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Progressive loss of synaptic integrity in human apoE4 targeted replacement mice and attenuation by apoE2

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Abstract

Inheritance of the *APOE4* allele is a well established genetic risk factor linked to the development of late onset Alzheimer's disease. As the major lipid transport protein in the central nervous system, apolipoprotein (apo) E plays an important role in the assembly and maintenance of synaptic connections. Our previous work showed that 7 month old human apoE4 targeted replacement (TR) mice displayed significant synaptic deficits in the principal neurons of the lateral amygdala, a region that is critical for memory formation and also one of the primary regions affected in Alzheimer's disease, compared to apoE3 TR mice. In the current study, we determined how age and varying *APOE* genotype affect synaptic integrity of amygdala neurons by comparing electrophysiological and morphometric properties in C57BL6, apoE knockout, and human apoE3, E4 and E2/4 TR mice at 1 month and 7 months. The apoE4 TR mice exhibited the lowest level of excitatory synaptic activity and dendritic arbor compared to other cohorts at both ages, and became progressively worse by 7 months. In contrast, the apoE3 TR mice exhibited the highest synaptic activity and dendritic arbor of all cohorts at both ages. C57BL6 mice displayed virtually identical synaptic activity to apoE3 TR mice at 1 month; however this activity decreased by 7 months. ApoE knockout mice exhibited a similar synaptic activity profile with apoE4 TR mice at 7 months. Consistent with previous reports that *APOE2* confers protection, the apoE4-dependent deficits in excitatory activity were significantly attenuated in apoE2/4 TR mice at both ages. These findings demonstrate that expression of human apoE4 contributes to functional deficits in the amygdala very early in development and may be responsible for altering neuronal circuitry that eventually leads to cognitive and affective disorders later in life.

Keywords

APOE; targeted; replacement; mice; synaptic; integrity

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1.1 Introduction

APOE4 carriers represent one quarter of the population in most developed countries and have the highest risk for contracting late onset Alzheimer's disease (AD) compared to non-*APOE4* carriers (Rubinsztein and Easton, 1999). In contrast, *APOE2* carriers represent approximately 15% of the population and theoretically deter Alzheimer's disease compared to presence of the *APOE3* allele (Corder et al., 1994). Humans are the only species to express three isoforms of apolipoprotein (apo) E, each differing by a single amino acid (Weisgraber, 1994). This singular difference has dramatic effects on apoE receptor kinetics (Bohnet et al., 1996) which may explain apoE isoform specific effects on synaptic transmission. Maintaining synaptic integrity is arguably the most important central nervous system (CNS) function needed to avoid AD. We define synaptic integrity as a functional synaptic unit with unimpaired neuronal transmission. The loss of synapses or decline in synaptic function is the strongest correlate of cognitive decline in AD (Terry et al., 1991). Multiple studies in animals show that deficits in synaptic transmission occur prior to the detection of any pathognomonic hallmarks of AD (i.e. plaques and tangles) (Buttini et al., 2002) (Mucke et al., 2000) (Raber et al., 2000). Since cognitive deficits can be detected in *APOE4* carriers very early in life (Acevedo et al., 2010) (Reiman et al., 2004) (Snowdon et al., 2000), determining when these deficits begin could provide very useful information for treating the disease. Furthermore, understanding how apoE2 expression confers protection against AD illuminates an entirely new research strategy for development of novel therapeutics.

Numerous groups have used the human apoE TR model to study the role of apoE in AD (Trommer et al., 2005) (Blain et al., 2006) (Osorio et al., 2007) (Yun et al., 2005) (Bales et al., 2009). Previously, we showed that young adult apoE4 TR mice displayed reduced excitatory synaptic transmission, dendritic arborization and spine density in the lateral amygdala compared to apoE3 TR mice, and that these deficits occurred in the absence of any pathological hallmarks such as gliosis, amyloid deposition or neurofibrillary tangles (Wang et al., 2005). In support of this, Dumanis et al. found that spine density was reduced in cortical layers II/III of apoE4 mice (vs. apoE3 mice) that became progressively worse with age (Dumanis et al., 2009). Behavioral studies demonstrate spatial memory deficits in young apoE4 TR mice compared to apoE3 mice (Grootendorst et al., 2005) that also became worse with age (Bour et al., 2008a) lending further support to apoE4 deficits in synaptic integrity. Furthermore, Trommer et al. found that young apoE4 mice exhibit reduced hippocampal long term potentiation (LTP) in the dentate gyrus compared to apoE3 mice (Trommer et al., 2004). In contrast, Korwek et al. found enhanced LTP in the CA1 field of apoE4 mice (Korwek et al., 2009), demonstrating that the effect of apoE4 on neuronal transmission is regionally specific.

The amygdala is a limbic structure that participates significantly in memory formation (Akirav and Richter-Levin, 2002) (Fried et al., 2001) (McGaugh, 2004) and like the hippocampus is one of the earliest structures to undergo neurodegeneration in AD (Hamann et al., 2002) (Cuenod et al., 1993). The amygdala also regulates neural processes that govern affective states, such as depression and anxiety which are very prevalent in AD. For example, Hamann et al. found significant impairment in fear conditioning in AD patients, suggesting a loss of synaptic integrity in the amygdala (Hamann et al., 2002). Behavioral studies in mice have also shown that apoE differentially impacts multiple measures of anxiety (Siegel et al., 2010) (Raber, 2007) (Bour et al., 2008b) (Grootendorst J, 2005).

