Increased Cortical and Thalamic Excitability in Freely Moving APPswe/PS1dE9 Mice Modeling Epileptic Activity Associated with Alzheimer’s Disease

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Amyloid precursor protein transgenic mice modeling Alzheimer’s disease display frequent occurrence of seizures peaking at an age when amyloid plaques start to form in the cortex and hippocampus. We tested the hypothesis that numerous reported interactions of amyloid-β with cell surface molecules result in altered excitation–inhibition balance in brain-wide neural networks, eventually leading to epileptogenesis. We examined electroencephalograms (EEGs) and auditory-evoked potentials (AEPs) in freely moving 4-month-old APPswe/PS1dE9 (APD9) and wild-type (WT) control mice in the hippocampus, cerebral cortex, and thalamus during movement, quiet waking, non-rapid eye movement sleep, and rapid eye movement (REM) sleep. Cortical EEG power was higher in APD9 mice than in WT mice over a broad frequency range (5–100 Hz) and during all 4 behavioral states. Thalamic EEG power was also increased but in a narrower range (10–80 Hz). Furthermore, APD9 mice displayed augmented cortical and thalamic AEPs. While power and theta–gamma modulation were preserved in the APD9 hippocampus, REM sleep-related phase shift of theta–gamma modulation was altered. Our data suggest that at the early stage of amyloid pathology, cortical principal cells become hyperexcitable and via extensive cortico-thalamic connection drive thalamic cells. Minor hippocampal changes are most likely secondary to abnormal entorhinal input.

Keywords: amyloid, auditory-evoked potential, EEG, hippocampus, local field potentials

Introduction

Alzheimer’s disease (AD) increases the prevalence of unprovoked seizures in the elderly population (Hauser et al. 1986; Amatniek et al. 2006; Lozsadi and Larner 2006). These isolated clinical observations gained renewed general interest when 2 recent experimental studies reported frequent occurrence of seizures in transgenic mice carrying mutated form of human amyloid precursor protein (APP) (Palop et al. 2007; Minkeviciene et al. 2009). Although the mechanisms underlying seizure generation in APP transgenic mice remain unexplored, several lines of evidence point to amyloid-β (Aβ) as an important trigger for epileptogenesis in these mice. First, despite 2 very different genetic constructs [APPswe + APPind mutation in one study (Palop et al. 2007) and APPswe + PS1dE9 mutation in another (Minkeviciene et al. 2009)], these 2 mouse lines with reported spontaneous seizures share a combination of mutations that favor accumulation of Aβ42 peptide in the brain. Second, the seizures peak at an age when amyloid plaques start to form in the cortex and hippocampus. Third, exogenous application of Aβ42 peptide enhances excitability in cortical and hippocampal slices of wild-type (WT) mice (Minkeviciene et al. 2009). Thus, either the amyloid plaques themselves or, more likely, high concentrations of soluble Aβ aggregates are candidates for the initial trigger for neuronal hyperexcitability and seizure activity.

Despite extensive use as an AD mouse model, few studies have studied the functional impact of amyloid accumulation on spontaneous electrical activity in APP transgenic mice in vivo. The above-mentioned studies (Palop et al. 2007; Minkeviciene et al. 2009) focussed on seizure activity and used mainly cortical screw electrodes in measuring electroencephalograms (EEGs). Several studies have used EEGs to characterize sleep states in APP transgenic mice (Wisor et al. 2005; Zhang et al. 2005; Jyoti et al. 2010), but those have paid less attention to the EEG changes and did not include frequencies higher than 40 Hz. To our knowledge, the only systematic study on EEG changes associated with APPswe mutation is our old study comparing APPswe or presenilin-1 (A246E) single mutant and double APP/PS1 mutant mice with their WT littermates (Wang et al. 2002). Unfortunately, none of these mouse lines had evidence for seizures (although the possibility cannot be excluded).

In order to assess the status of neuronal networks in seizure-prone amyloid-producing transgenic mice, the present study examined EEG, local field potentials (LFPs), and auditory-evoked potentials (AEPs) in freely moving APPswe/PS1dE9 (APD9) and WT control mice at the age of 4 months when first amyloid plaques are observed. This is an age by which about 60% of the mice should have had at least 1 seizure (Minkeviciene et al. 2009). The study focussed on the hippocampal CA1 and dentate gyrus (DG) fields, cerebral cortex, and thalamus, all areas highly relevant for epileptogenesis. We report several electrophysiological indices speaking for an altered excitation–inhibition balance in the cortex and thalamus, whereas the hippocampus shows only modest changes. In addition, we describe new electrophysiological phenomena (behavioral state-dependent theta–gamma coupling strength, shift of theta phase where gamma is maximal during rapid eye movement (REM) sleep, and clusters of high-frequency bursts (HFBs) during early sleep in the frontal cortex), which will help characterization of ongoing physiological processes and understanding of coupling mechanisms within and between brain regions.

