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Corticothalamic network dysfunction and behavioral deficits in a mouse model of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is associated with cognitive decline as well as seizures. Growing evidence indicates seizures contribute to cognitive deficits early in disease, but how they develop and impact cognition are unclear. To investigate potential mechanisms, we studied a mouse model that overexpresses mutant human amyloid precursor protein (APP) with high levels of A β . These mice develop generalized epileptiform activity, including nonconvulsive seizures, consistent with alterations in corticothalamic network activity. APP mice exhibited reduced activity marker expression in the reticular thalamic nucleus (nRT), a key inhibitory regulatory nucleus, and increased activity marker expression in downstream thalamic relay targets that project to cortex and limbic structures. Slice recordings revealed impaired cortical inputs to nRT that may contribute to corticothalamic dysfunction. These results are consistent with our findings of impaired sleep maintenance in APP mice. Finally, the severity of sleep impairments predicted the severity of deficits in Morris water maze, suggesting corticothalamic dysfunction may relate to hippocampal dysfunction, and may be a pathophysiological mechanism underlying multiple behavioral and cognitive alterations in AD.

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Disclosure Statement

The authors declare no competing financial interests.

Keywords

Alzheimer's disease; seizure; epilepsy; corticothalamic; APP; sleep fragmentation

1. Introduction

Alzheimer's disease (AD) is characterized by cognitive impairment and a prominent loss of memory. However, AD is also accompanied by a number of other cognitive and behavioral alterations including deficits in attention, cognitive processing, and sleep maintenance (Ballard, et al., 2001, Levinoff, et al., 2005, Moran, et al., 2005, Perry and Hodges, 1999, Petit, et al., 2004, Sahakian, et al., 1989, Yesavage, et al., 2003). The underlying mechanisms that give rise to impairments in diverse behavioral domains are unknown.

Several lines of evidence suggest that dysregulation of the corticothalamic network may be a common denominator that contributes to the cognitive and behavioral alterations associated with AD. The corticothalamic network regulates processes that include attention, cognitive processing, learning and memory, and sleep maintenance (Beenhakker and Huguenard, 2009, Briggs and Usrey, 2008, Jones, 2009, Rouiller and Welker, 2000), all of which are altered in AD. Moreover, dysregulation of tightly regulated activity in the corticothalamic network can give rise to non-convulsive seizures (Beenhakker and Huguenard, 2009, Ernst, et al., 2009, Meeren, et al., 2002, Paz, et al., 2011, Steriade and Contreras, 1998). Notably, non-convulsive seizures and other forms of epileptiform activity have been documented in both AD patients and transgenic mouse models of AD (Palop, et al., 2007, Vossel, et al., 2013), consistent with the hypothesis that dysregulation of the corticothalamic network may contribute to deficits in AD.

Conservative estimates indicate that AD patients exhibit a 5-10 fold increase in seizure incidence compared to age-matched controls (Amatniek, et al., 2006, Hauser, et al., 1986, Larner, 2010, Lozsadi and Larner, 2006, Vossel, et al., 2013). However, seizures and epileptiform activity in AD may be vastly underestimated due to subclinical epileptiform activity and non-convulsive seizures (Vossel, et al., 2013). Multiple mouse models of AD also exhibit a wide range of network dysfunction manifested as generalized epileptic discharges and spontaneous convulsive and non-convulsive seizures (Corbett, et al., 2013, Gurevicius, et al., 2013, Harris, et al., 2010, Hazra, et al., 2013, Lalonde, et al., 2005, Minkeviciene, et al., 2009, Palop, et al., 2007, Verret, et al., 2012, Vogt, et al., 2011). However, the precise mechanisms by which seizures arise in AD and develop into dysfunctional networks that incorporate a wide range of brain regions is unknown.

In transgenic mice expressing mutant human amyloid precursor protein (APP mice), non-convulsive seizures with cortical oscillations that are similar to absence seizures have been described (Palop, et al., 2007), consistent with dysregulation of corticothalamic network activity (Beenhakker and Huguenard, 2009, Coenen and Van Luijtelaar, 2003). Other studies in AD mouse models have indicated that the thalamus is directly engaged during certain seizures (Gurevicius, et al., 2013). These lines of evidence suggest that the corticothalamic network may be dysfunctional in AD. However, the corticothalamic network may be perturbed in a number of ways (Beenhakker and Huguenard, 2009, Menuz and Nicoll,

2008, Noebels, 2012, Paz, et al., 2011), and the precise nature of corticothalamic network dysfunction in APP mice is unknown.

In this study, we first used an immunohistochemical marker of neuronal activity to map the patterns of activity in the corticothalamic network in APP mice, allowing us to focus our studies on particular cortical and thalamic areas. We then performed electrophysiological recordings in corticothalamic slices from APP mice to verify that results from the mapping studies were indicative of alterations in neuronal and synaptic activity. Finally we assessed behavioral measures of both corticothalamic and hippocampal function. Our results suggest that alterations in the corticothalamic network in APP mice may contribute to impairments in several behavioral and cognitive domains in APP mice, and perhaps also in AD.

2. Materials and Methods

2.1 Transgenic Mice

Heterozygous transgenic mice and nontransgenic (NTG) littermates of line J20 were used for these studies at 4–6 months of age. These mice express human amyloid precursor protein (APP) carrying Swedish (K670N, M671L) and Indiana (V717F) mutations under control of the platelet-derived growth factor β -chain promoter, on a C57BL/6J background (Mucke, et al., 2000). Male and female mice were used for experiments, sex was balanced across genotypes unless noted otherwise. No sex-dependent effects were observed. Mice were group housed with *ad-libitum* access to food and water, and were kept on a 12:12 light cycle. One week before behavioral testing, mice were singly housed to reduce variability in performance. All experiments were performed under protocols approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University, Philadelphia, PA, USA.

