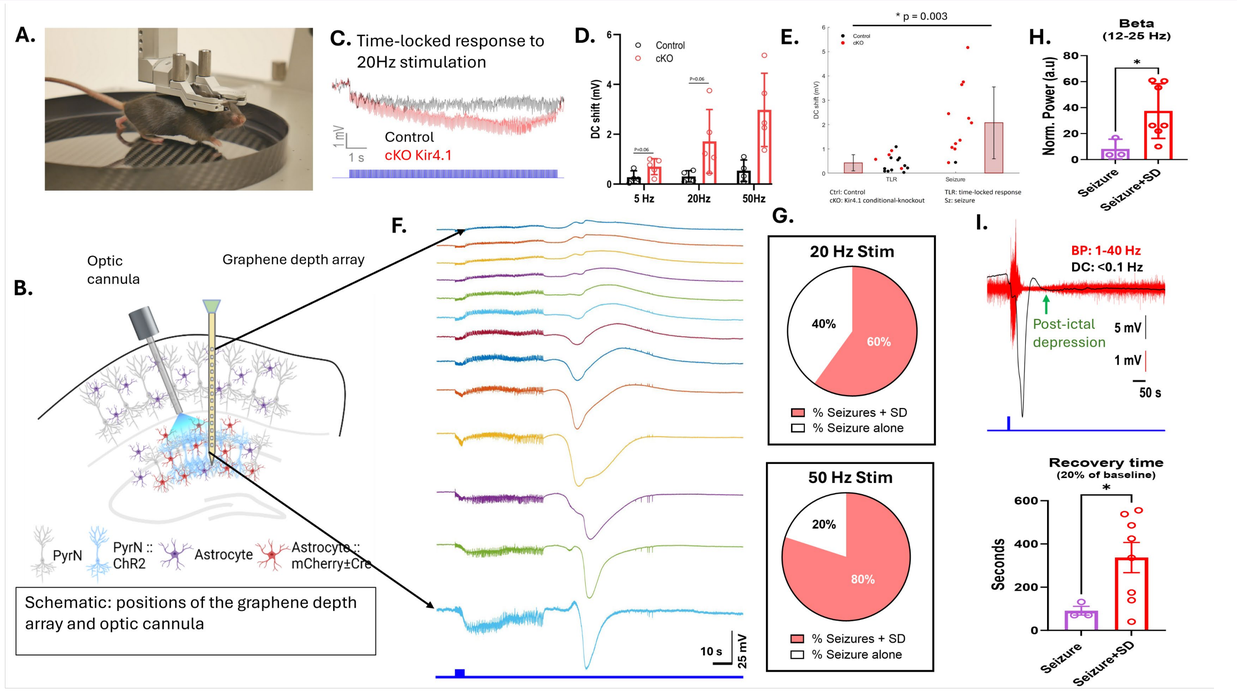
Spontaneous seizures tend to cluster. It has been shown that the seizure that terminates a cluster often has increased severity; generalisation and behavioural response (Kudlacek J et al 2021).

Greater seizure power and area of recruitment predicts SD occurrence (Tamin I et al 2021).

Do seizure-associated SD's terminate seizure clusters?