Herein we examine both the effect of age and *APOE* genotype on synaptic integrity using the human apoE TR mice. We used apoE2/4 heterozygous mice to measure the effect of apoE2 expression on synaptic integrity since apoE2 homozygous mice develop type III

hyperlipidemia (Sullivan et al., 1998), a chronic pro-inflammatory state that can confound interpretation of CNS phenotypes. Analysis of "wild type" C57BL6 (C57) mice provided us an opportunity to make evolutionary comparisons between murine and human apoE while apoE knockout (KO) mice were used to assess the absence of apoE on synaptic integrity in the amygdala. Overall, our data suggests that age and *APOE* genotype have a significant impact on regulating synaptic transmission and neuronal morphology.

1.2 Experimental procedures

Preparation of animals

The TR mice were created by gene targeting as described previously (Sullivan et al., 1997). Briefly, the construction of the TR mice differ from other types of apoE transgenic mice in that human *APOE* genomic fragments were used to replace the mouse *Apoe* gene via homologous recombination. All lines of apoE TR mice contain chimeric genes consisting of mouse 5' regulatory sequences continuous with mouse exon 1 (non-coding) followed by humans exons (and introns) 2–4. Thus, all three lines of apoE TR mice regulate apoE gene expression in the same fashion. The present study uses mice that have been backcrossed to C57B16 mice eight times and therefore are >99.6% C57B16. The animals are genotyped using an allele-specific PCR approach based on Hixson and Vernier (Hixson and Vernier, 1990). All mice are maintained on a normal chow diet (ND) consisting of 4.5% (w/w) fat and 0.2% (w/w) cholesterol (Prolab Isopro, Agway Inc., DeWitt, NY). The animals were handled in accordance with guidelines approved by the Duke and VA Animal Care and Use Committee, which includes minimizing the number of animals used and their suffering. All experiments were performed on age (1 or 7 months) and sex (male) matched animals for each genotype. TR mice were either homozygous for *APOE3* (3/3), *APOE4* (4/4) or heterozygous for *APOE2* and *APOE4* (2/4). Age and sex matched C57B16 and apoE KO mice were also used in this study.

Slice preparation

Acute coronal amygdala slices (350 μm thick) from the TR mice were prepared as described previously (Wang et al., 2005) (Klein and Yakel, 2006). Briefly, mice were anaesthetized with 200 mg/kg 2,2,2-tribromoethanol and decapitated. Brains were quickly removed and placed in ice-cold oxygenated artificial cerebral spinal fluid containing (mM): 126 NaCl, 2.5 KCl, 1.3 $MgCl_2$, 2.5 $CaCl_2$, 1 NaH_2PO_4, 25 $NaHCO_3$ and 10 glucose. Upon removal of the cerebellum, the brain was hemi-sected and the right hemisphere was glued to the Vibratome 1000 Plus (Vibratome, Campden, England) stage and immersed in cooled oxygenated artificial cerebral spinal fluid.

Electrophysiology

All experiments were conducted single-blinded, where the researcher performing the electrophysiological recordings and data analysis were unaware of the *APOE* genotype. After a 1 hour incubation period single slices were transferred to the recording chamber. Whole-cell patch-clamp recordings were performed in lateral amygdala neurons using patch pipettes with resistances of 3–5 MΩ filled with internal solution (IS) containing (in mM): 140 potassium gluconate, 0.5 $CaCl_2$, 2 MgATP, 2 $MgCl_2$, 5 EGTA, and 10 Hepes (pH 7.2–7.3). For morphological studies, 0.3% biocytin (Sigma) was added to the IS on the day of the experiment. Slices were superfused at room temperature (18–22°C) with artificial cerebral spinal fluid. We clamped cells at −70 mV and recorded spontaneous excitatory post-synaptic currents (sEPSCs) using an Axopatch 200B amplifier, filtered at 1 kHz, and sampled at 10 kHz using pClamp 10.1 software (Axon Instruments). We measured the sEPCSs interval and amplitude using Mini Analysis software (Synaptosoft, Inc. Decatur, GA) and statistical analyses was performed using Origin software (Microcal, Northampton,

MA, USA). Averaged data are presented as means ± S.E.M. Statistical significance ($P < 0.05$) was assessed using a Student's *t* test. Firing properties and electrical excitability were assessed using whole-cell current-clamp mode. Hyper- and depolarizing step pulses were delivered at 1 s durations ranging from −0.2 to 0.2 nA in 25 pA increments.

Biocytin staining

At the end of the recording session, the patch-pipette was gently retracted from the filled neuron and the brain slice was immediately transferred to a solution containing 4% paraformaldehyde in phosphate buffered saline, pH 7.4 (USB Corp., Cleveland, OH) and stored at 4° C. Slices were incubated for 1–3 days with avidin-biotin-peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories, Inc., Burmingham, CA) diluted in PBS containing 1% Triton X-100. Slices were then stained with diaminobenzidine (Vector Labs) solution freshly prepared in PBS. The reaction was stopped by rinsing with deionized water and the stained slices were mounted on gelatin subbed slides, air-dried overnight and cover slipped using standard techniques. The morphology of the biocytin-filled neurons was examined using a light microscope (either a Nikon EP3000 or Zeiss Axio ImagerD2), then traced with Neurolucida software (under a 40X objective) and analyzed with Neuroexplorer software (MicroBrightField, Colchester, VT).

