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EEG Signal Evaluation of Aldehyde Dehydrogenase Deficient Mice

DATE

This document is the final report of a research study conducted under the terms of a Research Agreement between NeuroDetective International Inc. and CLIENT, DATE.

This study was conducted between DATE and DATE by:

INTRODUCTION

This study evaluated sleep activity in two groups of mice, one genetically modified the other wildtype controls, supplied by the CLIENT. Each group consisted of 12 males, which were first screened for certain pathogens prior to the start of the study.

The study itself occurred in an AAALAC accredited vivarium. The animals were first instrumented with bilateral EEG electrodes and an EMG electrode. Following a 7-day recovery period, the mice were acclimated to the recording chambers and tethered to a biopotential cable for 2 days, after which polysomnographic recordings were sampled at 128 Hz for 48 h.

METHODS

NDI received 24 male mice (12 Aldh7a1 knockout, KO; and 12 wild-type controls; WT) aged 4-6 weeks into quarantine. During quarantine vivarium staff reported two mice with alopecia and one mouse with pinkish discharge from the penis that did not resolve. Mice were cleared for vivarium excluded pathogens [IDEXX folder] and transferred to a holding room on a 12:12 light dark cycle with ambient temperatures of 23 ± 1 °C. All mice had *ad lib* access to food and water.

For the initial cohort, 11 mice were anesthetized with ketamine–xylazine (IP; 87 and 13 mg/kg, respectively) and instrumented with bilateral EEG electrodes (Plastics One; Roanoke, VA) over the right and left frontal and parietal cortices and an EMG electrode over the cerebellum, which acted as a ground. In addition, a stainless steel EMG electrode was placed in the nuchal muscles. The electrodes were fixed with light-cured dental composite (Prime-Dental Mfg, Chicago, IL). One mouse from each cohort had complications from surgery and was excluded. After at least 7 days of recovery, mice were acclimated to the recording chambers and tethered to a biopotential cable for 48h after which polysomnographic recordings ensued for 48h [PSG Record*.kcd files]. Polysomnographic recording cables connected the head electrodes to commutators that were in turn led to amplifiers (Grass Model 15LT; Natus Neurology, Warwick, RI). The analog EEG and EMG signals from the amplifiers were converted to digital signals (128 Hz sampling rate) and recorded. EEG signals were filtered below 0.1 Hz and above 100 Hz with a 60 Hz notch filter. One week later, the experiment was repeated with a second cohort of 11 mice in a research design that was counterbalanced for chamber, commutators and recording cables.

Manual off-line sleep scoring using SleepSign for Animal (Kissei Comtec; Matsumoto, Nagano, Japan) determined time in state (wake, NREMS, REMS), state bout counts and bout durations [PSG Record*.raf files] in 10 sec epochs. NREMS was characterized by high amplitude EEG signals and low EMG activity. REMS was identified by regular low-amplitude EEG and minimal EMG activity. Wakefulness was determined when the signal exhibited low amplitude fast EEG and high EMG activity. Epochs containing suspect ictal EEG activity were tagged (scored as M) and extracted for separate analyses. EEG signals from mouse 106 indicated sickness (decreased EEG amplitude and transient bout transitions with the suppressed REMS and compromised light/dark rhythmicity), and records from 9 WT and 10 KO mice were used for subsequent analyses.

In addition, FFT analyses using a Hanning window and normalized NREMS EEG SWA (2h bins) and state-specific overall power spectra (12h bins; 1-20 Hz in 1Hz) were determined. Epochs containing ictal EEG signals were excluded from the state-specific FFT analyses. Suspect epileptiform discharge epochs were extracted from mouse 116 and 117 [Discharge Traces.pptx file], and categorized by amplitude, duration and spectral profiles [Discharge Data.xlsx file]. Given that only one WT and one KO, had few total suspect discharges, statistical comparisons were not warranted.

Statistical analyses were performed using SPSS v. 17 [Statistics\] for vigilance state duration and bout data and EEG SWA outcome measures were compared in 2h (NREMS EEG SWA) or 12h time blocks (all other state-specific outcomes) by 2 x 12 or 2 mixed ANOVAs with factors of strain (KOs vs. WT) and time using Day 1 and Day 2 averaged blocks. The use of 12h time blocks for state specific spectral analyses was decided *a priori* from 2h NREMS EEG SWA results. A 2 x 20 mixed ANOVA (strain x single Hz spectral frequencies) was used for analysis. When sphericity was violated, a Huynh-Feldt adjustment was used on repeated measures, but the original degrees of freedom are reported. Where appropriate, student's t-tests were used for pairwise comparisons of time matched bins. Genotype reconfirmation was successful in each experimental animal per tail tissues rendered to CLIENT after mice were euthanized.

RESULTS

(NOTE: All figures, descriptions of outcome measures, and traces are attached to this report as separate files. All raw data files of PSG recordings are available upon request.)

Suspect Cortical Discharges

A primary finding is that strain differences in discharge characteristics resembling seizure-like activity were negligible. One animal from each strain had suspect discharges that occurred during the dark period (when mice are predominantly awake). The characterization details are descriptive due to low incidence [Table 1].

On Day 1, m116 had two bouts of high amplitude 4 Hz peak [Spreadsheets/Discharge Data/m116 WT tab] bilateral seizure-like activity. This activity did not transfer to the EMG and was likely not myoclonic. The Day 1 bouts were separated by 16 seconds and were followed by a slight decomposition of the EEG as indicated by an ephemeral post-ictal decrease in EEG amplitude. Twenty-six h and 40 min later (on Day 2) a discharge with similar characteristics (e.g. duration and spectral frequency) occurred. The amplitude progressively increased across the three total discharges in m116. These ictal-type discharges are idiopathic, but could have arisen from the surgical procedure.

