Research Article

Opposing Roles of apolipoprotein E in aging and neurodegeneration

Eloise Hudry1, Jacob Klickstein1, Claudia Cannavo2, Rosemary Jackson2, Alona Muzikansky3, Sheetal Gandhi1, David Urick1, Taylie Sargent1, Lauren Wrobleski1, Allyson D Roe1, Steven S Hou1, Kishore V Kuchibhotla4, Rebecca A Betensky3, Tara Spires-Jones2, Bradley T Hyman1

Apolipoprotein E (APOE) effects on brain function remain controversial. Removal of APOE not only impairs cognitive functions but also reduces neurtic amyloid plaques in mouse models of Alzheimer’s disease (AD). Can APOE simultaneously protect and impair neural circuits? Here, we dissociated the role of APOE in AD versus aging to determine its effects on neuronal function and synaptic integrity. Using two-photon calcium imaging in awake mice to record visually evoked responses, we found that genetic removal of APOE improved neuronal responses in adult APP/PSEN1 mice (8–10 mo). These animals also exhibited fewer neuritic plaques with less surrounding synapse loss, fewer neuritic dystrophies, and reactive glia. Surprisingly, the lack of APOE in aged mice (18–20 mo), even in the absence of amyloid, disrupted visually evoked responses. These results suggest a dissociation in APOE’s role in AD versus aging: APOE may be neurotoxic during early stages of amyloid deposition, although being neuroprotective in latter stages of aging.

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Introduction

Apolipoprotein E (APOE) in the central nervous system (CNS) has been the focus of study for two reasons: it is the most abundantly expressed apolipoprotein in the CNS (Bjorkhem & Meaney, 2004; Huang & Mahley, 2014), and inheritance of the E4 allele of the APOE gene profoundly impacts the risk for Alzheimer’s disease (AD), exacerbating amyloid deposition and worsening cognition and synapse loss. We, therefore, sought to examine the effect of APOE depletion on neuronal function and synaptic integrity in adult or aged mice in both physiological and pathophysiological contexts.

As the primary CNS apolipoprotein, APOE is responsible for much of the regulation of the brain lipid metabolism, particularly the transfer of cholesterol and phospholipids from glial cells to neurons (Boyles et al, 1985; Pitas et al, 1987; Pfrieger & Ungerer, 2011). During adulthood, neurons rely on cholesterol from glial cells for many processes; thus APOE plays an important role in modulating synapse growth, stabilization, and renewal in a physiological context (Holtzman & Fagan, 1998; Mauch et al, 2001). APOE is also involved in removing cholesterol and lipids from the CNS, therefore controlling the clearance of cellular debris and promoting remyelination in the aged CNS and some neurodegenerative diseases (Mahley, 1988; Zlokovic, 2011; Cantuti-Castelvetri et al, 2018). Other functions of APOE in the neural tissue include buffering oxidative stress (Evolta et al, 2010; Chen et al, 2015) and preserving the integrity of the blood–brain barrier (Fullerton et al, 2001; Hafezi-Moghadam et al, 2007; Nishitsuji et al, 2011), further emphasizing the pivotal role of APOE in maintaining brain homeostasis.

Previous studies have observed that a complete lack of APOE in murine models leads to cognitive impairment when compared with wild-type mice (Gordon et al, 1995; Masliah et al, 1997; Kitamura et al, 2004; Trommer et al, 2004; Yang, Gilley et al, 2011a; Zerbi et al, 2014), whereas others failed to detect similar deficits (Hartman et al, 2001; Bour et al, 2008). A recent case study of a 40-yr-old man with a complete absence of APOE expression initially reported normal cognitive function (despite dramatic hypercholesterolemia [Mak et al, 2014]), but a second in-depth evaluation showed some evidence of cognitive impairment (Cullum & Weiner, 2015). Whether these discrepancies result from the use of different cognitive tasks or from the age of the animals and subjects included in each study is unclear, but there is no doubt that further investigation of the specific impact of APOE on neuronal function in vivo remains an important unmet goal.

In the context of disease, APOE was identified more than two decades ago as a significant modulator of the risk for late-onset AD (Wisniewski & Frangione, 1992; Corder et al, 1993, 1994; West et al, 1994; Hyman et al, 1996; Lippa et al, 1997). APOE is a well-established partner of amyloid β (Aβ) peptides, catalyzing Aβ oligomerization, aggregation in the parenchyma (Holtzman et al, 2000; Fagan et al, 2002; Hashimoto et al, 2012), clearance (Deane et al, 2008;
Castellano et al, 2011; Hudry et al, 2013), and recruitment to the synapse (Koffie et al, 2012). More recently, APOE has also been identified as a molecular trigger of the amyloid-dependent neuroinflammatory response via its role as a ligand for the triggering receptor expressed on myeloid cells 2 (TREM2) (Atagi et al, 2015; Yeh et al, 2016). Disruption of the murine apoe gene in AD transgenic models significantly delays the formation of the so-called “dense core” Thio-S–positive amyloid plaques (Bales et al, 1997; Irizarry, Cheung et al, 2000a), even though substantial load of diffuse amyloid and elevated concentrations of soluble Aβ peptides remain in the parenchyma (Irizarry, Rebeck et al, 2000b). These results suggest that a complete lack of APOE may have a beneficial impact on amyloidopathy, a hypothesis recently validated using an approach by antisense oligonucleotide-based knockdown of APOE in mouse models of amyloidosis (Huynh et al, 2017). However, the consequences of APOE genetic disruption on neuronal function and synaptic integrity are still being debated. The question remains if APOE can simultaneously protect and impair brain homeostasis. The present study aims to examine both APOE’s role in normal physiology and in Aβ-induced neurotoxicity. To do so, we investigate how APOE affects neuronal function and synaptic integrity rather than only focusing on amyloid changes.

In this study, we used two-photon calcium imaging in the visual cortex to measure visually evoked neuronal responses (Andermann et al, 2011; Grienberger et al, 2012; Kuchibhotla et al, 2014) and array tomography to assess synapse density at a single-synapse resolution (Koffie et al, 2012; Tai et al, 2012; Kay et al, 2013). We systematically evaluated the impact of the presence or absence of APOE on neuronal function and synaptic integrity in mice that develop plaques (APP/PSEN1 mice expressing both the human mutated Amyloid precursor protein, APP, and presenilin-1, PSEN1, genes) and those without increased amyloidosis (wild-type). Our results demonstrate that in the context of amyloid pathology, APOE enables Aβ-dependent neuronal dysfunction and synaptotoxicity and a dramatic protective effect is observed by ApoEnull. By contrast, APOE also appears as an important factor to preserve brain function during aging, even in the absence of amyloid deposition. These findings, therefore, dissociate APOE’s role towards amyloid neuropathological changes versus normal aging and warrant further consideration of the impact of APOE on neuronal function in addition to its effect on amyloid.

