Optimizing Gene Therapy for CLN3 Disease

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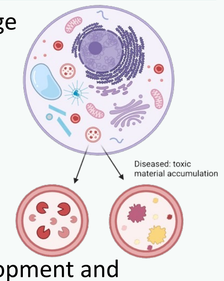
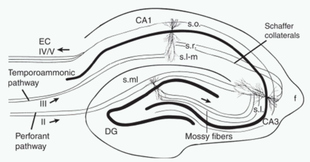
INTRODUCTION



- CLN3 Disease (Juvenile Batten) is a lysosomal storage disease characterized by the absence of functional CLN3 protein that leads to an accumulation of auto fluorescent lipofuscin in lysosomes
- The CLN3 gene encodes for a transmembrane protein of unknown function. We know it is Important for endocytosis, intracellular trafficking, and autophagy
- CLN3 is expressed in the whole brain in early development and primarily shifts towards the dentate gyrus(DG) of the hippocampus postnatally
  - ➢ The DG is involved in encoding short-term memory and learning
- The disease is passed down through autosomal recessive inheritance and is the most common form of childhood dementia
  - ➢ Characterized by early on-set blindness, anxiety, dementia, seizures, movement disorders, cardiac arrhythmias, and death by 3rd decade of life
- More than 85% of patients have a 1-KB deletion encompassing exons 7 and 8

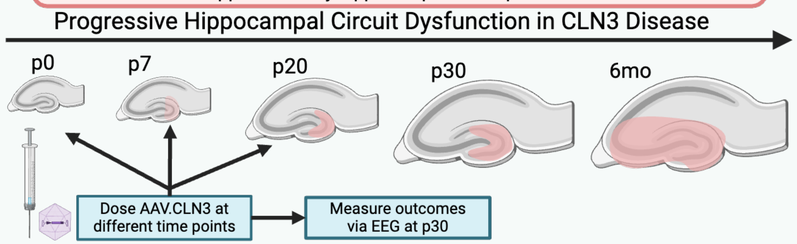
Previous findings in CLN3 -/- mice at 2 and 6 months: DG is hypoexcitable, faster EEG background activity, loss of hippocampal sharp wave ripples long before lysosomal storage accumulation begins



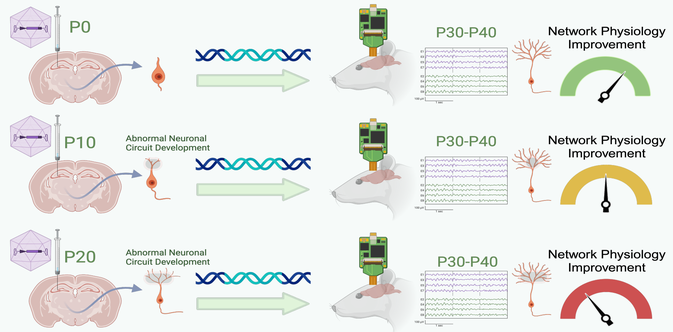
OBJECTIVES

Overall Goal: Develop network-directed therapies combined with gene replacement to improve outcomes in lysosomal storage disorders (LSDs)

Aim 1: What happens to early hippocampal development in CLN3 disease?



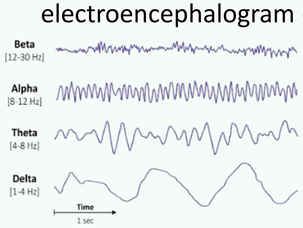
Aim 2: If gene therapy is given after onset of hippocampal dysfunction will it normalize circuit physiology?

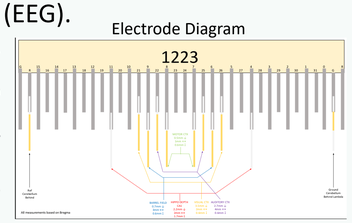


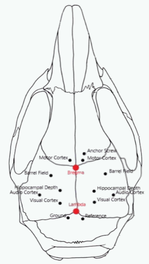
Hypothesis: Gene therapy will not efficiently improve network physiology because once abnormal development occurs, gene replacement will not reverse the damage.

METHODS

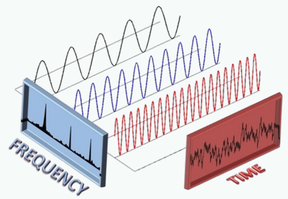
- To directly measure hippocampal activity and elucidate maturation of network dynamics in early development we use electroencephalogram (EEG).

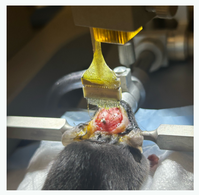


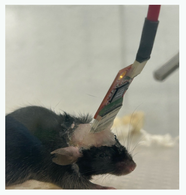




Waves frequencies, beta, alpha, theta, and delta correlate with a person's wakefulness.

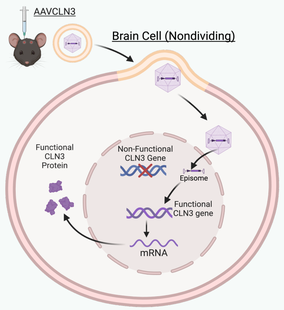




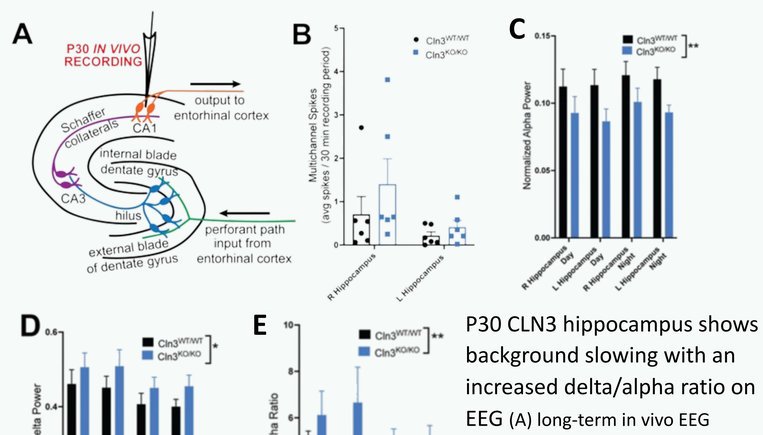


Fast Fourier transform decomposes the EEG signal into specific frequencies so we can correlate the ratios of slow, medium, and fast signals to cognitive features.

- Animals at p0, p10, and p20 are dosed with the AAV constructs via bilateral ICV injection
- We use a transgene expressed under control of 1665 bp of the endogenous CLN3 promoter, to restore physiologic expression levels and avoid overexpression



RESULTS



P30 CLN3 hippocampus shows background slowing with an increased delta/alpha ratio on EEG (A) long-term in vivo EEG recordings from the CA1 region of the hippocampus (B) Trend toward increasing interictal spikes, but not significant (C) Decreased fast alpha power (D) Increased slow delta power (E) Statistically significant increase in delta:alpha ratio

DISCUSSION

- Interictal spikes are synchronous discharges of a group of neuron that can signify a seizure potential, a clinical marker of CLN3
- Increased delta:alpha ratio is an established EEG measure of pathologic slowing and a clinical marker of brain injuries and strokes

FUTURE DIRECTIONS

Now that we have established EEG pathological slowing in CLN3 -/- mice as early as p30 , our next step is to determine if there is a time point in abnormal neuronal circuit development after which gene replacement cannot efficiently improve network physiology. We know that gene replacement in utero or at p0 improves network physiology. We will dose mice at different ages with the AAV and measure their network physiology using EEG.

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