Materials and Methods

Animals

The subjects were female APD9 mice (n = 10, weight 21 ± 0.4 g, mean ± SEM) and age-matched WT littermates (n = 7, weight 21 ± 0.6 g).
Their age at the time of recordings was 16–17 weeks. The APdE9 colony founders were obtained from D. Borchelt and J. Jankowsky (Johns Hopkins University, Baltimore, MD, USA), while the mice were raised locally at the Laboratory Animal Center in Kuopio, Finland. Mice were created by coinjection of chimeric mouse/human APPswe and human PS1-dE9 (deletion of exon 9) vectors controlled by independent mouse prion promoter elements. The 2 transgenes coinTEGRated and cosegregate as a single locus (Jankowsky et al. 2004). The line was originally maintained in a C3HeJxC57BL/6J hybrid background. By the time of the present work, the mice were backcrossed to C57BL/6J for 14 generations. The mice were housed in individual cages in a controlled environment (constant temperature 22 ± 1°C, humidity 50–60%, lights on 07:00–19:00 h). Animals had food and water available ad libitum. All animal procedures were carried out in accordance with the guidelines of the European Community Council Directives 86/609/EEC and approved by the Animal Experiment Board of Finland.

_Implantation of Electrodes for EEG/LFP and EMG Recording_

Surgery was performed under isoflurane anesthesia with the induction flow at 450 L/min (4.5%) and maintenance at 205–213 L/min (~2.0%). Animals were chronically implanted with a hippocampal tripolar electrode and a unipolar thalamic electrode (Formvar insulated stainless steel wire, diameter 50 μm, California Fine Wire Company, CA, USA), with a vertical tip separation of 400 μm in the following brain coordinates: the right septal hippocampus at AP −2.0 mm (from bregma), ML 1.3 mm (from the midline), and DV 1.2–2.0 mm (from the dura) and a unipolar thalamic electrode AP −1.0 mm, ML 1.3 mm, and DV 3.3 mm. In addition, 2 screw electrodes were fixed on the right frontal and occipital bones. The screws served also as anchors for dental acrylic cement and the connector (Mill-Max, NY, USA). After the surgery, the mouse received carprofen (5 mg/kg, i.p., Rimadyl, Vericore, Dundee, UK) for postoperative analgesia, and antibiotic powder (bacitracin 250 IU/g and neomycinsulfate 5 mg/g, Bacibact, Orion, Finland) was applied, if necessary, onto the wound. In addition, silver wires [perfluoralkoxy (PFA)-insulated, diameter 200 μm, A-M Systems, Sequim, WA, USA] were inserted into the neck muscles of the animal during surgery for electromyogram (EMG) recording.

_Video EEG and AEP Acquisition_

After a recovery period of 7 days, the animals were accustomed to the recording setup. EEG was recorded for an hour when the animal was freely moving in a metal box. Three hippocampal, 1 thalamic, and 1 frontal cortical channel was recorded and referred to the screw electrode above the cerebellum, which also worked as the animal ground. The connector was attached to a custom-made preamplifier, and the signal was further amplified with an AC amplifier (gain 1000, Grass, Quincy, MA, USA). During undisturbed 1 h daytime recording, about 1000 sweeps of 4 s were collected and digitized at 2 kHz per channel (DT2821 series A/D board; Data Translation, Marlboro, MA, USA). The signal was bandpass-filtered between 1 and 3000 Hz. The data were acquired by using Sciworx 5.0 program (DataWave Technologies, Loveland, CO, USA). The behavior of the animals was recorded using a camera (Live/Cam, Video IM Pro, Creative, Dublin, Ireland) that was positioned on top of the cage and synchronized with electrophysiological signals. AEPs were evoked using a pair of click tones (3 kHz, duration 10 ms, 70 dB, 500 ms between a pair of clicks, inter-stimulus interval 10 s). For AEP recordings, the mouse was continuously observed and all records for further analysis were obtained during immobility of the animal. A total of 30 responses were sampled and averaged.

_Estrous Cycle Analysis_

Vaginal smears were collected after each EEG recording session with a single-use cotton swap and transferred to a microscopic slide. Dry smears were stained with Giemsa solution (Giemsa Azur-Eosin-Methyleneblue solution; Merck, Dormstadt, Germany) and were examined microscopically. The stages were classified into diestrus, proestrus, estrus, and metestrus, following the classification of Nelson et al. (1982). We observed no correlation between the estrous cycle and the measured parameters. Therefore, we estrous cycle stage in further analysis.