Results

Disruption of visually evoked responses in transgenic mice model of amyloidosis

To establish how APOE modulates neuronal function and amyloid-dependent dysfunction in vivo, we recorded neuronal calcium transients triggered by visual stimulation in wild-type, APOEnull, APP/PSEN1, and APP/PSEN1/APOEnull mice (Fig 1A). The study of neuronal dysfunction in the visual cortex is relevant to AD, as deficits in central sensory processing have been reported in the disease, particularly at advanced stages (Cronin-Golomb et al, 1991; Bublak et al, 2011). The visual area V1 is also easily accessible for intra-vital calcium imaging (Andermann et al, 2011) and constitutes the output of a relatively simple circuitry downstream of the retina and the lateral geniculate nucleus within the thalamus (Seabrook et al, 2017), therefore facilitating the recording of neuronal responses to well-controlled sensory stimuli. In addition, two age groups were included in our study (8–10-mo-old “adult” mice and...
18–20-mo-old “aged” mice) to determine the impact of age, genotype, and the interaction of both parameters (Fig S1A). We could record robust neuronal responses to the visual stimuli in all experimental groups. In our stimulation protocol, responding cells were detected in the primary visual area (mean = 6.89 ± 1.05% for wild-type littermates; 13.76 ± 2.13% for the APP/PS1; 9.75 ± 2.3% for the APP/PSEN1 mice, Fig S1A and B). Within the same field of view, a wide range of different response patterns was observed, including nonresponsive cells (no correlation between calcium transients and visual stimulation), broadly responsive cells (increased firing simultaneously to any stimulus), and visually tuned neurons responding to a specific orientation and/or direction of the visual stimulus (Fig 1B). There was no statistical difference between the percentage of “responding cells” among the experimental groups (Fig 2A, *P = 0.0682), with variability depending whether or not the region of interest (ROI) considered was exactly located within the primary visual cortex area or at the edge of it (Fig S2).

When establishing our protocol, we observed that in contrast to the broadly tuned cells that responded to any stimuli, occasional neurons systematically fired when the stimuli went off (which we defined as “off-responders,” Fig 2B), a phenomenon previously described when recording visual responses in awake animals (Jin et al, 2008; Liang et al, 2008). When we calculated the percentage of “off-responding” neurons in comparison with the overall number of responding cells within the same fields of view, we observed a significant increased proportion of “off-responders” specifically in APP/PSEN1 mice (16.57 ± 4.68%, Kruskal–Wallis test, *P = 0.0068, Fig 2C), which tended to be exacerbated in aged as compared with adult transgenic APP/PSEN1 animals (even though the difference between “adult” and “aged” APP/PSEN1 did not reach statistical significance, Fig S3). Intriguingly, the percentage of off-responding neurons was much lower in all the other groups considered (wild-type: 4.32 ± 1.27%; APOE<sup>null</sup>: 4.68 ± 1.19%; and APP/PSEN1/APOE<sup>null</sup> mice: 3.2 ± 0.84%), and especially in APP/PSEN1/APOE<sup>null</sup> mice lacking apoe expression, suggesting that APOE<sup>null</sup> mice normalizes an alteration from normal physiology observed in APP/PSEN1 mice.

ApoE genetic ablation in adult APP/PSEN1 mice restores visual selectivity but sensitizes the brain towards age-associated neuronal dysfunction.

To gain further insight into the functional integrity of the visual network between wild-type, APOE<sup/null</sup>, APP/PSEN1, and APP/PSEN1/APOE<sup/null</sup> mice, we then compared the direction selectivity index (DSI) and orientation selectivity index (OSI) between these groups (Fig 3A and B). DSI and OSI represent quantifiable measurements of the ability of visual neurons to respond to a principal orientation or direction of a visual stimulus as compared with others, and higher DSI and OSI values correspond to a more specific tuning to the stimulation. Of importance, this functional feature (the tuning of visual neurons to stimulation) has been previously reported to be impaired in mouse models of amyloidopathy (Grienberger et al, 2012). After fitting a linear mixed model with genotype and “age” as fixed effect and “mouse” as random effect, we demonstrated that the log-adjusted DSI and OSI were significantly associated with the genotype (*P = 0.0013 for DSI and *P = 0.0076 for OSI) and with the age of the mice (*P = 0.0325 for DSI and *P = 0.0068 for OSI). In particular, the average of both DSI and OSI was lower in adult APP/PSEN1 mice as compared with wild-type animals (*P = 0.0002 and *P = 0.0008, respectively), therefore demonstrating impaired tuning to visual

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For the figures and tables, please refer to the original document.
stimulation in this model of amyloidosis. This was not the case between the control and APP/PSEN1/APOE<sup>null</sup> groups (P = 0.4091 and P = 0.1584). These results, therefore, demonstrated that the absence of APOE largely abrogates A<sub>β</sub>-dependent neuronal dysfunction in a mouse model of amyloidosis. However, APOE<sup>null</sup> mice still showed a mild decreased OSI average when compared with wild-type controls (P = 0.0294), implying that the complete lack of APOE itself can impact tuning performances.

Pair-wise comparisons between adult and aged mice (Fig S4A) revealed more prominent age-dependent decreases of the OSI values in APP/PSEN1/APOE<sup>null</sup> (P = 0.037), with a similar but non-significant decrease in APOE<sup>null</sup> (P = 0.109) mice. No age-dependent change was detected for the control or APP/PSEN1 groups, which respectively presented with high or low OSI means across the life-span of the mice. The DSI values showed fewer differences with only a significant decreased average of the DSI detected in aged APP/PSEN1 mice (P = 0.0445). The higher sensitivity of OSI to discriminate changes in visual tuning can be explained by the fact that the OSI was calculated based on the tuning discrimination between the "favorite" orientation as compared with three other orientations, whereas the DSI was calculated based on preferential firing for one direction as compared with the opposite one. When compared with age-matched littermates (Fig S4B), both adult and aged APP/PSEN1 mice were significantly impaired for OSI and DSI (P = 0.011 for DSI and P = 0.0012 for OSI when comparing adult APP/PSEN1 and littermates; and P = 0.022 for DSI and P = 0.042 for OSI when comparing aged APP/PSEN1 and littermates). However, only the OSI values were significantly diminished in aged APOE<sup>null</sup> mice as compared with aged controls (P = 0.04). The averaged OSI in aged APP/PSEN1/ApoE<sup>null</sup> mice was also lower than wild-type animals, but this difference did not reach significance (P = 0.084). These results demonstrated early and pronounced neuronal dysfunction in mice model of A<sub>β</sub> amyloidosis, a phenotype significantly improved in adult mice lacking APOE. However, age-dependent deficits were also detected in older APOE<sup>null</sup> and, to a lesser extent, APP/PSEN1/APOE<sup>null</sup> mice. We concluded that APOE is a necessary factor participating in amyloid-dependent loss of network integrity, although complete lack of APOE independently triggers mild neuronal dysfunction with age.