2.2 EEG

NTG and APP mice (4–6 months old, 4/genotype) were anesthetized for electrode implantation for video EEG monitoring. Teflon-coated silver wires (127 μ m in diameter) attached to a 6-pin Delran pedestal (Plastics One, USA) were implanted bilaterally into the subdural space over frontal and parietal cortices (from Bregma: 1.0 mm A-P, 1.5 mm M-L; –2.2 mm A-P, 2 mm M-L) along with a hippocampal depth electrode (–2.2 mm A-P, 2 mm M-L, 1.8 mm from the brain surface). Ground and reference electrodes were implanted directly behind Lambda on either side of midline. Mice were given at least 72 h recovery period before recordings were conducted. EEG recordings were performed on at least two different days for a minimum of 8 h each time using a Stellate Harmonie acquisition system (version 7.0a, Stellate, USA) with a sampling rate of 2 kHz, 10,000x gain, 1 kHz low-pass filter and 0.3 Hz high-pass filter, and the analysis performed on the data without further digital filtering. Native Stellate and Labchart Pro (AD Instruments Inc., USA) software were used for EEG signal processing. Movement artifacts and seizure events were excluded from EEG traces before processing for analysis of spike frequency. Frequency power analysis of EEG signals was calculated using the Welch method. The normalized mean power over frequency ranges of interest was calculated for left and right frontal cortical electrodes individually, then averaged across electrodes and animals. Before averaging, the power value

for individual frequency ranges of each electrode was normalized by the total power between 0.1-1000 Hz to account for differences in electrode impedance across animals.

2.3 In vitro slice electrophysiology

Mice were anesthetized, decapitated, and brains rapidly removed and immersed in an ice-cold sucrose cutting solution (in mM): 26 NaHCO₃, 216.2 sucrose, 10 glucose, 2.5 KCl, 1.25 NaH₂PO₄, 7 MgCl₂, and 0.5 CaCl₂ equilibrated with 95% O₂ and 5% CO₂, pH 7.4. 400- μ m-thick thalamocortical slices containing VB thalamus and nRT were prepared on a Leica VT1000S vibratome in ice-cold cutting solution. Slices were transferred to a chamber filled with ACSF (in mM): 130 NaCl, 26 NaHCO₃, 2.5 KCl, 1 MgCl₂, 2 CaCl₂, 1.25 NaH₂PO₄, and 10 glucose equilibrated with 95% O₂ and 5% CO₂, pH 7.4. Slices were incubated at 30 $\pm$ 0.5°C for 1 hr in ACSF to allow recovery prior to electrophysiological recordings. For recordings, slices were placed in a submerged recording chamber and perfused with ACSF at 30°C. Recording electrodes (resistance of 1–2 M Ω) were prepared using a micropipette puller (P30, Sutter Instruments, USA). Slice anatomy was identified using an upright differential contrast optic with wide-field epifluorescence microscope (BX51WI, Olympus, USA) equipped with infrared-differential interference contrast camera. Evoked field excitatory postsynaptic potentials (fEPSPs) were recorded using a concentric bipolar stimulating electrode and data acquisition was triggered with a digital stimulator. Evoked stimulation (0.04–0.3 μ A, 0.2 msec duration) was set to half to two-thirds maximum fEPSP, responses were evoked every fifteen seconds for 5 mins. Data were low-pass filtered at 4 KHz, digitized at 10 KHz (Digidata, pClamp, Molecular Devices, USA). For long range connections between cortex and thalamic nuclei, the stimulating electrode was positioned in deep cortical layers V/VI while recordings fEPSPs from nRT, or vice-versa. For local field population responses in thalamic nuclei and cortical areas, the stimulating electrode was placed close to recording electrode. Slice from which stable responses were unable to be evoked were discarded. fEPSP slopes were calculated as the maximal slope of the ascending phase, measured as the maximum slope between 50 and 90% of the evoked field response. Mean fEPSP slope measurements were used to compare between genotypes for any given area of interest.

2.4 In vivo tracer dye injection

To assess integrity of corticothalamic fibers, mice were injected with 0.27 μ L of the retrograde tracer Alexa Fluor® 488 conjugated Cholera toxin β subunit (Life technologies, Grand Island, NY, USA) using a 10 μ L Hamilton Gastight syringe with an attached 33-gauge 45° beveled needle (Hamilton; Reno, NV, USA). The needle was lowered through burr holes drilled over the cortical surface to deliver the tracer in the thalamus (–1.3 mm A-P, 1.9 mm M-L, 3.4 mm DV). Two weeks following injection, animals were deeply anesthetized, perfused with saline, and brains were quickly removed and placed into carbonated ice-cold sucrose cutting solution. 400- μ m-thick horizontal thalamocortical slices were prepared on a Leica VT1000S vibratome and transferred to 4% paraformaldehyde. Fixed slices were stored in 0.1 M phosphate buffer (pH 7.4) and mounted on glass slides for fluorescence imaging using Metamorph.

