Scoring Sleep and Wake Vigilance States in Rodents

A comparison between expert hand scoring, decision tree, and neural network data processing

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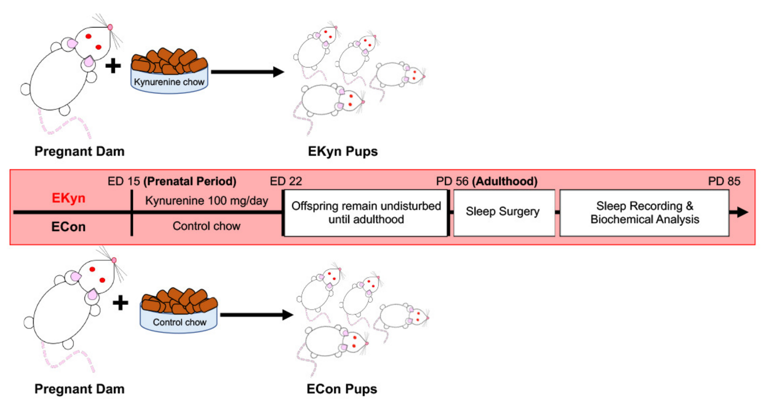
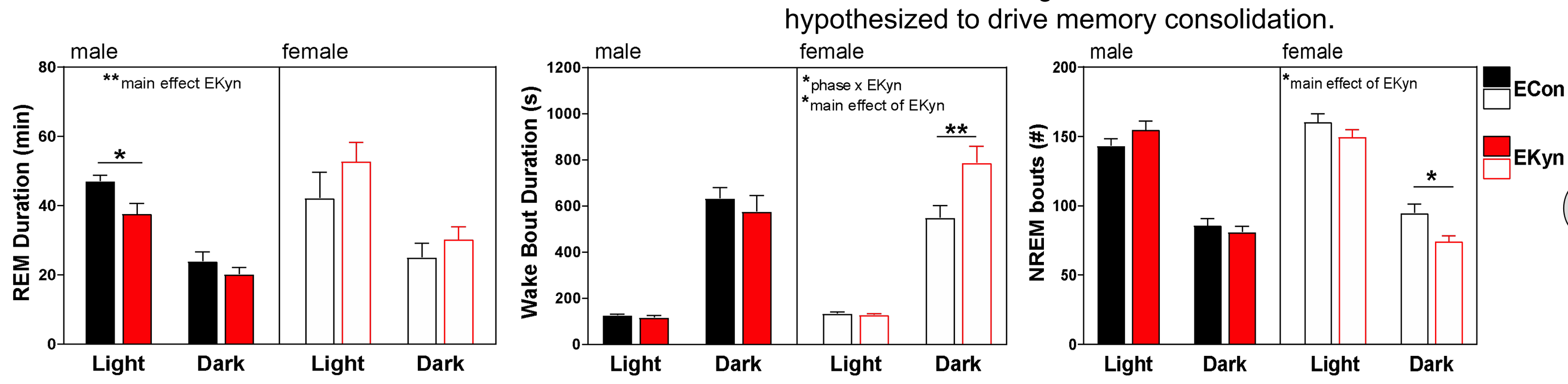
Introduction

- Sleep is a naturally reoccurring state of mind and body characterized by altered consciousness, relatively inhibited sensory activity, inhibition of all voluntary muscles, and reduced interactions with surroundings.
- Kynurenic acid (KYNA) is a tryptophan metabolite implicated in the pathology of neurocognitive disorders including schizophrenia, bipolar disorder, and neurodegeneration.
- When acutely elevated in rodents, KYNA is known to cause cognitive impairments and impaired sleep.(Pocivavsek et al., 2017).



•The three commonly accepted stages of sleep are Wake, NREM sleep, and REM sleep, which are characterized by electroencephalogram (EEG) and electromyogram (EMG) oscillations. NREM sleep is commonly referred to as slow wave sleep due to the low frequency delta (0.5-2 Hz) waves. During REM, or paradoxical sleep, the EEG oscillations appear similar to wake, but muscle tone and activity is absent.