In m117, the discharge activity was restricted to less than 5h of the dark period on Day 1 and manifested two different phenotypes. Initially a short duration (20-29 sec) high amplitude lower frequency (1 Hz peak) was observed in three bouts. The discharge dynamic transitioned to a more distributed high amplitude brief spike pattern with a 3.5 Hz peak from background signal over the 3-6 min duration of discharge episodes. Increases in maximum amplitude were undetectable due to signal clipping. Neither discharge pattern reoccurred on Day 2 nor transferred to EMG and could reflect a noise artifact. In sum, subclinical seizures were not detected in EEG signals as a trait in either WT or KO mice.

Sleep/Wake State Comparisons

Sleep/wake architectures of each strain were similar. The expected time in state differences between the 12h light and dark periods were detected in wake, NREMS and REMS (TIME: $F_{1,17}$=146.77 p<.001; $F_{1,17}$=138.54 p<.001; $F_{1,17}$=100.93 p<.001, respectively), while the main effects of STRAIN and TIME x STRAIN interactions were not statistically significant [Fig 1]. Likewise wake, NREMS and REMS bout counts (Fig 2; TIME: $F_{1,17}$=134.02 p<.001; $F_{1,17}$=137.45 p<.001; $F_{1,17}$=87.69 p<.001, respectively) and bout lengths (Fig 3; TIME: $F_{1,17}$=80.88 p<.001; $F_{1,17}$=16.01 p<.01; $F_{1,17}$=16.38 p<.01, respectively) indicated a significant main effect of TIME whereas, all STRAIN and STRAIN x TIME interactions in sleep/wake state dependent variables were not statistically significant. Thus, the sleep/wake phenotypes of the above outcome measures were not unique between WT and KO strains, and both strains exhibited standard murine sleep/wake rhythms.

Cortical EEG Spectral Comparisons

FFT analyses of EEG signals indicate state related changes between WT and KO animals. NREMS EEG spectra showed KO mice had higher spectral content compared to WT mice (STRAIN x Hz interaction: $F_{19,323}$=4.77 p=.026) and pairwise comparisons indicate that this occurred in the 1-2 Hz frequencies irrespective of time [Figs 4A and 4B]. The NREMS EEG SWA (1-4Hz) recapitulates that pattern (Fig 5; STRAIN: $F_{1,17}$=5.24 p=.035; TIME: $F_{11,187}$=19.84 p<.001). The REMS EEG spectra was also elevated in the KOs compared to WT (STRAIN x Hz interaction: $F_{19,323}$=4.77 p=.008). Pairwise comparisons indicate significance in the 2-4 Hz window during the light period (Fig. 6A) and 2-5 Hz window during the dark period (Fig 6B. Strain differences in spectral content were not detected in wake-scored epochs (Fig 7A and 7B). Moreover, the overall spectral content during waking was not significantly altered during the light or dark periods. This was not the case with spectral content during NREMS (TIME: $F_{1,17}$=84.77 p<.001) or REMS (TIME: $F_{1,17}$=6.22 p=.023) which increased in both strains during dark periods compared to the light periods (Figs 4A vs. 4B and 5A vs. 5B). The expected changes in wake, NREMS and REMS between Hz (Hz: $F_{19,323}$=360.62 p<.001; $F_{19,323}$=122.02 p<.001; $F_{19,323}$=114.64 p<.001, respectively) and Time x Hz interactions (Hz: $F_{19,323}$=3.74 p=.039; $F_{19,323}$=34.94 p<.001; $F_{19,323}$=12.02 p<.001, respectively) were also significant. In summary, a second key finding of this EEG phenotyping study is that, compared to WT mice, the KO mice had elevated low frequency spectral power during REMS and NREMS, which persisted during the light and dark phases.

Table 1. Discharge Data

Mouse	Discharge Count	Time	Duration (sec)	Max Amplitude (peak+nadir)	Interval (hr:min:sec)	Point Trace #
m116 WT	1	Day 1-12:43:30	38.64	6.968	00:00:15.92	1
	2	Day 1-12:44:21	92.201	8.821	26:36:39.19	1
	3	Day 2-15:25:31	50.86	9.979	end	2
m117 KO	1	Day 1-15:14:25	29.164	9.321	00:00:29.52	3
	2	Day 1-15:15:35	24.094	8.076	01:26:45.92	3
	3	Day 1-16:42:45	19.617	9.339	02:21:26.88	4
	4	Day 1-19:04:36	403.117	9.442	00:28:50.67	5
	5	Day 1-19:40:08	192.656	9.441	00:00:53.84	6
	6	Day 1-19:44:25	244.321	9.429	end	7
				red indicates signal clipping		

