

EEG Recordings and Activity Observations as Translational Biomarkers



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Background

Aims

- Disease-related insomnia and other sleep disturbances, as well as changes in EEG pattern, are widely reported in CNS disorders, including mental and neurodegenerative disease.
- Since conventional cable-based EEG recordings influence natural behaviour we have developed a system of wireless EEG recordings (both hippocampi and prefrontal cortex) using a wireless Neurologger system, combined with automated activity observations (PhenoTyper).
- This procedure was applied to assess age- and disease related phenotypes in wild type and transgenic Alzheimer mice.
- Our procedures provide powerful tools for translational research in animal models of CNS disorders.

- Assess alterations in gross locomotor activity, sleep pattern and EEG spectra in aging and transgenic AD mice.
- To characterise sleep / wake structures in light and dark phase without disrupting the activity of the animals.
- To verify procedures and establish relevant biomarkers.

Methods

Animals:

• Age study: 3 months (n=7), 6 months (n=7), 6 months (n=8), 12 months (n=8) and 15 month (n=10) old C57BL/6j mice were used to investigate age-related alterations in gross activity in light and dark phase.

• Alzheimer mice: Four transgenic (tg) groups [APP/PSEN1 double tg young (n=7), APP/PSEN1 double tg old (n=6) and PSEN1 littermates young (n=7), PSEN1 littermates young (n=10)] and were compared for alterations in their activity and sleep patterns. Effects of the genotype difference on sleep, activity and EEG patterns were assessed in young (5-6 months) and aged animals (19-20 months).



Fig-1. (A) PhenoTyper cage. (B) Videotracking with Ethovision. PhenoTyper cages: (30cm x 30cm x 35cm) with digital infrared sensitive video camera; Ethovision 3.0 (Tackxys, UK) was used as videotracking software. Activity (e.g. distance moved) and time spent in food and water zones can be assessed to compare the behavior of different groups of experimental animals.



Fig-2. Freely behaving mouse undergoing EEG recording. Wireless EEG recordings (New Behaviour) Light weight: 3g (approximately 10% of the body weight) EEG recorder with built-in-accelerometer to record body movements.

EEG Surgery

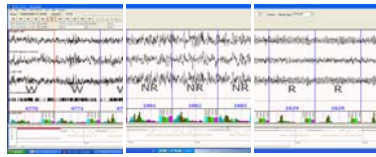
- Epidural gold screw electrodes were placed over the prefrontal cortex (PFx) and left and right hippocampi (LH/ RH).
- Reference and ground screws were placed at neutral zones (parietal and occipital regions).
- Screw electrodes and assembly pins were anchored and fixed to the skull by means of dental cement.
- Recovery period: Animals were kept in the home cage for up to 10 days before the recordings commenced.

SleepSign & MatLab based EEG analysis

- Vigilance stages were analyzed by automated scoring followed by visual correction.

Parameters analysed:

- Behavioural stage identification and power spectral analysis based on 4-sec bins.
- Identification of stages: Accelerometer activity, FFT-delta power and theta ratio:



Criterion set for automated scoring:
a) W (15 < crosscounts accelerometer < 400)
b) NR (250 < FFT-delta power > 100 AND 0 < crosscounts accelerometer < 15)
c) R (45 < FFT-theta ratio < 100 AND 0 < crosscounts accelerometer < 15)
d) Previous stage if none of the above is valid

Fig-3. Sample EEG stretches illustrating the different vigilance stage. Criteria are given on the right.

Data Analysis

Statistical comparisons of PhenoTyper behavioral data were made by repeated measures (RM) 2-way ANOVA, followed by RM 2-way ANOVA planned comparison to determine age effects, genotype effects and age/genotype by time interactions, with post-hoc Bonferroni test (GraphPad Prism 5.0). Significance was determined at the level of p<0.05. For vigilance state analysis, one way ANOVA was performed followed by Tukey post hoc tests for comparison between different groups. Data are expressed as mean ± SEM.

Neurologger

- Wireless EEG recorder, light weight :3g; built-in-accelerometer; sample rate: 200Hz
- Channels: Ch1: PFx; Ch2: Ref-1; Ch4: RH; Ch5: LH; Ch6: Ref-2

Results

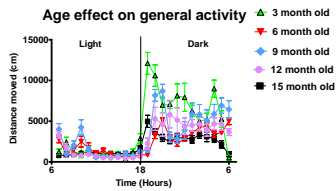


Fig-4. Mean total distance moved during the light and dark hours in the PhenoTyper cages. Age-related alterations in the mean distance moved was observed between young and old mice.

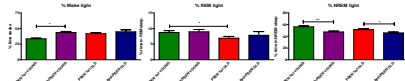


Fig-8. Time spent in Wake, REM and NREM stages during light hours (in %). Data are expressed as means plus SEM. Both young and old groups exhibited a significant effect of genotype on time spent in NREM sleep. In young animals, a significant effect of genotype was observed in the % time spent awake, and an age-related decrease in % REM was also identified in the PSEN control group.

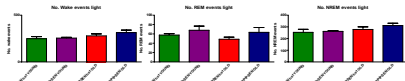


Fig-9. Mean number of Wake, REM and NREM events during light hours. Data are expressed as means plus SEM. No significant effect of age and genotype were observed.