•Spindles are bursts of corticothalamic neural oscillatory activity in the frequency range of 10-15 Hz with a duration greater than 0.5s and are hypothesized to drive memory consolidation.



Previously, male EKyn offspring, REM duration was decreased in the light phase. Additionally, in female EKyn offspring, sleep deficits in the dark phase were observed. (Rentschler et al. 2021).

Methods

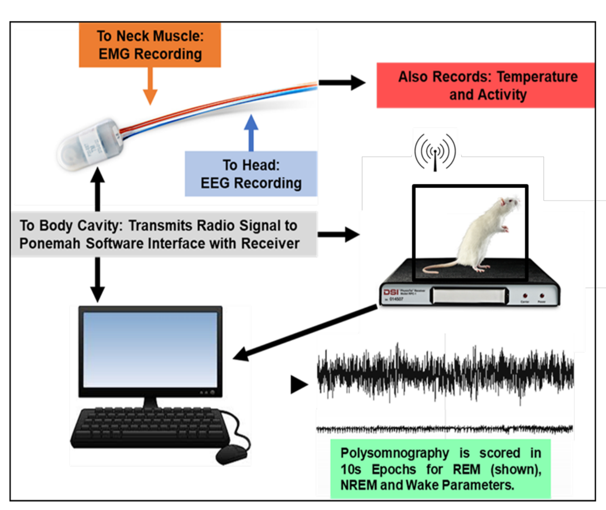
Prenatal Kynurenine Treatment as a Tool to Study Sleep Dysfunction in Psychotic Disorders

Pregnant Wistar dams were fed chow laced with 100 mg kynurenine (Sai Advantium, Hyperbad, India) from embryonic day (ED) 15-22, corresponding to the second trimester in human pregnancy. Embryonic kynurenine (EKyn) and embryonic control (ECon) offspring were left to mature into adulthood. The EKyn paradigm has been validated to study sleep dysfunction in psychotic disorders (Rentschler et al. 2021).

Electroencephalography (EEG) and Electromyography (EMG) Signal Acquisition

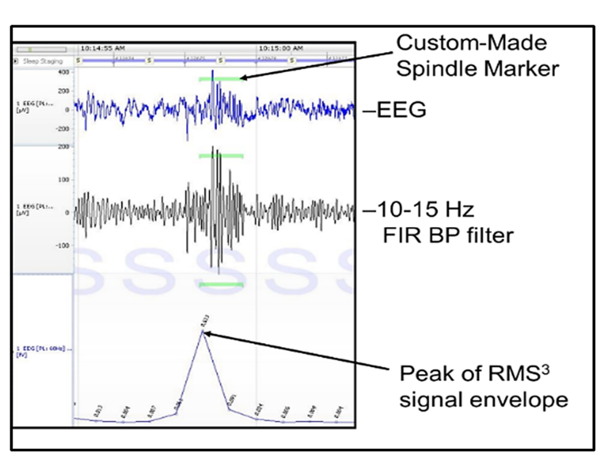
Under isoflurane anesthesia, animals were implanted with telemetry transmitters between postnatal days (PD) 56-85, or the rodent equivalent age to young adulthood in humans. EEG leads were inserted at 2.0mm anterior/+1.5mm lateral and 7.0mm posterior/-1.5mm lateral relative to bregma and secured 0.5mm into the skull. EMG leads were inserted into the dorsal cervical neck muscle approximately 1.0 mm apart. Animals recovered 7-10 days before experimentation.

Sleep data was recorded in a designated room where rats were undisturbed. EEG and EMG waveforms were simultaneously collected for a 4-day period for females and a 2-day period for males using Ponemah 6.10 software. This data was then imported to NeuroScore 3.0 for scoring by trained professionals. It was classified in 10-second epochs as either wake (low-amplitude, high-frequency EEG and high-amplitude EMG), NREM (high-amplitude, low-frequency EEG with low-amplitude EMG), or REM (low-amplitude, high-frequency EEG with very low EMG tone).