Fig 1. Time in State

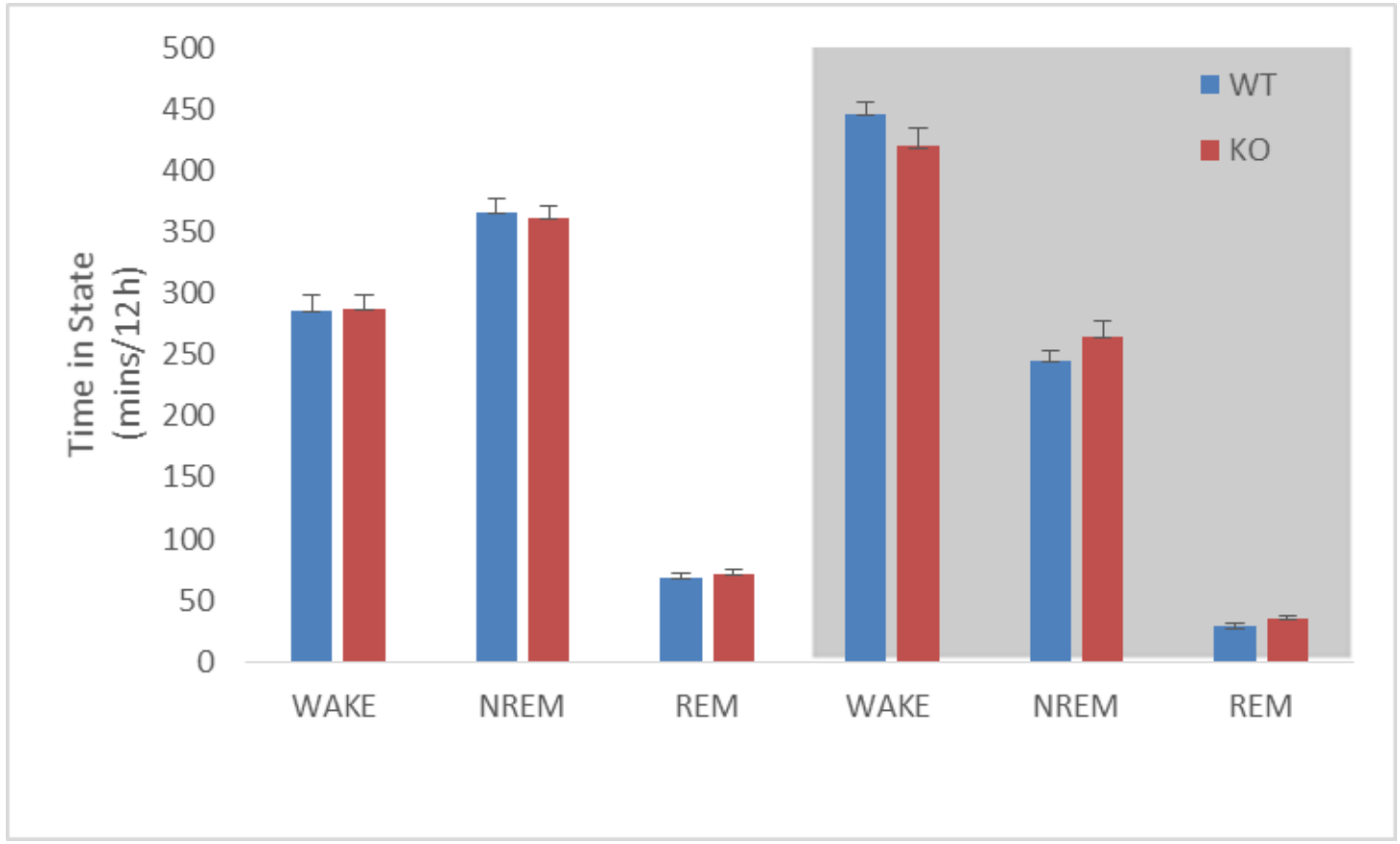


Fig 2. Bout Counts

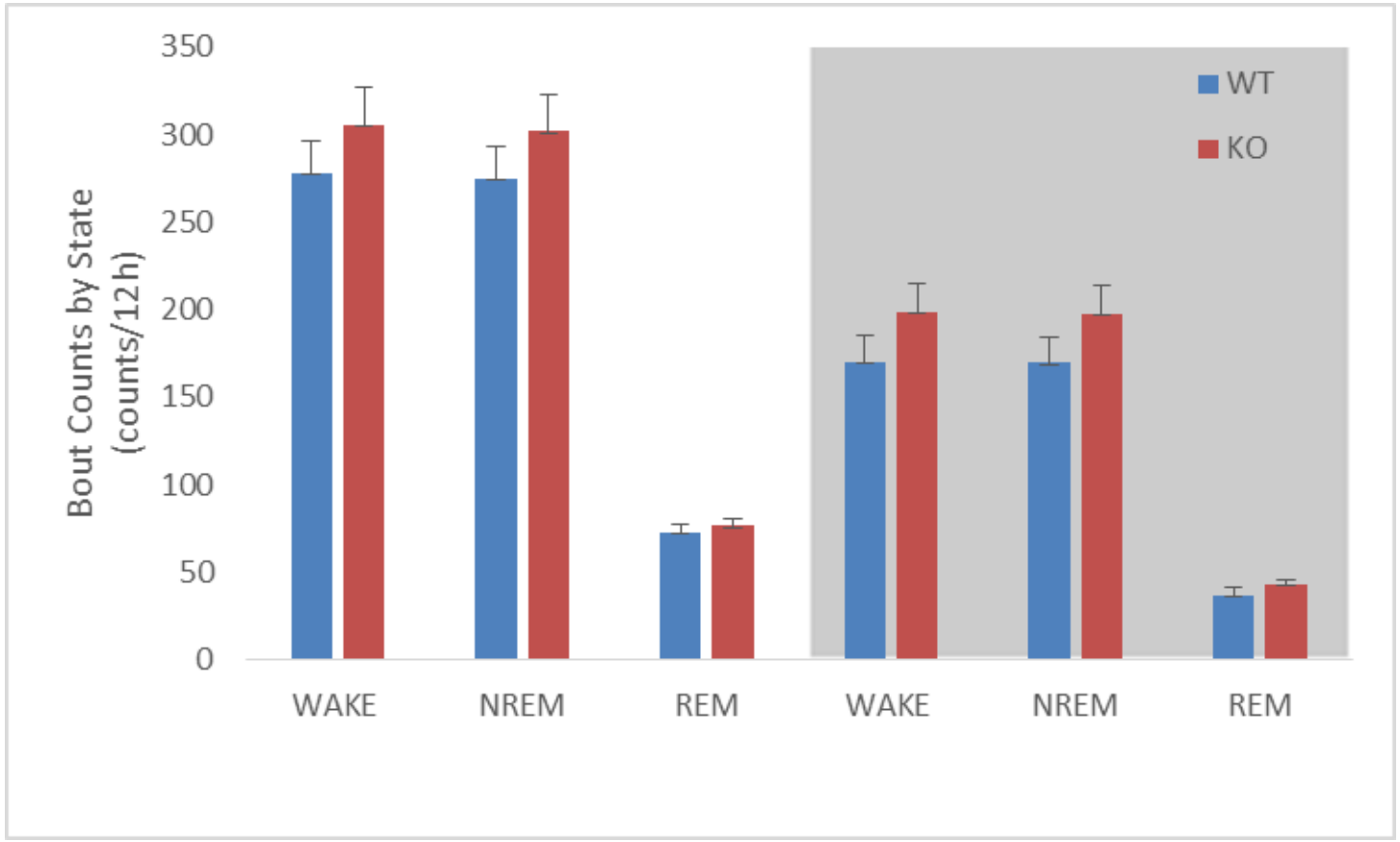


