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Deficits in synaptic function occur at medial perforant path-granule cell synapses prior to Schaffer collateral-CA1 pyramidal cell synapses in the novel TgF344-Alzheimer's Disease Rat Model

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Abstract

Alzheimer's disease (AD) pathology begins decades prior to onset of clinical symptoms, and the entorhinal cortex and hippocampus are among the first and most extensively impacted brain regions. The TgF344-AD rat model, which more fully recapitulates human AD pathology in an age-dependent manner, is a next generation preclinical rodent model for understanding pathophysiological processes underlying the earliest stages of AD (Cohen et al., 2013). Whether synaptic alterations occur in hippocampus prior to reported learning and memory deficit is not known. Furthermore, it is not known if specific hippocampal synapses are differentially affected by progressing AD pathology, or if synaptic deficits begin to appear at the same age in males and females in this preclinical model. Here, we investigated the time-course of synaptic changes in basal transmission, paired-pulse ratio, as an indirect measure of presynaptic release probability, long-term potentiation (LTP), and dendritic spine density at two hippocampal synapses in male and ovariectomized female TgF344-AD rats and wildtype littermates, prior to reported behavioral deficits. Decreased basal synaptic transmission begins at medial perforant path-dentate granule cell (MPP-DGC) synapses prior to Schaffer-collateral-CA1 (CA3-CA1) synapses, in the absence of a change in paired-pulse ratio (PPR) or dendritic spine density. N-methyl-D-aspartate receptor (NMDAR)-dependent LTP magnitude is unaffected at CA3-CA1 synapses at 6, 9, and 12 months of age, but is significantly increased at MPP-DGC synapses in TgF344-AD rats at 6 months only. Sex differences were only observed at CA3-CA1 synapses where the decrease in basal transmission occurs at a younger age in males versus females. These are the first studies to define presymptomatic alterations in hippocampal synaptic transmission in the TgF344-AD rat model. The time course of altered synaptic transmission mimics the spread of pathology through hippocampus in human AD and provides support for this model as a valuable preclinical tool in elucidating pathological mechanisms of early synapse dysfunction in AD.

Keywords

Alzheimer's disease; hippocampus; TgF344-AD rat model; synaptic transmission; long-term plasticity; spine density

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Introduction

Human AD is characterized by several key features including increased soluble amyloid beta in plasma (A β), increased hyperphosphorylated tau (p-tau) in cerebrospinal fluid (CSF) (Fagan et al., 2014), gliosis, amyloid plaques, neurofibrillary tau tangles (NFTs), neuronal cell loss, and memory impairment (Jack et al., 2010; Sperling et al., 2011). It is now recognized that there is a long presymptomatic phase prior to the onset of memory loss during which AD pathology targets neurons in selectively vulnerable medial temporal lobe structures critical for memory encoding and spatial navigation (Khan et al., 2014; Killiany et al., 2002; Stranahan and Mattson, 2010). Thus, synaptic function is altered years to decades prior to clinical presentation of symptoms (Sperling et al., 2011).

In the medial temporal lobe, AD pathology begins in the entorhinal cortex (EC) and spreads via functionally connected synapses to hippocampus (Braak and Braak, 1995, 1991; Gómez-Isla et al., 1996; Llorens-Martín et al., 2014), a highly organized structure that relays information from EC via three glutamatergic synapses, collectively known as the tri-synaptic pathway (Amaral, 1993). Specifically, primary inputs are received via perforant path projections from EC to the granule cells in the dentate gyrus, while area CA1 is the primary output pathway for information processed by the hippocampal circuit. Current evidence from animal studies strongly support that EC-to-hippocampal synaptic weakening results from a pathologic increase in toxic soluble species of A β and p-tau (Jucker and Walker, 2013; Spiess-Jones and Hyman, 2014). Intervention during this long presymptomatic phase of AD is projected to have the greatest clinical efficacy, as it may prevent the collapse of neural networks critical to memory (Hyman et al., 1984; Sperling et al., 2011).

Since the generation of the first transgenic AD mouse (Games et al., 1995), creation of a rodent model that fully recapitulates human AD has remained elusive for over two decades. While studies using transgenic mouse models with disease causing autosomal dominant human mutations in amyloid precursor protein (APP) and/or presenilin 1 or 2 have shed light on many mechanistic underpinnings of certain aspects of AD, these models receive criticism in that they do not fully recapitulate all cardinal AD pathology (e.g. lack NFTs and neuronal loss) (Braidy et al., 2012; Do Carmo and Cuello, 2013; Hall and Roberson, 2012; LaFerla and Green, 2012). It is now thought that rat models will have greater therapeutic translational efficacy as they are closer to humans in evolution, morphology, genetics, and physiology (Gibbs et al., 2004; Jacob and Kwitek, 2002; Lin, 1995; Yang et al., 2004). As such, the newly developed TgF344-AD rat model harboring two human transgenes causing familial AD (FAD), APP^{swe} and PS1^{E9}, driven by the mouse prion promoter (Cohen et al., 2013), is recognized as the most comprehensive and clinically relevant rodent model of AD to-date (Do Carmo and Cuello, 2013; Saraceno et al., 2013). Importantly, in addition to having increased soluble A β , A β plaques, and gliosis, TgF344-AD rats develop neurofibrillary tangles (NFTs) and overt neuronal loss, which are not present in mouse models harboring the same transgenes (Elder et al., 2010; Hall and Roberson, 2012), or even in rats with the same transgenes driven by a different promoter (Do Carmo and Cuello, 2013). Specifically, beginning at 6 months of age, increased levels of soluble A β , p-tau levels, and gliosis are present in both male and female TgF344-AD rats prior to amyloid

plaques, NFTs, cell loss, and behavioral impairment on hippocampus dependent learning tasks, reported to begin at 15 months (Cohen et al., 2013). Additionally, recent data demonstrate increased tau pathology in hippocampus, amyloid plaques in EC and hippocampus, simultaneous with decreased cerebrovasculature function (Joo et al., 2017) in 9 month TgF344-AD animals. These findings follow the pathological timeline reported in Cohen et al., 2013, and support preclinical human data showing a correlation between vascular dysfunction and increased plasma A β levels (van Dijk et al., 2007). Thus, the more faithful recapitulation of human AD enhances the impact of experimental findings using this model. Detailed electrophysiological studies of early synapse dysfunction and hippocampal regional vulnerability in the TgF344-AD rat model are absent from current literature. Therefore, at 6, 9, and 12 months, we assessed basal glutamatergic synaptic transmission, paired-pulse ratio, long-term potentiation (LTP), and spine density in male and ovariectomized female TgF344-AD rats at the beginning and end of the hippocampal trisynaptic pathway (medial perforant path to dentate granule cells (MPP-DGC) and CA3-CA1 synapses, respectively). Identifying when the initial signs of synaptic dysfunction occur in this preclinical model, which more fully recapitulates human AD, will provide a framework for revealing novel mechanisms and therapeutic targets during the earliest phase of the disease.

Materials and Methods

Animals

All animals used in these studies were bred in-house at UAB. All breeding and experimental procedures were approved by the University of Alabama at Birmingham's Institutional Animal Care and Use Committee and follow the guidelines outlined by the National Institutes of Health. The original breeding pair was acquired from Terrance Town at the University of Southern California, who developed this transgenic rat model (Cohen et al., 2013). TgF344-AD males harboring the APP (*APP^{swe}*) and delta exon 9 mutant human presenilin-1 (*PS1 E9*) were bred to wildtype (WT) F344 females (Envigo, Indianapolis, IN (previously Harlan Laboratories)) to prevent inbreeding depression and to ensure proper care of offspring. Rats were maintained under standard laboratory conditions (12 hour light/dark cycle, lights off at 14:00 h, 22°C, 50% humidity, food (Harlan 2916; Teklad Diets, Madison, WI) and water *ad libitum*). Rats were housed in same-sex groups of four or less at weights of ~300 grams or two per cage when ~400 grams, using standard rat cages (7 inch (height) $\times$ 144 in² (floor)). TgF344-AD and Wildtype (WT) littermate male and female rats were aged to 6, 9, and 12 months for experiments.