1.3 Results

1.3.1 ApoE2 expression attenuates loss of excitatory transmission associated with apoE4 expression in young adult mice

Spontaneous excitatory post-synaptic currents (sEPSCs) were recorded from pyramidal-like neurons in the lateral amygdala from male wild type (C57), apoE KO, apoE3, E4, and E2/4 mice at 7 months of age using whole-cell patch-clamp electrophysiology. At least 2–4 neurons were analyzed from 10–12 animals per genotype. Neurons were classified based on their firing properties in response to positive (depolarizing) current injection (Faber et al., 2001). Both accommodating and regular firing neurons, characteristic of pyramidal neurons, were used for sEPSC analysis (representative image of a pyramidal neuron in Figure 2A). Fast-spiking neurons, which are characteristic of inter-neurons, comprised less than 1% of the total neurons tested and were eliminated from analysis.

The sEPSC amplitudes and intervals were analyzed from 2 minute continuous recordings. Representative traces from each cohort are illustrated in Figure 1A. A comparison of mean sEPSC amplitudes and intervals are presented in Figure 1B and further summarized in Table 1. The mean amplitudes are similar between each cohort; however, we observed significant differences in the mean interval between each cohort. First, in addition to replicating our previous finding (Wang et al., 2005) where apoE4 mice displayed an increase in the mean sEPSC interval compared to apoE3 mice at 7 months of age, we also observed a similar increase in the interval in apoE KO mice. Secondly, apoE2/4 mice displayed a significant decrease in the mean sEPSC interval compared to apoE4 mice. The sEPSC interval in the C57 mice was most similar to the apoE2/4 mice. Finally, the mean sEPSC interval in the apoE3 mice was significantly less than all other cohorts, suggesting an enhancement in excitatory activity conferred by human apoE3.

1.3.2 Neuronal morphological analysis as a function of age and APOE genotype

Differences in morphological properties of neurons can provide one explanation for different sEPSC frequencies. Thus, we analyzed biocytin-filled neurons from each cohort (originating from the sEPSC studies) for quantitative differences in morphological properties. The apoE3 neurons showed the most complex branching pattern of all genotypes, whereas the apoE4 and apoE2/4 mice exhibited the least complex pattern (Figure 2B) and were significantly

less complex than apoE3. Scholl analysis, which represents the geometrical organization of dendritic arborization, further revealed that apoE4 and E2/4 mice displayed a significant reduction in dendritic length and number of nodes (Table 2) compared to apoE3 mice (Fig. 3). The morphological properties of apoE KO and C57 neurons were not significantly different than apoE3 neurons (Fig. 3, Table 2), although there was a trend toward a decrease in dendritic arbor.

1.3.3 Pubescent apoE4 TR mice display reduced excitatory activity

We recorded sEPSCs from pyramidal-like neurons in the lateral amygdala from C57, ApoE KO, apoE3, E4, and E2/4 mice at 1 month of age to determine whether the synaptic integrity phenotype observed at 7 months was present at an earlier stage of development. We observed no significant difference in the mean amplitude between the various cohorts (Figure 4). However, apoE4 TR mice showed an increase in the mean sEPSC interval that was significantly higher than all other cohorts, except the apoE KO mice. Further comparison of each cohort (between 1 and 7 months) showed that apoE3 synaptic activity did not change with age but the mean sEPSC interval significantly increased in all other cohorts, with the largest increase occurring in the apoE4 mice.

We assessed morphological properties in the apoE3, E4 and E2/4 mice at 1 month of age and observed no statistically significant difference in these cohorts of mice (Table 3). Further comparison of each apoE cohort between 1 and 7 months showed that total dendritic length did not change with age in the apoE3 TR mice but significantly decreased in apoE4 and apoE2/4 mice. All three cohorts also showed a significant decrease in soma area between 1 and 7 months.

Discussion

In the current study, we assessed age-dependent electrophysiological and morphological properties of principle neurons within the lateral amygdala in male human apoE TR, apoE KO, and C57 mice. Our results revealed that the human apoE TR mice display differential effects on synaptic integrity compared to each other, as well as to C57 and apoE KO mice. First, expression of apoE4 results in significantly lower dendritic arbor and excitatory transmission compared to non-apoE4 mice by 7 months of age. Second, co-expression of apoE2 with apoE4 attenuates the loss in synaptic activity, but does not alter dendritic length. This result supports the hypothesis that expression of apoE2 has a protective effect on synaptic integrity. Third, the apoE KO mice display reduced excitatory transmission similar to apoE4 mice, while C57 mice are more similar to apoE2/4 mice at 7 months. Fourth, a significant reduction in excitatory activity is present in apoE4 TR mice as early as 1 month of age while the loss of dendritic arbor has not yet begun to manifest. Finally, all cohorts (except apoE3 mice) show significant reductions in excitatory transmission with age (i.e. 1 to 7 months).