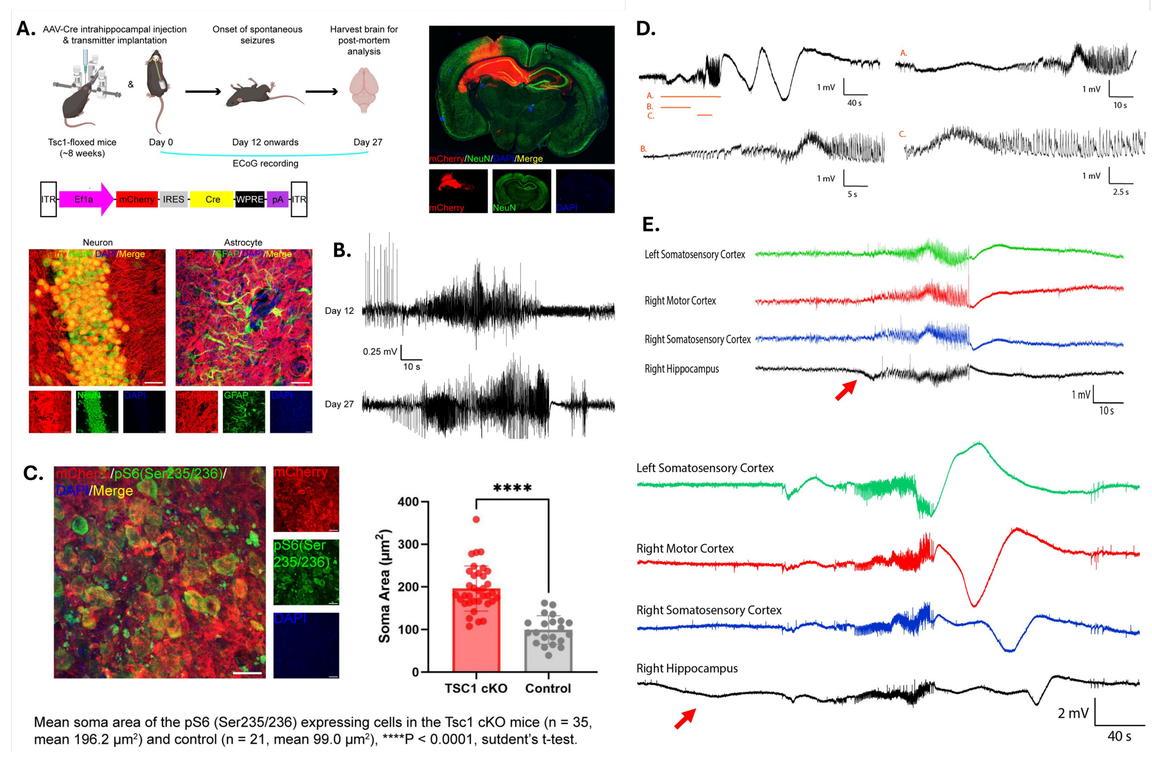
References
1. Kudlacek J et al 2021. PMID: 33771663
2. Tamim I et al 2021 Nature Communications. PMID: 33850125
3 Bonaccini Calia A et al 2022. Nature Nanotechnology. PMID: 34937934
4. Loonen et. al., 2019 BRAIN. PMID: 30649209

Graphene micro-transistor recordings allow high fidelity recordings of DC shifts and Seizure associated Spreading Depolarisations



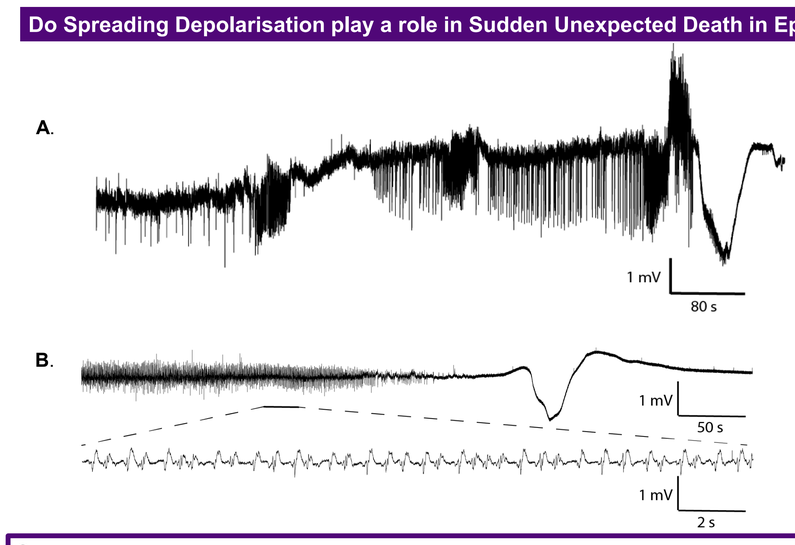
We used high fidelity DC-coupled graphene micro-transistor arrays to investigate stimulation induced DC shifts, seizures and SD's in awake cKO Kir4.1 mice. **A**. Awake head-fixed mouse in a Neurotar frame. **B.** Experimental schematic; Kir4.1 knockout in astrocytes and Channelrhodopsin (ChR2) expressed in excitatory neurones within the hippocampus. **C&D**. Larger DC shifts observed following brief trains of optogenetic stimulation in cKO mice compared to control. **E**. Optogenetic stimulation that resulted in large DC shifts triggered seizures. **F.** Many triggered seizures were associated with an SD. **H**. Seizures with higher power were associated with SD. **G.** The frequency of optogenetic stimulation influenced the probability of a seizure-associated SD. **I.** Seizures associated with SD had a significantly longer period of post-ictal depression.