ApoE genetic ablation does not impact global amyloid burden but affects the aggregation state of A<sub>β</sub>

To evaluate how the "rescue effect" observed in mice lacking APOE correlated with the level of amyloid pathology, we performed a
stereological analysis of the load and density of amyloid plaques in the cortex of APP/PSEN1 and APP/PSEN1/APOE<sup>null</sup> mice. Conventional anti-Ab immunolabeling detecting all amyloid aggregates revealed that neither the burden nor the density of deposits was significantly different between APP/PSEN1 and APP/PSEN1/APOE<sup>null</sup> (Fig 4A–C). By contrast, a dramatic decrease in the amount of fibrillar deposits stained by Methoxy-XO<sub>4</sub> was observed in mice lacking APOE (Fig 5D; P < 0.0001 and P = 0.0004, respectively, for Methoxy load and density between APP/PSEN1 and APP/PSEN1/APOE<sup>null</sup> mice), in agreement with previous reports showing that APOE significantly affects the aggregation state of Ab neurotoxic species (Irizarry et al, 2000a; Holtzman et al, 2000; Fagan et al, 2002).
Figure 5. Ab-dependent synaptic loss and neuritic dystrophies in adult APP/PSEN1 and APP/PSEN1/APOEnull mice.
(A) Representative images of array tomography ribbons after immunostaining for Ab (1C22), presynaptic terminals (antisynapsin I, red), postsynaptic densities (anti-PS95, green), and nuclei (DAPI, blue). It is possible to appreciate how diffuse is the amyloid in the brain of APP/APOEnull mouse as compared with the compact staining observed in the APP mouse. Scale bar = 10 μm. (B) Scatter dot plots summarizing the density of PSD95 (left panel) and synapsin (right panel)-positive puncta in the cortex of wild-type, APOEnull, APP/PSEN1, and APP/PSEN1/APOEnull mice. A significant decrease in synaptic density was only observed in the close vicinity of amyloid plaques in AD transgenic mice. n = 6 mice/group (10–12 mo “adult” cohort); Two-way ANOVA and Tukey’s post hoc test. *P < 0.05 and ***P < 0.001. (C) Representative images of neuritic dystrophies (arrows) around amyloid plaques observed in APP/PSEN1 and APP/PSEN1/APOEnull mice, showing that most sprouting axons are found in mice expressing endogenous murine apoE. Scale bar = 50 μm. (D) Scatter dot plot summarizing the stereological quantification of the number of dystrophies observed per area of amyloid (evaluated by immunohistological staining). n = 6 mice per group (10–12 mo “adult” cohort); Mann–Whitney test. *P < 0.005.
Consequently, the ratio of amyloid load between “all deposits” and dense core plaques was significantly higher in APP/PSEN1/APOE<sup>null</sup> as compared with APP/PSEN1 mice (Fig 4D). Within each genotype, the amyloid load progressed between adult and aged mice, but the increase in Methoxy-XO<sub>4</sub>-positive plaques was not as prominent as the changes observed when both diffuse and compact deposits were analyzed altogether (Fig S5). To complete this analysis, we determined the concentrations of Aβ40 and Aβ42 in the TBS and formic acid (FA) fractions of the brains, and we observed a general decrease in the amounts of Aβ species in APP/PSEN1/APOE<sup>null</sup> mice as compared with APP/PSEN1 [Fig 4E–G; P < 0.0001, respectively, for TBS soluble Aβ40 and Aβ42, P = 0.0004 and P = 0.019, respectively, for FA soluble Aβ40 and Aβ42]. Interestingly, these differences were essentially due to the substantial levels of amyloid peptides in aged APP/PSEN1 animals (Fig S6A–C), whereas the amounts of both soluble and insoluble Aβ40/42 were comparable between the adult cohorts (for which the lack of APOE alleviates Aβ-dependent neuronal dysfunction). These data emphasize the weak correlation that exists between the global amount of amyloid and Aβ-dependent neuronal dysfunction. These data support the hypothesis that the improved visual tuning observed in APP/PSEN1/APOE<sup>null</sup> animals (8.51 × 10<sup>8</sup> ± 76.51 × 10<sup>7</sup> PSD95 puncta/mm<sup>3</sup> and 3.54 × 10<sup>8</sup> ± 6.83 × 10<sup>7</sup> synapsin puncta/mm<sup>3</sup>) and APOE<sup>null</sup> (9.83 × 10<sup>8</sup> ± 2.42 × 10<sup>7</sup> PSD95 puncta/mm<sup>3</sup> and 9.89 × 10<sup>8</sup> ± 3.54 × 10<sup>7</sup> synapsin puncta/mm<sup>3</sup>) mice (Fig S9B), with an averaged density of PSD95 and synapsin puncta close to plaques of 6.83 × 10<sup>8</sup> ± 2.76 × 10<sup>7</sup>/mm<sup>3</sup> and 7.24 × 10<sup>8</sup> ± 3.14 × 10<sup>7</sup>/mm<sup>3</sup>, respectively, as compared with 9.99 × 10<sup>8</sup> ± 3.21 × 10<sup>7</sup> and 10.30 × 10<sup>8</sup> ± 3.26 × 10<sup>7</sup> synapses/mm<sup>3</sup> far from plaques (P < 0.0001). This decrease in synaptic density in the direct vicinity of amyloid in APP/PSEN1 mice was also significant compared with wild-type (9.77 × 10<sup>8</sup> ± 3.63 × 10<sup>7</sup> × 10<sup>8</sup> synapsin puncta/mm<sup>3</sup> and 10.82 × 10<sup>8</sup> ± 4.46 × 10<sup>7</sup> synapsin puncta/mm<sup>3</sup>; P < 0.0001) and APOE<sup>null</sup> (9.83 × 10<sup>8</sup> ± 2.42 × 10<sup>7</sup> × 10<sup>8</sup> synapsin puncta/mm<sup>3</sup> and 9.89 × 10<sup>8</sup> ± 3.54 × 10<sup>7</sup> synapsin puncta/mm<sup>3</sup>; P < 0.0001) controls. Interestingly, no drop of the synaptic density was detected close to plaques in APP/PSEN1/APOE<sup>null</sup> mice (8.51 × 10<sup>8</sup> ± 76.51 × 10<sup>7</sup> × 10<sup>8</sup> synapsin puncta/mm<sup>3</sup> and 9.53 × 10<sup>8</sup> ± 7.03 × 10<sup>7</sup> synapsin positive puncta/mm<sup>3</sup>), which was significantly higher than the synaptic density measured in the vicinity of amyloid deposits in APP/PSEN1 mice (P = 0.0345), and comparable with wild-type and APOE<sup>null</sup> animals. These results, thus, imply that the presence of both Aβ and APOE is necessary to observe synaptic collapse in AD transgenic mice and that abolishing apoE expression suffices to rescue amyloid-dependent synaptic loss and the functional impairments observed in APP/PSEN1 mice. Intriguingly, when the accumulation of Aβ at the synapse in APP/PSEN1 and APP/PSEN1/APOE<sup>null</sup> was measured as the percentage of pre- and post-synaptic densities co-localizing with amyloid, no significant difference could be observed between those groups (Fig S9A), thus challenging the concept of APOE acting as a chaperone mediating Aβ accumulation at the synapse. In addition, the synaptic volume decreased significantly close to plaques in APP/PSEN1 mice, a parameter that remained unchanged in the absence of APOE (Fig S9B).