Genotyping

Transgene incorporation was verified twice for each animal, first using weaned tail-clips (collected at postnatal day 21) and second using cerebellar tissue collected on the day of sacrifice for electrophysiology recordings. *APP^{swe}* and *PS1 E9* transgene expression was confirmed using polymerase chain reaction (PCR) and the Terra^{TM} PCR Direct Polymerase Mix (Clontech Laboratories, Inc. Mountain View, CA). PCR analysis and cycling parameters as follows: denaturation at 94°C for 3 min, followed by 30 cycles of 30 sec at 94°C, 30 sec at 58°C, 60 sec at 72°C, and finally 5 min at 72°C before holding at 4°C. Primers used for

probing the *APP^{swe}* and *PS1 E9* allele were: APP_forward 5'-CCGAGATCTCTGAAGTGAAGATGGATG-3', PS1_forward 5'-CAGGTGGTGGAGCAAGATG-3', and PRP_reverse (targets the genomic sequence found in both the mouse prion promotor and the rat genome) 5'-GTGGATACCCCTCCCCCAGCCTAGACC-3'. Amplified PCR products were separated by gel electrophoresis in a 1.5% agarose gel and visualized by ethidium bromide staining. Animals were excluded from the study if the PCR genotyping results from the weaned tail clip and adult cerebellar sample did not match, and this occurred in only 4 out of 223 cases.

Surgery

Ovarian hormones rapidly alter synaptic transmission, dendritic spine density, and learning and memory in female rats (Hajszan et al., 2007; Smith and McMahon, 2006, 2005; Vedder et al., 2013; Woolley, 1998; Woolley and McEwen, 1993). It was therefore necessary to control for cycling hormones in these initial characterization studies. At 6, 9, and 12 months of age, TgF344-AD and WT littermate female rats were bilaterally ovariectomized (OVX) under 2.5% isoflurane in 100% oxygen, using aseptic conditions. Because rats were at different stages in the estrous cycle when they underwent OVX, we used a postoperative window of 10–14 days to allow for depletion of endogenous ovarian hormones before being used in experiments, as previously published (Smith et al., 2016, 2010, Smith and McMahon, 2006, 2005, Vedder et al., 2014, 2013). Importantly, LTP magnitude at CA3-CA1 synapses in OVX rats 10–14 days post OVX is not different from ovary intact cycling rats at diestrus (when plasma E2 is lowest in ovary intact cycling rats) (Smith et al., 2009). This confirms that 10–14 days of E2 deprivation and the OVX surgery does not negatively affect synaptic function.

Slice Preparation

Animals were deeply anesthetized via inhalation anesthesia using isoflurane, rapidly decapitated, and brains removed. Coronal slices (400 μ m) from dorsal hippocampus were prepared using a vibratome (1000 Plus). To preserve neuronal health and limit excitotoxicity, slices were sectioned in low Na⁺, sucrose-substituted ice-cold artificial cerebrospinal fluid (aCSF) containing [in mM: NaCl 85; KCl 2.5; MgSO₄ 4; CaCl₂ 0.5; NaH₂PO₄ 1.25; NaHCO₃ 25; glucose 25; sucrose 75 (saturated with 95% O₂, 5% CO₂, pH 7.4)]. Slices were held at room temperature for 1 hr in standard artificial cerebrospinal fluid (aCSF) [in mM: 119.0 NaCl, 2.5 KCl, 1.3 MgSO₄, 2.5 CaCl₂, 1.0 NaH₂PO₄, 26.0 NaHCO₃, 11.0 Glucose (saturated with 95% O₂, 5% CO₂, pH 7.4)] before transfer to submersion chamber for recordings.

Electrophysiology

Extracellular field excitatory postsynaptic potentials (fEPSPs) recorded from the dendritic region were performed in a submersion chamber perfused with standard aCSF warmed to 26–28°C. All data were obtained using the electrophysiology data acquisition software pClamp10 (Molecular Devices, LLC, Sunnyvale, CA.) and analyzed using Origin 2016 (OriginLab), Graphpad Prism 7 (GraphPad Software, Inc.) and SPSS 22 (IBM Corp.). For MPP-DGC synapses, baseline fEPSPs were generated by stimulating the medial perforant path input onto dentate granule cells (MPP-DGC; 0.1 Hz for 200 μ s) using a twisted

insulated nichrome wire (A-M Systems, Inc., Sequim, WA) electrode placed in MPP within 200–300 μm of an aCSF-filled glass recording electrode. Correct electrode placement was confirmed visually and by the generation of paired-pulse depression (PPD), characteristic of MPP-DGC synapses (Colino and Malenka, 1993; McNaughton and Barnes, 1977) at an interstimulus interval of 50 ms throughout the duration of the experiment. For CA3-CA1 synapses, Schaffer collateral axons were stimulated using a twisted insulated nichrome wire electrode placed in CA1 stratum radiatum within 200–300 μm of an aCSF-filled glass recording electrode, and paired-pulse facilitation (PPF) characteristic of this synapse (Wu and Saggau, 1994) was recorded. Baseline fEPSPs were obtained by delivering 0.1 Hz stimulation for 200 μs to generate fEPSPs of ~ 0.5 mV in amplitude ($\sim 50\%$ of maximal response). At both pathways, only experiments with $\leq 8\%$ baseline variance were included in the final data sets.

Input/Output Curves. After a stable 10 min baseline, input-output (I/O) curves were generated by increasing the stimulus intensity (10 μA increments) until a maximal fEPSP slope was obtained, usually at 200 μA stimulus intensity. Initial slope of the ten fEPSPs generated at each stimulus intensity were averaged and plotted as a single value. When I/O curves were generated from multiple slices from a single animal, they were averaged together to represent the data from that single animal. Statistical significance was determined using unpaired Student's *t*-test at the maximal stimulus intensity (200 μA ; * $p < 0.05$).

Paired-Pulse Ratio (PPR). After a 10 min stable baseline, pairs of stimulation were delivered at 10, 20, 50, 150, 200, and 400 millisecond (ms) inter-stimulus intervals (ISIs). PPR was calculated by dividing the initial slope of the second event by the initial slope of the first event. ISIs from TgF344-AD vs WT rats were analyzed with a repeated measure general linear model (GLM) (* $p < 0.05$). Greenhouse-Geisser Corrected values were used for all cohorts except 6 month male PPR at MPP-DGC and 6 month female PPR at MPP, which assumed normal sphericity as determined by Mauchly's Test of Sphericity.

Long-term potentiation (LTP). At MPP-DGC synapses, following a 10 min stable baseline (0.1 Hz, 200 μs with stimulation intensity set to elicit initial fEPSP amplitude of $\sim 50\%$ maximum response), NMDA receptor (NMDAR)-dependent LTP was induced using high-frequency stimulation (HFS, 100 Hz, 1 s duration $\times$ 4, 60s interval). Inhibitory drive onto dentate granule cells is very strong (Coulter and Carlson, 2007) and as such, LTP induction at MPP-DGC synapses required GABA_AR antagonism to allow sufficient NMDAR activation (Eadie et al., 2012; Steward et al., 1990; Wu et al., 2001) (Supplemental Fig.1). Picrotoxin (100 μM) was present during collection of baseline transmission and for the duration of the experiment. Separate control experiments were performed in the absence of picrotoxin, and as shown (Supplemental Fig. 1), we are unable to induce LTP at MPP-DGC synapses in the absence of GABA_AR blockade. At CA3-CA1 synapses, following a 10 min stable baseline (0.1 Hz, 200 μs with stimulation intensity set to elicit initial fEPSP amplitude of $\sim 50\%$ maximum response), NMDAR-dependent LTP was induced using 4 bouts of theta-burst stimulation (TBS) with each bout consisting of 5 pulses at 100Hz repeated 10 times at 200 ms intervals, and each bout separated by 20 ms (Barnes et al., 1996; Kumar et al., 2007; Watabe and O'Dell, 2003). Weak TBS consisting of only 2 bouts was used in separate

experiments to ensure the above TBS protocol did not saturate LTP. Note that because some slices were used as part of a separate study, the extracellular aCSF contained DMSO (0.1%) in all LTP experiments at CA3-CA1 synapses for recordings from both WT and TgF344-AD rats. Statistical significance was determined using unpaired Student's *t*-test by comparing the average of the fEPSP slope from the last 5 min of the recording (44–59 min) to baseline for each genotype (* $p < 0.05$).