Fig 3. Mean Bout Durations

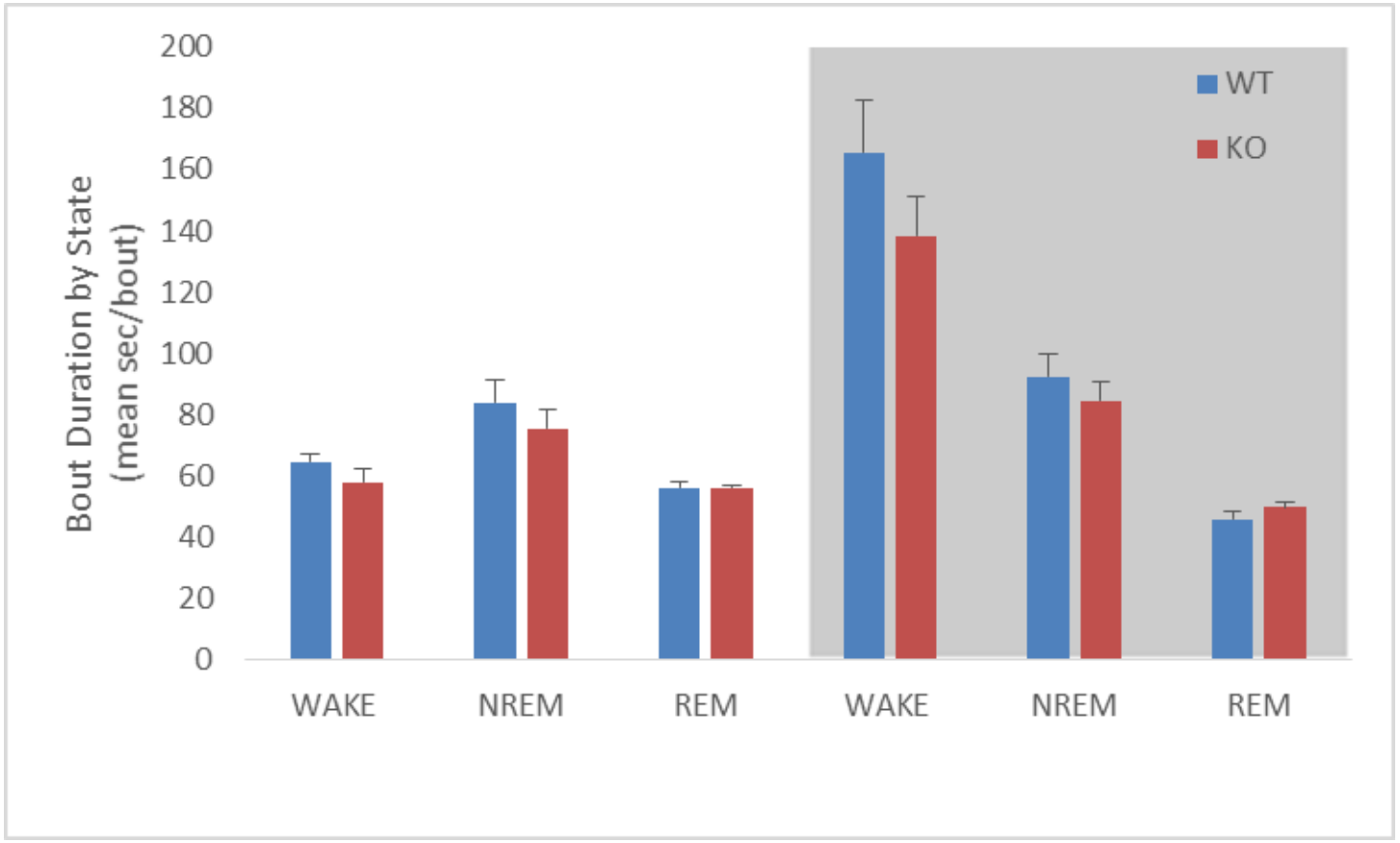
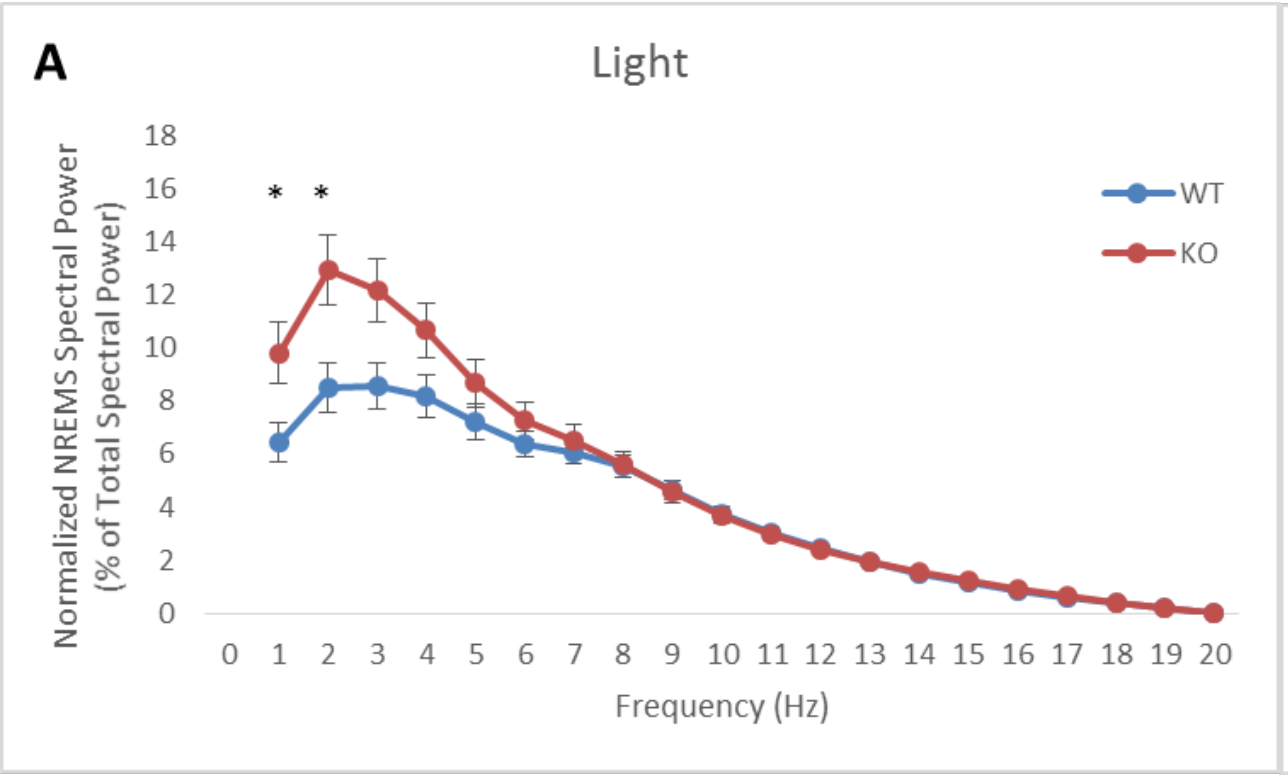