NREM Spindle Detection and Analysis

A band pass filter for 10-15 Hz frequency was applied to the EEG signal in Neuoscore. A root mean square transform was cubed and applied to the band pass filtered signal for more precise spindle detection. NREM spindles were scored manually with a custom-made scoring marker to detect spindle frequency and duration. Data for spindle detection was analyzed at the first 4 hours of the light phase, when NREM sleep is most frequent for rodents. Periodogram power spectra were determined using Fast Fourier Transform to detect the peak spindle frequency for each subject. Peak to peak (p2p) calculations were used to determine average spindle amplitude.



Spindle detection in Neuroscore software was made possible using a bandpass (BP) filter and a cubed RMS transform.

Decision Tree and Neural Network Development

To automate the scoring process, decision trees and neural networks were developed. After importing data that included hand-scored sleep stage, EEG, and EMG (mean, max, min, p2p, and standard deviation) for 4 days of each rodent, a decision tree was created in the Python scikit-learn package. The accuracy and classifications from the decision tree were exported for analysis. An artificial neural network was developed in python to classify the sleep stage into bins of sleep, REM, and NREM. These scores were saved in a .csv file which then was analyzed in Matlab and Python

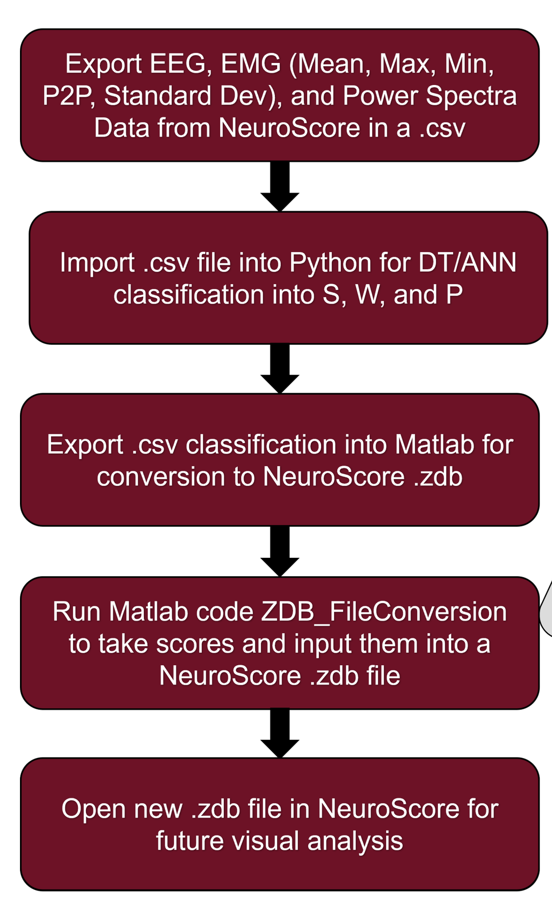
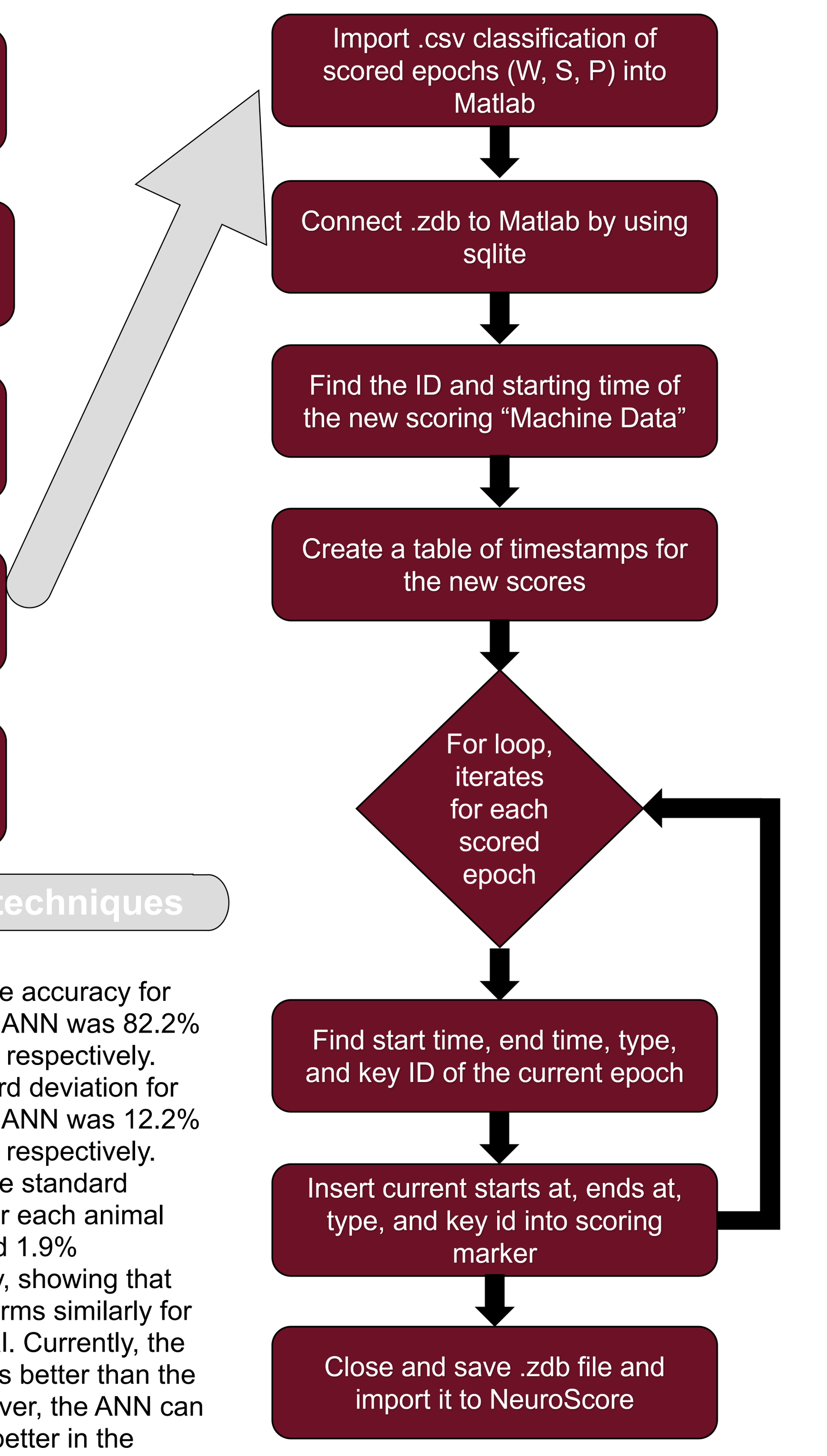
Importing Data Into NeuroScore using Matlab/SQLite connection