Depolarization during stimulus used to induce LTP. At MPP-DGC synapses, the steady-state depolarization reached during the tetanus, which facilitates NMDAR activation, was calculated on averaged HFS sweeps from each LTP experiment included in the LTP data set. The first 200 ms from each group was compared during the 4th round of HFS. Stimulation artifacts were subtracted from the traces, and the depolarization was measured by the deviation from baseline at 170 ms (the time-point at which the deflection reaches steady state). At CA3-CA1 synapses, the 4th round of TBS used to induce LTP was averaged among all LTP experiments in each experimental group. Area under the curve (AUC) was measured during depolarization at CA3-CA1 synapses, as TBS does not cause a steady-state deviation from baseline due to the fewer number of pulses delivered in each train (5 pulses in TBS versus 100 pulses in HFS). For consistency and rigor, SSD and AUC analyses were performed for both types of LTP inducing stimulation, HFS and TBS. Because depolarization does not reach steady state during theta burst stimulation, at CA3-CA1, synapses steady-state was arbitrarily defined at 80 ms from baseline for analysis (when the combined contribution from both AMPAR and NMDAR-mediated currents are the greatest). The last round of stimulation was used for analysis at each synapse, as this is when depolarization is greatest. Steady-state depolarization was calculated in OriginLab 2016 and AUC was calculated in Graphpad Prism 7. Statistical significance was determined using one-tailed unpaired Student's *t*-test (* $p < 0.05$; ** $p < 0.01$).

Histology

Dendritic spine labeling was obtained using the FD Rapid Golgi Stain Kit (FD Neuro Technologies, Inc., Ellicott City, MD) and followed manufacturer's instructions. Briefly, brains were rapidly dissected after isoflurane anesthesia, rinsed with MilliQ water, and placed in a light impermeable glass vial filled with equal volumes of solution A and B for 9 days at room temperature. The brain was then transferred to solution C for 72 hrs. According to the manufacturer's instructions, both Solutions A+B and Solution C (contents proprietary) were replaced after the first 24 hr immersion. Regions just anterior and posterior to hippocampus were removed and the remaining brain section embedded in 3% agar. 150 μ m sections were collected in solution C using a vibratome (Vibratome Series 1000, TPI, and St. Louis, MO) and were mounted onto 3% gelatin coated slides. Slices were placed in a 10-unit staining dish with detachable handles and submerged in Milli-Q water twice for four minutes each and then moved to a 1:1 mixture of solution D and E for ten minutes. Mounted slices were dehydrated for 4 min 1x each in 50%, 75%, 95%, and finally 100% EtOH. Slides were cleared in xylene 3x for 4 min each and were cover-slipped with Eukitt (Sigma-Aldrich Co. LLC). Brightfield confocal z-stack images were acquired with a 100x oil immersion objective (1.4 na) using an Olympus BX51 microscope and Stereo Investigator (MBF Bioscience), and were acquired at the same depth in dorsal hippocampus that

electrophysiological recordings were made. A 10 μm segment was outlined using Reconstruct Software Version 1.1.0.0 (Fiala, 2005) from z-stacked images (15–30 images per stack, 0.05 μm optical section thickness). Only protrusions with a clearly discernible spine head and neck were counted (Risher et al., 2014; Smith and McMahon, 2005). DGCs were selected for analysis if the secondary and tertiary apical dendrites were intact in the upper blade (ectal limb) and could be tracked back from the middle molecular layer to the soma in the granule cell layer. CA1 pyramidal cells were selected for analysis if the apical dendritic arbor was intact and tertiary dendrites in stratum radiatum were distinguishable and could be tracked back to an identified secondary dendrite. Dendritic spines on 3–6 segments from 3 different sections were averaged together to represent mean density for each animal. Statistical significance was determined using unpaired Student's *t*-test on averaged spines from each genotype (* $p < 0.05$).

Statistical Analysis

Experimenter was blind to genotype during experimental procedure, data collection, and analysis. Genotype was revealed only at final analysis. Sex and age were not interleaved, therefore all statistical analyses for each experiment were performed within each experimental cohort (e.g. 6 month male TgF344-AD vs WT littermate) and not across cohorts. Results reported at mean $\pm$ SEM with significance set at $p < 0.05$ (*) determined by unpaired Student's *t*-test assuming unequal variance, and repeated measures GLM as described above. Sufficient power was determined with G*Power 3.1.9.2 (Franz Faul, University Kiel, Germany). Outliers were determined with a Grubb's test (GraphPad Software, Inc.) and significant outliers were removed.

Results

Basal synaptic transmission is altered during the presymptomatic phase and targets MPP-DGC synapses before CA3-CA1 synapses

We first determined if basal synaptic transmission is pathologically altered and whether EC inputs to DG dysfunction prior to CA3-CA1 synapses, similar to synaptic pathology in human AD (Khan et al., 2014). Males and females were raised to 6, 9, and 12 months of age and extracellular dendritic field potential recordings (fEPSPs) at MPP-DGC and CA3-CA1 synapses from acute hippocampal slices were performed (Fig. 1). It was important to OVX females 10–14 days prior to recordings to control for fluctuations in ovarian hormones over the four day rodent estrous cycle, since proestrous levels of 17β estradiol is associated with increased spine density, NMDAR transmission, and LTP magnitude (Hajszan et al., 2007; Smith et al., 2010; Smith and McMahon, 2006, 2005; Vedder et al., 2013; Woolley, 1998; Woolley and McEwen, 1993). Input-output (I/O) curves were generated by incrementally increasing the stimulus intensity (0–200 μA , 10 μA intervals) to ask if maximal synaptic strength is decreased in TgF344-AD rats compared to wild-type (WT) littermate controls, at each age and in each sex.

Maximal fEPSP slope at MPP-DGC synapses in TgF344-AD rats was significantly decreased compared to WT in males (* $p < 0.05$; Fig. 1 A.1) and females (* $p < 0.05$; Fig. 1 C.1) beginning at 6 months of age and this continued through 9 and 12 months of age, in

both sexes (Fig. 1 A.2, 9 month males, $*p < 0.05$; A.3, 12 month males, $**p < 0.01$, C.2, 9 month females, $*p < 0.05$; C.3; 12 month females, $*p < 0.05$). These results show that MPP-DGC basal transmission is decreased as early as 6 months in TgF344-rats. In contrast, maximal transmission at CA3-CA1 synapses is unchanged at 6 months in TgF344-AD males ($p > 0.05$; Fig. 1 B.1) and females ($p > 0.05$; Fig. 1 D.1). By 9 months of age, there is a significant deficit in Tg males ($*p < 0.05$; Fig. 1 B.2) that continues at 12 months ($*p < 0.05$; Fig. 1 B.3). Significant deficits in the I/O curve at CA3-CA1 synapses in TgF344-AD females do not appear until 12 months ($*p < 0.05$; Fig. 1 D.3). These data show that the time-course for deficits in maximal basal transmission at CA3-CA1 synapses occurs later at CA3-CA1 synapses than at MPP-DGC synapses in TgF344-AD rats. Additionally, these data suggest that decreased maximum transmission occurs at CA3-CA1 synapses in TgF344-AD males at a younger age than it does in females.

