Amyloidopathy in mice as a model of cortical network dysfunction in AD

Steven C Leiser*, Maninder Chopra, David A Gurley, Karin Agerman, Anna Bogstedt, Michael C Quirk, James J Doherty, Johan Sandin, Edward P Christian
Department of Neuroscience, AstraZeneca Pharmaceuticals, 1800 Concord Pike, Wilmington, DE 19850
Neuroscience, AstraZeneca R&D, 151 85 Södertälje, Sweden

Nov 16, 2010

651.12/I3

Introduction

The progression and pathology of Alzheimer's disease (AD) is characterized by a number of neurobiological and cognitive changes. Outside of the symptoms of impaired cognition, neuronal changes associated with AD progression can be expressed as altered physiological functions including sleep patterns, changes in EEG activity and event related potential (ERP) responses as well as increased epileptiform and/or seizure activity. Thus EEG based measures of neurological function offer a number of translatable, physiological endpoints that are likely indicative of disease progression in both AD patients and animal models.

Continuing with a systematic *in vivo* neurophysiological characterization of Tg2576 mice ('Leiser et al., 2009), we demonstrate here with surface EEG recordings that Tg2576 mice have reduced Delta and elevated Theta power with a persistently higher dominant frequency (DF) and time spent in that specific frequency (~8Hz). While WT mice have a DF in the Delta band, Tg2576 mice have a DF within the Theta band that persists for the duration of the recording sessions suggesting a level of hyperexcitability in the brains of this Aβ over expressing mouse model. We also confirmed presence of epileptiform waves with no myoclonus.

In precedence, here we demonstrate this phenotype in parietal surface EEG (previous report used hippocampal LFP), yet show a divergence of this phenotype in frontal surface EEG, where changes did not reach significance in Tg2576 vs. WT mice. Furthermore, to assess whether the Theta rhythm was correlated to locomotor activity (LMA), we compared total normalized power at 6-8 Hz to the total distance traveled for every 60 seconds of a two hour recording session during the active (day) cycle. The findings demonstrate conclusively that the elevated high-Theta rhythm is not caused by or secondary to locomotor activity.

Recent evidence shows an elevated Theta frequency in AD patients (e.g. references 2-6), which, similar to this work, occurs prior to cognitive decline/dementia. In conclusion, the surface EEG in these mice have similar hallmarks to EEG recordings from Alzheimer's patients. Our findings thus implicate EEG changes as important translational biomarkers for testing therapeutic strategies in the treatment of AD.

Methods

Surgery

Female mice were anesthetized using isoflurane, skull from Nasofrontal to Occipital was exposed, and a Pinnacle Technology (Lawrence, Kansas) prefabricated 8201 mouse EEG/EMG Headmount positioned to lie primarily between Bregma and Lambda was anchored by four screws that also acted as electrodes that recorded Frontal (EEG2) and Parietal (EEG1) EEG/ECoG. The headmount assembly was cemented onto the skull with dental acrylic. Animals were allowed to recover from surgery for 7-10 days.

Recording

Locomotor Activity (LMA), video, and EEG data were collected simultaneously from up to 8 mice for 2 hours each recording session. EEG was recorded using a data acquisition and conditioning system (Dax, Pinnacle Technology) that recorded 2 EEG signals from a headmounted preamplifier delivering 100x amplification, eliminating 87% of the artifact due to animal movement with 400Hz sampling rate and filtered 1-50Hz. Both EEG1 and EEG2 are differential recordings and share a Common EEG electrode located parietally. EEG 1 has a posterior bilateral placement while EEG 2 provides a cross cortical measurement between the Common EEG (parieto-occipital) and EEG 2 (Frontal).

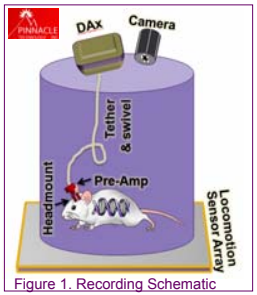

Figure 1. Recording Schematic

EEG Analyses

The EEG data was collected using the Pinnacle Technology Acquisition software in EDF format. This was converted to Spike2's (CED) SMR format for analyses. EEG was interpolated to 128 Hz and bandpassed filtered from 1-64 Hz. Power Spectral Densities (PSDs) were created for each EEG for the entire 2 hour recording with an FFT size of 256 (~0.5Hz bins). The power in particular bands, e.g. Delta (0.5-4.2), Theta (4.7-8.9), and Gamma (20-45), were calculated by summing all relevant frequency bins. The Theta-Delta ratio was calculated by dividing the total power in Theta by the total power in Delta. All analyses were performed on an individual mouse basis and for each EEG (EEG1 and EEG2) for each of the Recording Days. EEG power was also normalized by dividing the raw power in each bin by the total power (sum of the power in all of the bins). The individual mouse data was averaged to assess the population response of EEG1 and EEG2 on any given Recording Day. Additionally Dominant Frequencies (DFs) were calculated for each mouse. A Spike2 script performed this analysis and yielded DFs for each EEG for every 30 seconds. To obtain a DF, a PSD is created for the specified time window (30 sec) and the frequency at which maximum power occurs is the DF. The average DF for each mouse for each EEG for each day is calculated by averaging all DFs from all time bins (0-2 hours) in that given recording. The population DF, for WT and TG2576 mice separately, is calculated by averaging these single DF values for each mouse. Statistical analyses by way of an unpaired (TG vs. WT) or paired (MRK vs. VEH) t-test, where applicable, are also performed across these individual DF and PSD values.