2.5 Sleep monitoring

Sleep-wake behavior was monitored in real time using HomeCageScan software (CleverSys, Inc., Reston, VA, USA), which allows for automated detection of rodent behaviors including sleep-wake based on movements, body postures, and duration/frequency of activities. The HomeCageScan software sleep detection algorithm has also been used previously for studying sleep-wake patterns in rodents (Hazra, et al., 2014, Martinowich, et al., 2011). Each platform is fitted with an individual LED/IR sensitive camera capturing 640x480 pixels at 30 frames per sec, automatic 12 hr LED light/IR light controller for light/dark cycles, and video multiplexer for simultaneous multiple video acquisition to a Windows-based computer running HomeCageScan software. For statistical analysis, sleep-wake data were binned in one hour bins and exported to Excel. Because mice are nocturnal and sleep primarily during the light phase, sleep-wake behavior during the light phase was analyzed. Average sleep bout length was determined by normalizing the total duration of sleep time over number of awakens for each respective bin.

2.6 Morris Water Maze

Mice were tested using our published protocol (Corbett, et al., 2013). Briefly, before testing began, mice were pre-trained for one day by placing them on the platform in the pool. For the cued platform phase, a black and white striped rod was placed vertically in the center of the platform. The duration and distance traveled to reach the platform were measured in two sessions/day for two successive days. Each session consisted of two trials in which mice were dropped from different locations, and the platform was located in different quadrants, to assess visual acuity and motor performance. Hidden platform training involved two sessions/day for five consecutive days. Each session consisted of three trials. The platform location remained constant and mice entered the pool from different locations. 24 hours after the last training trial, mice were given a 60 s probe trial test in which the platform had been removed from the pool. Performance in the cued, hidden, and probe trial portions of the maze was video-tracked and analyzed using WaterMazeScan of the TopScan software suite (CleverSys Inc, Reston, VA, USA). No mice exhibited notable floating or thigmotaxic behavior that affected performance. The experimenter was blinded to genotype during testing.

2.7 Immunohistochemistry

For brain harvesting, mice were flush-perfused transcardially with DEPC-treated saline. Hemibrains were frozen on dry ice for biochemistry or postfixed in 4% paraformaldehyde for immunohistochemistry. Tissue preparation and immunohistochemistry were performed as previously described (Chin, et al., 2007, Corbett, et al., 2013). Coronal sections (30 μ m thick) were cut using a freezing sliding microtome and collected into ten subseries throughout the rostral-caudal extent of the brain for immunohistochemical studies. Sections were stained with avidin-biotin/immunoperoxidase using rabbit anti-FosB (Santa Cruz, Dallas, Texas, USA) and biotinylated goat anti-rabbit antibodies (Vector Laboratories, Burlingame, CA, USA) with diaminobenzidine as a chromagen, or fluorescently immunolabeled with anti-NeuN (Millipore, Billirica, MA, USA) and Alexa-fluor 594 secondary antibody (Thermo Fisher, Waltham, MA, USA). Cell counts were used to

quantify FosB and NeuN in thalamus. In cortex and CA1, optical density was used to quantify FosB due to the high density of FosB immunoreactive cells. Quantification was performed using ImageJ processing and analysis software.

2.8 Statistical Analysis

Statistical analyses were performed with Prism 5.0 software. All differences between means were assessed using a two-tailed, unpaired Student's t-test. A one-way ANOVA with Tukey's post-hoc test was used to assess FosB immunoreactivity in the somatosensory cortex. Regression analysis was used to detect correlations.

3. Results

3.1 APP mice display epileptiform activity and behavioral abnormalities consistent with corticothalamic network dysfunction

APP mice exhibit aberrant network activity including spontaneous epileptiform spikes, convulsive seizures, and non-convulsive seizures (Palop, et al., 2007, Roberson, et al., 2011, Sanchez, et al., 2012, Verret, et al., 2012). The non-convulsive seizures exhibited by APP mice are accompanied by spike wave discharges associated with corticothalamic dysfunction (Palop, et al., 2007). Consistent with previously published reports, we observed spontaneous epileptic spiking activity and seizures in APP mice (Fig 1A-C). We also observed 7-10 Hz oscillations resembling spike-and-wave discharges (Fig 1D), which are typical in animals with absence epilepsy (Bosnyakova, et al., 2006, Midzianovskaia, et al., 2001, van Luijtelaa and Coenen, 1986, Velazquez, et al., 2007). The number of seizures per animal varied greatly (between 1-5 per APP mouse with seizures). Two APP mice didn't exhibit any seizures during the duration of the recordings, but did exhibit epileptic spikes. Abnormal spike discharges were detected in the cortical and hippocampal depth electrodes in every APP mouse analyzed (Fig. 1A-C). Consistent with previous reports (Roberson, et al., 2011), spiking frequencies in APP mice were $\sim 167 \pm 40$ spikes/h (mean $\pm$ S.E.M; Fig. 1C).

We analyzed the spectral power values of spontaneous oscillatory brain activity across different frequencies (Fig 1E), including delta (1-4 Hz), theta (4-8 Hz) alpha (8-13 Hz), beta (13-30 Hz), and both low and high gamma (30-100 Hz) frequency bands (Belluscio, et al., 2012, Bojak, et al., 2013, Buzsaki and Wang, 2012, Carracedo, et al., 2013, Holgado, et al., 2010, Lorincz, et al., 2008, Takatsuki, et al., 2005, Traub, et al., 1999). Characterization of cortical EEG power analysis was performed from EEG recordings during the light phase. We found that APP mice exhibit a significant decrease in the power of theta oscillations, and a trend for an increase in delta oscillations, compared to NTG mice (Fig. 1E).