_Histology_

At the end of the experiment, the animals were deeply anesthetized with Equithesin solution (0.1 mL/10 g) (chloral hydrate 425 mg + phenobarbital 60 mg/mL, 1.75 mL + propylene glycol 3.5 mL + ethanol 100% 1.2 mL, filled till 10 mL volume with distilled water) and perfused with ice-cold saline for 5 min at 10 mL/min, followed by 4% paraformaldehyde solution for 9 min at 10 mL/min. The brain was removed and left for immersion postfixation for 4 h in 4% paraformaldehyde and after that in 30% sucrose solution for 24 h. The brains were stored in a cryoprotectant at −20°C until slicing. Coronal sections (thickness 35 μm) were cut with a freezing slide microtome. Every second hippocampal section was chosen for double staining. Amyloid pathology was verified by Congo red staining, and the electrode locations were confirmed by glial fibrillary acidic protein (GFAP) staining. Mouse anti-GFAP was bought from Sigma-Aldrich (MO, USA; 1:1000) and goat antimouse with horse radish peroxidase from Pierce Biotechnology (Rockford, IL, USA; 1:500). In addition, section stains were obtained for the N-terminal human Aβ-specific antibody W02 (Genetics, Switzerland) to visualize diffuse amyloid deposits. Amyloid plaques were examined in septal hippocampal sections using an Olympus microscope at ×10–20 magnification to verify that transgenic animals were expressing amyloid pathology, as described previously (Shemer et al. 2006). Consistent with earlier studies (Garcia-Alloza et al. 2006; Minkeviiciene et al. 2009), amyloid plaques only started to build up in 5-month-old APdE9 mice (corresponding to ~0.1% amyloid plaque load at this age, whereas amyloid load can exceed 20% of the cortical volume in aged APP/PS1 mice). W02 antibody staining for diffuse Aβ deposits (Fig. 1A) revealed plaques in the neocortex and hippocampus. Of note, even the first plaques were often Congo red-positive and surrounded by reactive astroglia (Fig. 1B).

The electrode location was verified by GFAP-positive gliosis around the electrode (Supplementary Fig. S4). In addition, we used well-known hippocampal electrophysiological markers to verify electrode location: phase of theta for maximum gamma oscillation (Montgomery et al. 2009), phase of theta for ripples (Isomura et al. 2006), and depth profiles of auditory-evoked response (AEP; Brankack and Buzsaki 1986).

_EEG, LFP, and AEP Data Analysis_

All signals were normalized to amplification, and offline analyses were conducted using MATLAB (Mathworks, Natick, MA, USA; R2006a). Each 70 min recording of LFP during spontaneous behavior was preprocessed. To eliminate sweeps with artifacts, we first calculated the distribution for averaged power spectrum values between 80 and 90 Hz for each individual channel and for each 4 s sweep. Then, outlying sweeps were excluded using iterative implementation of the Grubbs test for outliers (MATLAB routine “deleteoutliers.m” by Brett Shoelson). This effectively removed occasional artifacts related to bad contact, jerky movements, or animal handling. In the second step, we assigned a behavioral state (movement, quiet wakefulness, REM sleep) for each sweep and for each channel based on a semiautomated procedure. The behavioral states were determined based on the following variables: (1) alpha (10–16 Hz) by gamma (30–80 Hz) power ratio (alpha/gamma), (2) theta (7–9 Hz) by delta (1–4 Hz) power ratio (theta/delta), and (3) the muscular electrical activity amplitude (EMG, 1–100 Hz). The sweeps with EMG amplitude above the mean were assigned movement, whereas all remaining waking time was assigned quiet wakefulness. An alpha/gamma ratio above the mean characterized NREM sleep. If between 2 NREM epochs the ratio remained above 25% of smallest values, those epochs were merged together. A theta/delta ratio above the mean by 2 SDs characterized REM sleep, and 2 REM epochs were merged together if the ratio remained above the mean in between. In addition, the preceding 4 s sweep had to be classified as NREM sleep; otherwise, sweeps with high theta/delta ratios were assigned to wakefulness. The automated scoring was further verified by visual inspection of the
established protocol (Csicsvari et al. 1999). Hippocampal LFPs were then averaged for each behavioral state and corrected if necessary. Welch’s averaged modified periodogram method of spectral estimation (default MATLAB parameters) was used to fit a least-squares FIR filter model. The filtered background (filtered background subtraction) was used to reduce the baseline activity in the thalamus and cortex, respectively. The amplitudes of the AEPs were calculated by dividing the response amplitude after the first stimulus (A1, conditioning stimulus) and were converted into percentage by multiplying the values by 100 (ratio = A2/A1 × 100%). The data from the 2 genotypes were compared using Student’s t-test for independent samples as the test of choice because it is generous against departures from normality, even for small sample sizes (Miller 1997; Ramsey and Schafer 2001). Occasionally (for some subsignificant data), we also employed nonparametric statistic, but that did not yield substantially different results. The threshold for acceptance of significance was set to 0.05. The results are presented as means ± SEM.