The absence of APOE decreases Aβ-associated neuritic anomalies and glial reactivity

To complete our evaluation of the impact of APOE on Aβ-dependent neurotoxic effects in the microenvironment of amyloid plaques, we performed a stereological analysis of the density of neuritic dystrophies around each deposit (Fig 5C) and Aβ-dependent glia reactivity in the cortex of APP/PSEN1 and APP/PSEN1/APOE<sup>null</sup> animals (adult cohort). Although numerous abnormal neurites were generally associated with amyloid plaques in APP/PSEN1 mice, they were seldom in APP/PSEN1/APOE<sup>null</sup> animals (Fig 5D, P = 0.043). In addition, when we evaluated the number of reactive...
astrocytes (Glia fibrillary acidic protein, GFAP, positive) and microglia (Iba1 positive) clustered around amyloid aggregates in APP/PSEN1 and APP/PSEN1/APOE<sup>null</sup> mice, we observed a significant decreased density of both glial cell types in animals lacking APOE (Fig 6, \( P = 0.0006 \) for microglia and \( P = 0.0012 \) for astrocytes). These results not only establish APOE as an essential modulator of amyloid deposition but also demonstrate its strong impact on a large panel of Aβ-associated neurotoxic events and downstream inflammatory reactions in the local vicinity of amyloid plaques. Whether or not these effects are a direct consequence of the change in the aggregation state and toxicity of Aβ or indirectly related to the impact of a lack of APOE on microglia/astroglia function remains to be determined.

Discussion

Neuronal dysfunction and loss of synaptic integrity are tightly associated with aging and AD cognitive decline, but understanding the molecular bases of those changes and identifying possible modulators remain as important challenges to overcome. APOE, the major apolipoprotein of the brain and most important genetic contributor to the sporadic form of AD (Wisniewski & Frangione, 1992; Corder et al, 1993, 1994; West et al, 1994; Hyman et al, 1996; Lippa et al, 1997), is a well-established partner of amyloid β peptides and has been shown to directly impact the aggregation, deposition, and clearance of those neurotoxic species (Holtzman et al, 2000; Fagan et al, 2002; Deane et al, 2008; Castellano et al, 2011; Hashimoto et al, 2012; Koffie et al, 2012; Hudry et al, 2013). Although those findings clearly emphasize the pivotal role of APOE in AD neuropathological changes, the field is still divided as to whether or not clinical benefit could be achieved by reducing the levels of APOE (Koldamova et al, 2005; Bien-Ly et al, 2012; Liao et al, 2014; Lane-Donovan et al, 2016; Zheng et al, 2017). In addition, previous studies have described that a complete lack of APOE in a physiological context may lead to a loss of brain function during aging. The question, therefore, is how can we disambiguate the function of APOE in maintaining brain resilience during aging while also participating in Aβ-induced neurotoxicity?

By recording visually evoked neuronal responses in awake mice, we were able to demonstrate that a complete absence of APOE restores neuronal function in adult APP/PSEN1 animals (decreased “off-responding” cells and increased OSI and DSI), so that the efficacy of induced responses is comparable between wild-type and APP/PSEN1/APOE<sup>null</sup> animals. In addition, the lack of APOE in AD mice preserves synaptic and neuritic integrity around plaques and decreases amyloid-associated glial immunoreactivity. Despite these beneficial effects, the idea of knocking-down APOE expression to improve AD neuropathological hallmarks remains controversial because previous reports have shown that APOE<sup>null</sup> animals present with some cognitive deficits, electrophysiological alterations, and functional connectivity changes when compared with wild-type mice (Masliah et al, 1997; Kitamura et al, 2004; Trommer et al, 2004; Yang et al, 2011a; Zerbi et al, 2014). Here, we confirm that genetic ablation of APOE impairs visually evoked responses of aged APOE<sup-null</sup> and APP/PSEN1/APOE<sup>null</sup> mice as compared with controls. Taken together, we demonstrate that apoE genetic disruption alleviates Aβ-associated neurotoxicity early but sensitizes the brain towards age-dependent neuronal dysfunction. Discrepancy between our results and previous reports may result from the experimental settings used, as we assessed neuronal function using a relatively simple sensory circuit within the brain as compared with more complex behavioral tasks.