Whole cell recordings in brain slices from 1 month old mice revealed significant differences in excitatory transmission (i.e. sEPSC interval) between apoE4 mice and all other cohorts examined (except apoE KO mice). Although the frequency of synaptic transmission was lowest in the apoE4 mice at 1 month, we did not detect any statistically significant difference in neuronal morphology compared to apoE2/4 or apoE3 mice. Dumanis et. al found significant differences in neuronal morphology (i.e. spine number) within cortical layers II/III of 1 month apoE TR mice (Dumanis et al., 2009). This same study, however did not detect any morphological differences in the dentate gyrus at 1 month. Thus, the effect of *APOE* genotype on neuronal morphology appears to vary from one brain region to another. Comparisons between 1 and 7 month old apoE2/4 and apoE4 mice showed an increase in the sEPSC interval and a decrease in dendritic arbor with no change in the apoE3 mice. We did

not extend morphological analysis to the C57 or KO mice as no significant differences were observed in the sEPSC interval at 1 month when compared to apoE3 mice.

The apoE4-associated deficit in synaptic activity in the lateral amygdala is consistent with previous electrophysiological studies performed in the hippocampus of apoE TR mice. For example, LTP in the dentate gyrus was significantly lower in apoE4 mice compared to apoE3 and C57 mice at 2–4 months (Trommer et al., 2004). In contrast, apoE4 mice displayed enhanced LTP in the CA1 field (Korwek et al., 2009). These regional differences are likely due to the complex circuitry of the hippocampus, where the dentate and CA1 comprise distinct circuitries and differing synaptic connections with various brain regions.

The reduction in synaptic activity in apoE KO is similar to two previous reports showing synaptic alterations in the hippocampus (Krugers et al., 1997) (Veinbergs et al., 1999) however differs from two other studies which found no differences in LTP, behavior or neuronal morphology, between apoE KO mice and controls (C57 mice) (Anderson et al., 1998) (Fagan et al., 1998). We did not detect any difference in neuronal morphology in 7 month old apoE KO mice compared to C57 or apoE3 TR mice. It is possible that synaptic deficits that occur in apoE KO mice do not appear until later in life, as Ji et al found that spine density and dendritic length was reduced in apoE KO mice at 12 months (Ji et al., 2003), although this finding is refuted by a study which found no differences between apoE KO and C57 mice at 12 months (Anderson et al., 1998). These contrasting studies highlight the inherent problems associated with using apoE KO mice to study the function of human apoE isoforms, as compensatory changes in other proteins and the extreme hyperlipidemic phenotype of apoE KO mice make it difficult to draw comparisons with human apoE expressing mice. Analysis of C57 mice showed that they were most similar to apoE2/4 and E3 mice at 1 month; however, by 7 months the C57 mice showed a significant reduction in excitatory activity while the apoE3 mice remained unchanged. This indicates that murine apoE functions differently than human apoE3 in the mouse brain with respect to excitatory transmission in the amygdala. This is not surprising given that murine and human apoE are very distant in evolutionary terms (e.g. only 70% amino acid homology between mouse and human apoE) (Weisgraber, 1994).

The two major findings from this study are; one, the age dependent increase in mean sEPSC interval in all cohorts except the apoE3 TR mice, and two, the attenuation of synaptic activity (mean sEPSC interval) by apoE2 in the apoE2/4 mice. These results indicate a significant *APOE* genotype effect on synaptic transmission in the TR mice. Analogies can be drawn with human cognitive studies which show that both age and *APOE* genotype have a significant impact on cognitive ability (Riley et al., 2002) (Snowdon et al., 2000). Our results also support the idea that the *APOE* alleles function in a co-dominant manner, otherwise the phenotype in the apoE2/4 mice would be indistinguishable from the apoE4 mice. Our results also support human gene association studies for relative risk of AD between varying *APOE* genotypes (Roses et al., 1995). For example, *APOE2/4* heterozygotes have a significantly lower risk of developing AD compared to *APOE4/4* homozygotes (Saunders et al., 1993) (Corder et al., 1993).

In our earlier study (Wang et al., 2005) we were able to show a correlation between reduced synaptic transmission and reduced dendritic arbor in the apoE4 mice at 7 months. Herein we found 1 month old apoE4 mice displayed reduced synaptic transmission compared to apoE2/4 and apoE3 mice, however we did not detect any differences in neuronal morphology between the three cohorts. Since we were unable to measure spines in the 1 month cohorts it is possible that varying numbers of spines could account for the difference in synaptic activity. We are encouraged by the study in similar cohorts of mice (1 month TR mice) which found varying spine counts, albeit in a different brain region (Dumanis et al.,

2009). We plan to use Golgi staining techniques in future studies to aid in determining the number of spines, since we were unable to reliably count spines in any of these cohorts due to technical limitations with imaging the biocytin-filled cells.

We were able to measure other characteristics of neuronal morphology, however, and confirmed our original finding in the 7 month cohort which showed significant differences between apoE3 and E4 mice. Analysis of the apoE2/4 mice, however, showed no difference in dendritic length compared to apoE4 mice, which again could be explained by varying spine number. In the current study we elected not to use apoE2 homozygous mice in order to avoid any potential confounding effects of hyperlipidemia on synaptic integrity (Sullivan et al., 1998). Future analysis of functional and morphological outcomes using hemizygous apoE TR mice (i.e. E2/E0 and E4/E0) could provide insight into the individual contribution of each isoform; however the apoE2/0 mice would be expected to develop type III hyperlipidemia, a similar confounding variable present in apoE2 and apoE KO homozygous mice.