**Results**

The sleep–wake cycle during the 70 min recording session did not differ between the genotypes (P > 0.2 for all behavioral states). Of the 999 collected 4 s sweeps, the majority was

**Figure 1.** Representative histological sections from a transgenic APdE9 mouse. (A) W02 antibody staining for diffuse Aβ deposits (squares) revealed plaques in the neocortex and hippocampus. (B) Even these early amyloid deposits were surrounded by activated astrogia as revealed by combined Congo red + GFAP staining. Scale bar for (A) is 1.0 mm and for (B) 100 μm.

**Figure 2.** Representative example of PSD across frequencies between 1 and 100 Hz of a single WT mouse EEG for each behavioral state. Note the logarithmic scales on both axes.
categorized as NREM sleep (WT: 499 ± 19 and TG: 519 ± 16). Active movement (WT: 250 ± 23 and TG: 270 ± 17) and quiet waking (WT: 226 ± 34 and TG: 178 ± 19) were equally prevalent, whereas REM sleep was detected in the smallest number of sweeps (WT: 16 ± 7 and TG: 26 ± 6).

**Brain Region-Specific Increase of PSD in APdE9 Mice**

The effect of overexpression of mutated APP and PS1 genes on EEG/LFP power spectrum was assessed for all behavioral states (Fig. 2) and for 4 recording sites (frontal cortex, CA1 stratum radiatum/lacunosum moleculare, DG, and thalamus). We chose to run multiple t-tests for each 1 Hz bin. Notwithstanding the possibility to inflate the type I error due to multiple comparisons, the benefit of seeing clear frequency range boundaries and obvious correlative structure of P-values inside a particular frequency range outweighed those concerns. Whereas NREM sleep PSD showed no difference in the hippocampus (CA1 or DG) between APdE9 transgenic (TG) and WT littermates, there was a significant PSD increase in a broad frequency range in the thalamus and frontal cortex (Fig. 3A,B and Supplementary Fig. S1). In particular, the power increase in alpha, beta, and gamma frequency ranges was highly significant (P < 0.01). The only notable difference between the cortex and the thalamus in this genotype comparison was a more narrower significant gamma range (up to 70 Hz in the thalamus and 120 Hz in the cortex) and a significant increase in theta oscillation in TG mice in the cortex, which was less apparent in the thalamus (Fig. 3A,B). A similar pattern of increased EEG/LFP power was found during other behavioral states (Fig. 3C).

**Gamma Oscillations**

Next, we wanted to more closely study the genotype differences in gamma oscillations, which are known to be sensitive for alternations in excitation-inhibition balance and which showed a strong genotype effect in our recordings. We first calculated the envelope of gamma oscillations for each behavioral state and each channel and built histograms to test their distributions. One possibility for the increased gamma PSD in TG mice is that they may have bouts of higher/lower gamma activity embedded into normal oscillations. However, the distributions of gamma PSDs in the analyzed sweeps showed little deviation from Gaussian distribution, making this unlikely.

It is well established that oscillatory activities assigned to different frequency bands are not completely independent. For example, theta phase is known to modulate gamma power in rodent hippocampal and cortical circuits (Bragin et al. 1995; Chrobak et al. 2000; Buzsaki et al. 2003). To test how gamma oscillations interact with theta, we measured an MI as described previously (Tort et al. 2008, 2010). First, we calculated bicoherence (Kramer et al. 2008) in order to estimate frequency bands of interest (Fig. 4A). We chose to test how theta (6–10 Hz) phase modulates gamma (40–100 Hz) amplitude.

We found that MI was far above chance level (threshold of significance was set to P = 0.01) in the hippocampus (both CA1 and DG), but not in the frontal cortex or thalamus (Fig. 4C). In addition, we observed no cortical gamma modulation by hippocampal theta (neither in the 40–100 Hz nor in the 100–200 Hz range; data not shown). Furthermore, the significant hippocampal theta–gamma modulation was dependent on the behavioral state. Because of the small number of sweeps categorized as REM, we first computed analysis of variance (ANOVA) for theta–gamma modulation between the 3 remaining states, which proved to be highly significant (F1,3,15,6 = 24.3, P < 0.001, ANOVA with Greenhouse–Geisser correction; Fig. 4B,D). The Bonferroni post hoc test for the repeated measures variable affirmed that this modulation was strongest during movement and weakest during NREM sleep (P < 0.01). REM sleep was rare during our sampling period, and in 40% animals we failed to collect a sufficient number of REM sweeps for the statistics. Yet, when we performed an ANOVA for the remaining mice, we got a highly significant effect of the behavioral state (F1,2,7,3 = 55.7, P < 0.001). As evident from Figure 4D and confirmed by Bonferroni post hoc test, the MI was higher during REM sleep than in any other state (P < 0.002). Furthermore, the MI was higher during movement than during quiet waking (P = 0.03) or NREM sleep (P < 0.05). In neither statistical design did we find a significant genotype effect or genotype by state interaction. In addition, we detected that the theta phase with the highest gamma power was shifted during REM sleep compared with other behavioral states (Fig. 4B). We quantified this phase of modulation shift between NREM and REM sleep in the hippocampus (Fig. 4E). In WT mice, the mean phase shift in CA1 was 49 ± 6° and slightly smaller in DG 32 ± 5°, whereas in TG mice, this shift was twice smaller (27 ± 5° and 16 ± 6°, respectively). The 2-way ANOVA with genotype and electrode location as fixed factors revealed a significant genotype effect (F1,11 = 7.7, P = 0.018), whereas the location effect only approached significance (F1,11 = 4.2, P = 0.065).