Data in the human population are essentially missing, with only one case study published so far reporting “below-average” cognition and memory performances in an individual with a complete lack of APOE protein in the brain (Cullum & Weiner, 2015). Because of the relatively young age of the individual, it is hard to predict if...
the absence of APOE will lead to exacerbated cognitive decline later on, similar to what is observed in animal models. Alternative strategies to inhibit the detrimental impact of APOE in disease while preserving its physiological role in maintaining brain homeostasis have already been attempted. For example, intraarterial injections of Aβ12-58P, a modified Aβ peptide homologous to the binding side of APOE on Aβ40/Aβ42 peptides that crosses the blood–brain barrier, is able to disrupt the interaction between APOE and Aβ, reduce the formation of fibrillar aggregates, alleviate t pathology in triple transgenic mice (PS1M146V, APPSwe, and tau- P301L), and improve behavioral deficits (Yang et al, 2011b; Liu et al, 2014). Reducing the amount of APOE specifically in the brain tissue (and not in the periphery) could be another alternative. The recent characterization of the bEKO mouse model by Lane-Donovan and colleagues (Lane-Donovan et al, 2016), which fortuitously lacks APOE in the brain but shows normal levels in the plasma, suggests that the Aβ brain phenotype may be, as these animals do not show any cognitive deficits as compared with APOE”null mice. However, the clinical translation of this discovery may prove difficult to achieve considering that various neural cell types express APOE (astrocytes, microglia, and endothelial cells). Interestingly, the work by Zheng and colleagues demonstrated that the sole deletion of APOE in microglia, and endothelial cells. Importantly, the “rescue effect” observed in the absence of APOE did not directly correlate with a significant change in the global amount of amyloid pathology in the brain of our adult cohort (assessed by conventional immunostaining or by quantification of Aβ by ELISA), but rather with a specific decrease in dense-core Methoxy-positive deposits in APP/PSEN1/APOE”null mice (both in adult and aged groups). Of note, the initial load of fibrillar amyloid at baseline is especially abundant in the model we used, the APP/ PSEN1 strain, because of the presence of the Swedish APP (KM670/ 671N) and β41 PS1-mutated transgenes. It is, therefore, conceivable that the impact of APOE deficiency may be less significant in other models of amyloidopathy that only carry a mutated APP allele. Nevertheless, the shift in the aggregation state of amyloid between APP/PSEN1 and APP/PSEN1/APOE”null has been reported in other studies (Bales et al, 1997; Irizarry et al, 2000a) and essentially reproduces the morphological difference between β-sheet deposits (labeled with Thioflavin-S) mostly observed in AD patients and diffuse plaques detected in non-demented individuals (Dickson, 1997; Urbanc et al, 2002). This conformational change of extracellular amyloid is relevant to the disease as fibrillary neurotic plaques are associated with deleterious effects on the surrounding neuropil, such as the presence of dystrophic neurites and recruitment of reactive glial cells (Masliah et al, 1990; Knowles et al, 1999; Vehmas et al, 2003), which are absent around more diffuse aggregates observed in cognitively normal individuals and in our APP/PSEN1/APOE”null animals. The underlying mechanisms remain incompletely understood, but in vitro and in vivo studies have previously shown that APOE forms stable complexes with Aβ (Naslund et al, 1995), directly co-deposits in plaques (Namba et al, 1991), modulates Aβ oligomerization (Hashimoto et al, 2012; Gar et al, 2014), and converts amyloid protofibril to fibril (Hori et al, 2015), which all could result in the observed changes in plaque morphology. Further comparison of the profile of Aβ oligomers, which are especially concentrated around amyloid plaques and have been shown to trigger neurotoxicity (Walsh & Selkoe, 2007; Arbel-Ornath et al, 2017; Yang et al, 2017), may give additional clues to understand the beneficial impact of the lack of APOE in Aβ-dependent neuronal function and synaptotoxicity.

It is possible that some of the protective effects we observed in APP/PSEN1/APOE”null mice arise independently from the change observed in the aggregation state of Aβ. In particular, the decreased gial reactivity in APP/PSEN1/APOE”null mice could result from a direct impact of APOE on the biology of microglia and astrocytes in the context of AD, even in the presence of Aβ neurotoxic species. The APOE-TREM2 pathway has recently been identified as a major switch triggering a phenotypic change in microglia in the context of neurodegeneration (Atagi et al, 2015; Yeh et al, 2016; Krasemann et al, 2017). Upon activation of this cascade, microglial cells become chronically inflammatory and lose their homeostatic signature, a phenomenon that is not present when the endogenous expression of apoE was shut down specifically in microglia. Considering that activated neuroinflammatory microglia have also been shown to control the conversion of resting (A2) to reactive astrocytes (A1) (Liddelow et al, 2017), the inhibition of the TREM2-APOE pathway in APP/PSEN1/APOE”null mice could explain the overall decrease of gial reactivity independently of the decreased density of neurotic plaques in those mice.

Finally, our array tomography data emphasize the importance of APOE in driving Aβ-associated synapse loss. While the density of synapsin and PSD95 puncta was dramatically reduced in the vicinity of amyloid deposits in APP/PSEN1 mice (by about 30%, as we have previously reported [Koffie et al, 2009; Kay et al, 2013]), no such reduction of pre- or post-synaptic elements was detected in APP/ PSEN1/APOE”null mice. Still, the lack of APOE neither prevented the recruitment of Aβ at the synapse nor the decreased synaptic volume characteristic of amyloidosis mouse models. As the synapti- c aptic size is generally accepted as an indicator of connection strength (Kasai et al, 2010; Penzes et al, 2011), it is possible that synaptic stability remains compromised in APP/PSEN1/APOE”null mice. In addition, we have previously reported that the different APOE isoforms differentially modulate the recruitment of Aβ at the synapse in human AD brains (Koffie et al, 2012), therefore acting as a chaperone toward neurotoxic amyloid peptides. The fact that Aβ still co-localizes with synaptic terminals in the absence of APOE implies that other factors may be involved to shuttle those neurotoxic species at the synapse. Among others, apolipoprotein J (ApoJ, or clusterin) is a likely candidate, as it is also known to in- teract with Aβ and modify its aggregation and deposition (Mulder et al, 2014; Miners et al, 2017). Whether or not this is the case remains to be determined, as we did observe a rescue of neuronal function in the absence of APOE alone, therefore indicating that the pres- ence of Aβ at the synapse is necessary but not sufficient to trigger synaptocollapse. Because our findings place APOE as a pivotal factor triggering amyloid synaptotoxicity, it is possible that its association with Aβ is necessary for it to adopt a toxic conformation or allows the interaction between Aβ and specific cellular receptors.
(such as APP itself, as recently reported [Wang et al., 2017], TREM2 [Yeh et al., 2016], or any other APOE receptors [Holtzman et al., 2012; Lane-Donovan & Herz, 2017]) to initiate various deleterious molecular cascades in neurons or even in glial cells.