The mechanism responsible for the reduction in synaptic transmission associated with apoE4 is currently unknown, but several possibilities may account for this effect. The lateral amygdala receives excitatory/glutamatergic afferents originating from the cortex and thalamus (McDonald et al., 2002) and age-dependent pruning of dendrites and spines in cortical and thalamic afferents may contribute to the loss of excitatory activity. Since loss of synaptic integrity within the cortical limbic system is thought to begin very early in AD, potential disruption of these connections in the apoE4 TR mice could explain the reduction in synaptic transmission. While inhibitory inputs can also impact the degree of excitatory activity, our previous report showed no differences in the amplitude or frequency of spontaneous inhibitory synaptic currents (sIPSCs) between apoE3 and E4 (Wang et al., 2005). It is plausible that the reduction in sEPSCs observed in apoE4 mice is due to the concomitant decrease in dendritic length of the post-synaptic cell. However, this does not explain our result seen in apoE2/E4 mice.

We had previously suggested that one explanation for the observed apoE4 deficit (at 7 months) was due to a deficiency in lipids required for neuronal remodeling (Wang et al., 2005). The idea that apoE might regulate synaptic transmission via the distribution of lipids in neuronal membranes was first proposed by Mahley et al. (Mahley, 1988) and Poirier et al. (Poirier, 1994). It is now known that synaptic plasticity depends on glial derived cholesterol (Goritz et al., 2002) which is delivered by apoE-containing HDL particles, and that phospholipids are required for maintaining synaptic plasticity (Koudinov and Koudinova, 2001) (Hering et al., 2003) (Bourre et al., 1989) . Therefore, apoE4 (relative to apoE3 and E2) may be deficient in delivery of essential lipids for maintaining synaptic plasticity.

The observed apoE4 deficit in synaptic integrity could also be due to insufficient levels of functional apoE4 protein which would result in a rate-limiting supply of lipids required for synaptic plasticity. We and others recently showed that protein levels are lowest in the hippocampus and cortex of 3–4 month old male apoE4 mice compared to apoE3 and E2 mice (Sullivan et al., 2009) (Riddell et al., 2008). Another group found a trend towards lower apoE protein expression in the amygdala of female apoE4 TR mice (compared to apoE3 and apoE2 mice) however, no analysis of male TR mice was performed (Siegel et al., 2010). Future studies are needed to determine the effects of gender and age on apoE protein levels.

Human *APOE4* carriers display signs of cognitive impairment by the second decade of life (Bourre et al., 1989, Murthy et al., 2002)[41] (Ganguli et al., 2000) (Scarmeas et al., 2005) and on into middle age (Blair et al., 2005) (Wishart et al., 2006). Amygdalar volumes are

also significantly reduced in probable AD patients based on MRI morphometry (Cuenod et al., 1993) (Basso et al., 2006). Determining when cognitive impairment begins in *APOE4* carriers may reveal insight into the etiology of late onset AD and improve strategies for intervention. Here we provide the first electrophysiology studies in the human equivalent of "pre-adolescents" (i.e. pubescent 1 month old TR mice) which reveal an age and *APOE* genotype effect on synaptic transmission in the amygdala. Ongoing and future studies in our lab aim to investigate the long-term impact of apoE isoforms on synaptic integrity in aged animals.

Abbreviations

AD	Alzheimer's disease
apo	apolipoprotein (refers to the protein or isoform)
APOE	designation for human apolipoprotein E gene
CNS	central nervous system
C57	murine wild type strain
sEPSC	spontaneous excitatory post-synaptic currents
KO	knock out
LDLR	low density lipoprotein receptor
LTP	long term potentiation
TR	targeted replacement

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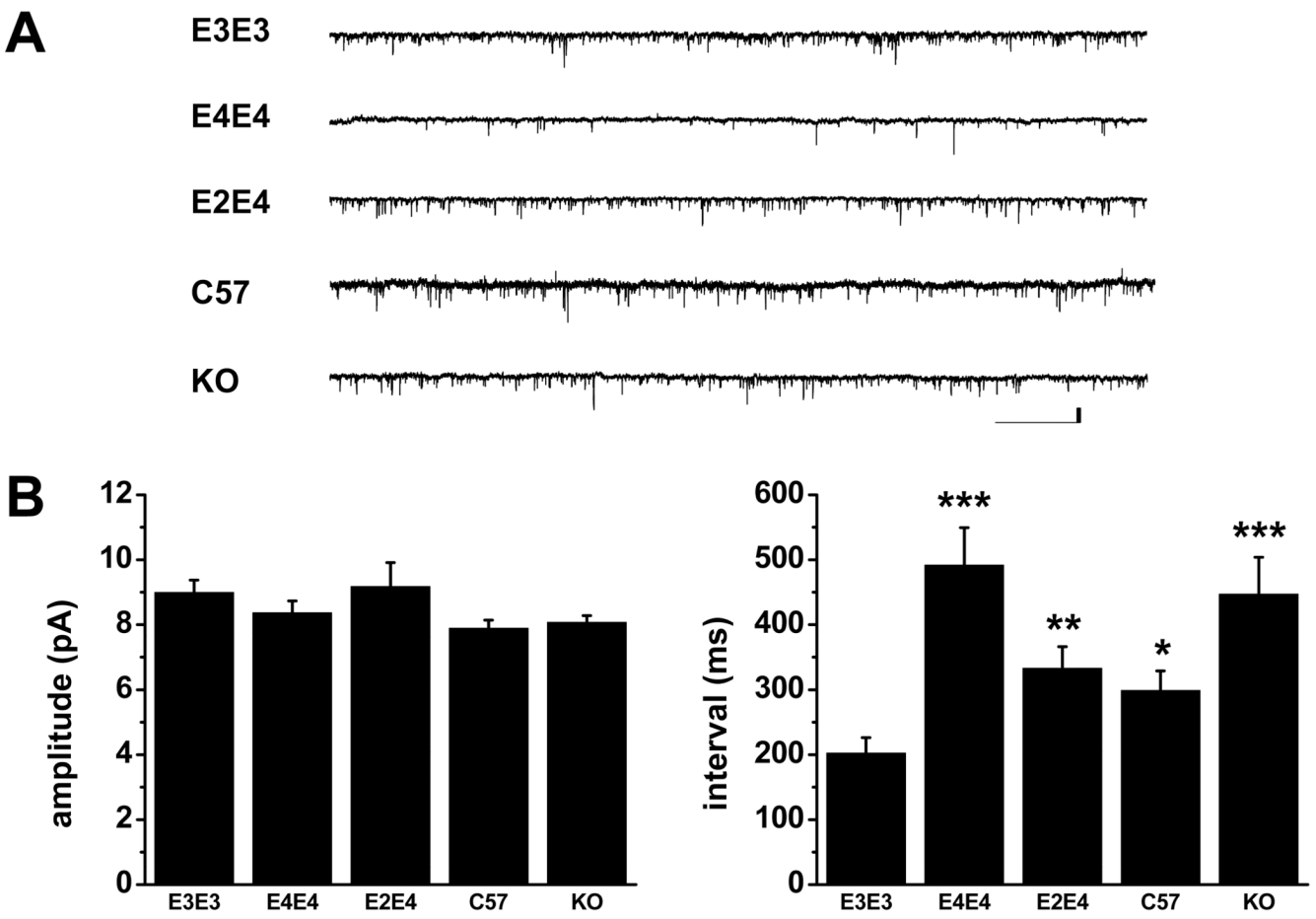