Paired-pulse ratio (PPR) is not altered during the presymptomatic phase in TgF344-AD rats

To determine whether altered presynaptic release probability might be responsible for the decreased basal synaptic transmission in TgF344-AD rats, PPR, an indirect measure of presynaptic neurotransmitter release probability (Dobrunz and Stevens, 1997), was analyzed. Paired-pulse stimulation results in paired-pulse depression (PPD) at MPP-DGC synapses, as these synapses have high initial release probability (McNaughton and Barnes, 1977). Paired-pulse facilitation (PPF) occurs at CA3-CA1 synapses, reflecting their low initial release probability (Dobrunz and Stevens, 1997). No difference in PPR at MPP-DGC synapses (initial slope fEPSP2/fEPSP1) was found at any inter-stimulus interval (ISI, 10–400 ms) tested in the 6, 9, and 12 month TgF344-AD vs WT cohorts, in either sex ($p > 0.05$) as determined by repeated measures general linear model (GLM). As expected, PPR was altered by the ISI applied (Fig. 2 A.1, $F(2.86, 34.29) = 57.07$, $p < 0.01$; A.2, $F(2.48, 39.73) = 88.27$, $p < 0.01$; A.3, $F(2.16, 28.07) = 29.64$, $p < 0.01$; C.1, $F(6, 36) = 64.95$, $p < 0.01$; C.2, $F(2.66, 42.48) = 28.38$, $p < 0.01$; C.3, $F(2.55, 48.42) = 41.94$, $p < 0.01$). However, there was no effect of genotype, regardless of ISI [main effect of genotype Fig. 2 A.1, $F(1, 12) = 0.091$, $p = 0.77$; A.2, $F(1, 16) = 0.043$, $p = 0.84$; A.3, $F(1, 13) = 2.59$, $p = 0.13$; C.1, $F(1, 6) = 0.012$, $p = 0.92$; C.2, $F(1, 16) = 2.03$, $p = 0.17$; C.3, $F(1, 19) = 0.13$, $p = 0.73$; Genotype*Intensity interaction, Fig. 2 A.1, $F(2.86, 34.29) = 1.06$, $p = 0.38$; A.2, $F(2.48, 39.73) = 0.87$, $p = 0.45$; A.3, $F(2.16, 28.07) = 0.54$, $p = 0.60$; C.1, $F(6, 36) = 0.89$, $p = 0.51$; C.2, $F(2.66, 42.48) = 0.78$, $p = 0.50$; C.3, $F(2.55, 48.42) = 0.22$, $p = 0.86$]. The same was true at CA3-CA1 synapses. Again, PPR changes with varying ISIs, (Fig. 2 B.1, $F(2.24, 24.61) = 82.29$, $p < 0.01$; B.2, $F(2.08, 47.90) = 46.45$, $p < 0.01$; B.3, $F(1.74, 31.27) = 7.43$, $p = 0.003$; D.1, $F(2.45, 36.71) = 57.53$, $p < 0.01$; D.2, $F(1.94, 33.04) = 28.45$, $p < 0.01$; D.3, $F(1.37, 17.70) = 5.89$, $p = 0.018$). There was no effect of genotype, regardless of ISI [main effect of genotype Fig. 2 B.1, $F(1, 11) = 0.30$, $p = 0.60$; B.2, $F(1, 23) = 1.08$, $p = 0.31$; B.3, $F(1, 18) = 0.49$, $p = 0.49$; D.1, $F(1, 15) = 0.012$, $p = 0.92$; D.2, $F(1, 17) = 1.03$, $p = 0.32$; D.3, $F(1, 13) = 1.86$, $p = 0.20$; Genotype*Intensity Fig. 2 B.1, $F(2.24, 24.61) = 0.46$, $p = 0.66$; B.2, $F(2.08, 47.90) = 0.65$, $p = 0.53$; B.3, $F(1.74, 31.27) = 0.49$, $p = 0.59$; D.1, $F(2.45, 36.71) = 2.24$, $p = 0.11$; D.2, $F(1.94, 33.04) = 1.27$, $p = 0.295$; D.3, $F(1.37, 17.70) = 0.56$, $p = 0.52$]. These results suggest that the decrease in basal synaptic strength observed at MPP-DGC and CA3-CA1 synapses in Tg vs WT rats (Figs. 1 A.1-A.3, B.2-B.3, C.1-C.3, D.3) is likely not a consequence of a

change in presynaptic function, supporting a possible postsynaptic locus, although additional studies are needed.

MPP-DGC LTP magnitude is increased in 6 month TgF344-AD rats

Studies examining the impact of AD pathology on LTP magnitude have yielded conflicting results, likely due to differences in method of exogenous A β delivery, Tg-rodent model used, and/or the disease stage at which recordings were performed (Marchetti and Marie, 2011; Spires-Jones and Hyman, 2014). To determine if the novel comprehensive TgF344-AD rat model has alterations in LTP during the early phase of the disease, we investigated NMDAR-dependent LTP at MPP-DGC and CA3-CA1 synapses. Because MPP-DGC basal synaptic transmission is decreased by 6 months of age in both sexes (Figs. 1 A.1, C.1), we began our studies at this age to determine whether the LTP magnitude was also changed at this early presymptomatic time-point. We found that the magnitude of LTP induced with high frequency stimulation (HFS) is significantly increased at MPP-DGC synapses in both 6 month TgF344-AD male and OVX females when compared to WT littermate controls (Fig. 3A male WT: $123 \pm 4\%$ of baseline fEPSP slope vs Tg: $140 \pm 7\%$ of baseline fEPSP slope, $*p < 0.05$; Fig. 3B female WT: $115 \pm 8\%$ of baseline fEPSP slope vs Tg: $139 \pm 10\%$ of baseline fEPSP slope, $*p < 0.05$). In contrast, at CA3-CA1 synapses, when the I/O curve is unaffected at 6 months, the magnitude of NMDAR-dependent LTP induced with Theta Burst Stimulation (TBS) was not significantly different in TgF344-AD males or in OVX TgF344-AD females compared to WT littermate controls (Fig. 3C male WT: $139 \pm 9\%$ of baseline fEPSP slope vs Tg: $131 \pm 5\%$ of baseline fEPSP slope, $p > 0.05$; Fig. 3D female WT: $147 \pm 5\%$ of baseline fEPSP slope vs Tg: $145 \pm 7\%$ of baseline fEPSP slope, $p > 0.05$). Together these results show that the LTP magnitude is altered at MPP-DGC synapses prior to CA3-CA1 synapses during the presymptomatic phase in the TgF344-AD rat, and occurs when basal transmission is also altered at MPP-DGC synapses.

Depolarization during LTP induction is increased at MPP-DGC but not CA3-CA1 synapses

Sufficient dendritic depolarization must be obtained to relieve the voltage-dependent Mg²⁺ block from NMDARs during LTP induction. Therefore, to determine whether heightened dendritic depolarization during LTP inducing stimulation might be linked with the increased LTP magnitude at MPP-DGC synapses, we assessed steady-state depolarization in 6 month TgF344-AD rats compared to WT littermate controls (Fig. 4), and analyzed this in two ways, as we have done previously (Franklin et al., 2014; Smith and McMahon, 2006). We measured the deflection from baseline at 170 msec after the stimulus artifact when the deflection is stable, and also measured area under the curve (AUC). We found that steady-state depolarization during HFS at MPP-DGC was significantly increased in TgF344-AD males (Fig. 4 A.1-A.2; $*p < 0.05$) and females (Fig. 4 B.1-B.2; $**p < 0.01$) compared to WT littermates. In contrast, steady-state depolarization is not different during TBS-induced LTP at CA3-CA1 synapses in 6-month-old TgF344-AD males (Fig. 4 C.1-C.2; $p > 0.05$) or females (Fig. 4 D.1-D.2; $p > 0.05$) compared to WT littermates, consistent with unaltered LTP magnitude at CA3-CA1 synapses (Fig. 3C and D). Area Under the Curve (AUC) (Fig. 4 A.3,B.3,C.3,D.3) analysis yielded similar results with a significant increase in depolarization in TgF344-AD females (Fig. 4 B.3; $p < 0.01$) at MPP-DGC synapses and a near significant effect in males (Fig. 4 A.2; $p=0.08$). Again, no difference in depolarization during theta burst

was found at CA3-CA1 synapses ($p > 0.05$) in males (Fig. 4 C.3) or females (Fig. 4 D.3). Together, these results suggest that the increased LTP magnitude at MPP-DGC synapses could be due to facilitated depolarization during the tetanus which does not occur at CA3-CA1 synapses in 6-month-old TgF344-AD rats where LTP magnitude is unaffected. It is also important to note that the studies performed at MPP-DGC synapses required pharmacological blockade of GABA_ARs to successfully induce LTP (Supplemental Fig. 1), while those at CA3-CA1 synapses were performed with inhibition intact.