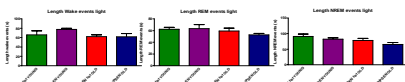


Fig-10. Mean duration of Wake, REM and NREM events during light hours. Data are expressed as means plus SEM. No significant effect of age and genotype were observed.

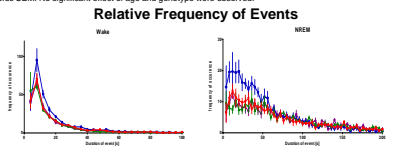


Fig-11. Relative occurrence and distribution of Wake, REM and NREM per duration of events. Data are expressed as means plus SEM. Aged APP/PSEN mice show sleep fragmentation as indicated by a significantly increased number of shorter epochs in both Wake (P<0.001 for epoch lengths from 8 to 12 seconds) and NREM (P<0.001 for epoch lengths from 4 to 60 seconds).

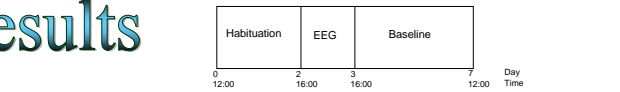


Fig-5. Activity and EEG recording protocol in the PhenoTyper cages: 2 days habituation period, 1 day (24 hours) EEG recording via the Neurologger chip, followed by 4 days of baseline recording for observing the activity during light and dark period.

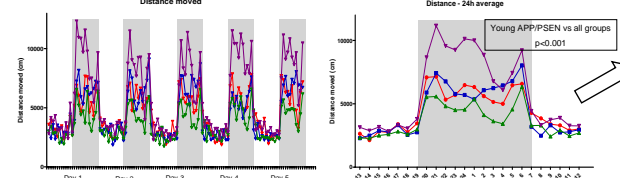
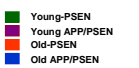


Fig-6. Mean distance moved by different group of mice in the PhenoTyper. Line graph represents the distance moved during the 5 days (excluding 2 days of habituation) in a 1-hour bins. Young APP/PSEN animals exhibit significantly higher activity in the dark phase. A two-way ANOVA indicates a significant effect of group, phase and an interaction. Specifically, post-tests reveal that young APP/PSEN animals (p<0.001), exhibit significantly higher activity in the dark phase.



Habituation-EEG recording - Post recording Distance - Light/Dark

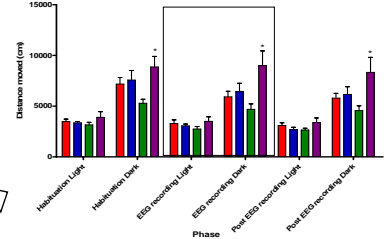
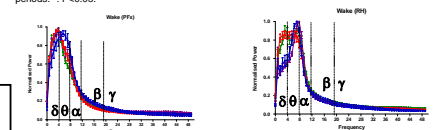
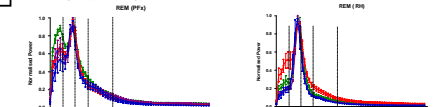


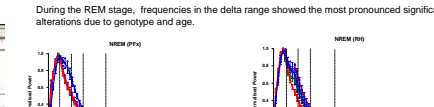
Fig-7. Mean distance moved during different experimental periods and light-dark hours of PhenoTyper recordings. Data are expressed as mean ± SEM. There were no significant differences in activity within groups during different experimental periods (habituation / EEG recording / post EEG recording). This indicates that the attachment of the Neurologger did not affect the gross activity of mice in light and dark periods. * P<0.05.



During the awake stage, frequencies in delta & theta range showed significant alterations due to genotype and age.



During the REM stage, frequencies in the delta range showed the most pronounced significant alterations due to genotype and age.



During NREM, mostly frequencies in the theta, alpha and beta range showed significant alterations due to genotype and age.

Fig-13. Normalized EEG power spectra from prefrontal cortex (PFx) and right hippocampus (RH) for different vigilance stages in the light phase. Data are expressed as means±SEM. PSEN littermates (PSEN) served as controls for animals with both APP and PSEN mutations (APP/PSEN).

Vigilance Stage	PFx			RH		
	Young PSEN / Old PSEN	Young APP/PSEN / Old APP/PSEN	Old PSEN / Old APP/PSEN	Young PSEN / Young APP/PSEN	Young PSEN / Old APP/PSEN	Old PSEN / Old APP/PSEN
Wake	NS	Theta	Delta and Theta range	Theta	Delta	Delta
REM	Delta	Delta and Beta	Beta	Delta	NS	Delta and Beta
NREM	Alpha	NS	Delta, Theta and Alpha	Beta	Theta and Beta	Theta and Beta

Table 1. An overview of frequencies affected by genotype and aging during different vigilance stages in the APP/PSEN vs PSEN Alzheimer groups. NS = Non-significant.

Conclusions

1. The use of PhenoTyper and wireless EEG recordings were found to be sensitive tools for translational research. The combined use of these approaches can uncover age- and Alzheimer-disease related biomarkers in sleep & awake states, and surface electroencephalic activity.
2. During the dark phase, young control animals (3m) showed highest activity while 15-month old animals had significantly reduced activity.
3. When comparing APP/PSEN mice with PSEN littermates, age- and genotype related alterations were observed in circadian activity; and delta, theta and alpha frequencies were affected in different behavioral stages.