Figure 1.

A. Representative traces of whole-cell patch-clamp recordings illustrating sEPSCs in lateral amygdala neurons from designated cohorts at 7 months of age. Scale bar is 10 pA, 2 ms. B. Comparison of mean ± SEM amplitude (left) and interval (right) of sEPSCs at 7 months of age (C). No significant differences in amplitude occurred. Compared to E3/E3, significant differences in the sEPSC interval were observed in C57 (* $p<0.05$), E2/E4 (** $p<0.005$), E4/E4 and KO (*** $p<0005$). There was also a significant difference ($p<0.01$) between the E4/E4 and E2/E4 cohorts. See Table 1 for N values.

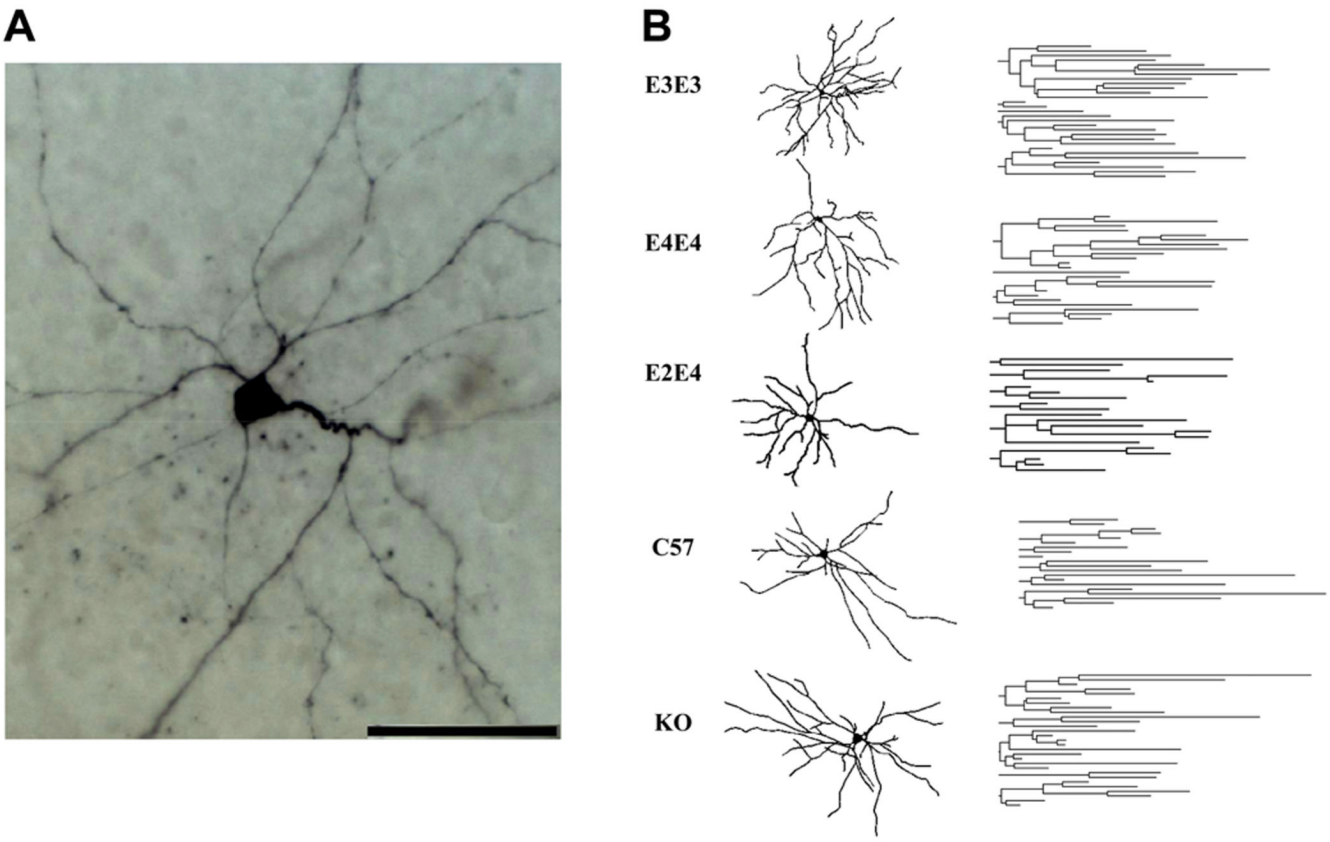

Figure 2.

