Modelling seizure-induced and extinction learning-dependent neuronal activation in pediatric epilepsy

Christina Hansen[1], Khalil Abed Rabbo[2], Kathryn Laprade[2], Amanda Hernan PhD[2], James Stafford PhD[2]

[1]Larner College of Medicine, University of Vermont; [2]Department of Neurological Sciences, University of Vermont

Introduction

- Lifetime incidence of epilepsy is 1 in 26 individuals, with many diagnoses occurring during childhood.[1]
- Pediatric epilepsy strongly correlates with behavioral, cognitive, and psychiatric comorbidities which can be more detrimental to quality of life than the actual seizures.[2]
- Early life seizures are associated with learning and memory deficits due to lasting modification of neuronal activation patterns in a manner that is brain-region specific.[3]
- Impairment of fear extinction learning is an established hallmark of early life seizures (Fig. 1).[3] However, it is unclear how neural ensembles engaged by seizures are implicated in subsequent cognitive impairment to produce fear-extinction deficits.
- Brain-region specific differences in seizure-induced neural network changes may reflect epigenomic mechanisms acting in disparate regulatory regions of the epileptic brain.

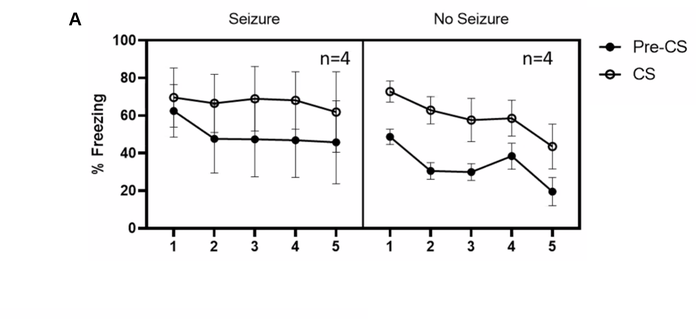


Figure 1. Evaluated fear extinction learning in an early life seizure model. A) ELS group demonstrate reduced subsequent fear extinction and a more stable freezing response. In contrast, non-ELS group shows robust fear extinction over subsequent trials. B) ELS group also demonstrates reduced CTX/CS discrimination. These findings suggest cognitive deficits in learning after ELS that persist beyond the early developmental period. CS= conditioned stimulus; CTX= context stimulation.

Objective

- We sought to characterize brain-region specific neuronal activation in an early life seizure mouse model.
- We compared regional patterns of neuronal engagement in early life seizure (ELS) and later activation during fear extinction learning.

Methods

- A transgenic mouse model was engineered, linking nuclear surface GFP luminescence to neuronal activation in a flurothyl-induced generalized seizure (Fig 2).
- Dual immunofluorescent staining of both ELS-tagged neurons (GFP) and NeuN, a pan-neuronal marker (neuronal nuclear antigen), was performed to localize regions of neuronal activation and compare activation amongst anatomic regions implicated in prior seizure models.[3,4]
- After seizure induction, the immediate early gene c-Fos served as an independent marker of neuronal activation in fear extinction.
- Dual immunofluorescent staining of GFP and cFos was used to evaluate the degree of co-activation between seizure and fear extinction states.

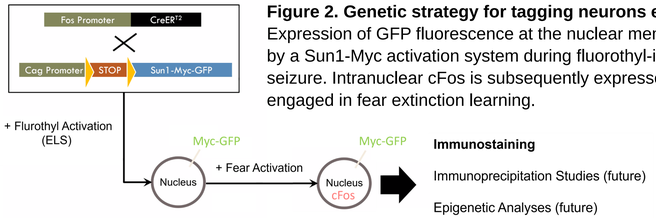


Figure 2. Genetic strategy for tagging neurons engaged by ELS. Expression of GFP fluorescence at the nuclear membrane is driven by a Sun1-Myc activation system during fluorothyl-induced grand-mal seizure. Intranuclear cFos is subsequently expressed among neurons engaged in fear extinction learning.