Knockout of the Tsc1 protein in adult hippocampus results in spontaneous seizures, and seizure associated spreading depolarisations.



A. Experimental schematic. Tsc-1 is a protein that regulates MTOR activity and is commonly mutated and/or dysfunctional in focal cortical dysplasia. We knocked out Tsc1 expression in adult mice in multiple cell types using a viral vector under the EF1a promotor. **B.** This resulted in spontaneous seizures arising ~11-13 days after injection of virus. **C.** Post-hoc analysis demonstrated that the soma of neurons was significantly increased in mice where Tsc1 had been knocked out. **D.** Example of a seizure with a recurrent SD recorded using a wireless OSI DC-coupled telemetry device. **E**. Similar to cKO of Kir4.1, cKO of Tsc1 resulted in both generalised seizures and generalised seizures associated with spreading depolarisation. Note red arrows indicate a slow pre-seizure DC shift in the hippocampal lead, the presumed seizure focus.

Do Spreading Depolarisation play a role in Sudden Unexpected Death in Epilepsy (SUDEP)?



In SUDEP-prone transgenic mice it has been proposed that seizure-associated SD mediates SUDEP by inhibiting respiratory control centres within the brainstem (Loonen et. al., 2019 BRAIN). Our experimental design is not suited to investigate this hypothesis directly. In rare cases when mice died following seizure activity, an SD was always observed. Whether this is cause, correlation or consequence of death requires further investigation. **A.** A cluster of 3 seizures from a cKO Kir4.1 mouse. The final seizure was associated with an SD and the mouse died following this seizure. **B**. In a cKO Tsc1 mouse a cluster of seizures in quick succession (5 in 15 minutes) evolved into a period of status epilepticus (SE) and a terminal SD.

Summary

1. Astrocytes are often dysfunctional in epilepsy and down-regulate Kir4.1 potassium channels.
2. cKO of Kir4.1 in the hippocampus of adult mice results in spontaneous seizures and a lowered threshold for optogentically induced seizures – which have a high rate of seizure-associated SDs.
3. DC-coupled wireless video telemetry demonstrates that spontaneous seizure-associated SDs occur ~ 50% of all seizures in this model.
4. Seizure-associated SD have a profound impact on the post-ictal period.
5. Seizure-associated SD increase the inter-seizure interval suggesting that they may 'reset' the brain, transiently reducing the likelihood of transition to seizure.
6. cKO of Tsc1 in the hippocampus of adult mice results in dysplastic neurons, spontaneous seizures and seizure-associated spreading depolarisations.
7. In rare examples of SUDEP seizure-associated spreading depolarisations were always observed; whether these are a cause or consequence of death remains to be known and is an area of our future research.

Email: r.wykes@ucl.ac.uk
rob.wykes@manchester.ac.uk

WykesNeuroLab.com
X: LabWykes

Worshipful Company of Pewterers.
Medical Research Council
Manchester Epilepsy Research Network @mern_UoM
CENTRE FOR NANOTECHNOLOGY IN MEDICINE
EPILEPSY RESEARCH INSTITUTE UK