Acknowledgement

We would like to thank the following for superior support: Megan Miller, Amy Hehmeyer, Denis Hehman, Donna Soltung, and the RST department at AZ R&D Wilmington, DE.

References

1. Leiser SC, Chin J, Bowlby MR, Perl R, Dunlop J, Randall AD, Pangalos M, Reinhart P. EEG in mouse models of Alzheimer's disease as a translational in vivo biomarker. Program No. 729.22. 2009 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2009. Online.
2. Prichep LS. Quantitative EEG and electromagnetic brain imaging in aging and in the evolution of dementia. Ann N Y Acad Sci. 2007 Feb;1097:156-67.
3. Prichep LS, *et al*. Neurobiol Aging. 1994 Jan-Feb;15(1):85-90.
4. Prichep LS, *et al*. Prediction of longitudinal cognitive decline in normal elderly with subjective complaints using electrophysiological imaging. Neurobiol Aging. 2006 Mar;27(3):471-81.
5. Stomrud E, *et al*., Slowing of EEG correlates with CSF biomarkers and reduced cognitive speed in elderly with normal cognition over 4 years. Neurobiol Aging. 2010 Feb;31(2):215-23.
6. Shin J. Theta rhythm heterogeneity in humans. Clin Neurophysiol. 2010 Mar;121(3):456-7.

Clear Differences in EEG of Tg2576 mice compared to Wild-type (WT) mice: Parietal more than Frontal EEG/ECoG Reveals Greater Theta/ Delta Separation in Tg2576

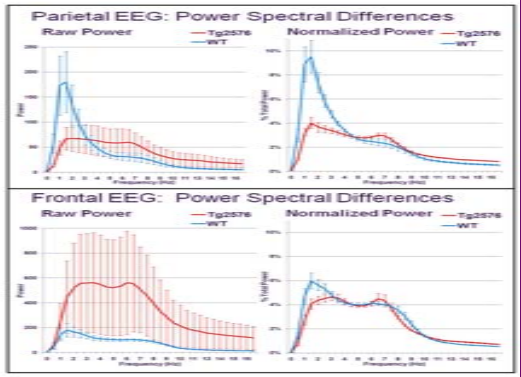

Figure 2. Parietal EEG and Frontal EEG Power Spectral Density plots for 22 recordings from 9 WT mice and 23 recordings from 9 TG mice. Plotted are mean ±SEM (error bars).

Tg2576 have Lower Delta and Higher Theta than WT Significantly Greater Theta/Delta Ratio

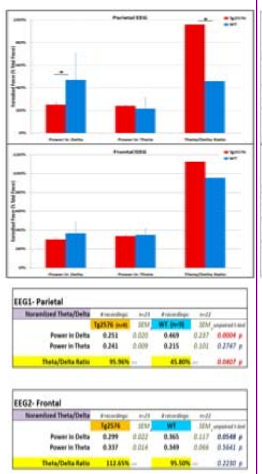

Figure 3. Mean Parietal EEG and Frontal EEG Power Spectral Density plots for 22 recordings from 9 WT mice and 23 recordings from 9 TG2576 mice.
Plotted are mean ±SEM (error bars).

Tg2576 mice have Higher Dominant Frequency & Time in Frequencies ≥6Hz than WT mice

Figure 4. Mean Parietal EEG and Frontal EEG Dominant Frequencies and Percent Time in DF≥6Hz for 15 recordings from 7 WT mice and 14 recordings from 6 TG mice.

Theta Rhythm Not Coupled to Locomotor Activity. TG2576 have Higher Theta but Not Different in LMA.