After the data were scored by the decision tree and the neural network into W, S, and P, corresponding to Wake, NREM, and REM, they were imported back into NeuroScore for visualization and analysis. Matlab code was developed which inserted this data of epoch scores(.csv) into a NeuroScore database (.zdb) for human visualization. The .zdb is a SQLite database with 14-16 tables containing information such as epoch number, epoch start/end time, epoch type (Wake, Sleep/NREM, Paradoxical/REM), key id, notes, deleted status, internal properties, and scoring for the NeuroScore file. The Matlab code imports the machine scored data (.csv), opens a connection to SQLite to connect to the .zdb, inserts the machine scores into the .zdb, and closes the connection to SQLite. This approach enabled the machine data to be visualized in NeuroScore.

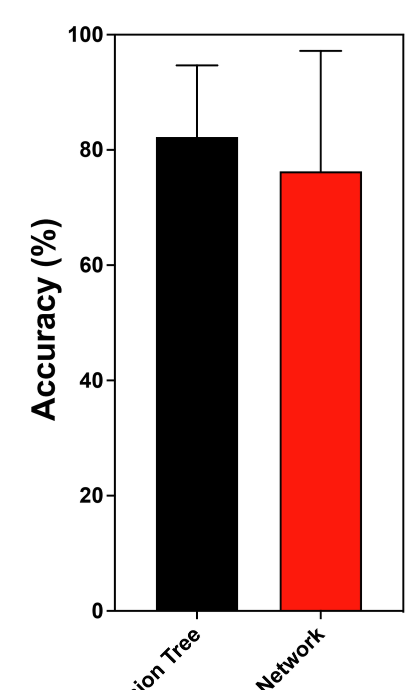
Statistical Analysis

Data were analyzed using GraphPad Prism 8.0 (GraphPad, La Jolla, CA). Two-way ANOVA was used to assess spindle dynamics between female ECon and EKyn animals relative to estrous cycle day. Student's T-test was used to assess accuracy of the decision tree method vs. the machine learning method. All data are mean ± SEM.