**High-Frequency Oscillations (HFOs) in the Hippocampus and Cortex**

HFOs (>100 Hz) have been linked to epileptic activity in humans and in animal models of epilepsy (Jiruska et al. 2010), and previously our laboratory reported that APdE9 mice have a high prevalence of unprovoked seizures (Minkeviciene et al. 2009). Therefore, we explored HFOs in detail. Several types of HFOs were prominent in mouse EE/LFP recordings (Fig. 5A,B). The first type represents a typical hippocampal ripple oscillation, which appeared in the CA1 area during NREM sleep as compact oscillations in the frequency range of 120–200 Hz (Fig. 5A). The general manifestation and localization of hippocampal ripples appeared to be normal in TG mice. A different type of HFO was detected in the frontal cortex. Occasionally, at early stages of NREM sleep, we observed bursts that had a broad frequency range (100–600 Hz) and short duration (<50 ms; Fig. 5B). The bursts appeared repetitively approximately every 100 ms and formed clusters of 3–5 s (Fig. 5C,D). Usually, several clusters appeared in a row with an interval of 6–10 s (Fig. 5C). During 1 h recording of spontaneous activity, we detected from 0 to 15 such clusters in mice of both genotypes. Although the HFO cluster was a rare event, notably, there was little overlap between the timing of cortical HFO clusters and hippocampal ripples (at least within ±10 s), which rules out the possibility that cortical HFOs are induced by ripples either through volume conduction or through multisynaptic connections (Supplementary Fig. S2). In contrast, a similar and usually parallel HFO cluster was detectable in the thalamus, although with a lower...
amplitude. Finally, we compared cortical HFO cluster amplitude and duration between the genotypes. Although due to the small number of events the difference was nonsignificant, there was a trend for HFO clusters in TG mice to have a higher amplitude and/or a longer duration compared with WT mice (Fig. 5E; mean duration WT: 1.4 ± 0.2 s, TG:

Figure 3. Increased PSD in the frontal cortex and thalamus, but not in the hippocampus in APdE9 mice. (A) The top row illustrates the mean power with SEM during NREM sleep. For better visualization, the spectrum was “whitened” (multiplied by \( f^2 \)). (B) Bin by bin (1 Hz) \( P \)-values for the genotype comparison during NREM (the gray dashed line marks significance level of \( P = 0.05 \)). (C) \( P \)-values for the genotype comparison during the remaining behavioral states. Note that all axes are logarithmic and \( P \)-values are reversed.

Figure 4. Theta-phase modulation of gamma amplitude is behavioral state- and genotype-dependent. Bicoherence was calculated for the frequency range 1–100 Hz to detect interacting frequency bands (A, an example from hippocampal LFP during movement). (B) Representative example of theta–gamma phase–amplitude plot in the hippocampus of a WT mouse during various behavioral states. (C) Observed MI during NREM sleep compared with shuffled data in the 4 studied brain sites (TH, thalamus). For hippocampal LFP, the MI was far above the surrogate MI threshold of \( P = 0.01 \), whereas in the cortex and thalamus, no significant theta–gamma modulation was observed. (D) The strength of MI depends on behavior, but not on genotype. (E) The shift of theta phase at which gamma power peaks during REM sleep when compared with NREM sleep was significantly attenuated in TG animals (\( P = 0.02 \)). Data represent mean ± SEM.
1.9 ± 0.2 s, \(P = 0.10\) and mean amplitude WT: 0.18 ± 0.01 mV, TG: 0.21 ± 0.02 mV, \(P = 0.18\).