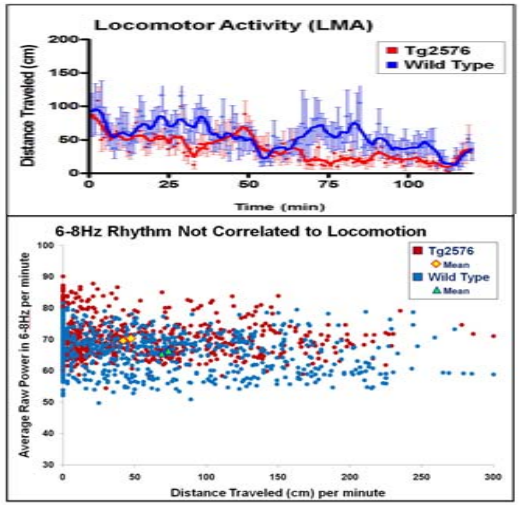

Figure 5. Top: Average locomotor activity (LMA) from 8 TG and 6 WT mice from one recording session show similar patterns of activity irrespective of genotype. Bottom: The raw power in 6-8Hz for each mouse for every 1 minute (y-axis) compared to the LMA activity (distance traveled in cm, DTcm) for that same minute (x-axis) shows that LMA activity does not influence theta power for either TG (red) or WT (blue) mice. This measurement was performed using Parietal EEG.

Tg2576 Elevated Theta Rhythm Ameliorates with Chronic MRK560 Treatment

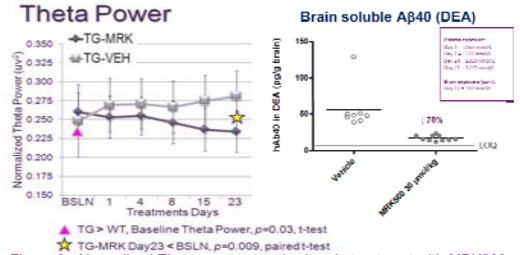

Figure 6. Normalized Theta power reveals chronic treatment with MRK560 progressively reduces theta to a max reduction shown at day 23 when Aβ 40 levels are reduced by 70%.

Gamma Power Increased with Chronic MRK560

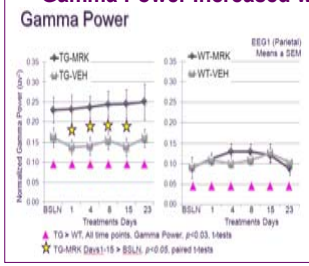

Figure 7. Normalized Gamma power averaged for n= 6 TG-MRK, 5 TG-VEH, mice reveals chronic treatment with MRK560 increases gamma power, albeit moderately, compared to Baseline in Tg2576 mice while gamma power remains steady in all other cohorts. Interestingly gamma power is already elevated in Tg2576 mice compared to WT mice.

Results

Spectral changes were measured in both EEG recordings from each mouse: EEG1, which represents activity primarily over parietal cortex, and EEG2, which represents activity primarily spread across the Frontal cortex. Power Spectral Densities (PSD) were calculated for a total of 22 recordings from 9 WT mice and 23 recordings from 9 TG mice. These recordings were from 7 sessions over 4 different days. Line graphs of the PSDs illustrate the 0.5Hz bins used in all analyses (**Figure 2**). Raw is clearly elevated in the higher frequencies in TG mice compared to WT mice. Additionally, a peak in the PSD can be observed at 6-8Hz. Raw EEG power shows high variability across mice so for direct comparisons, power was normalized. This was derived for each recording for each mouse by dividing the power in each frequency bin (0.5Hz) by the total power for that recording.

Normalized power reveals a dominant Delta frequency band component in WT mice with only a moderate amount of Theta power, whereas TG mice clearly have diminished Delta and elevated Theta frequency components. The average power in Delta and Theta are displayed in **Figure 3** and tabulated in **Table 1**. Parietal EEG reveals Delta is significantly greater in WT mice compared to TG mice. Interestingly and in contrast to previous studies [1], there was not a significant difference in peak Theta frequency likely due to low sample size. The Theta/Delta ratio was significantly different and approached a 1:1 (100%) ratio in TG mice. Interestingly no differences were noted in the population average of normalized power from the Frontal EEG recordings.

Hyperexcitability in Tg2576 mice was also evaluated by calculating the Dominant Frequency (DF) in PSDs every 30 seconds in each recording and averaging these DFs for that recording. This assesses the particular frequency component that dominated the brain during that recording. A consistently higher DF is revealed in TG (~5Hz) vs. WT mice (~3Hz) in the Parietal EEG (**Figure 4** and **Table 2**). Interestingly once again, the Frontal EEG recordings did not demonstrate this phenomenon. In addition to the average DF for a recording, a temporal component (percent of time spent in frequencies DFs) was measured to determine if higher DFs persist over the course of the recording or if they are more transient. This analysis yielded, again only in Parietal EEG, that TG mice have an average percent time spent in DF≥6Hz of 46.4% compared to 14.9% in WT mice.

