Effects of genotype, sex and age in seizure-like activity in transgenic mice

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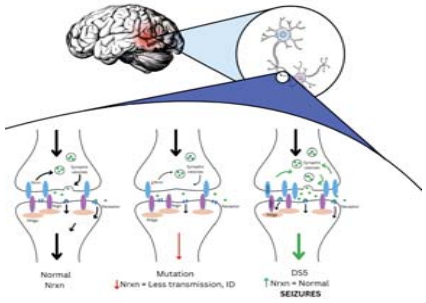
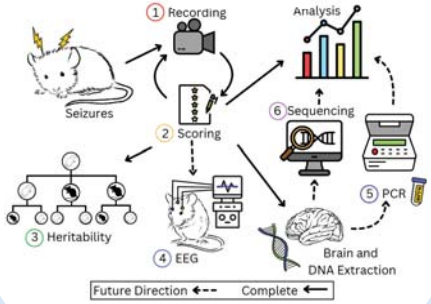
INTRODUCTION

Epilepsy affects 1% of the population[1] and is a condition involving neurons firing faster than normal in the brain, resulting in involuntary sensations and movement [2]. It can be caused by developmental issues, brain injury or genetic factors [2] and triggered by stress, toxins or brain injury [3]. Epilepsy is equally frequent in males and females [4]. It occurs most frequently in adults over 60 [5], or children (due to genetics) [6]. Neurexin-1 is a presynaptic molecule involved in cell adhesion; mutation in this gene is associated with various neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) [7]. Nrxn-1 interacts with proteins like post-synaptic Neuroligins [5] and membrane protein MDGA-2 [8]. Nrxn-1 model mice at the Brown Lab mouse colony at Dalhousie have been observed to have seizures mainly during cage changes, prompting this study.



METHODS



RESULTS

Mouse Model

- Wild Type (Wt) - baseline C57BL6/J mice (Nrxn+/+)
- Transgenic (Tg) - Nrxn-1+/- (knockdown - lower protein expression) [9]
- DS5/- - knockout Nrxn-1 + modification at splice site 5 - increased Nrxn-1 expression[9].
- DS5/DS5 - two Splice Site 5 Nrxn-1 modified alleles.

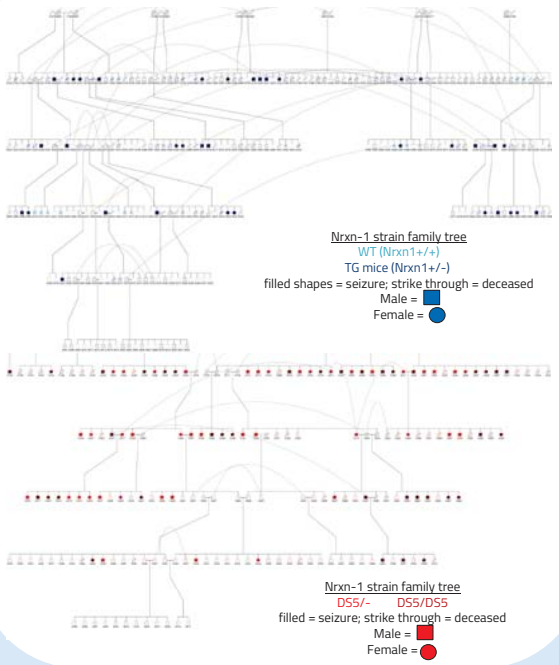
Measuring Seizure Phenotypes in Mice

- Based on Racine Scale [10]

Table 1 - The Brown Lab Scale, with descriptions of convulsion observations of mice, based upon revised scale in Racine paper.

Score	Descriptor	Further elaboration	Hypothetical EEG (Based on Racine paper)
0	Baseline	Normal - no seizure	Wake rhythm
1	Whisker trembling	Whisker movements, possibly alongside normal motor movements	Increased amplitude of EEG, spike-wave discharges
2	Sudden Behavioral arrest	Stopped moving, staring off into space, some tail rigidity	
3	Facial jerking	Nose jerks, facial movements more severe than nose twitching, occurring after behavioral arrest	
4	Neck jerks	Head movements/twitches Sitting down No "arm reaches"	sharp spikes, followed by spike-wave discharges
5	Clonic seizure	Sitting position No loss of balance Arm reaches	high frequency small amplitude rhythmic waves, almost identical to active wake EEG
6	Clonic/Tonic clonic - Belly	Seizures on belly with arm reaches Loss of balance including leaps forward but not uncontrolled jumping May "flatten" against the floor	high amplitude polyspikes, spike-wave discharges
7	Clonic/Tonic-clonic - Side	Falling onto side Arm reaches	
8	Clonic/Tonic-clonic - wild jumping	Leaps through the air Spastic limb jerking	
9	Tonic extension	Extension of forelimbs (rapid twitching while on side) Usually it is a long recovery period after a level 8 seizure	almost flat EEG, gradual diminishing
10	Seizure leading to death of animal	During recovery period Death	Flatlining EEG

Non-Mendelian, Age-Related Inheritance



Genotype-Dependent Phenotype with No Sex Differences

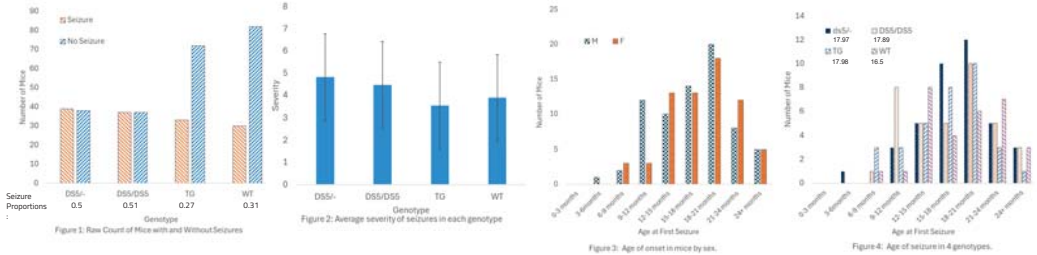


Figure 1: Raw Count of Mice with and Without Seizures
Figure 2: Average severity of seizures in each genotype
Figure 3: Age of onset in mice by sex.
Figure 4: Age of seizure in 4 genotypes.

CONCLUSION

- A new scale may be more useful for assessing spontaneous seizures as opposed to kindling/chemically induced ones
- There is no sex effect for proportion, seizure intensity or age of onset
- Both DS5/- and DS5/DS5 mice have a higher seizure population proportion compared to the Tg and Wt mice, indicating an issue with the rescue model, as well as the association of Nrxn-1 with seizure activity.
- The family tree analysis does not indicate a Mendelian pattern of inheritance; however, it shows older mice as having a high proportion of seizures, indicating an age effect. This may be related to the accumulation of Nrxn-1 with age.
- Both the Nrxn-1 knockdown mice and the DS5 rescue mice can provide valuable insight into seizure occurrence and requires more exploration to determine these mice as a potential spontaneous seizure model.

FUTURE DIRECTION + LIMITATIONS

- Limitations:
  - Not every seizure was caught on camera, shorter seizures may have been missed.
  - Age of onset was indeterminate
  - No constant observation
- Future Direction
  - EEG (4)
  - Sequencing (5) and Expression Levels (6)

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