Fig 4. NREMS Power spectra



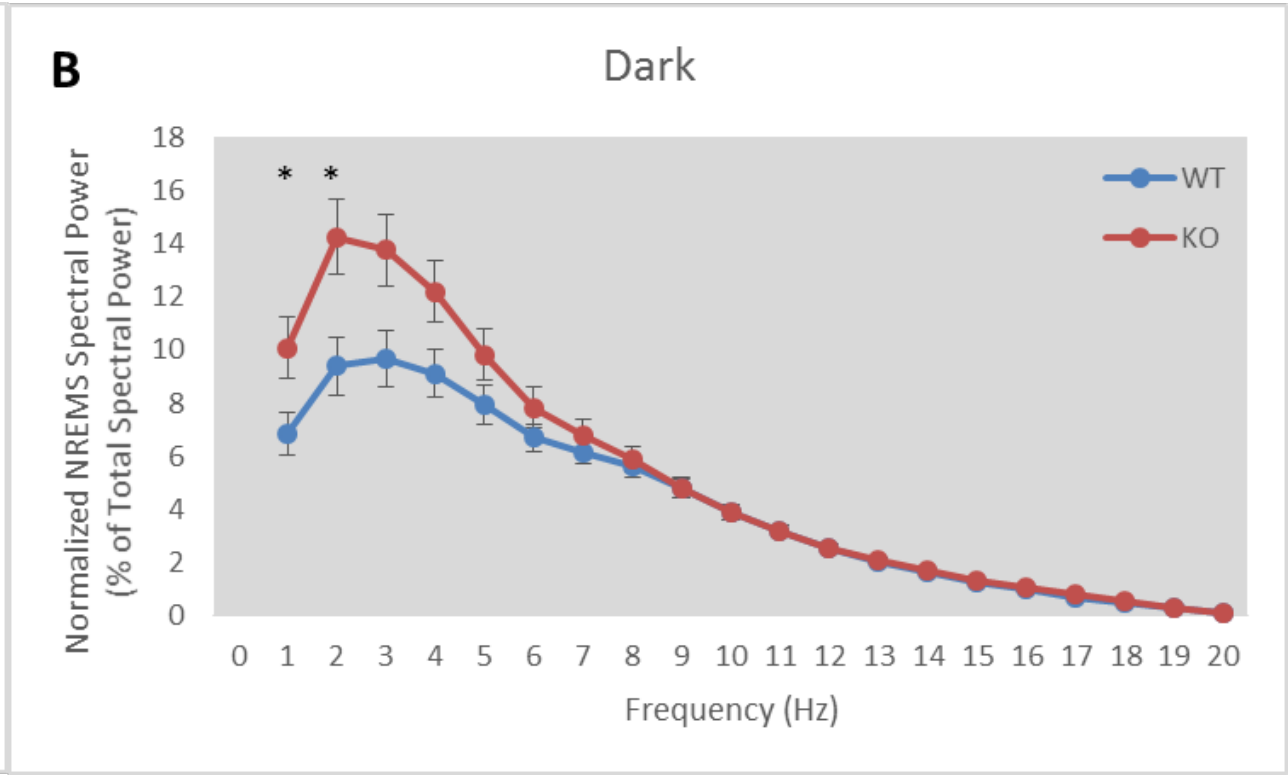


Fig 5. NREMS EEG SWA