Coding Protocol and Computational Efficacy

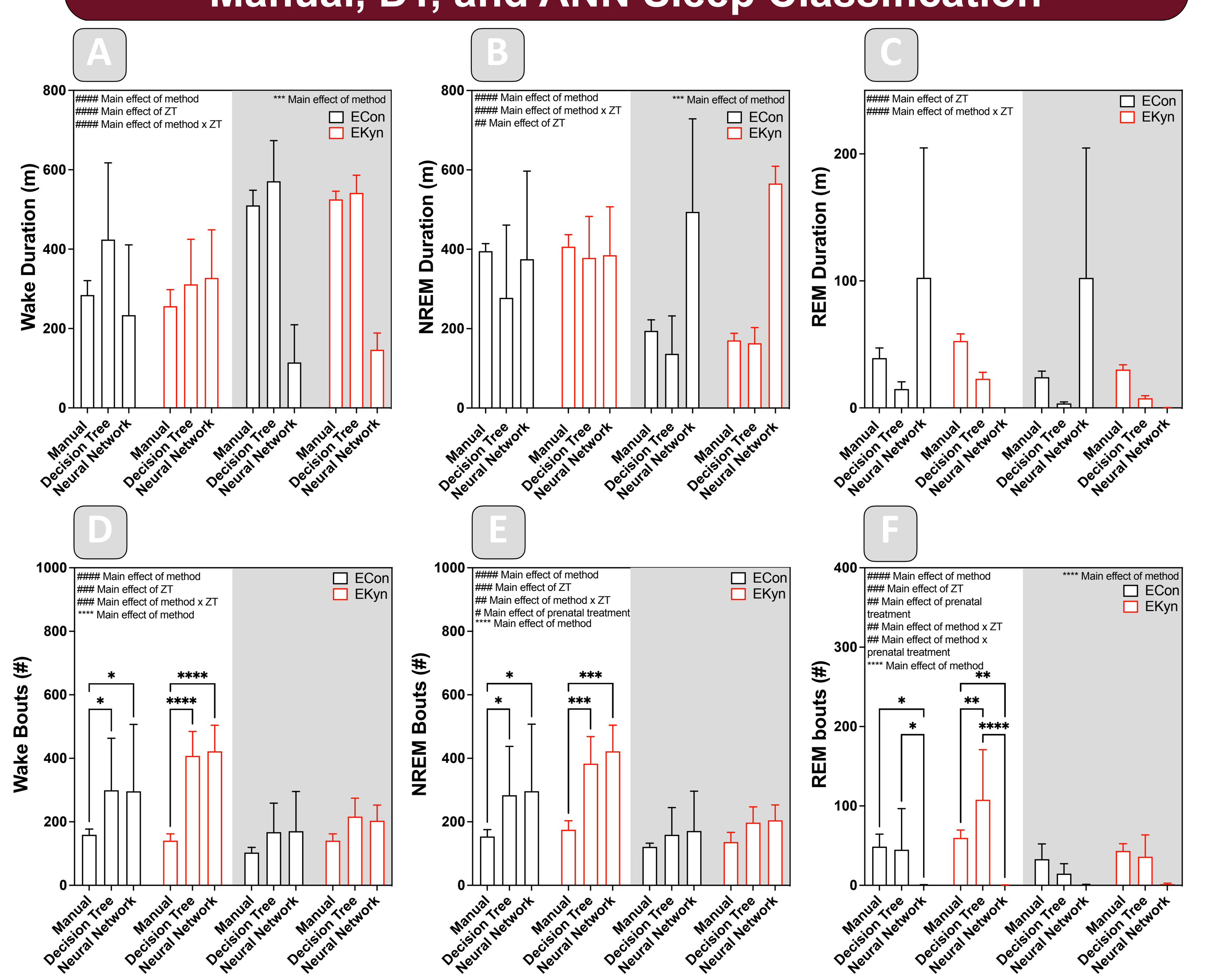



Comparison of sleep scoring techniques



The average accuracy for the DT and ANN was 82.2% and 76.3%, respectively. The standard deviation for the DT and ANN was 12.2% and 20.5%, respectively. The average standard deviation for each animal was 1.3 and 1.9% respectively, showing that the AI performs similarly for each animal. Currently, the DT performs better than the ANN, however, the ANN can be trained better in the future to increase its accuracy.