APP mice also exhibited spontaneous intermittent seizures (Fig. 1B, F). Recordings revealed synchronous leading spikes across all the EEG channels at the onset of recorded seizures (Fig 1F), suggesting that the origin of the ictal event may involve deep brain structures that function as a common synchronizer (Bertram, et al., 2001). Notably, seizures with deep brain structure origins typically involve the corticothalamic network (Bertram, et al., 2001, Steriade and Contreras, 1998).

To test whether APP mice exhibit behavioral alterations consistent with corticothalamic dysfunction, we assessed sleep maintenance, as dysregulation of corticothalamic activity leads to impairments in sleep maintenance and an increase in awakenings from sleep (Anderson, et al., 2005, Beenhakker and Huguenard, 2009, McCormick and Bal, 1997, Steriade, 2003). We assessed the duration of sleep and number of awakenings during the light phase, when mice primarily sleep (Anaclet, et al., 2009, Crocker and Sehgal, 2010, Hoyer, et al., 2013), using HomeCageScan. We found that APP mice exhibited an increased number of awakenings (Fig. 1*G*) even though APP and NTG mice slept similar durations of time (Fig. 1*H*). Consistent with these findings, APP mice also exhibited shorter sleep bout durations compared to NTG controls (Fig. 1*I*).

3.2 Chronic neuronal activity is altered in key components of the corticothalamic network in APP mice

The function of the corticothalamic network is dependent on a dynamic interplay between the reticular thalamic nucleus (nRT), thalamic relay neurons, and downstream target regions including cortex and limbic structures (Fig 2*A*). Deep layers of cortex provide the primary input source to nRT (Beenhakker and Huguenard, 2009, Destexhe, et al., 1998), although other brain regions also provide input (Cavdar, et al., 2008). Output from nRT is GABAergic and restricted within the thalamus (Beenhakker and Huguenard, 2009, McCormick and Bal, 1997). Downstream thalamic relay nuclei connect reciprocally to nRT as well as target brain regions. Our finding that APP mice exhibit impairments in sleep maintenance suggested that the tightly regulated activity in the corticothalamic network may be dysregulated. Notably, corticothalamic dysfunction can also result in pathological conditions including absence epilepsy (Beenhakker and Huguenard, 2009), a type of non-convulsive seizure such as those exhibited by APP mice. Several modes of corticothalamic dysregulation have been found to exist (Fig 2*A*), but whether any of them might underlie the alterations exhibited by APP mice has not been addressed.

To investigate the mode of corticothalamic dysfunction in APP mice, we mapped neuronal activity in key components of the corticothalamic network by examining the expression pattern of FosB in key components of the network. FosB is an activity-dependent transcription factor with a half-life on the order of weeks, making it an excellent marker of chronic activity (McClung, et al., 2004, Nestler, et al., 2001). We found that APP mice express fewer FosB-immunoreactive (IR) cells in the nRT compared to NTG controls (Fig 2*B,C*). Immunostaining for NeuN, a marker of neuronal nuclei, demonstrated that there is no difference in the number of neurons in nRT between NTG and APP mice, indicating that cell loss was not a contributing factor for decreased FosB expression in the nRT of APP mice (Fig 2*D, E*). We also found a similar reduction in FosB-IR cells in another well-characterized transgenic mouse model of Alzheimer's disease that overexpress mutant APP as well as mutant presenilin (PSAPP mice), resulting in high levels of A β (Holcomb, et al., 1998) (Fig 2*F*). In contrast, transgenic mice from Line I5A that overexpress wild-type human APP and do not exhibit a high A β load (Mucke, et al., 2000) do not display a significant change in the number of FosB-IR cells in the nRT compared to NTG controls (Fig 2*G*).

We also assessed whether a chronic decrease in nRT activity might affect expression of

FosB in downstream thalamic relay nuclei. The nRT provides tonic inhibition to thalamic relays nuclei throughout the thalamus (Cavdar, et al., 2008, Cope, et al., 2005, Pinault, et al., 1998, Steriade and Contreras, 1995) and therefore decreased activity in nRT might result in disinhibition of these neurons. APP mice did not exhibit a greater number of FosB-IR neurons in the ventral posteromedial nucleus that relays sensory information from the head to somatosensory cortex (Fig 2*G*), but did in ventral posterolateral nucleus (Fig 2*H*), a thalamic nucleus that relays sensory information from the body to somatosensory cortex. Correspondingly, we found that APP mice exhibited increased FosB-IR in somatosensory cortex (Fig 2*J*). A greater number of FosB-IR neurons in thalamic relay nuclei that target limbic regions was observed in APP mice as well. APP mice exhibited an increase in

FosB-IR neurons in the paraventricular nucleus (Fig 2*J*), which projects to deep layers of entorhinal cortex (Beckstead, 1978, Segal, 1977) as well as an increase in the number of

FosB-IR neurons in the thalamic reuniens nucleus (Fig 2*K*), which projects to both entorhinal cortex and the CA1 region of hippocampus (Cavdar, et al., 2008, Dolleman-Van Der Weel and Witter, 1996). No apparent change in the number of FosB-IR neurons was observed in the lateral geniculate nucleus (Fig 2*L*), suggesting that corticothalamic network dysfunction in APP mice involves dysregulation of specific thalamic nuclei.