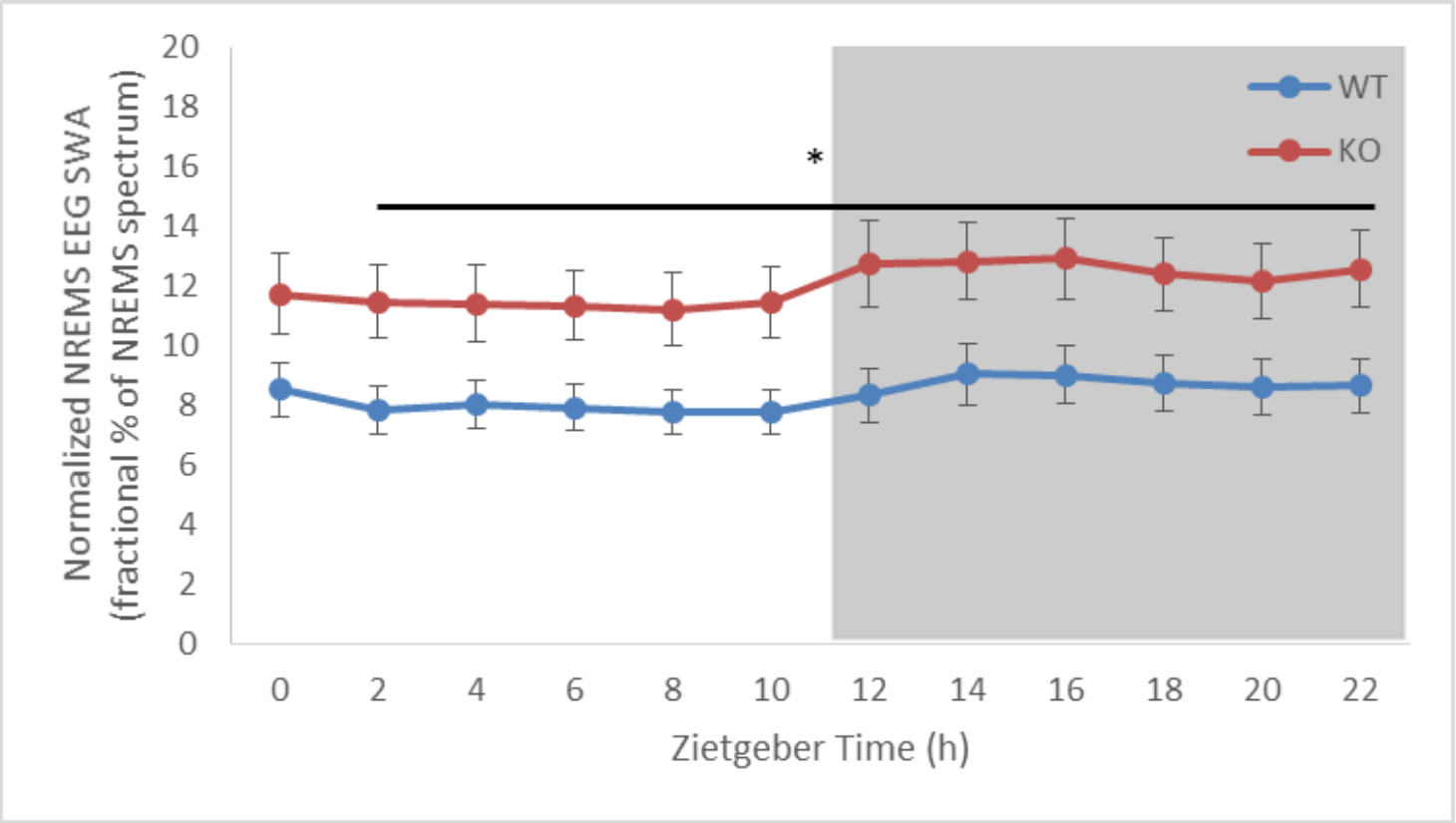


Fig 6. REMS Power spectra

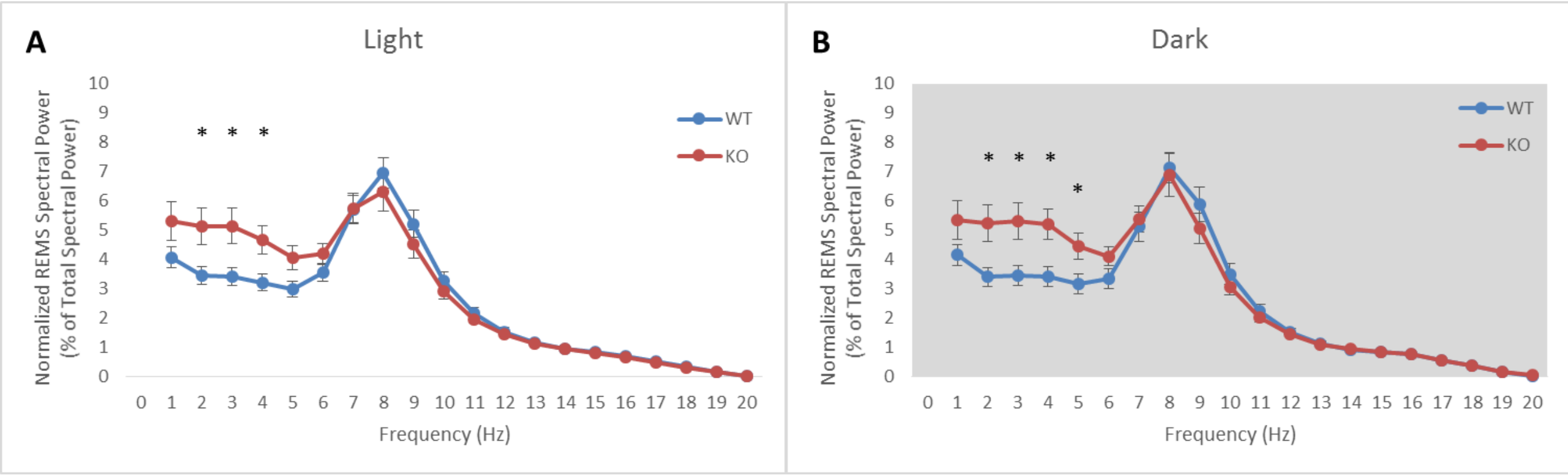