Manual, DT, and ANN Sleep Classification

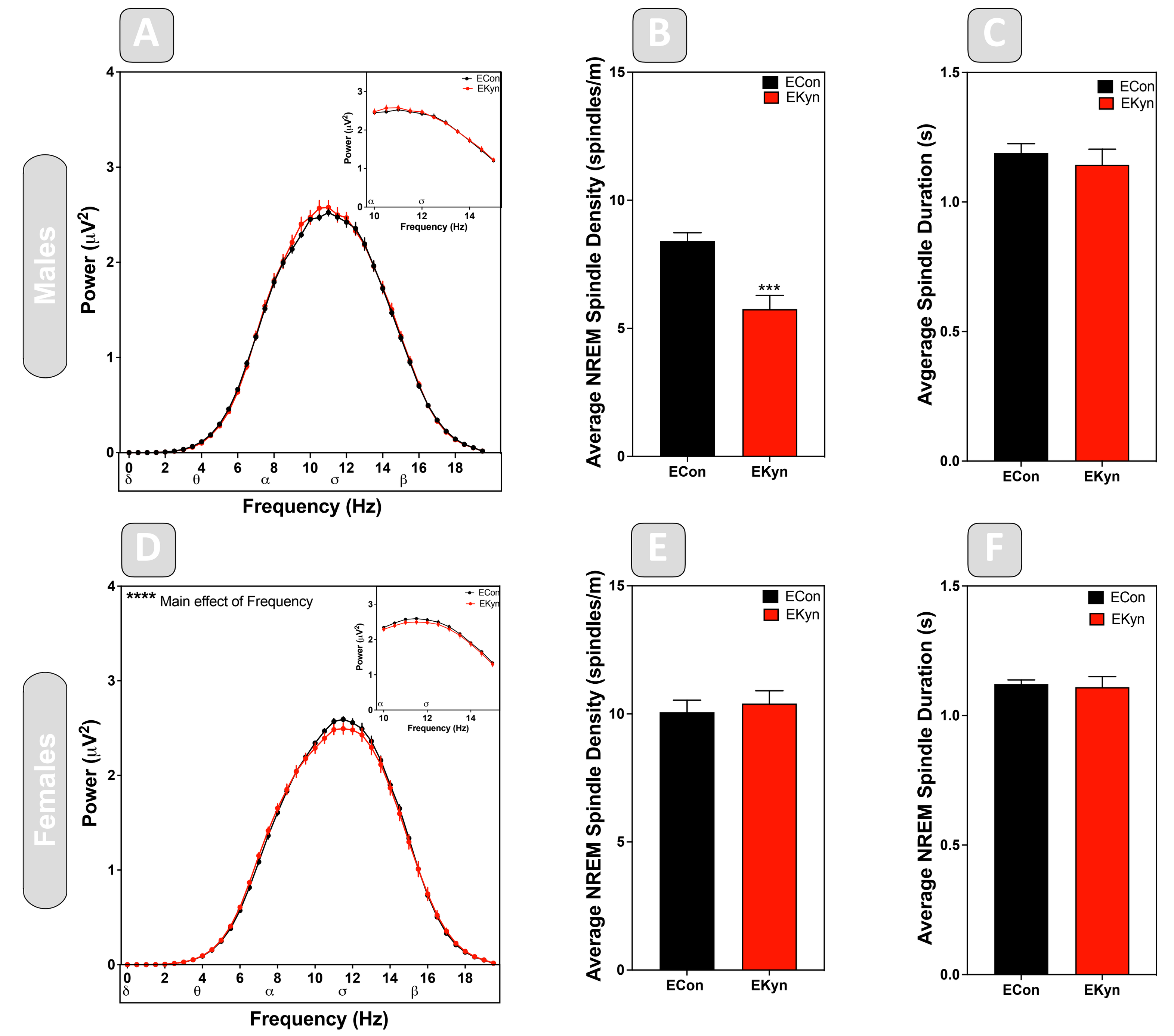


(A) Wake Duration **(B)** NREM Duration **(C)** REM Duration **(D)** Wake Bouts **(E)** NREM Bouts **(F)** REM Bouts
All data are from females.

Automated sleep scoring classification systems are more accurate during the dark phase when compared to expert scoring.

Two way ANOVA, Fisher's LSD Test, **** P < 0.0001;
Three-way ANOVA, Bonferroni's post-hoc test: ## P < 0.008, ###P <0.001, ####P < 0.0001; Data are +- SEM; N=7-8 per group.

Manual Spindle Analysis for EKyn offspring



Previously, in male EKyn offspring, the average NREM spindle density was shown to be decreased. Female spindle data was analyzed to see if these differences were sex specific or held between both genders. No deficit was found in the average NREM spindle density in females. The female power spectra data show that in EKyn offspring, the power is decreased in the 10-15 Hz range.

(A) Male FFT Spindle Spectra **(B)** Male Average NREM spindle density **(C)** Male Average Spindle Duration **(D)** Female FFT Spindle Spectra **(E)** Female Average NREM spindle density **(F)** Female Average Spindle Duration

Three-way ANOVA, Bonferroni's post-hoc test: ****P < 0.0001; Student's T-test; Data are ± SEM; N=7-8 per group.

Future Directions

Matlab Spindle Detection Automation

The spindles in this work were hand scored, which takes an extensive amount of time for a trained professional. In addition to the automation of the sleep state scoring, the automation of sleep spindles using Matlab or other software can be developed to process the data more efficiently and reliably and eliminate intra-personal variability. A sleep spindle detection protocol in Matlab, as described in a Uygun et al. 2019 Sleep paper, was received to be adapted to fit the precise needs of our lab.

Data Processing and Scoring in Matlab to eliminate NeuroScore

Methods can be developed to export the data scoring and processing steps to software such as Matlab, instead of using NeuroScore for this process. Eliminating NeuroScore from the process will allow the data to be processed more efficiently, effectively, and precisely.