3.3 Aberrant thalamic FosB expression patterns are related to impairments in sleep maintenance

To gain insight into whether the aberrant patterns of FosB expression might contribute to the impairments in sleep maintenance we observed in APP mice, we examined the relationship between FosB expression and awakenings from sleep. We found that FosB-IR in thalamic relay neurons (Fig 3*A*) as well as in somatosensory cortex (Fig 3*B*) correlated with the number of awakenings from sleep during the light phase in APP mice. These results are consistent with the hypothesis that hyperactivity in key brain regions of the corticothalamic network may be associated with sleep maintenance deficits in APP mice.

3.4 Cortical input to nRT is reduced in APP mice

The use of FosB to map patterns of neuronal activity is useful for identifying key brain regions of interest. We then investigated whether the altered expression of FosB in APP mice was due to functional alterations in specific circuits, by performing evoked field potential recordings in cortico-thalamo-cortical slice preparations. The majority of corticothalamic projections are concentrated in deeper layers of somatosensory cortex (Agmon and Connors, 1991, Agmon, et al., 1993, Cruikshank, et al., 2010); therefore, slices with visible cortical layer 4 barrels and identifiable nRT structure were used in recordings (Fig 4*A*). Compared to NTG controls, APP mice exhibited a decrease in the amplitude of evoked responses within the nRT following stimulation of deep layers of somatosensory cortex (Fig. 4*B*). No differences between NTG and APP mice were observed in evoked cortical responses following electrical stimulation of thalamocortical fibers in the thalamic ventral basal nucleus (Fig. 4*C*), indicating that corticothalamic, but not thalamocortical, connections were impaired. When evoked field potentials in nRT were recorded after direct stimulation of nRT, no differences in the average evoked field potentials (Fig. 4*D*) or input-output ratios (Fig. 4*E*) were observed in APP mice compared to NTG controls, indicating

that intrinsic excitability within the nRT is not impaired. Similar results were obtained when we recorded evoked field potential responses in midline thalamic relay nuclei after direct stimulation- compared to NTG mice, no differences in the slope of the average evoked field potential (Fig. 4F) or input-output ratio (Fig. 4G) were observed in APP mice. These results support the hypothesis that decreased cortical output may contribute to reduced activity in nRT, and perhaps downstream disinhibition of thalamic relay targets.

3.5 Anatomical corticothalamic connections are intact in APP mice

To determine whether impaired cortico-nRT connections may be attributed to anatomical alterations, we used a retrograde tracer, cholera toxin-B, to specifically highlight the projections from cortex to thalamus. No overt anatomical differences were observed in the corticothalamic fibers between APP and NTG mice (Fig. 5).

3.6 Cortical signal propagation is decreased in APP mice

Transgenic AD mice exhibit decreased levels of functional Nav1.1 α subunits in cortex (Corbett, et al., 2013, Verret, et al., 2012). Nav1.1 α is expressed throughout the cortex (Catterall, 2000) Allen Brain Atlas), including GABAergic interneurons (Ogiwara, et al., 2007) and glutamatergic pyramidal neurons, particularly in deep layers of somatosensory cortex (Ogiwara, et al., 2013). Decreased functional Nav1.1 α could contribute to impaired generation and propagation of action potentials and to the observed reduction in cortical input to nRT. To investigate whether propagation dynamics of neuronal signaling were impaired in the cortex of APP mice, we measured the velocity of lateral propagation in cortical layer 2/3 of somatosensory cortex in NTG and APP mice (Fig 6A). This region has robust lateral connections, facilitating an examination of signal propagation. Indeed, we found that lateral propagation velocity in cortical layer 2/3 was decreased in APP mice compared to NTG controls (Fig. 6B-D).

3.7 Dysfunction in the corticothalamic network and hippocampus are related in APP mice

To assess whether dysfunction of the corticothalamic network might contribute to hippocampal dysfunction in APP mice, we first investigated whether limbic structures directly targeted by thalamic relay nuclei also exhibited alterations in FosB expression. Compared to NTG controls, APP mice exhibited increased FosB-IR in entorhinal cortex, including layers 4-6 (Fig 7A,B), an area that receives input from the paraventricular nucleus (Beckstead, 1978, Segal, 1977) and the nucleus reuniens (Dolleman-Van Der Weel and Witter, 1996), both of which showed increased FosB expression in APP mice (see Fig 2). Moreover, the number of FosB-IR neurons in the paraventricular nucleus (PVT, Fig 7C) and the nucleus reuniens (Fig 7D) correlated with FosB-IR in layers 4-6 of ERC. In addition to projecting to entorhinal cortex, the nucleus reuniens also projects to the CA1 region of the hippocampus (Cavdar, et al., 2008, Dolleman-Van Der Weel and Witter, 1996, Loureiro, et al., 2012). Compared to NTG mice, APP mice exhibited increased expression of FosB in the CA1 pyramidal cell layer (Fig 7E,F). Notably, FosB-IR in the nucleus reuniens correlated positively with FosB-IR in the CA1 pyramidal cell layer of APP mice (Fig 7G), suggesting a common underlying mechanism. Finally, we examined whether the magnitude of alterations in corticothalamic-driven behaviors related to the

magnitude of deficits in hippocampal behavior in APP mice. We found that the number of awakenings during the light phase, a measure of impaired sleep maintenance, correlated positively with the distance required to find the hidden platform in Morris water maze (Fig 7H).

4. Discussion

In this study, we found that APP mice exhibit alterations in the expression of neuronal activity markers in specific components of the corticothalamic network, which were confirmed with electrophysiological assessment of circuit function. Our results suggest that impaired cortical signal propagation leads to reduced inputs to nRT and subsequent disinhibition of thalamic relay nuclei and their downstream targets. Such alterations are consistent with the impairments in sleep maintenance we observed in APP mice, as well as the occurrence of nonconvulsive seizures, suggesting that the dysfunction in the corticothalamic network may be a common denominator in the repertoire of impairments associated with Alzheimer's disease.