Materials and Methods

Animals

APPswe/PS1dE9 (APP/PSEN1) mice (The Jackson Laboratory [Jankowsky et al., 2004; Reiserer et al., 2007]) were used for the study. This mouse model expresses the human mutant APP and presenilin genes respectively containing the Swedish mutation K594N/M595L and the exon 9 deletion. Amyloid deposition starts as early as 3-month old in this model, with most amyloid plaques being of fibrillar nature because of the presence of the ΔE9 PS1 mutation leading to an increase in Aβ42/40 ratio. To generate APP/PSEN1/APOEnull mice, one APP/PSEN1 hemizygous transgenic animal was crossed with an APOEnull breeder (The Jackson Laboratory). The resulting APP/PSEN1/APOEnull‘ generation was then crossed with another APOEnull breeder to generate APP/PSEN1/APOEnull and APOEnull animals. The resulting APP/PSEN1/APOEnull offspring was then bred with APOEnull mice to generate all the animals used in this study. Controls included littersmates from the APP/PSEN1 colony altogether with C57BL/6 (appropriate controls of the APOEnull mice). We had previously verified that these control groups showed similar responses to visual stimulation and, therefore, could be merged together. Genotyping for APOE and APP were performed by PCR following the protocol given by The Jackson’s Laboratory. A detailed description of the cohort is presented in Fig S1. All animal experiments were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care following the guidelines set forth by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Viral vector construction and production

The yellow cameleon cDNA (YC3.6) was cloned to an AAV2 backbone, under a hybrid CMV immediate-early enhancer/chicken β-actin promoter/exon1/intron and before the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE). High titer of AAV serotype 8 were produced using a conventional triple transfection approach by the PENN Vector Core (University of Pennsylvania). Viruses were tittered by quantitative PCR, and the final concentrations of the AAV viral stocks used for this study reached $4 \times 10^{12}$ vg/ml.

Intracortical AAV injection and cranial window implantation

Surgical procedures were performed as described previously (Hudry et al, 2010; Kuchibhotla et al, 2014; Arbel-Ornath et al, 2017), with minor modifications. Mice were anesthetized by intraperitoneal injection of ketamine/xylazine (100 mg/kg and 50 mg/kg, respectively, body weight) and positioned on a stereotactic frame (Kopf Instruments). A 3-mm cranial window was opened above the right primary visual cortex V1 (coordinates from $\lambda +0.5$ mm anteroposterior, 2.7 mm lateral, and 0.6 mm dorso-ventral). After removing the skull, 3 μl (12 $\times 10^{10}$ vg) of AAV8-CBA-YC3.6 viral suspension was injected at a rate of 0.15 μl/minute in the layer 2/3 neurons using a 33-gauge sharp needle attached to a 10-μl Hamilton syringe (Hamilton Medical). The window was then closed with a 5-mm-diameter cover glass and secured with a mixture of dental cement and Krazy Glue. A custom-made stainless steel headpost (Ponoko) was finally fixed to the skull using C&B Metabond dental cement (Parkell) to allow recording in awake mice. After a recovery period of one month, the mice were habituated to the head-fixation device (Thorlabs posts and Altos head clamps) by running freely on a circular treadmill (Ponoko) for 15 min a week before the first imaging.

Recording of visually evoked neuronal responses

For in vivo calcium imaging, an Olympus Fluoview FV1000MPE multiphoton laser scanning system mounted on an Olympus BX61WI microscope and an Olympus 25× dipping objective (NA = 1.05, Olympus) were used, with the emission path shielded from external light contamination. A DeepSee Mai Tai Ti:sapphire mode-locked laser (Mai Tai; Spectra-Physics) generated two-photon excitation at 860 nm, and detectors containing three photomultiplier tubes (Hamamatsu) collected emitted light in the range of 460–500, 520–560, and 575–630 nm. CFP and YFP photomultipliers (PMTs) settings remained unchanged throughout the different imaging sessions, but laser power was adjusted as needed. The mice were imaged awake and directly placed under the objective. To detect visually evoked neuronal responses, time courses of calcium transients were recorded in head-fixed awake animals while a 19-inch LCD monitor (Viewsonic VP930B) displayed the visual stimuli in front of the left eye (screen–eye distance, ~20 cm; screen-midline angle, 60°). Visual stimuli were drifting (2 Hz) sine-wave gratings (80 or 100% contrast, black and white) presented for 7 s. Eight stimuli (at 45° orientation increments) were presented sequentially in counter-clockwise order with a 7-s pause between stimuli, and 10 cycles of visual stimulation were presented during each recording. For each imaging session, two to three cortical frames (254 μm × 254 μm, scan rate 2.3 Hz, 0.429 s/frame, depth of 200–300 μm) were taken.

Image processing and analysis

In vivo imaging data were analyzed using custom-written scripts in Fiji (National Institutes of Health: http://fiji.sc/) and MATLAB (MathWorks). Images were aligned for shifts in the $x$-$y$ plane using the StackReg function of Fiji, neuronal cell bodies (identified by the presence of neuritic processes) were manually selected (ROIs), YFP:CFP ratios were created and spatially locked laser (Mai Tai; Spectra-Physics) generated two-photon excitation at 860 nm, and detectors containing three photomultiplier tubes (Hamamatsu) collected emitted light in the range of 460–500, 520–560, and 575–630 nm. CFP and YFP photomultipliers (PMTs) settings remained unchanged throughout the different imaging sessions, but laser power was adjusted as needed. The mice were imaged awake and directly placed under the objective. To detect visually evoked neuronal responses, time courses of calcium transients were recorded in head-fixed awake animals while a 19-inch LCD monitor (Viewsonic VP930B) displayed the visual stimuli in front of the left eye (screen–eye distance, ~20 cm; screen-midline angle, 60°). Visual stimuli were drifting (2 Hz) sine-wave gratings (80 or 100% contrast, black and white) presented for 7 s. Eight stimuli (at 45° orientation increments) were presented sequentially in counter-clockwise order with a 7-s pause between stimuli, and 10 cycles of visual stimulation were presented during each recording. For each imaging session, two to three cortical frames (254 μm × 254 μm, scan rate 2.3 Hz, 0.429 s/frame, depth of 200–300 μm) were taken.
of responses in all other directions, $\Sigma \Delta R$ other (0 ≤ $\Delta SI$ ≤ 1; 1 = perfectly orientation-tuned). DSI was calculated as $\Delta R$ max divided by the sum of that direction and the antiparallel direction, $\Delta R$ max + $\Delta R$ orthogonal (0 ≤ $\Delta SI$ ≤ 1; 1 = perfectly direction-tuned). The higher the values for OSI and DSI, the better the neuron tuned to a specific visual stimulus.