It is important to evaluate the relation of high theta rhythm in Tg2576 mice to locomotor activity (LMA). LMA was collected simultaneously with the EEG. The average LMA for TG and WT mice for a particular recording (**Figure 5**, top) shows that these mice traverse similar distances over the course of the recording. Both start out with higher LMA, likely due to heightened exploratory behavior, but this declines noticeably within ~10 minutes The trace reveals that although both WT and TG mice seem to gradually decrease LMA, there is a transient increase in LMA for WT mice at 75 minutes that did not occur for TG mice. However to directly assess the relation of LMA and theta power, the raw 6-8 Hz power was calculated for each mouse in 1 min bins and independently correlated to the LMA activity (distance traveled in cm, DTcm) in the same time epoch (**Figure 5**, bottom). This analysis shows that LMA activity does not account significantly for theta power variability for either TG or WT.

For the MRK560 study, importantly, Theta power differed significantly between TG and WT mice at baseline (**Figure 6**). This separation, an important benchmark necessary to compare treatment effects was not present in in in the frontal lead, and thus is not shown. Additionally, only mice that consistently had artifact-free signals for the duration of the 23 day study were included in the final analyses. This resulted in 12 MRK560-treated TG mice (TG-MRK) completing the study, comprised of 3 of the 8 MRK560-treated WT mice (WT-MRK), 3 of the 9 vehicle-treated TG mice (TG-VEH), and 3 of the 8 vehicle-treated WT mice (WT-VEH).

Theta power (**Figure 6**) was significantly greater in TG mice (averaged for 6 TG-MRK and 5 TG-VEH) compared to WT mice (n=3) at Baseline (p=0.03, unpaired t-test). TG mice had 34% more Theta power than WT mice and 11% lower delta power (delta power was not significantly different). MRK560 treatment progressively lowered Theta power in the TG mice compared to Baseline by 2.6% on Day 1, 2.1% on Day 4, 5.6% on Day 8, 9% on Day 15, and 10% on Day 23. Theta power was significantly reduced in TG-MRK mice by Day 23 compared to Baseline (p=0.009, paired t-test). Conversely, vehicle-treated TG mice exhibited a modest increase in Theta power throughout the study as the mice aged, with an increase of 8.4% on Day 1, 9.0% on Day 4, 7.3% of Day 8, 10.7% on Day 15, and 13.2% on Day 23 compared to Baseline. Delta power remained relatively unaffected in other cohorts. At termination (day 23) the MRK560 exposure in the brain was 267 nmol/L and Aβ level was reduced by 70%. Assessing the Theta/Delta Ratio, as Dominant Frequency, Percent Time in DF≥6Hz, and DF Distributions (not shown) averaged across mice yields similar trends with chronic MRK560 treatment to those observed in the assessment of Theta power alone.

Tg2576 mice (either MRK- or VEH- treated) had significantly greater Gamma power (**Figure 7**) than WT mice. TG mice, throughout the study (TG > WT, all time points, p<0.03, time-matched unpaired t-tests). Normalized Gamma power averaged for each cohort revealed chronic treatment with MRK560 moderately, but progressively increased gamma power, compared to Baseline in TG mice (TG-MRK Days 1-15 > BSLN, p<0.05, time-matched paired t-tests), while gamma power was steady in other cohorts.

Conclusions

ECoG from Tg2576 mice exhibited consistently aberrant EEG rhythms relative to wt litter mates:

- **Enhanced theta (4.7-8.9 Hz) power, coinicident with reduced delta (0.5-4.2 Hz) power**
- **A persistently higher Dominant Frequency (DF) as well as total time spent in higher frequencies, particularly a frequency band centered around 8Hz.**
- **Chronic treatment with the γ-secretase inhibitor MK-560 reduced elevated theta power in Tg2576 mice compared to wild-type mice.**

Taken together, these results suggest network hyperexcitability in this Aβ over-expressing mouse model. They further suggest that measures of aberrant cortical EEG as an attractive endpoint to test pharmacological interventions designed to reverse Aβ-mediated pathophysiology

Earlier studies with hippocampal depth electrodes reported similar abnormal EEG activity in Tg2576 mice (Leiser et al. 2009). The current study demonstrates this phenotype in ECoG recordings predominantly over parietal cortex relative to frontal cortex. The ability to detect such EEG abnormalities from selected surface leads enhances the utility of this model to study potentially translatable biomarkers for clinical EEG endpoints in Alzheimer's Disease (AD) patients.

In conclusion, Tg2576 mice display EEG/ECoG abnormalities reminiscent of similar alterations in EEG recorded from AD patients. Specifically, the current results are consistent with clinical observations that AD is associated with an altered dominant frequency in the EEG (specifically theta). In AD, abnormalities in cortical EEG appear coincident with, or even precede, the earliest stages of dementia. EEG changes in these Aβ over expressing mice may provide a specific biomarker corresponding to disease state, and testing novel therapeutic strategies

AstraZeneca
life inspiring ideas