LTP magnitude is unaltered at MPP-DGC and CA3-CA1 synapses in TgF344-AD male or female rats at 9 and 12 months

To assess if MPP-DGC LTP is altered at 9 and 12 months, HFS-LTP was examined in males and OVX females. Unlike the increased LTP magnitude observed at 6 month MPP-DGC synapses (Fig. 3 A, B), by 9 months of age, potentiation at these same synapses in TgF344-AD male and OVX female is comparable to WT littermates (Fig. 5. A.1, male WT: $117 \pm 8\%$ of baseline fEPSP slope vs Tg: $130 \pm 7\%$ of baseline fEPSP slope; $p > 0.05$; C.1, female WT: $133 \pm 11\%$ of baseline fEPSP slope vs Tg: $139 \pm 11\%$ of baseline fEPSP slope; $p > 0.05$). The same result is observed at 12 months (Fig. 5 A.2, male WT: $121 \pm 6\%$ of baseline fEPSP slope vs Tg: $129 \pm 4\%$ of baseline fEPSP slope; $p > 0.05$; C.2, female WT: $143 \pm 10\%$ of baseline fEPSP slope vs Tg: $127 \pm 9\%$ of baseline fEPSP slope; $p > 0.05$). It is important to note that while the LTP magnitude at MPP-DGC synapses was not different between genotypes at 9 and 12 months, we observed an increased probability of inducing LTP in slices from TgF344-AD rats compared to WT (9 mo WT males 23.1%, 9 mo Tg males 38.1%, 9 mo WT females 35.7%, 9 mo Tg females 71.4%, 12 mo WT males 53.8%, 12 mo Tg males 83.3%, 12 mo WT females 61.1%, 12 mo Tg females 81.8%). Thus, adaptive mechanisms may be in place to overcome the decreased basal transmission in the face of worsening AD pathology to maintain synaptic plasticity.

To determine if the CA3-CA1 LTP magnitude was altered during any of the presymptomatic ages assessed in TgF344-AD rats, TBS-LTP was additionally examined in males and OVX females at 9 and 12 months of age (Fig. 5 B.1, B.2, D.1, D.2). There is no difference between genotypes in either sex at 9 months of age (Fig. 5 B.1, male WT: $138 \pm 7\%$ of baseline fEPSP slope vs Tg: $132 \pm 4\%$ of baseline fEPSP slope; $p > 0.05$; Fig. 5 D.1, female WT: $136 \pm 8\%$ of baseline fEPSP slope vs Tg: $140 \pm 6\%$ of baseline fEPSP slope; $p > 0.05$). Even at 12 months of age, TBS-LTP at CA3-CA1 synapses remains intact in TgF344-AD rats (Fig. 5 B.2, male WT: $121 \pm 6\%$ of baseline fEPSP slope vs Tg $129 \pm 4\%$ of baseline fEPSP slope; $p > 0.05$; Fig. 5 D.2, female WT: $129 \pm 5\%$ of baseline fEPSP slope vs Tg $126 \pm 7\%$ of baseline fEPSP slope; $p > 0.05$). These data show that TBS can reliably induce LTP at CA3-CA1 synapses in both TgF344-AD and WT rats during the presymptomatic phase. To ensure that our TBS protocol did not saturate the LTP magnitude at CA3-CA1 synapses and therefore mask a potential genotype difference, a weak TBS protocol was used to induce LTP in 12-month-old animals, as the I/O curve is significantly decreased in TgF344-AD rats at this age in both sexes (Figs. 1 B.3, D.3). Reducing TBS to 2 bouts still did not reveal genotype differences in LTP magnitude in 12 month TgF344-AD males (Supplemental Fig. 2A, WT: $119 \pm 5\%$ of baseline fEPSP slope; Tg $125 \pm 8\%$ of baseline fEPSP slope; $p > 0.05$) or females (Supplemental Fig. 2B, WT: $138 \pm 10\%$ of baseline fEPSP slope; Tg $125 \pm 7\%$ of

baseline fEPSP slope; $p > 0.05$) compared to WT littermates, and suggests our TBS protocol was not saturating, and was not masking a potential deficit in LTP magnitude in TgF344-AD rats.

Spine density is unaltered

Whether spine loss is responsible for the observed decrease in basal synaptic transmission (Fig. 1) is unknown. To investigate this, spine density was analyzed at 9 months, when basal synaptic transmission is significantly decreased at MPP-DGC synapses (Fig. 1 A.2, C.2), and is just beginning at CA3-CA1 synapses (Fig. 1 B.2) in TgF344-AD rats compared to WT. Spine density of middle molecular layer (MML) DGC dendrites was not significantly different between TgF344-AD vs WT males (Fig. 6 A.1, B.1; $p > 0.05$) or between acute-OVX TgF344-AD and WT females (Fig. 6 A.2, B.2; $p > 0.05$). These results suggest tertiary dendritic spine density on DGCs remain intact by at least 9 months of age in the TgF344-AD model, despite the significant decrease in basal synaptic transmission. Similarly, spine density on CA1 pyramidal cell tertiary dendrites was not significantly different in 9-month male (Fig. 6 C.1, D.1; $p > 0.05$) and acute-OVX female (Fig. 6 C.2, D.2; $p > 0.05$) TgF344-AD rats compared to WT littermates. Together, these results suggest that decreased maximum basal synaptic transmission occurring at 9 months (Fig. 1 A.2, B.2, C.2) is not a consequence of a loss of synapses, and suggests the possibility of a loss of postsynaptic AMPARs. Together, these data show that the time-course for altered synaptic physiology precedes gross morphological changes in spine density at MPP-DGC and CA3-CA1 synapses in the comprehensive TgF344-AD model.

Discussion

The goal of this study was to investigate whether alterations in hippocampal synaptic efficacy precede reported hippocampus dependent learning and memory deficits in the novel comprehensive TgF344-AD rat model. Together our data show that at ages when soluble A β , hyper-p-tau, and gliosis are increased, but prior to reported hippocampal-dependent behavioral deficits (Cohen et al., 2013), basal synaptic strength is weakened and LTP is pathologically altered. Specifically, the maximum strength of basal excitatory transmission is decreased as early as 6 months of age at MPP-DGC synapses in TgF344-AD rats compared to WT littermate controls. This decreased basal synaptic connectivity at MPP-DGC synapses occurs prior to weakened maximum synaptic transmission at CA3-CA1 synapses, which begins at 9 months in males and 12 months in females, demonstrating a sex bias at CA3-CA1 synapses. Importantly, the decreased strength of basal transmission occurs in the absence of a change in the PPR or dendritic spine density at either synapse. The magnitude of NMDAR-dependent LTP is pathologically enhanced only at 6 months at MPP-DGC synapses in both sexes, while at CA3-CA1 synapses, the LTP magnitude is unchanged. Overall, these results show that hippocampal synaptic dysfunction occurs earlier at synapses emanating from EC during the presymptomatic phase of the disease, recapitulating what is known to occur in human hippocampus in subjects with AD (Khan et al., 2014). Furthermore, the finding that synaptic dysfunction occurs prior to reported learning and memory deficits corroborates results from human fMRI studies during preclinical AD that

synaptic function is altered prior to cognitive deficits (Khan et al., 2014; Killiany et al., 2002).