The first indications that network dysfunction is highly prominent in APP mice were present in the EEG recordings, both in terms of the presence of seizures and spike-and-wave discharges as well as the alterations in spectral power values of spontaneous oscillatory brain activity across different frequencies. Similar findings in spectral power values have been reported by others in the same mouse line, a different mouse line APP/PS1 (APPswe, PSEN1dE9) carrying the mutant human APP transgene along with mutant human presenilin, and in rats in which A β was injected into the hippocampus (Rubio, et al., 2012, Villette, et al., 2010, Wang, et al., 2002). Early studies show that prominent theta power enhancement in human cortical EEG to play an important role in various working and episodic memory tasks and overall memory consolidation (Guderian, et al., 2009, Klimesch, 1999, Schmiedt, et al., 2005). Theta rhythms also have been shown to facilitate the formation of long-range functional networks, sometimes referred as theta synchronization (Buzsaki and Draguhn, 2004, Mellem, et al., 2013, von Stein and Sarnthein, 2000). During cognitive processing, including visual and auditory stimuli, theta synchronization/coupling helps in recruitment of various neural network and information co-ordination among distinct neuroanatomical structures (Kawasaki, et al., 2014). EEG studies in AD patients have also shown altered delta and theta power (Fraga, et al., 2013, Huang, et al., 2000, Jeong, 2004, van Deursen, et al., 2008), although they also exhibit a decrease in alpha and beta frequency band power (Fraga, et al., 2013, Jeong, 2004). In our APP mice, we did see a trend for decreased alpha frequency, but the difference between genotypes did not reach statistical significance.

Alzheimer's disease is associated with deficits in sleep maintenance, attention, and cognitive processing (Ballard, et al., 2001, Levinoff, et al., 2005, Moran, et al., 2005, Perry and Hodges, 1999, Petit, et al., 2004, Sahakian, et al., 1989, Yesavage, et al., 2003), which are all dependent on corticothalamic function (Beenhakker and Huguenard, 2009, Briggs and Usrey, 2008, Jones, 2009, Rouiller and Welker, 2000). While degeneration of cortical and thalamic neurons may be the primary mechanism of corticothalamic dysfunction in later stages of AD (de Jong, et al., 2008), here we provide evidence that symptoms attributable to

corticothalamic dysfunction occur at an age with no neuronal loss in our mouse model of AD (Mucke, et al., 2000). A recent study also demonstrated that by 3-4 months of age, there was impaired cholinergic excitation in layer 6 corticothalamic neurons of the TgCRND8 mouse model of AD, which has implications for the ability of acetylcholine to regulate attention (Proulx, et al., 2015). TgCRND8 mice are similar to the mice used in this study in that they express also mutant human APP with Swedish and Indiana mutations, but transgene expression is driven by the PrP promoter and these mice exhibit more aggressive amyloidosis (Chishti, et al., 2001). In addition to the deficits in sleep maintenance, attention, and cognitive processing in AD, corticothalamic dysfunction may also contribute to the development of certain types of non-convulsive seizures exhibited by AD patients (Vossel, et al., 2013) and mouse models including both APP mice and PSAPP mice that also express mutant human presenilin 1 (Minkeviciene, et al., 2009, Palop, et al., 2007). Our data demonstrate dysfunction of specific corticothalamic components in APP mice, and suggest that corticothalamic dysfunction is a pathophysiological mechanism that may underlie a wide range of physiological and behavioral alterations associated with AD.

Notably, corticothalamic dysfunction occurs independently of plaque pathology. Plaque deposition typically begins in the molecular layer of the dentate gyrus, as demonstrated in Mucke et al., 2000. However, the results presented here do not correspond at all to the presence of plaques, as similar effects were observed in younger mice with no plaque deposition at all (4 months old), as in slightly older mice (6 months old) in which plaque deposition has just begun. Indeed, we found that alterations in FosB expression in nRT and in sleep-wake patterns manifest as early as 2-3 months of age in J20 APP mice (unpublished observations), which is well before the beginning of plaque formation. This timeframe is particularly interesting, as the results in this study therefore indicate that network dysfunction is an early event that occurs prior to and independently of plaque pathology.

Understanding corticothalamic dysfunction in AD may lead to improvements both directly in corticothalamic-mediated behavioral alterations and in also behaviors less directly controlled by the corticothalamic network, as the effect of corticothalamic dysfunction is known to alter a wide range of brain regions (Chan, et al., 2006, Chan, et al., 2004, Ernst, et al., 2009, Jung, et al., 2013, Paz, et al., 2011). For example, in atypical absence epilepsy (absence epilepsy that is not confined to corticothalamic components), network dysfunction in the corticothalamic network invades vulnerable brain regions, such as the hippocampus, ultimately inducing damage (Chan, et al., 2006, Chan, et al., 2004, Jung, et al., 2013). This has been demonstrated in a mouse model of atypical absence epilepsy that has both corticothalamic dysfunction and hippocampus-dependent cognitive deficits (Chan, et al., 2006, Chan, et al., 2004). Although the mechanisms underlying the damage of other brain regions are not precisely known, a possibility is that hyperactive thalamolimbic nuclei directly engage dysfunction in limbic targets. The nucleus reuniens contributes to hippocampus-dependent memory in mice (Loureiro, et al., 2012), so altered function of this thalamic nucleus may also contribute to hippocampus-dependent memory deficits in APP mice. Furthermore, behaviors mediated by the corticothalamic network, such as attention and sleep maintenance, also play important roles in hippocampus-dependent memory (Diekelmann and Born, 2010, Fishbein, 1971, Fishbein and Gutwein, 1977, Hennevin, et al., 1995, Lawrence and Sahakian, 1995, Sahakian, et al., 1989, Smith, 1985, Stickgold, 2005) and

therefore disruption of these corticothalamic-mediated behaviors may further exacerbate hippocampus-mediated cognitive deficits in AD.