A. High power image of a representative pyramidal-like lateral amygdala neuron from an apoE3 TR mouse at 7 months of age (scale bar, 50 μm). B. Camera lucida reconstructions (left) and dendrograms (right) corresponding to representative neurons from the indicated cohorts. Quantitative morphological analysis is presented in Table 2.

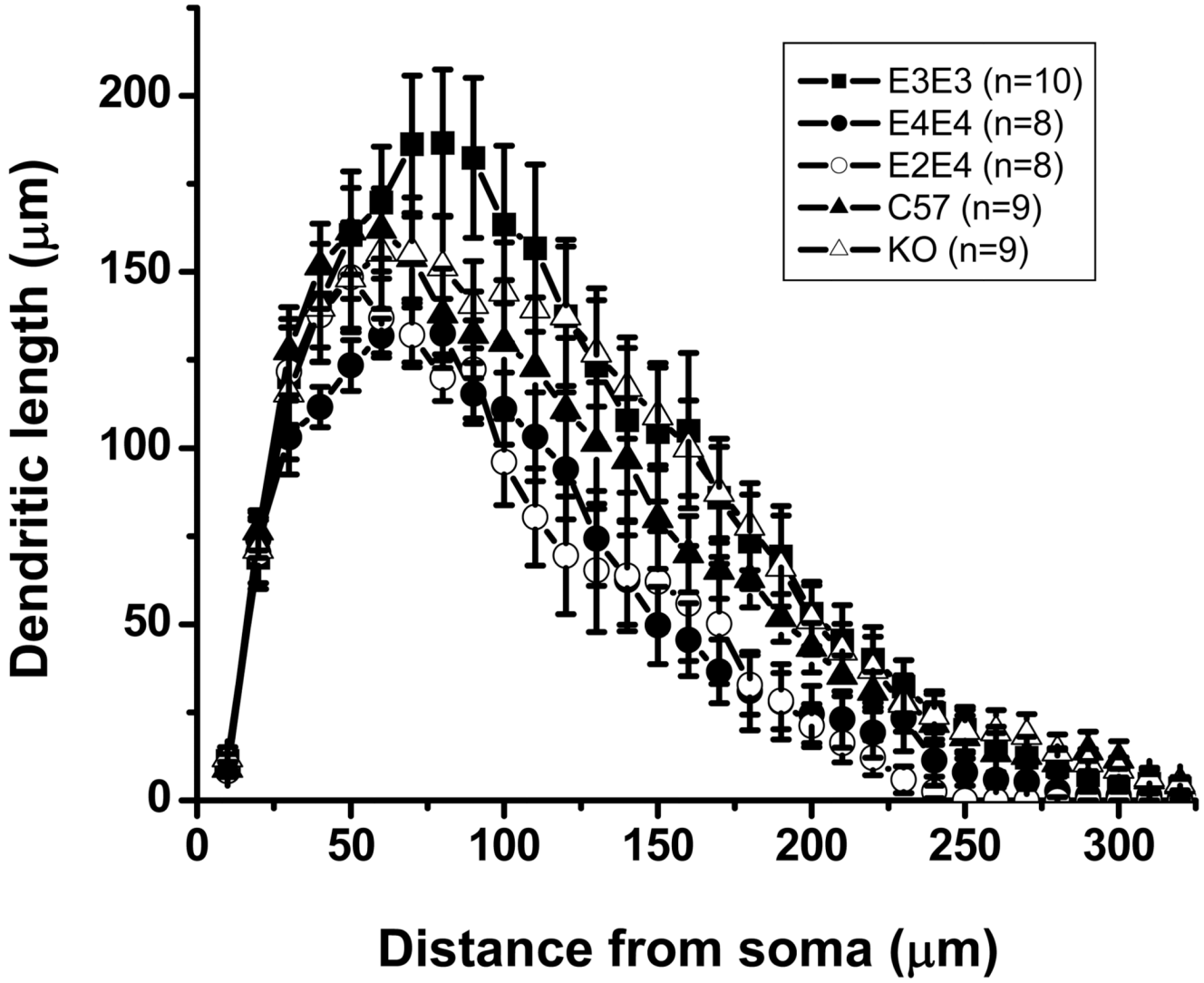

Figure 3.
Scholl analysis of averaged lateral amygdala neurons from the indicated ApoE cohort at 7 months of age representing the dendritic length and the number of dendritic intersections crossing each 10 μm radius progressively more distal to the soma. See Table 2 for N values.