Elevated soluble A β oligomers (A β o), hyperphosphorylated tau species, and gliosis are present at 6 months of age in TgF344-AD rats (Cohen et al., 2013), when the decrease in basal synaptic strength is observed at MPP-DGC synapses. Importantly, all of these pathological changes are implicated in early AMPAR dysfunction in AD models (Almeida et al., 2005; Hoover et al., 2010; Hsieh et al., 2006). Our findings that basal synaptic transmission is pathologically decreased in TgF344-AD rat hippocampal synapses agree with previous literature from mouse models overexpressing human APP Swedish and Indiana mutations, which all find a deficit in the I/O relationship prior to plaque formation (Hermann et al., 2009; Hsai et al., 1999; Ye et al., 2010). However, the mechanism(s) contributing to the decrease in I/O we observe is not yet clear, but is likely due to altered AMPAR transmission as a result of increased A β oligomers (A β o). AMPARs become internalized in the presence of soluble A β o (Hsieh et al., 2006; Kamenetz et al., 2003; Shankar et al., 2007) and as such, increased A β o at these ages in the TgF344-AD rats provides one possible mechanism for a decrease in the I/O curve at TgF344-AD MPP-DGC and CA3-CA1 synapses. Importantly, abnormally phosphorylated tau is implicated in impaired AMPAR subunit trafficking to the postsynaptic density (Hoover et al., 2010), which could also contribute to the decrease in basal transmission observed in the I/O curve. While the many potential relationships between how tau and A β work copathogenically to alter neuronal function in AD are still being elucidated, it is thought that elevated soluble A β leads to downstream tau hyperphosphorylation and mislocalization to somatodendritic compartments where it drives A β -induced downregulation of surface AMPARs (Hoover et al., 2010; Ittner et al., 2010; Miller et al., 2014; Zempel et al., 2010). Thus, decreased AMPAR transmission caused by the increasing AD pathology is likely contributing to the loss of synaptic connectivity, an interpretation supported by no change in presynaptic release probability and dendritic spine density.

Because inhibitory transmission was not blocked during the generation of I/O curves, we cannot rule out that enhanced inhibitory drive, and therefore altered excitation/inhibition (E/I) balance, may be responsible for the observed decrease in basal synaptic strength. Epileptic/seizure activity is associated with AD (Amatniek et al., 2006; Hauser et al., 1986) and rodent studies in Tg AD mice have confirmed altered E/I balance (Palop et al., 2007; Verret et al., 2012; Yoshiike et al., 2008). While some argue for reduced inhibitory drive (Limon et al., 2012; Palop et al., 2007; Verret et al., 2012), the TgAPP/PS1 E9 mouse model, which harbors the same transgenes driven by the same promoter as TgF344-AD rat, exhibits enhanced GABA_AR mediated inhibition, and low dose GABA_AR inhibition significantly improves performance on the Morris-Water Maze (Yoshiike et al., 2008). It is also important to note that gliosis in the form of activated microglia and astrocytes are present at the ages assessed and could contribute to the decreased I/O observed. Microglia and astrocyte function are highly plastic and exist on a continuum from protective to damaging depending on length and severity of pathological insult (Hamby and Sofroniew, 2010; Town et al., 2005). Whether activated microglia are neuroprotective or damaging during presymptomatic pathology in the TgF344-AD rat should be further explored. Indirect evidence for glial control of AMPAR subunit composition suggests that astrocytes can buffer

neuronal excitotoxicity by regulating the GluR2-subunit of AMPARs (Van Damme et al., 2007). Whether one or all of above mentioned mechanisms contribute to decreased TgF344-AD basal transmission between 6 and 12 months of age reported in this study remain to be explored.

The majority of studies that have analyzed presynaptic release probability in rodent models of AD have focused on CA3-CA1 synapses and most report normal PPF, while at MPP-DGC synapses results are conflicting, as the exact pathway stimulated in each study was not always identified (Marchetti and Marie, 2011). One study reported normal PPD at MPP-DGC synapses in young asymptomatic mice (Houeland et al., 2010). Three other studies at MPP-DGC synapses report reduced PPF (which is characteristic of lateral perforant path (LPP) connections to dentate from EC) in already symptomatic hAPPJ20 mice (Harris et al., 2010; Palop et al., 2007; Roberson et al., 2011). Therefore, our results showing unaltered PPR at MPP-DGC and CA3-CA1 synapses recapitulate findings in other AD models prior to behavioral deficits and support a postsynaptic mechanism for decreased basal synaptic transmission at these synapses. Synaptic inhibition was not blocked during I/O or PPR experiments and therefore a contribution for increased inhibitory drive cannot be ruled out as a factor for decreased I/O we observe.

Studies examining the impact of AD pathology on LTP have yielded conflicting results and differences in method of exogenous A β delivery, Tg-rodent model used, and disease stage are likely responsible for these discrepancies (for review see Spires-Jones and Hyman, 2014). The majority of LTP studies in AD rodent models have been done at CA3-CA1 synapses. Our finding of unchanged LTP magnitude at 6 months at CA3-CA1 synapses is consistent with previous studies in pre-plaque rodent models of AD (Hanson et al., 2015; Qi et al., 2014) and is a consistent finding in AD mouse models (Marchetti and Marie, 2011). Recently, it has been reported TgF344-AD rats develop plaques in entorhinal cortex and hippocampus at 9 months of age (Joo et al., 2017). Plaque staining in 9 month TgF344-AD dentate and CA1 have been individually confirmed by our lab (data not shown). The maintenance of LTP at CA3-CA1 synapses, pre-to-post plaque deposition, compared to WT littermates recapitulates results from multiple mouse models of AD (Fitzjohn et al., 2001; Hsai et al., 1999). Our results show that CA3-CA1 synapses are able to maintain plasticity at WT levels while pathology progresses. A proposed mechanism to preserve plasticity during early AD pathology is adaptive changes to glutamate receptor subunit sensitivity during LTP induction at these synapses. Specifically, Hanson et al. 2015 shows that LTP at CA3-CA1 synapses is of the same magnitude in WT vs PS2APP mice, however, the underlying mechanism is different such that there is a recruitment of extrasynaptic GluN2B-subunit containing receptors during the tetanus in the Tg-AD mice that maintains LTP magnitude at normal levels (Hanson et al., 2015). Detailed studies using whole-cell and field potential recordings of NMDAR function during LTP induction and maintenance should be explored at early ages in the TgF344-AD rat model.

Interestingly, our results at MPP-DGC synapses at 6 months show that LTP magnitude in TgF344-AD rats is greater than WT, supporting previous studies using APP^{swe}/PS1^{E9} mice and B6.152H Tg-AD mice which show enhanced LTP in dentate compared to wildtype littermates (Poirier et al., 2010; Yoshiike et al., 2008). Although the enhanced LTP at MPP-

DGC synapses at 9 and 12 months is lost in TgF344-AD rats, LTP induction appears to be facilitated as AD pathology progresses since successful LTP appears to occur more often in slices from Tg rats. The reasons for this remain unknown, but are likely a result of compensatory mechanisms recruited to maintain the ability of MPP-DGC synapses to undergo long-term changes in synaptic efficacy in the face of increasing pathology. As mentioned above, presymptomatic PS2APP mice displayed “normal” LTP, yet unlike WT mice, LTP from PS2APP mice relies on activation of extrasynaptic GluN2B-NMDARs to occur. Additionally, it should be noted that changes in basal transmission do not always correlate with a change in LTP magnitude. The I/O curve measures the maximum strength of basal synaptic transmission, which can be influenced by synapse number, postsynaptic AMPAR density, presynaptic glutamate release, and/or GABAergic tone. LTP, on the otherhand, is a measure of the ability of excitatory synapses to undergo a long-term change in synaptic efficacy. This is usually mediated by insertion of postsynaptic AMPA receptors. As paired-pulse ratio (an indirect measure of presynaptic release probability) and spine density are not changed, decreased synaptic AMPAR expression and/or increased GABAergic tone could lead to decreased maximum transmission measured in the I/O curves while individual synapses maintain their ability to insert synaptic AMPARs following LTP inducing stimulation. Since basal transmission is decreased at 6 months, enhanced LTP in TgF344-AD rats at MPP-DGC synapses could result from altered excitation/inhibition balance, altered resting membrane potential, or a change in passive membrane properties, all of which will be investigated in future studies.