**Abnormal AEPs in APdE9 Mice**

To further assess the nature of altered inhibitory control in TG mice, we recorded cortical and thalamic AEPs, employing the gating paradigm, with 2 short tones presented as pairs. Typically, the amplitude of the second response is dramatically suppressed in comparison with the first one, with a maximum reduction observed at the 500 ms inter-stimulus interval (Bickford-Wimer et al. 1990). Such an interval would be too long for a direct \(\gamma\)-aminobutyric acid (GABAA)-mediated feedback inhibition and is likely to involve sustained GABAB-mediated mechanisms (Hershman et al. 1995). The cortical AEPs of both TG and WT mice had the following 3 components: N1, P2, and N3 (Fig. 6). The AEP amplitude was in
general higher in TG mice than in WT mice. The amplitude of the earliest component (about 15 ms after tone onset) was significantly higher in TG mice than in WT mice (during both conditioning and test stimulus, $P<0.05$). In addition, the AEP component around 40 ms was also significantly higher in TG mice. The thalamic response was similar to the cortical response, but appeared slightly earlier and also tended to be higher in TG mice. The amplitude of later components (around 30 and 65 ms) in response to conditioning stimulus was also significantly higher in TG mice than in WT mice ($P<0.05$).

The cortical and thalamic AEPs showed a robust habituation upon tone repetition (Fig. 6). The paired-pulse ratios of the single components (N1, P2, and N3) and complex components (N1–P2 and P2–N3) were similar in WT and TG mice. Only the earliest cortical component N1 was less suppressed in TG (54 ± 6%) than in WT mice (34 ± 7%), with difference close to significance ($t = -2.1, P = 0.054$).

**Discussion**

We found that electrical brain activity as measured by EEG/LFP is drastically altered in the frontal cortex and thalamus of APdE9 mice compared with their WT littermates, but only subtle differences were observed in the hippocampus. Increased EEG power was observed over the entire gamma frequency band in TG mice in the frontal cortex and thalamus, and this was independent of the behavioral state. In addition, there was a significant increase in lower frequencies (including theta) in the TG mouse cortex. Furthermore, APdE9 mouse displayed increased cortical and thalamic AEPs and a trend toward increased transient HF activity (>100 Hz) in the frontal cortex. In contrast, only minor alternations were observed in the hippocampus. While power and theta–gamma modulation are preserved in TG mice, the alternation of REM sleep-related theta–gamma phase shift speaks for some underlying changes also in the hippocampus.

Despite the extensive use of APP (or APP/PS1) transgenic mice as a model of AD, only a few studies have addressed possible EEG alterations in these mice. While characterization sleep phenotypes in AD mouse models have been explored in more than 10 studies, those were heavily focussed on the sleep pattern and largely ignored EEG abnormalities in these mice (reviewed in Platt et al. 2011). Those few studies that included analysis of EEG/LFP did not include frequencies above 40 Hz and had limited number of electrodes and/or behavioral states (Wang et al. 2002; Wisor et al. 2005; Zhang et al. 2005; Jyoti et al. 2010). Therefore, our current study is the first extensive recording of spontaneous electrical brain activity in freely moving AD mice during a complete set of behavioral states, covering a broad frequency range and 3 brain structures. Up to date, the most reproducible finding in AD mouse models is the increased power of theta oscillation and no change in cortical delta power. In addition, enhanced alpha–beta oscillations have been reported (Wang et al. 2002; Jyoti et al. 2010). Thus our finding of enhanced cortical theta–gamma power is largely compatible with these earlier findings.

In contrast, the findings in APP transgenic mice appear almost opposite to the general pattern of EEG slowing that has emerged over 2 decades of EEG studies in AD patients. Compared with cognitively normal elderly subjects, AD patients usually demonstrate increased delta and theta power and a parallel decrease in alpha and beta power (Jeong 2004). This slowing of EEG has largely been attributed to the loss of cortical cholinergic projections from the basal forebrain (Jeong 2004), although decreased cortical glucose metabolisms may also contribute to this EEG slowing (Diers et al. 2000). Notably, degeneration of cholinergic projections is very modest in APP transgenic mice compared with the human disease. For instance, although minor changes in vesicular ACh release and G-protein coupling of muscarinic ACh receptors can be observed in APdE9 mice already at 7 months of age (Machova et al. 2008), conventional ACh markers such as AChE and ChAT activity do not decline before 17–19 months of age (Savonenko et al. 2005; Machova et al. 2008). Secondly, besides expressing AD-related Aβ in excess, APdE9 mice as all APP transgenic mice overexpress the human APP protein with all its cleavage products. Some of the EEG changes may rather reflect consequences of APP overexpression than Aβ accumulation, as shown in our earlier study in separate APPswe and PS1(A246E) mouse lines (Wang et al. 2002). Another important difference between human clinical EEG and that of the mouse models is technical. Conventionally, the clinical EEG has a low pass of 50 Hz, which does not allow assessment of gamma activity. Interestingly, a relatively recent study including higher EEG frequencies reported a state-independent increase in the 30–70 Hz gamma band in AD patients compared with mild cognitive impairment or control subjects, which is in agreement with our findings in APdE9 mice (van Deursen et al. 2008).