Although corticothalamic dysfunction may alter other brain regions, this alone cannot explain the wide range of epileptiform activity exhibited by APP mice. We hypothesize that corticothalamic dysfunction contributes to the epileptiform activity and associated cognitive deficits exhibited by APP mice in parallel with other factors, such as direct effect of A β in the hippocampus. It is also likely that corticothalamic dysfunction underlies other behavioral alterations that are independent of epileptiform activity, such as deficits in attention and sleep maintenance (Beenhakker and Huguenard, 2009, Briggs and Usrey, 2008, Jones, 2009, Rouiller and Welker, 2000). These behavioral alterations may also contribute to other detrimental processes associated with AD, including memory deficits.

Decreased activity in the nRT results in corticothalamic-mediated absence epilepsy in some mouse models (Menuz and Nicoll, 2008, Paz, et al., 2011). In contrast, nRT hyperactivity is more commonly established as a mechanism that causes absence epilepsy (Beenhakker and Huguenard, 2009). The corticothalamic dysfunction displayed by APP mice is unique and different than the dysfunction described in typical mouse models of absence epilepsy. Hence, corticothalamic dysfunction in APP mice is likely due to a unique pathophysiology in the context of AD, with many molecular and physiological alterations present that are specific to the multifaceted changes that occur in AD.

The nRT plays a critical role in deinactivating thalamic T-type calcium channels, which are critical for sleep maintenance (Anderson, et al., 2005, Beenhakker and Huguenard, 2009). Therefore, the reduced activity in nRT that we observed in APP mice could explain both corticothalamic-mediated seizures (Menuz and Nicoll, 2008, Paz, et al., 2011) and sleep maintenance deficits (Beenhakker and Huguenard, 2009) exhibited by APP mice. Notably, treatment with ethosuximide, a T-type calcium channel inhibitor (Collins, et al., 2008, Goren and Onat, 2007) that is used as an antiepileptic drug to treat certain types of corticothalamic-mediated epilepsy, had no effect on epileptiform activity in APP mice (Sanchez, et al., 2012). Ethosuximide is effective in suppressing nonconvulsive seizures caused by increased activity in nRT, hence the studies showing a lack of efficacy in APP mice are consistent with our finding of reduced activity of nRT. Together, our data support a model of corticothalamic dysfunction in APP mice in which impaired cortico-nRT circuitry contributes to nRT hypoactivity. Because the nRT is important for tonic inhibition of thalamic relay nuclei (Contreras and Steriade, 1995, Cope, et al., 2005, Pinault, et al., 1998), we hypothesize that reduced activity of nRT disinhibits thalamocortical and thalamolimbic nuclei causing hyperactivity in their cortical and limbic targets. Supporting this hypothesis, we found increased FosB expression in the VPL, nRE, and PVT thalamic nuclei as well as their cortical and limbic targets.

An important question that remains to be answered is whether restoring normal corticothalamic function can improve physiological and behavioral abnormalities in APP mice. Normalizing activity within the nRT of APP mice, perhaps through deep brain stimulation, optogenetics, or Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) may be of utility. Treatments that improve function of the corticothalamic

network in AD may improve a wide range of molecular, physiological, and behavioral impairments associated with AD. This study highlights the importance of gaining a comprehensive understanding of the pathophysiological mechanisms underlying AD in the search for novel therapeutic strategies.

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- APP mice exhibit sleep fragmentation, similar to AD patients
- Severity of sleep fragmentation and spatial memory correlate in APP mice
- Activity marker expression suggests hypoactive nRT in APP mice
- Impaired input to nRT may contribute to cortical hyperexcitability in APP mice
- Corticothalamic dysfunction may impair multiple behavioral and cognitive domains

Figure 1. Aberrant neuronal activity and sleep maintenance deficits in APP mice

A, EEG recordings from left and right frontal cortices (LFC, RFC), hippocampal depth (HIP), and parietal cortex (PC) in NTG mice of 4-6 months of age demonstrate normal brain activity, whereas age matched APP mice exhibit spike discharges (arrowheads) and intermittent seizures (B) Gray shaded area is described in panel F. C, APP mice exhibit increased average spiking or epileptiform discharge frequency (167.5 ± 39.82) compared to NTG controls (12.81 ± 2.302 ; $n = 4-5/\text{genotype}$; $\bar{Q}=10$). E, Spectral power analyses revealed a decrease in theta frequency oscillations. F, Magnified view of shaded area in panel B reveals that in APP mice, the leading spike (bracketed by dotted lines) at the onset of the seizure appears synchronously across all the EEG channels. G, Sleep analysis by HomeCageScan software revealed increased awakenings from sleep in APP mice although total sleep duration during the light phase was unaltered (H) ($n = 29-32/\text{genotype}$; $\bar{O}=22$, $\bar{Q}=38$). I, The average sleep bout length was shorter in APP mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 2. Chronic neuronal activity is altered in key components of the corticothalamic network in APP mice

