Neuronal Primary Cilia in Postnatal Brains & Alzheimer's Disease Mice EEG Patterns Under Fear Conditioning

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Objectives:

- To observe further effects of neurodegeneration on the hippocampus
- To identify early detection behaviors significant of neurodegenerative disease
- To develop a connection between these behaviors and recognition techniques

Introduction:

- Alzheimer's Disease (AD) is a neurodegenerative condition caused by the abnormal accumulation of amyloid β plaque. While small amounts of amyloid β plaque is to be expected with increased age, AD presents amyloid precursor protein (APP), a familial linked gene promoting the formation of plaque at inappropriate rates and times, further causing early neuronal death and tissue depreciation.(1,4)
- APP23 transgenic mouse models contain the human APP Swedish double mutation with hemizygous expression. It's found in the neocortical and hippocampal positions, areas indicative of plaque aggregation and memory loss.(3)
- Mice present the same pathology as human AD including plaque formation, hyperphosphorylated tau, neuronal death, and memory deficits before 24 months.(3)
- Within the hippocampal cortices, hair-like micro projections called primary cilia regulate higher cognitions, neurodevelopment, and physiological functions.
- Burst suppressions are moments of high electrical activity indicative of movement or a sharp change in focal activity.

Figure 1: Identifying APP23 (250bp) and wildtype (521bp) genotype in subject using gel electrophoreses

Methods:

- A colony of Jax mice, *Mus musculus*, were raised, weaned, and tagged between 17 and 21 days old in a sterile environment.
- Polymerase chain reaction and gel electrophoresis was done on sample tissue of each individual to assign a wildtype or APP genotype.
- **<u>Evaluation routes</u>**:
  1. Brain tissue was isolated using perfusion and cryoprotectant. Slices were examined under a Nikon A1R HD confocal microscope after applying an immunofluorescent stain.
  2. An EEG/EMG mount was surgically secured to the top of the skull. After recovery, the following recordings were made using Ethovision and Sirenia Acquisition of the basal neuronal activity:
     - **Sleep**: 24 hours sleep and activity recording with no outside stimuli
     - **Trace fear**: 2 training sessions were done 1 hour apart where a neutral tone was sounded for 15 seconds followed by a mild foot shock 30 seconds later and repeated for 7 cycles.
- Burst data and model were designed and detected by the UNH Department of Mathematics.

Hypothesis:

- The younger APP23 mice establish the connection between stimuli better than the wildtype mice.
- If more burst suppressions are present for APP23 mice, than their genotype is more successful than the control. In early development, the APP23 mice show to have more active neurons and, therefore, are more intelligent that the control.

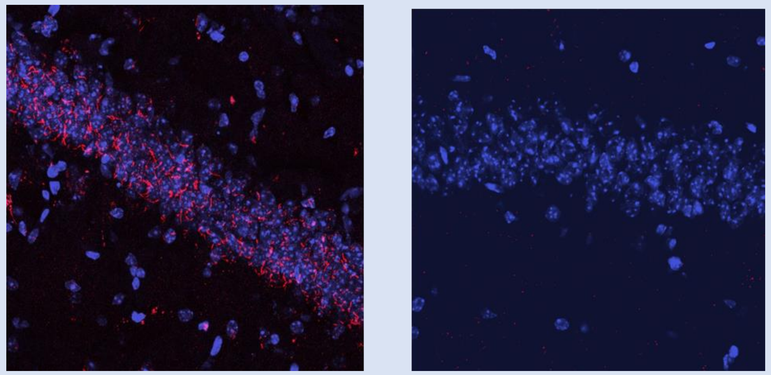

Figure 2: APP mice hippocampus CA1 primary cilia; Primary cilia marker AC3 (red), nuclei stained by DAPI (blue)

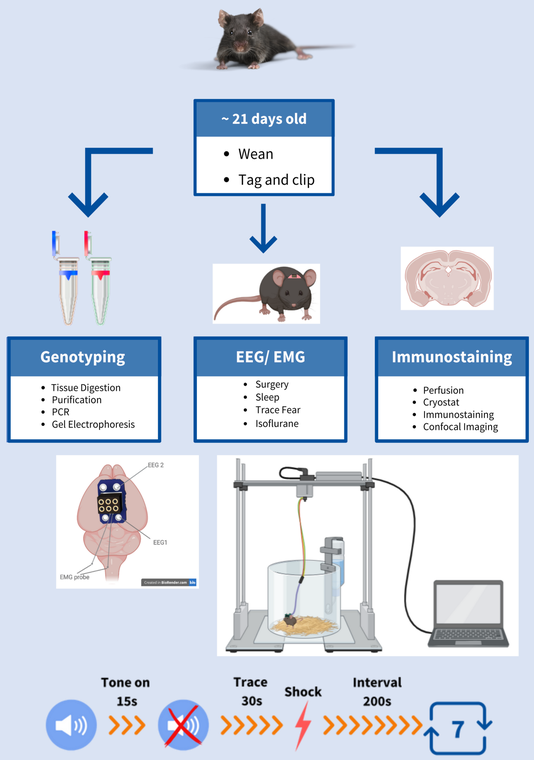

Figure 3: Methodological flow chart and trace fear sequence typically followed for the basic data retrieved per subject

Preliminary Results:

- Bands were identified at 250bp and 521bp for the APP23 and wildtype genotypes, respectively.
- Primary cilia were present in the hippocampal area in the wildtype and transgenic mice, but not in the knockout mice.
- There were no significant bursts differing between the control and APP mouse in Cycle 1 or Cycle 7 other than at the moment of shock deliverance.
- The APP mouse showed a significant burst suppression after the tone stimuli but prior to the time of the shock stimuli in Cycle 5.

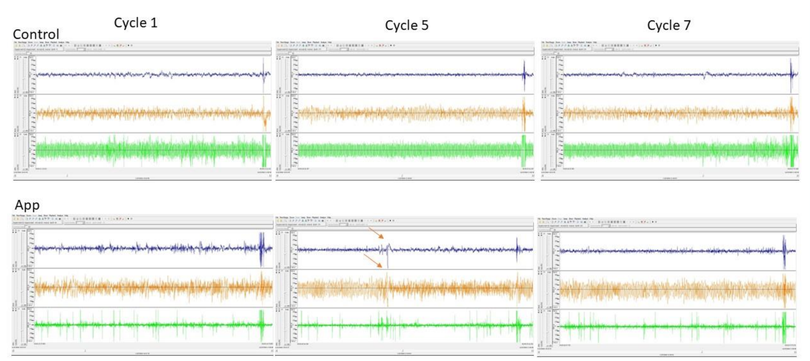

Figure 4: Burst data using Sirenia Acquisition of control and APP23 genotypes under trace fear conditioning.

Discussion & Conclusion

- Current data support the hypothesis and predictions, but more data is needed.
  - The APP23 mice showed apprehension at the release of the tone prior to the shock stimuli whereas the wildtype mice did not.
    - Indicative of learning response
  - Observationally, the APP23 mice tended to act more reactive surroundings than the control after the stimuli cycles were complete.
- Continuity of this data trend creates potential for a biomarker for early detection of neurodegeneration, broadly aiming towards disease prevention/ treatment efforts.
- **<u>Future Direction</u>**
  - Continue trace fear collection for more data
  - Support with behavioral tests and hippocampal confocal imaging
  - Evaluate from a comparative evolution standpoint

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