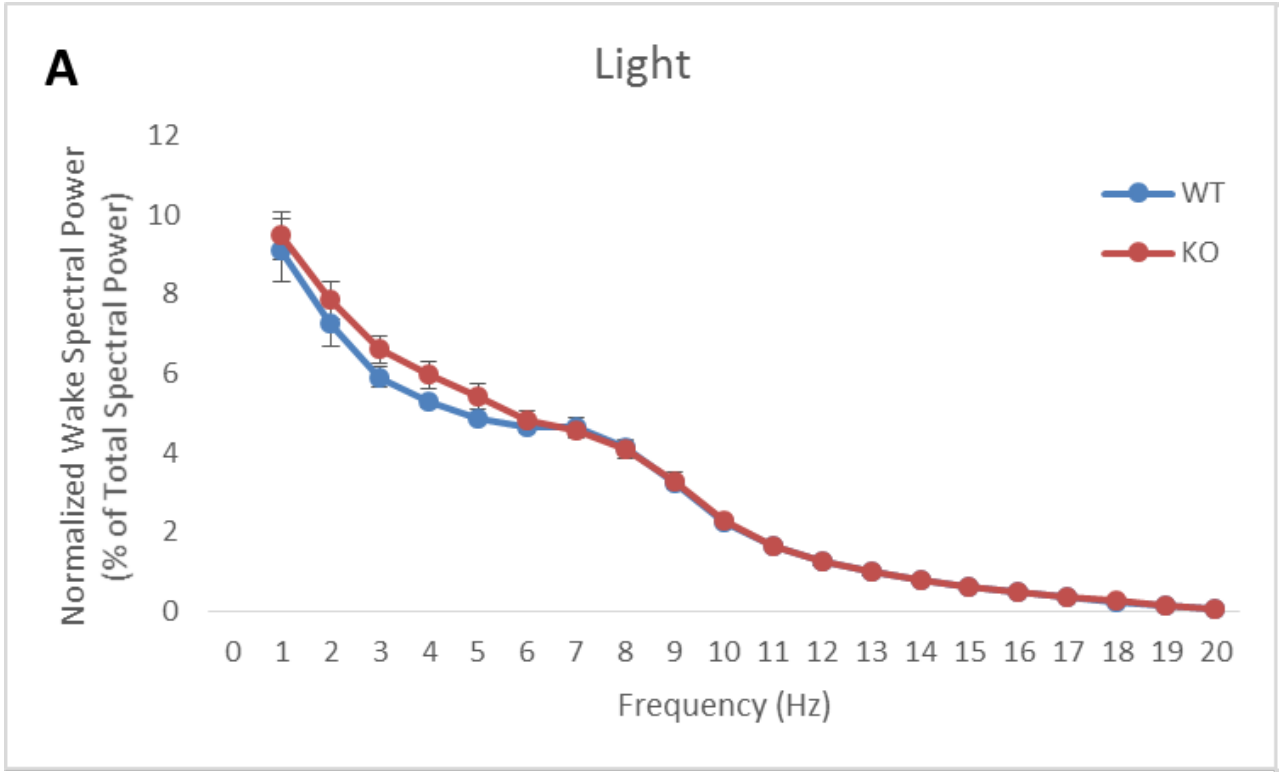
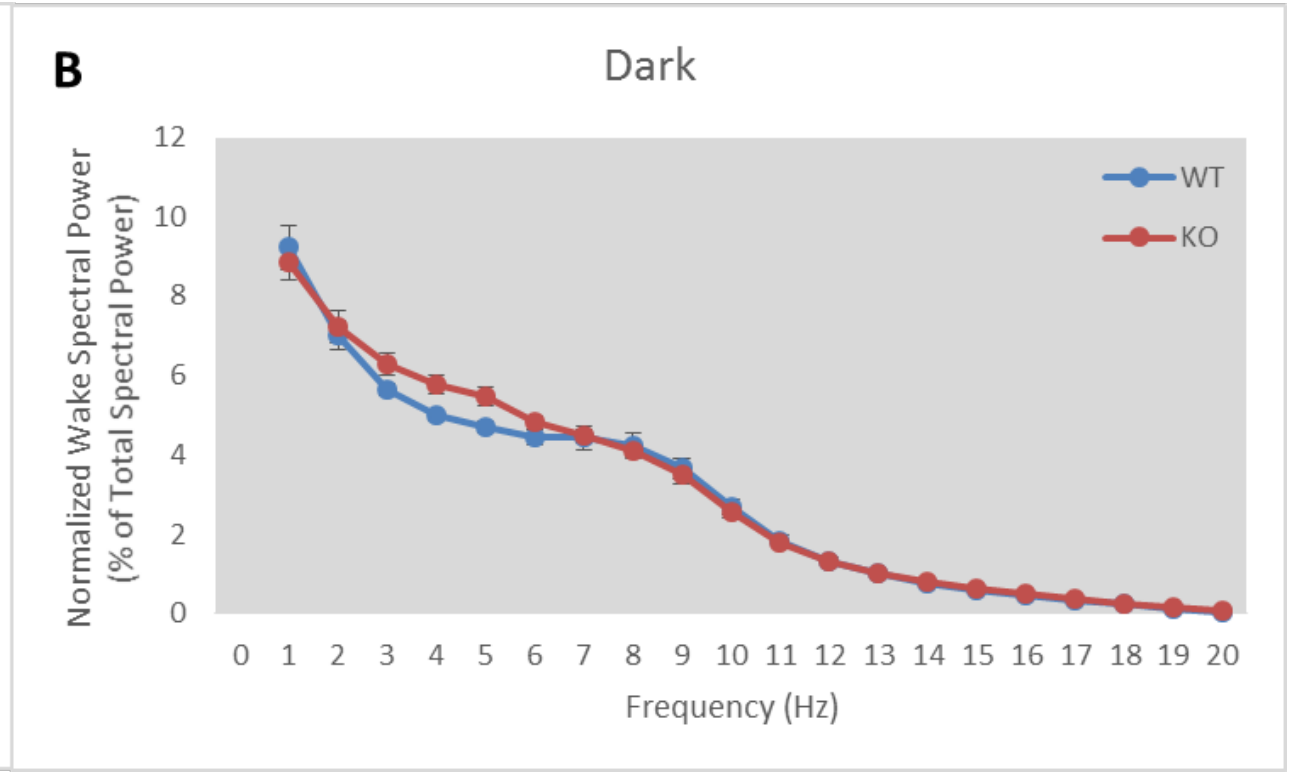