A, Models of normal and abnormal corticothalamic function: 1, Normal circuitry balances activity in nRT and thalamic relay nuclei (TR). 2, Increased nRT activity exacerbates circuit oscillations and give rise to spike wave discharges and seizures (Beenhakker and Huguenard 2009). 3, Increasing T-type calcium channels in TR induces pure absence epilepsy (Ernst et al., 2009). 4, Decreased nRT activation may disinhibit TR and increase its activity (Menuz and Nicoll, 2008, Paz, et al., 2011). B, Immunostaining demonstrating FosB-IR cells in nRT of NTG and APP mice (*inset*, co-stain of FosB with hematoxylin to demonstrate nuclear staining). C, Quantification reveals decrease in numbers of FosB-IR cells in nRT of APP mice relative to NTG mice (n = 11-12/genotype; ♂=11, ♀=12). D, Immunostaining of NeuN-positive neurons in nRT of NTG and APP mice. E, Quantification of NeuN immunostaining demonstrates no obvious cell loss in nRT of APP mice relative to NTG mice. F, FosB-IR in nRT is also reduced in PSAPP mice, another well characterized mouse model with high levels of A β , relative to NTG littermate controls (n = 5/genotype; ♂=10). G, FosB-IR in nRT is not altered in transgenic mice that overexpress wild-type APP that do not produce high levels of A β (n = 7-9/genotype; ♂=7, ♀=9). H-M, FosB-IR is not altered in the ventroposteromedial thalamic nucleus (VPM, H), but is increased in ventroposterolateral thalamic nucleus (VPL, I) of APP mice compared to NTG mice.

FosB-IR is increased in all cortical layers of somatosensory cortex (J) as well as in the paraventricular nucleus (PVT, K) and nucleus reuniens (nRE, L), but not in the lateral geniculate nucleus (LGN, M) of APP mice compared to NTG mice (n = 19/genotype; ♂=14, ♀=24). *p<0.05, **p < 0.01, ***p < 0.001.

Figure 3. Aberrant thalamic FosB expression patterns are related to impairments in sleep maintenance

Regression analyses were performed using only data from APP mice; NTG data points are shown for reference only. A, FosB-IR in thalamic relay nuclei correlates positively with the number of awakenings during the light phase in APP mice. B, FosB-IR in all layers of somatosensory cortex correlates positively with the number of awakenings during the light phase in APP mice.

Figure 4. Reduced cortical input to nRT does not alter the intrinsic excitability of key thalamic nuclei in APP mice

A, Image of a typical thalamocortical slice used for electrophysiological recordings. B, Stimulation of deep layers of cortex revealed a significant reduction in the slope of evoked field responses in nRT of APP mice. Left panel shows traces, right panel shows quantification. C, Evoked field responses recorded in cortex after stimulation of VPL revealed no differences in slope measurements between NTG and APP mice. Left panel shows traces, right panel shows quantification. D-F, Direct stimulation within nRT and recording in nRT (D) revealed no differences in the slope of the evoked field responses (E) or input–output measurements (F) in APP and NTG mice. G-I, Direct stimulation and recording within thalamic relay nuclei (G) also revealed no differences in the slope of evoked field responses (H) or input–output measurements (I) in APP and NTG mice. (n = 6-8/genotype; $\sigma^2=17$, $\phi=18$). $**p < 0.01$.

Figure 5. No overt anatomical abnormalities of corticothalamic connections are observed in APP mice

Corticothalamic connections were assessed in brain slices from NTG and APP mice after stereotaxic injection of the retrograde tracer cholera toxin β into the thalamus in vivo. Images from brain slices demonstrate intact anatomical connections (indicated by white arrows) in two representative NTG and APP mice (n=4/genotype; ♂=4; ♀=4).

Figure 6. Lateral cortical signal propagation in layer II/III is reduced in APP mice

A, Representative picture of an acute brain slice illustrating the bipolar stimulation electrode and two extracellular recording electrodes (Rec1 and 2) used to measure lateral signal propagation velocity in cortical layer II/III (dashed lines). B, APP mice exhibit reduced lateral propagation velocity compared to NTG controls. C-D, Extracellular recording traces from cortical layer II/III illustrate the delay for the signal (gray shading) to propagate between electrode 1 and 2 in a NTG mouse (C) and an APP mouse (D). (n = 6-7/genotype; ♂=5, ♀=8), *p < 0.05.

Figure 7. Corticothalamic network dysfunction and hippocampal dysfunction are related in APP mice

Regression analyses were performed using only data from APP mice; NTG data points are shown for reference only. A-B, Image of FosB-IR in entorhinal cortex (ERC) and quantification in layers 4-6 in NTG and APP mice. C, Relationship between FosB-IR in the paraventricular nucleus (PVT) and in layers 4-6 of ERC in APP mice. D, Relationship between FosB-IR in the nucleus reuniens (nRE) and in layers 4-6 of ERC in APP mice. E-F, Image and quantification of FosB-IR in CA1 of NTG and APP mice. G, Relationship between FosB-IR cells in nRE and FosB-IR in CA1 in APP mice. H, Regression analysis demonstrated a significant relationship between the number of awakenings from sleep during the light phase and the distance required to locate the hidden platform in the Morris water maze in APP mice. ($n = 19/\text{genotype}$; ♂=14, ♀=24), * $p < 0.05$, *** $p < 0.001$.