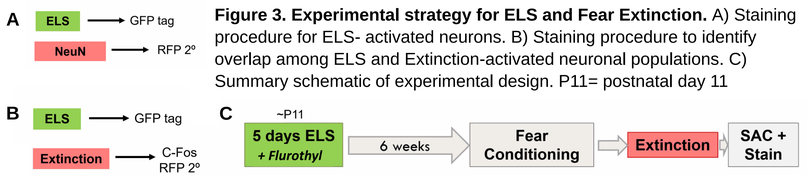
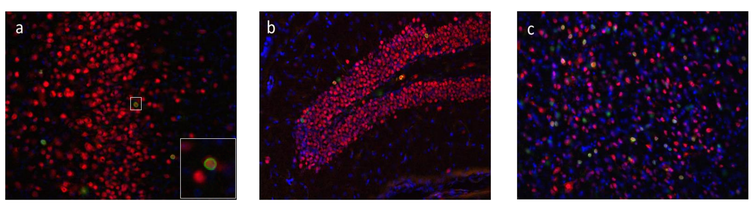


Figure 3. Experimental strategy for ELS and Fear Extinction. A) Staining procedure for ELS- activated neurons. B) Staining procedure to identify overlap among ELS and Extinction-activated neuronal populations. C) Summary schematic of experimental design. P11= postnatal day 11

Results



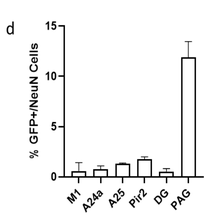


Figure 4. Dual immunostaining of GFP with NeuN in an early life seizure model. Relatively few neurons are activated in ELS in select brain regions. Immunostaining for seizure-activated neurons was performed using a GFP antibody (green), NeuN neuronal marker (red) and DAPI nuclear counterstain (blue). NeuN-positive neurons of the periaqueductal gray demonstrated greatest seizure activation as measured by GFP co-localization (Fig 4c,d), compared to forebrain regions such as piriform cortex (Fig 4a,d) or midbrain regions such as dentate gyrus (Fig 4b,d). 20x magnification. Fig 4d, n = 6. M1= primary motor cortex; A24a= prelimbic area; A25= infralimbic area; Pir2= piriform cortex; DG= dentate gyrus; PAG= periaqueductal gray.

Results

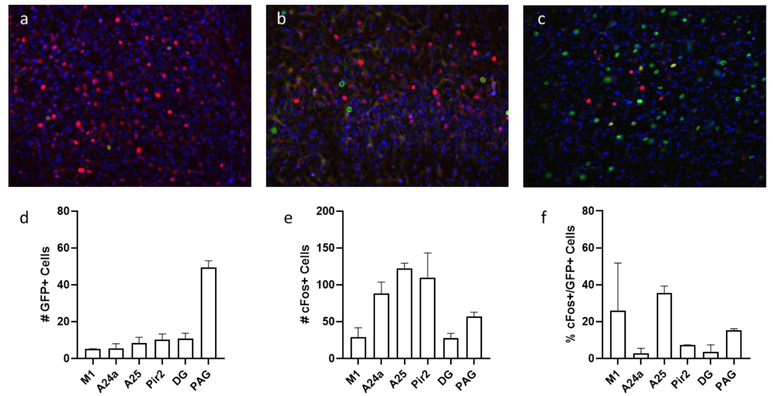


Figure 8. Dual immunostaining of ELS-GFP with cFos after fear extinction. Immunostaining for seizure activated neurons was performed using the GFP antibody (green), cFos antibody for neurons activated in the conditioned fear response (red), and DAPI nuclear counterstain (blue). cFos-labelling of fear-activated cells was most frequent in forebrain regions (Fig 8e), including the infralimbic area (Fig 8a) and piriform cortex (Fig 8b). GFP-labelling of seizure-activated cells was most abundant in the periaqueductal gray (Fig 8c, d). Co-localization of GFP and cFos cell populations was limited (< 40%) but strongest in the infralimbic area and periaqueductal gray (Fig 8f). 20x magnification. M1= primary motor cortex; A24a= prelimbic area; A25= infralimbic area; Pir2= piriform cortex; DG= dentate gyrus; PAG= periaqueductal gray.

Conclusions and Future Directions

- This study establishes a reliable immunofluorescent method for visualization of neuronal activation in seizure and extinction learning models.
- Extinction learning appears to engage ELS-activated neurons in a brain-region specific manner, with greatest incidence of overlapping activation in the infralimbic prefrontal cortex and midbrain periaqueductal gray.
- In future, we aim to:
  - Compare how regional neuronal engagement varies among seizure-exposed, non-seizure, and handling-only groups.
  - Correlate seizure-induced neuronal activation and extinction with epigenetic modifiers hypothesized to participate in brain region-specific regulation.
  - Determine if targeted epigenetic intervention can ameliorate cognitive deficits associated with early life seizures.

References

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