During the last 2 decades, our knowledge has substantially improved concerning the role of brain rhythms during behavior and of circuit/cellular mechanisms underlying rhythm generation (Wang 2010). As a general rule, local circuits are capable of generating fast oscillations (like beta–gamma), but for generation of slower oscillations (like theta), larger systems are needed (Wang 2010). The list of possible mechanisms contributing to oscillation includes resonance properties of cells due to ion channel expression (White et al. 1995; Pike et al. 2000; Hu et al. 2002; Richardson et al. 2003), recurrent excitation (Traub et al. 1992), excitation–inhibition interaction (Borgers and Kopell 2003; Hansel and Mato 2003), negative feedback (Wang 1999), kinetics of GABA receptors (Whittington et al. 1995; Wang and Buzsaki 1996; Wang 2010; Capogna and Pearce 2011), bursting cells (Higgs and Spain 2009), or pacemaker cells (Alonso and Llinas 1989; Llinas et al. 1991). While changes in a combination of oscillatory mechanisms may well explain the present broad frequency range of EEG alternations in APdE9 mice, it is unlikely that several rhythmogenic mechanisms would be affected simultaneously at this early stage of amyloid pathology. As a single pathological event underlying these EEG alternations, the interneuron pathology is a feasible candidate, especially involvement of basket cells. Indeed, there is evidence for the lack of perisomatic GABAergic synapses around amyloid plaques in human AD brains and in APdE9 mice (García-Marin et al. 2009). However, the interneuron pathology alone cannot account for the broad-band EEG changes in our study. It is well established that interneurons primarily determine the power of faster oscillations such as gamma (Mann and Paulsen 2005; Wang 2010; Whittington et al. 2011), which was also confirmed by our previous studies in mice varied interneuronal pathologies (Gurevicius et al. 2004, 2007, 2009). To our knowledge, increased excitation of principal cells is the only mechanism that may account for the increased power of
rhythms in a broad band ranging from theta to gamma as well as for increased amplitude of AEPs. For example, increasing cortical excitation through basal forebrain activation with N-methyl-D-aspartate (NMDA) increases gamma and theta in the cortico area (Cap and Jones 2000). In parallel, basal forebrain activation with NMDA also increases state of wakefulness (Cap and Jones 2000). The identity of projection neurons stimulated by NMDA is not known, but plausible candidates are basal forebrain cholinergic cells which have extensive projections to all cortical regions. Therefore, this may suggest that the increase drive from basal forebrain is behind cortical electrophysiological profile observed in our study. However, cortical acetylcholine levels are modulated state dependently (Marrosu et al. 1995), while we observed similar EEG changes in all behavioral states in the present study. Moreover, previous studies in ApD9 female mice showed decreased acetylcholine release as early as around 7 months of age (Machova et al. 2008). Recently, basal forebrain noncholinergic (most likely GABAergic) tonic neurons with long cortical projections have been described (Lin et al. 2006). Although prefrontal cortical activity was affected by synchronization of those cell ensembles, the oscillation frequencies between 5 and 100 Hz were not equally affected, and the modulation was behavioral state-dependent. Therefore, activation of these projections alone cannot account for the broad-band EEG changes in our study. The most plausible explanation for the current EEG and AEP findings remains to be a local hyperexcitability of cortical pyramidal cells themselves. This idea is corroborated by our previous finding that ApD9 mice have increased resting membrane potential of cortical pyramidal cells, which make them hyperexcitable (Minkeviciene et al. 2009).

In addition to conventional oscillation in the cortex, we also observed some HF phenomena. Although some properties of HF bursts in our study resemble pathological ripples (Jirouška et al. 2010), it is unlikely that they are related to epileptogenesis. First, burst clustering in a periodic manner has not been described for pathological ripples. Second, we observed similar clusters of bursts in WT control animal that do not express seizures (Minkeviciene et al. 2009). We argue that HF bursts observed in our study represent a fast transient event by which action potentials of different neurons can be synchronized with a high temporal precision (Supplementary Fig. S3). A similar event with analogous spectral characteristics has been described in association with sleep spindles and high-voltage spike-and-wave discharges (Kandel and Buzsaki 1997). However, sleep spindles cannot be the driving force behind clusters of HF bursts because they usually last only for approximately 1 s. It is known that epileptogenesis may “hijack” the thalamocortical networks for sleep spindle generation with spike–wave discharges as a result (Beenhakker and Huguenard 2009). However, we observed no spike–wave pattern associated with the HF burst, the burst frequency was far above 3 Hz, and our WT mice have never been reported to have seizures. Furthermore, in contrast to a weak association between sleep spindles and hippocampal ripples (Sirota and Buzsaki 2005), we observed a little overlap between hippocampal ripples and the cluster of HF bursts (Supplementary Fig. S2). Therefore, at present, we cannot identify the driving force for the cluster generation (with a periodicity of 10 Hz). The driving force may not be local to the cortex since LFP oscillation at approximately 10 Hz waxes and wanes a few times during a single cluster of HF bursts. In any event, a trend toward increased transient HF activity in ApD9 mice is in line with increased excitability of principal cells of transgenic animals.