The absence of a change in dendritic spine density shows that functional changes in synaptic transmission precede structural changes. The time-line for gradually accumulating soluble A β and hyperphosphorylated tau reported by Cohen et al., 2013, coincide with a decrease in basal transmission and pathologically enhance LTP at MPP-DGC synapses in the absence of spine loss at 9 months of age. Furthermore, the decreased basal transmission observed at both MPP-DGC and CA3-CA1 synapses is not a consequence of a loss in synapse density, and must be due to some other mechanism, such as loss of AMPARs from the synapses or enhanced inhibition as mentioned above. Altered glial function may also contribute to these findings. Disease stage dependent switch in microglial function progresses from protective in early stages and damaging in later stages as shown in the PS1/APP mouse (Jimenez et al., 2008). As markers for gliosis are increased beginning at 6 months in the TgF344-AD rat (Cohen et al., 2013), and spine density remains intact by 9 months, these data may suggest the increased glial response is protective at these ages, prior to a cytotoxic switch which could mediate synapse loss (Hong et al., 2016).

The majority of previous studies in Tg-AD rodent models have used males or mixed male/female animals. This is surprising given the vast gender disparity that exists in AD with two-thirds of those diagnosed being women (Alzheimer Association, 2016) and long-term ovarian hormone loss during natural or surgically induced menopause is a risk factor for the development of AD (Barron and Pike, 2012; Jamshed et al., 2014; Mielke et al., 2014). Importantly, replacement of the primary ovarian estrogen, 17 β estradiol (E2), during the critical window decreases memory deficits and AD risk in menopause (Lee et al., 2014; Silverman et al., 2011; Wharton et al., 2011). In transgenic AD mouse models, long-term ovariectomy (OVX) accelerates amyloid and tau accumulation and worsens memory

performance, while E2 replacement reverses these detrimental effects (Carroll et al., 2007; Carroll and Pike, 2008; Levin-Allerhand et al., 2002; Xu et al., 2006; Yue et al., 2005). Collectively, these data suggest that maintaining plasma E2 levels over the lifespan could be key to delaying disease onset or slowing its progression when ovarian E2 is lost in menopause. In these initial characterization studies, TgF344-AD males develop basal synaptic transmission deficits at CA3-CA1 synapses at 9 months while they do not appear in females until 12 months of age. As E2 regulates amyloid processing, tau, and glial uptake of glutamate (Goodenough et al., 2005; Liang et al., 2010; Liu et al., 2008; Nord et al., 2010; Shy et al., 2000; Zhang et al., 2008; Zhao et al., 2011), these data support the possibility that E2 has neuroprotective effects, even though females in this study had undergone short-term OVX. However, future studies using intact cycling females and OVX females with and without hormone replacement are needed to fully understand the risks and benefits of ovarian hormone loss and replacement on AD pathology and synaptic dysfunction as the disease progresses.

Conclusions

Because the hippocampus is one of the first and most extensively affected brain regions by AD pathology, identifying synaptic dysfunction in this network during presymptomatic disease is critical to minimizing cognitive deficits as AD pathology advances. Using TgF344-AD rats and extracellular dendritic fEPSP recording, we found changes in synaptic function occur at MPP-DGC prior to CA3-CA1 synapses, and months before reported hippocampal dependent behavioral deficits (Cohen et al., 2013). Thus, this model more fully recapitulates what is believed to occur in human AD. This model provides a next-generation tool for dissecting the earliest changes in network dysfunction in DG, information absent in current literature. Only when the earliest synaptic changes are revealed, in a clinically relevant model, can new interventions to slow disease progression be developed. Our data showing deficits in basal transmission, unaltered presynaptic release probability, normal CA3-CA1 LTP and pathologically enhanced MPP-DGC LTP magnitude predict a loss of postsynaptic AMPARs, altered passive membrane properties, and/or altered excitation/inhibition balance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

TgF344-AD synaptic strength is decreased at MPP-DGC synapses prior to CA3-CA1 synapses in both sexes. (A) At MPP-DGC synapses, basal synaptic strength was significantly decreased in TgF344-AD males (blue triangles) compared to WT males (black squares) at (A.1) 6 months ($n = 9$ slices/8 animals WT, $n = 7$ slices/6 animals Tg; $*p < 0.05$), at (A.2) 9 months ($n = 11$ slices/11 animals WT, $n = 11$ slices/11 animals Tg; $*p < 0.05$), and at (A.3) 12 months ($n = 11$ slices/10 animals WT, $n = 11$ slices/11 animals Tg; $**p < 0.01$). (B) At CA3-CA1 synapses, basal synaptic strength was not different between TgF344-AD males compared to WT males at (B.1) 6 months ($n = 10$ slices/10 animals WT, $n = 8$ slices/7 animals Tg, $p > 0.05$), but was significantly decreased at (B.2) 9 months, ($n = 11$ slices/11

animals WT, $n = 11$ slices/11 animals Tg; $*p < 0.05$), and at (B.3) 12 months ($n = 9$ slices/9 animals WT, $n = 13$ slices/13 animals Tg; $*p < 0.05$). (C) At MPP-DGC synapses in OVX females, basal synaptic strength was decreased in TgF344-AD females (red triangles) compared to WT littermates (black circles) at (C.1) 6 months ($n = 6$ slices/6 animals WT, $n = 8$ slices/7 animals Tg; $*p < 0.05$), at (C.2) 9 months ($n = 10$ slices/10 animals WT, $n = 6$ slices/6 animals Tg; $*p < 0.05$), and at (C.3) 12 months ($n = 13$ slices/11 animals WT, $n = 5$ slices/5 animals Tg; $*p < 0.05$). (D) At CA3-CA1 synapses, basal synaptic strength in TgF344-AD females compared to WT littermates is not altered at (D.1) 6 months ($n = 9$ slices/8 animals WT, $n = 10$ slices/10 animals Tg; $p > 0.05$), or at (D.2) 9 months ($n = 12$ slices/11 animals WT, $n = 8$ slices/8 animals Tg; $p > 0.05$), but is significantly decreased at (D.3) 12 months ($n = 11$ slices/11 animals WT, $n = 7$ slices/7 animals Tg; $*p < 0.05$). Data represent mean $\pm$ SEM. Significance determined by unpaired Student's t -test at 200 μ A. (Insets) Example traces from TgF344-AD and WT age/sex matched littermates at 200 μ A stimulus intensity. Scale bar 0.5 mv, 5ms.

Fig. 2.

Paired-pulse ratio is unaltered at MPP-DGC and CA3-CA1 synapses in TgF344-AD rats compared to WT littermates in both sexes. (A, B) PPR was not altered at MPP-DGC synapses (A.1, 6 months: $n = 8$ slices/7 animals WT, $n = 7$ slices/7 animals Tg; A.2, 9 months: $n = 10$ slices/9 animals WT, $n = 9$ slices/9 animals Tg; A.3, 12 months: $n = 6$ slices/6 animals WT, $n = 9$ slices/9 animals Tg) or at CA3-CA1 synapses (B.1, 6 months: $n = 11$ slices/8 animals WT, $n = 10$ slices/6 animals Tg; B.2, 9 months: $n = 13$ slices/13 animals WT, $n = 12$ slices/12 animals Tg; B.3, 12 months: $n = 10$ slices/9 animals WT, $n = 12$ slices/12 animals Tg) in TgF344-AD males versus WT littermates. (C, D) The same is true at CA3-CA1 synapses (C.1, 6 months: $n = 6$ slices/6 animals WT, $n = 5$ slices/5 animals Tg; C.2, 9 months: $n = 11$ slices/11 animals WT, $n = 6$ slices/6 animals Tg; C.3, 12 months: $n = 13$ slices/11 animals WT, $n = 7$ slices/7 animals Tg) and at CA3-CA1 synapses (D.1, 6

months: n = 8 slices/7 animals WT, n = 13 slices/10 animals Tg; D.2, 9 month: n = 12 slices/11 animals WT, n = 8 slices/8 animals Tg; D.3, 12 months: n = 6 slices/6 animals WT, n = 9 slices/9 animals Tg) in TgF344-AD females versus WT littermates. Data represent mean $\pm$ SEM. Statistical analysis was determined by repeated measures GLM; $p > 0.05$ for all data sets.