**Tissue collection and processing**

Mice were euthanized by CO2 asphyxiation and tissue collected for immunohistochemical and biochemical analysis. For the mice that underwent in vivo imaging, the right hemisphere (containing the surgical site) was fixed by immersion in 4% paraformaldehyde and 15% glycerol in PBS for 48 h before cryoprotection with 30% glycerol in PBS. A few 1-mm³ pieces of tissue were cut across the left hemisphere for array tomography. Because the surgical procedure and the injection of AAV-CBA-YC could have compromised the hemisphere for array tomography. Synaptoneurosomes preparation and Western blotting

− liquid nitrogen and stored at −80°C. Mice were performed on immunolabeled sections using an Odyssey CLx imager of 70% of cortical area, images were captured each time an amyloid deposit was encountered. Those images were then analyzed using the integrated Density function of Fiji software (http://fiji.sc/) and divided to GAPDH signal intensity. The samples from each batch of SNS were first normalized to the signal observed in the controls (Littermates), before performing statistical analyses on all SNS preparations.

**Sequential brain extraction**

Sequential brain extraction was performed as described previously (Hashimoto et al, 2012) from one cerebral hemisphere, after dissection of the olfactory bulb and cerebellum. The tissue was initially homogenized in 10 volumes of TBSI (Tris-buffered saline with protease inhibitor cocktail; Roche) with 25 strokes on a mechanical Dounce homogenizer and centrifuged at 100,000 g for 30 min at 4°C. The supernatant was collected as TBS-soluble fraction. The resulting pellet was consecutively extracted with TBS buffers containing 2% Triton and 2% SDS (with protease inhibitors), alternating homogenization and ultracentrifugation steps (100,000 g for 30 min). The final pellet was solubilized in 500 μl of FA by sonication. After a final ultracentrifugation step, the FA-soluble fraction was desiccated and the pellet resuspended in 100 μl of DMSO. The content in the most soluble (TBS) and insoluble (FA) Aβ species was analyzed in this study. The concentrations of Aβ40, Aβ42, and Aβ oligomers were respectively quantified with a mouse/human ELISA kit (Wako) and a human Amyloid β oligomers (82E1-specific) assay (IBL international).

**Immunohistology**

After cryoprotection of the brain in 30% glycerol for 48 h, 40-μm-thick floating sections were cut on a freezing microtome in the sagittal plane. Floating sections were successfully permeabilized in 0.5% Triton in TBS for 30 min, blocked in 5% normal goat serum in TBS for 1 h, and incubated with primary antibody overnight at 4°C in 25% NGS and 0.1% Triton in TBS (see Table 1 for a complete list of primary antibodies used). The sections were then washed with TBS and incubated with appropriate Alexa-Fluor 488- or Alexa-Fluor 568-conjugated secondary antibodies diluted in 25% NGS and 0.1% TritonX in TBS. After another round of washing, the sections were mounted onto slides and coverslipped with VECTASHIELD Mounting Medium with DAPI (Vector Labs). For the counterstaining of amyloid with Methoxy-XO4, the floating sections were incubated for 15 min in a solution of 1 μg/ml of Methoxy-XO4 (diluted in TBS) before mounting the slices with Fluoromount-G (No DAPI, SouthernBiotech).

**Stereology-based quantitative analyses**

All pathology quantification was carried out blinded until the last statistical analyses. Stereology-based studies of amyloid-associated neuritic dystrophies, reactive astrocytes, and microglia were performed on immunolabeled sections using an Olympus BX52 epifluorescent microscope equipped with motorized stage, DP70 digital CCD camera, and CAST stereology software (Olympus). The cortex was outlined under low-power objective (4×), and dystrophies, astrocytes, and microglia counts were made using 20× high numerical aperture (1.2) objective. Using a meander sampling of 70% of cortical area, images were captured each time an amyloid deposit was encountered. Those images were then analyzed using

**Synaptoneurosomes preparation and Western blotting**

Synaptoneurosomes preparations were based on procedures described by Tai et al (2012) with minor modifications. Briefly, an entire mouse hemisphere (about 250 mg of frozen tissue, without cerebellum and olfactory bulbs) was homogenized in 1.2-ml cold Buffer A (25 mM Tris, pH 7.5, 120 mM NaCl, 5 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 1 mM dithiothreitol, and Complete protease inhibitors; Roche), before being filtered through 80-μm nylon filters (Millipore). An aliquot of the filtrate was supplemented with SDS to 1%, boiled for 5 min, and centrifuged at 15,000 g for 15 min, and the supernatant was collected as total extract. The other portion was further filtered through 5-μm pore filters (PALL Acrodisc) and centrifuged at 1,000 g for 15 min at 4°C to pellet synaptoneurosomes. The supernatant, corresponding to the cytosolic extract, was further centrifuged at 100,000 g for 20 min to remove microsomes or other extracellular vesicles. The synaptoneurosomal pellet was washed once with cold Buffer A and centrifuged again at 1,000 g for 10 min. The pellet was extracted with 0.5 ml Buffer B (50 mM Tris, pH 7.5, 1% SDS, and 2 mM dithiothreitol) and boiled for 5 min. After centrifugation at 15,000 g for 15 min, the supernatant was collected as synaptoneurosomal extract. Protein content was measured by BCA assay (Pierce). The protein concentration was adjusted to 10 μg/10 μl for all Western blot samples, including Laemmli blue buffer and reducing agent. The samples were electrophoresed on 4–12% Bis-Tris gels in MES running buffer (Invitrogen). After transferring on nitrocellulose membrane (GE Healthcare), the blots were incubated with appropriate Alexa-Fluor 488- or Alexa-Fluor 568-conjugated secondary antibodies diluted in 25% NGS and 0.1% TritonX in TBS. After running and washing, the sections were mounted onto slides and coverslipped using VECTASHIELD Mounting Medium with DAPI (Vector Labs). For the counterstaining of amyloid with Methoxy-XO4, the floating sections were incubated for 15 min in a solution of 1 μg/ml of Methoxy-XO4 (diluted in TBS) before mounting the slices with Fluoromount-G (No DAPI, SouthernBiotech).
Fiji, counting the number of GFAP-positive astrocytes, Iba1-positive microglial cells or neuritic dystrophies (visible after immunostaining for neurofilaments) close to a plaque (<50 μm) and reporting this number to the surface of the plaque considered. For the quantification of amyloid load and amyloid density, Alexa-568-anti-Amyloid and Methoxy-XO4-positive plaques were imaged using a NanoZoomer-XR digital slide scanner (Hamamatsu) under a ×20 objective. After conversion of .ndpi files to tiff format, the number of deposits and their surface were determined using a custom-written script based on the "Analyze particle" function of Fiji (National Institutes of Health: http://fiji.sc/). The total surface occupied by amyloid and the total number of plaques were then reported to the cortical area of each section considered.