Fig 7. Wake Power spectra.





NDI-project Data Output

Outcome Measure	Spreadsheet	Statistics	Table/Figure
Discharge Amplitude	Discharge Data	n/a	Table 1
Discharge Duration	Discharge Data	n/a	Table 1
Discharge Interval	Discharge Data	n/a	Table 1
Discharge Counts	Discharge Data	n/a	Table 1
Discharge Spectrum	Discharge Data	n/a	m116 WT (1-3); m117 KO (1-6)
REM-Time in	Time in State, Bout and Durations 12 h bins	WNR Time in State 12h	Figure 1
REM-Bout Counts	Time in State, Bout and Durations 12 h bins	WNR Bout Counts 12h	Figure 2
REM-Bout Duration	Time in State, Bout and Durations 12 h bins	WNR Bout Duration 12h	Figure 3
REM-Power Spectrum	EEG Normed Power Spectra 12 h bins	EEG spectrum 2h	Figure 6
NREM-Time in	Time in State, Bout and Durations 12 h bins	WNR Time in State 12h	Figure 1
NREM-Bout Counts	Time in State, Bout and Durations 12 h bins	WNR Bout Counts 12h	Figure 2
NREM-Bout Duration	Time in State, Bout and Durations 12 h bins	WNR Bout Duration 12h	Figure 3
NREM-SWA	NREMS EEG SWA 12 h bins	NREM SWA 2h	Figure 5
NREM-Power Spectrum	EEG Normed Power Spectra 12 h bins	EEG spectrum 2h	Figure 4
Wake-Time in	Time in State, Bout and Durations 12 h bins	WNR Time in State 12h	Figure 1
Wake-Bout Counts	Time in State, Bout and Durations 12 h bins	WNR Bout Counts 12h	Figure 2
Wake-Bout Duration	Time in State, Bout and Durations 12 h bins	WNR Bout Duration 12h	Figure 3
Wake-Power Spectrum	EEG Normed Power Spectra 12 h bins	EEG spectrum 2h	Figure 7