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Fig. 3.

NMDAR-dependent long-term potentiation (LTP) is selectively enhanced at MPP-DGC synapses in 6 month TgF344-AD rats compared to WT littermates. (A, B) The magnitude of High Frequency Stimulation (HFS) induced LTP at MPP-DGC synapses was significantly increased in (A) TgF344-AD males (blue triangles) compared to WT littermates (black squares) ($n = 13$ slices/13 animals WT, $n = 7$ slices/7 animals Tg; $*p < 0.05$), and in (B) OVX TgF344-AD females (red triangles) compared to WT littermates (black circles) ($n = 7$ slices/7 animals WT, $n = 12$ slices/10 animals Tg; $*p < 0.05$) at 6 months. (C, D) The magnitude of Theta-Burst Stimulation (TBS) induced LTP is not different at CA3-CA1 synapses at 6 months in (C) TgF344-AD males compared to WT littermates ($n = 9$ slices/7 animals WT, $n = 6$ slices/6 animals Tg; $p > 0.05$) or in (D) OVX TgF344-AD females compared to WT littermates ($n = 8$ slices/7 animals WT, $n = 12$ slices/10 animals Tg; $p > 0.05$). Data represent mean $\pm$ SEM. Significance determined by unpaired Student's t -test.

Fig. 4.

Depolarization during tetanus is increased during the LTP inducing stimulation at MPP-DGC but not CA3-CA1 synapses in TgF344-AD rats compared to WT littermates. (A.1, B. 1) Averaged traces during HFS from experiments in Fig. 3A-B show increased steady-state depolarization (defined as the difference between baseline and when the deflection reached steady state) during 4th round of HFS tetanus at MPP-DGC synapses in TgF344-AD male (A.1, blue trace) and female (A.2, red trace) slices compared to WT (black traces). Scale bar 0.1mv, 25ms. (A.2) Pooled data from LTP experiments in Fig. 3A show the HFS steady-state

depolarization is significantly increased in TgF344-AD males ($n = 11$ WT, 0.100 ± 0.002 , $n = 7$ Tg, 0.152 ± 0.003 ; $*p < 0.05$). (A.3) Area Under the Curve (AUC) was additionally measured ($n = 11$ WT, 32.81 ± 3.0 , $n = 7$ Tg, 42.65 ± 5.8 ; $p = 0.08$). (B.2) Pooled data from LTP experiments in Fig. 3B show the HFS steady-state depolarization is significantly increased in TgF344-AD females ($n = 7$ WT, 0.088 ± 0.001 , $n = 10$ Tg, 0.159 ± 0.003 ; $**p < 0.01$). (B.3) AUC was also measured ($n = 7$ WT, 28.24 ± 2.1 , $n = 10$ Tg, 40.98 ± 3.4 ; $**p < 0.01$). (C.1,D.1) Averaged traces during the 4th bout of TBS from experiments in Fig. 3C-D show depolarization during tetanus is not altered at CA3-CA1 synapses in either TgF344-AD males (C.1, blue trace) or females (D.1, red trace) compared to WT littermates (black traces). Scale bar 0.25 mv, 25 ms. (C.2) Pooled data from LTP experiments in Fig. 3C do not show a difference in CA3-CA1 steady-state depolarization during tetanus in 6 month TgF344-AD males ($n = 7$ WT, 0.61 ± 0.043 , $n = 5$ Tg, 0.63 ± 0.010 ; $p > 0.05$). (C.3) AUC was not different ($n = 7$ WT, 29.79 ± 2.0 , $n = 5$ Tg, 29.49 ± 2.1 ; $p > 0.05$). (D.2) Pooled data from LTP experiments in Fig. 3D do not show a difference in CA3-CA1 steady-state depolarization during tetanus in 6 month TgF344-AD females ($n = 7$ WT, 0.56 ± 0.013 , $n = 10$ Tg, 0.54 ± 0.032 ; $p > 0.05$). (D.3) AUC was not significant ($n = 7$ WT, 32.05 ± 2.1 , $n = 10$ Tg, 31.43 ± 2.2 ; $p > 0.05$). Data represent mean $\pm$ SEM. Significance determined by unpaired Student's *t*-test.

Fig. 5.

NMDAR-dependent LTP is intact at MPP-DGC and CA3-CA1 synapses in 9 and 12 month TgF344-AD males and females. (A.1, A.2) LTP magnitude is not different between genotypes in 9 month (A.1, $n = 3$ slices/3 animals WT, $n = 8$ slices/8 animals Tg; $p > 0.05$) and 12 month males (A.2, $n = 7$ slices/6 animals WT, $n = 5$ slices/4 animals Tg; $p > 0.05$) at MPP-DGC synapses. (B.1-B.2) LTP magnitude is not different after TBS at CA3-CA1 synapses in (B.1) 9 month TgF344-AD males compared to WT littermates ($n = 12$ slices/9 animals WT, $n = 16$ slices/12 animals Tg; $p > 0.05$) or (B.2) 12 month males ($n = 11$ slices/

10animal WT, n = 13 slices/13 animals Tg; $p > 0.05$). (C.1-C.2) LTP magnitude is not different at MPP-DGC synapses in OVX females at 9 (C.2, n = 5 slices/4 animals WT, n = 5 slices/5 animals Tg; $p > 0.05$) and 12 months (C.2, n = 11 slices/7 animals WT, n = 9 slices/6 animals Tg; $p > 0.05$). (D.1-D.2) The same is true at CA3-CA1 synapses, where there is no difference in LTP magnitude between genotypes in OVX females at 9 (D.1, n = 14 slices/12 animals WT, n = 14 slices/10 animals Tg; $p > 0.05$) and 12 months (D.2; n = 9 slices/8 animals WT, n = 12 slices/12 animals Tg, $p > 0.05$). Data represent mean $\pm$ SEM. Significance determine by unpaired Student's *t*-test.

Fig. 6.

Dendritic spine density is unaltered on DGCs and CA1 pyramidal cells in TgF344-AD rats compared WT littermate controls. (A.1, A.2) Representative confocal images of Golgi-stained DGC middle molecular layer (MML) apical dendrites from 9-month male and OVX female TgF344-AD rats and WT littermates. (B.1) The number of MML apical dendritic spines per 10 μm is not different between genotypes in 9 month males (WT 17.8 ± 1.1 spines per 10 μm, 34 sections from 7 animals vs TgF344-AD 20.0 ± 0.8 spines per 10 μm, 46 sections from 10 animals; $p > 0.05$) or (B.2) females (WT $14.0 \pm 0.7/10$ μm, 57 sections/11 animals vs TgF344-AD $13.8 \pm 0.8/10$ μm, 61 sections/13 animals; $p > 0.05$) compared to WT littermates. (C.1, C.2) Representative confocal images of Golgi-stained CA1 pyramidal cell tertiary dendrites located in stratum radiatum from 9-month male and OVX female TgF344-AD rats and WT littermates. (D.1) The number of CA1 tertiary dendritic spines per 10 μm is not different between genotypes in 9 month males (WT $19.7 \pm 1.2/10$ μm, 36 sections/7 animals vs TgF344-AD $19.0 \pm 0.7/10$ μm, 47 sections/10 animals; $p > 0.05$) or (D.2) TgF344-AD females (WT $15.4 \pm 0.6/10$ μm, 59 sections/11 animals vs TgF344-AD $15.7 \pm 0.8/10$ μm, 64 sections/13 animals; $p > 0.05$). Data represent mean $\pm$ SEM. Significance determined by unpaired Student's *t*-test. Scale bar, 10 μm.