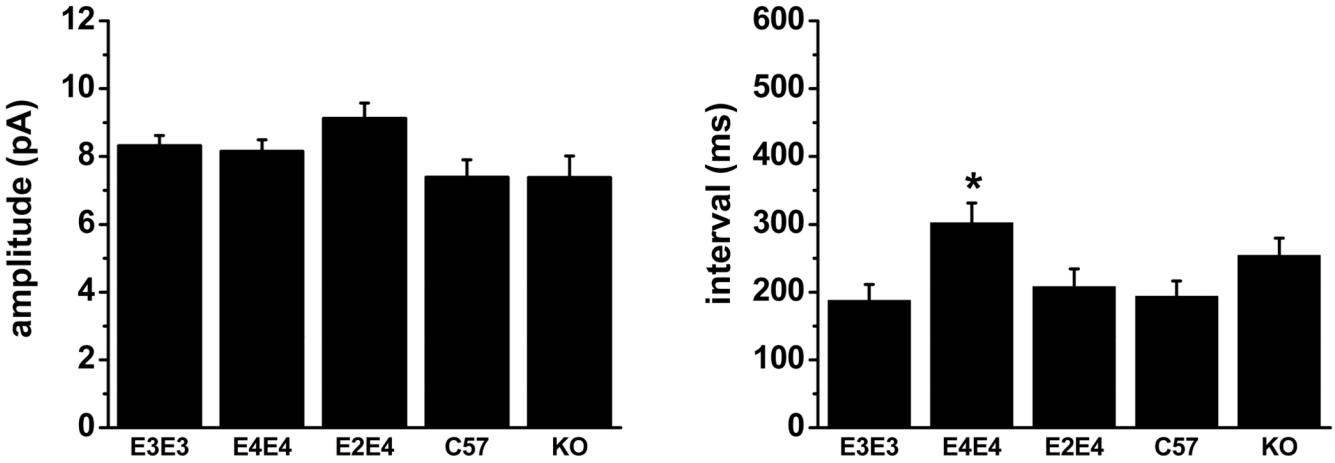

Figure 4.

Comparison of mean ± SEM amplitude (left) and interval (right) of sEPSCs at 1 month of age. The mean interval was significantly lower in apoE4 TR mice compared to apoE3 ($p<0.005$), apoE2/E4 ($p<0.05$) and C57 ($p<0.01$). See Table 1 for N values.

Table 1

Summary of mean sEPSC amplitudes and intervals from pyramidal-like neurons in the lateral amygdala.

	1 month			7 months		
Genotype	**# of mice (neurons)**	**Amplitude (pA)**	**Interval (ms)**	**# of mice (neurons)**	**Amplitude (pA)**	**Interval (ms)**
E3/E3	11 (23)	8.3 ± 0.3	188 ± 24*	12 (31)	9.0 ± 0.4	202 ± 24
E4/E4	12 (28)	8.2 ± 0.3	302 ± 29	10 (26)	8.4 ± 0.4	492 ± 58***
E2/E4	8 (24)	9.1 ± 0.5	208 ± 26**	12 (23)	9.2 ± 0.7	333 ± 33**
C57	6 (20)	7.4 ± 0.5	194 ± 23***	11 (26)	7.9 ± 0.3	299 ± 30*
KO	8 (19)	7.4 ± 0.6	254 ± 26	12 (30)	8.1 ± 0.2	447 ± 57***

At 7 months of age, significant differences in mean ± SEM sEPSC intervals compared to apoE3/E3 are noted (*p<0.05, **p<0.005 and ***p<0.0005. There was also a significant difference between apoE4/E4 and apoE2/E4 (p<0.01) at 7 months. At 1 month of age, significant differences in mean ± SEM sEPSC intervals compared to apoE4/E4: *p<0.005, **p<0.05) and ***p<0.01. Within each cohort, there was a significant difference in mean interval between 1 month and 7 months (p<0.05) for all groups except apoE3.

Table 2

Morphological properties of pyramidal-like neurons in the lateral amygdala from 7 month old ApoE TR mice

	E3/E3 (n=10)	E2/E4 (n=7)	E4/E4 (n=8)	C57 (n=9)	KO (n=8)
Soma area (μm^2)	110 ± 12	102 ± 6	114±13	140±14	132 ± 13
Number of primary dendrites	4.5 ± 0.3	4.7 ± 0.4	4.8 ± 0.4	4.4 ± 0.4	4.6 ± 0.5
Number of nodes Total	18.0 ± 1.9	14.7 ± 0.4*	12.6 ± 1.5*	17.4 ± 1.4	16.8 ± 1.7
dendritic length (μm)	2635 ± 322	1696 ± 122*	1697 ± 144*	2247 ± 158	2677 ± 280

Significant differences are in comparison to apoE3/E3 (*p<0.05).

Table 3

Morphological properties of pyramidal-like neurons in the lateral amygdala from 1 month old ApoE TR mice

	E3/E3 (n=10)	E2/E4 (n=7)	E4/E4 (n=10)
Soma area (μm^2)	182 ± 17	150 ± 9	178 ± 16
Number of primary dendrites	5.3 ± 0.5	5.1 ± 0.3	5.6 ± 0.3
Number of nodes	19.8 ± 1.8	21.6 ± 1.7	17.9 ± 1.4
Total dendritic length (μm)	2862 ± 225	2799 ± 270	2423 ± 230

There were no significant differences between the three apoE cohorts; however, apoE4/E4 exhibited a trend toward a decrease in the number of nodes and total dendritic length.