Conclusions

Analysis of DT, ANN, and Hand Scoring Comparisons

The average accuracy for the DT and ANN was 82.2% and 76.3%, respectively. The average standard deviation of accuracy between each day for the decision tree and neural network was 1.3% and 1.9%, showing that the AI scored each animal in a similar fashion. The AI had considerable trouble with one animal, 17E-5, perhaps due to the sleep data not fitting with the other animals recorded. Excluding this animal, the accuracy jumped to 84.7% and 81.3% for the DT and ANN respectively. The DT and ANN did not classify REM sleep accurately, possibly because this is the least classified sleep state in rodents, on average around 5-10% of the recording. Different methods of the ANN training could be implemented in order to improve the classification of REM and mimic manual scoring.

Importing Automated Data into NeuroScore (DSI)

Another goal of this project was to import the scored data set back into NeuroScore for visual analysis. By editing the .zdb with an SQLite connection in Matlab, the classified data set can be snuck into .zdb with a new key id. This new scoring is available as "Machine Data" in NeuroScore.

Sleep Spindles are differentially impaired in males, not females, females have higher average spindle density

Spindle data were analyzed for females in order to compare the data to previously published data for male spindles. In males, sleep spindles are differentially impaired by prenatal conditions. EKyn females showed a lower power spectra for spindles, especially in the 10-15 Hz range.

Applications

The paradigm of AI scoring and importing scorings back into NeuroScore can be used to increase efficiency and efficacy, eliminating intra-personal variability for sleep labs. The data analysis of sleep will be improved, allowing for more experiments to occur and processing the data faster.

Acknowledgements

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