The thalamic electrophysiological profile in our recordings closely resembles that of cortical EEG. Although the power spectrum density was increased over a broad frequency range in the thalamus of ApD9 animals, this happened in a slightly narrower range than in the cortex (less significant theta changes and smaller gamma range). These changes may arise independently in these 2 brain regions or could be driven by strong cortico-thalamic connections. Previous studies in ApD9 showed the highest amyloid plaque load in neocortical areas, followed by the amygdala and hippocampus, whereas only scattered plaques were found in the thalamus (Minkeviciene et al. 2009). Although the amyloid plaques themselves do not directly link to excitability, they also indicate a local increase in soluble amyloid species. Therefore, we speculate that thalamic hyperexcitability in ApD9 mice is driven by amyloid pathology in the neocortex.

To our knowledge, this is the first report on behavioral-dependent theta–gamma modulation in the mouse hippocampus. The gamma power was most strongly modulated during REM sleep, followed by movement, quiet waking, and NREM. Of note, despite the smallest theta–gamma modulation during NREM sleep, MI remained far above chance level also during NREM. One possible explanation for the state-dependent theta–gamma modulation may be state-dependent variation in gamma power, that is, increase in MI is due to augmentation of gamma oscillations. However, this is highly unlikely as we used MI based on an adaptation of the Kullback–Leibler distance, which has been shown to be insensitive to the absolute values of amplitude envelope (Tort et al. 2010). Another possibility for the behavioral state-dependent strength of MI is medial septum (MS) modulation of hippocampal LFPs. As shown in recent experimental and modeling studies, the reduced theta oscillations and theta–gamma coupling may stem from reduced drive from MS (White et al. 2000; Wulff et al. 2009). Interestingly, a recent study described a coherent neuronal ensemble in MS that enhanced hippocampal theta and gamma oscillations in behavioral state-dependent manner (Zhang et al. 2011). This most likely encompasses slow-firing cholinergic neurons with highest firing rate during REM sleep, intermediate during waking, and lowest during slow-wave sleep. Although we observed no significant alternations in the power of any single oscillation in the hippocampus of ApD9 mice, we did saw changes in theta–gamma coupling. In particular, theta–gamma modulation was affected during REM sleep, but not during other behavioral states. In WT animals, the theta phase with the highest gamma power was shifted during REM sleep compared with other behavioral states, but this shift was twice smaller in TG animals. A couple of recent studies reported that a small subpopulation of CA1 pyramidal cells start to fire action potential at an earlier theta phase during REM sleep than during exploratory behavior (Senior et al. 2008; Sirota et al. 2008; Mizuseki et al. 2011). Mizuseki et al. (2011) suggest that this subpopulation resides in the deep CA1 pyramidal cell layer and is more strongly driven by direct entorhinal input than superficial CA1 pyramidal cells. Since entorhinal cortex (EC) is among the first brain regions to express amyloid plaques in ApD9 mice (Garcia-Alloza et al. 2006; Minkeviciene et al. 2009), it is tempting to speculate that amyloid accumulation will first affect the
subpopulation of deep CA1 pyramidal cells, thus attenuating the theta-gamma phase shift during REM sleep. Another explanation for the phase shift in the theta-gamma modulation is the change in coupling between CA1-CA3 versus CA1-EC during different behavioral states. Recently, it was hypothesized that CA3 and EC “bind” with CA1 during different phases of theta (Colgin et al. 2009). Gamma oscillations maximal on the descending portion of the theta wave synchronize CA1 with CA3, whereas gamma peaking near the theta trough synchronizes CA1 with EC. Since CA3–CA1 gamma coherence is significantly decreased during REM sleep compared with waking (Montgomery et al. 2008), it is reasonable to assume that averaged theta phase for maximal gamma would correspondingly shift between these states. However, at present, there are no experimental data on CA1–EC coupling during sleep.

The magnitude (effect size) of electrophysiological changes observed in our recordings suggests that in APdE9 mice, alternations in cortex precede those in the hippocampus or thalamus. This fits well with the fact that the highest amyloid plaque load in APdE9 mice (as is the case in AD brains) is present in neocortical areas (Garcia-Alloza et al. 2006; Minkeviciene et al. 2009). At the early stage of amyloid pathology, cortical principal cells become hyperexcitable and via extensive cortico-thalamic connection also drive thalamic cells. Similarly, as judged from minor electrophysiological abnormalities in the APdE9 mouse hippocampus, alternation there appears later than in the neocortex and is most likely driven by abnormal entorhinal input. This chain of events reflects underlying network reorganization, which may lead to epileptic seizures (Palop et al. 2007; Minkeviciene et al. 2009).

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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