Array tomography

Sample collection and tissue processing

Array tomography analyses were performed as previously described (Hudry et al., 2013; Kay et al., 2013). Five to six pieces of cortical tissue (1 mm³) were dissected and fixed for 3 h in 4% paraformaldehyde and 2.5% sucrose in 0.01 M PBS. After dehydration in ethanol, the samples were incubated in LR White resin (Electron Microscopy Sciences) overnight at 4°C before polymerization at 53°C. Ribbons of 20–40 ultrathin (70 nm thick) serial sections were cut with a Histo Jumbo diamond knife (Diatome) on an ultracut microtome (Leica) and mounted on glass coverslips.

Staining and imaging

Ribbons were incubated in glycine (50 mM glycine in 1× TBS) for 5 min and blocked in blocking solution (0.05% Tween and 0.1% fish gelatin [Sigma-Aldrich] in 1× TBS) for 1 h before antibody staining. All antibodies (summarized in Table 1) were spun for 4 min at 10,000 g before being applied. Mouse 1C22 against oligomeric Aβ (1:200, donated by Dominic Walsh [Yang et al., 2015]), rabbit anti-synapsin-1 (1:50, Rb X Synapsin I, AB1543P; Millipore) and guinea pig anti-PSD95 (1:50, Anti-PSD95, 124014; Synaptic Systems [Koffie et al., 2009]) primary antibodies in block solution were added and incubated at 4°C overnight. Incubation with secondary antibodies diluted 1:50 in block solution (Alexa Fluor™ 488 donkey anti-mouse IgG [H+L], Invitrogen; Alexa Fluor™ 594 donkey anti-rabbit IgG [H+L], Invitrogen; and Alexa Fluor™ 647 goat anti-guinea pig IgG [H+L], Invitrogen) was performed for 1 h at room temperature, before counterstaining with DAPI for 5 min. Shandon Immu-Mount (Thermo Fisher Scientific) solution was used to mount the slides on polysine microscope slides (VWR International). Serial sections of the ribbon were imaged with a Zeiss AxioImager Z2 epifluorescent microscope, first at 10× to obtain a tilescan of the entire ribbon, then with a 63 × 1.4 NA Plan Apochromat objective for high-resolution images. Images were acquired with a CoolSnap digital camera and AxioImager software with array tomography macros (Carl Zeiss, Ltd). In short, two distinct areas were selected on each 10× tilescan. Once the areas were selected on two serial slices of the ribbon, the AxioImager software was able to find the same areas in each one of the subsequent slices and image them at 63×.

Data analyses

Image stacks were aligned using ImageJ (National Institutes of Health open software; multistackreg macro [Thevenaz et al., 1998]). ROI (10 μm × 10 μm) were cropped on the stack near plaques (<10 μm from the plaque edge) and far away from plaques (>35 μm from the edge of the halo). When no plaques were present (in wild-type and APOE<sup>null</sup>) Table 1. List of antibodies used for the study.

| Antigen                  | Species | Polyclonal/Monoclonal | Manufacturer          | Cat. No |
|--------------------------|---------|-----------------------|-----------------------|---------|
| Antibodies used for Immunohistology (IH)/WB |         |                       |                       |         |
| Aβ (N-terminal)          | Rabbit  | Polyclonal            | IBL/Tecan             | 18584   |
| Aβ 1-12 (clone BAM10)    | Mouse   | Monoclonal            | Sigma-Aldrich         | A3981   |
| Iba1                     | Rabbit  | Polyclonal            | Wako                  | 019-1974 |
| GFAP                     | Mouse   | Monoclonal            | Sigma-Aldrich         | G3893   |
| Neurofilament (SMI-311R) | Mouse   | Monoclonal, IgM, IgG1 | BioLegend             | 837801  |
| GABA B receptor 1        | Mouse   | Monoclonal, IgG 2a    | Abcam                 | ab55051 |
| NMDAR 1                  | Rabbit  | Monoclonal            | Abcam                 | ab109182|
| NMDAR2B                  | Rabbit  | Monoclonal            | Abcam                 | ab65783 |
| NMDAR2A                  | Rabbit  | Monoclonal, IgG       | Millipore             | ab9484  |
| GABA A receptor α1       | Rabbit  | Polyclonal            | Abcam                 | ab33299 |
| PSD95                    | Rabbit  | Monoclonal            | Cell Signaling        | 34505   |
| GAPDH                    | Mouse   | Monoclonal            | Abcam                 | ab9484  |
| Actin                    | Mouse   | Monoclonal            | Sigma-Aldrich         | A4700   |
| Antibodies used for array tomography |         |                       |                       |         |
| Oligomeric Aβ (1C22)     | Mouse   | Monoclonal            | Dr. Walsh’s lab       | Yang, O’Malley et al (2015) |
| Synapsin 1               | Rabbit  | Polyclonal            | Millipore             | AB1543P |
| PSD95                    | Guinea-pig | Polyclonal           | Synaptic Systems      | 124014  |
mice), ROI were randomly selected on the stack. Custom algorithms were used to threshold the crops in IMAGEJ/FIJI (Schindelin et al, 2012). Custom MATLAB macros were used to remove puncta that were only found in a single section, detect synapses, quantify the numbers and sizes of synaptic puncta, and determine which synaptic puncta were co-localized with Aβ. Synaptic density was calculated as the number of puncta per volume of tissue sampled (synapses/mm³).

Statistical analyses

A detailed summary of the statistical analyses performed is presented in the Supplemental Information 1 “Statistical analyses,” including the test chosen for each analysis, exact P-values and confidence intervals. The statistical software SAS was used to analyze the relationship between adjusted DSI/OSI and genotype, age, and the interaction between them. A linear mixed model was fitted to the DSI and OSI values after log transformation, with these factors (genotype or age) as fixed effects and mouse as random effect. Significance was set for P-values < 0.05.

Array tomography data were analyzed using GraphPad Prism software, and P < 0.05 was considered significant. Shapiro–Wilks test was used to check for normal distribution of data. Brown–Forsythe test (with ANOVA or the nonparametric equivalent Kruskal–Wallis test) and F-test (with t test or the nonparametric equivalent Mann–Whitney test) were used to check for equality of variance of the data. Synaptic density in crops was measured and averaged for each mouse. A two-way ANOVA test followed by a Tukey’s multiple comparisons post hoc test was used to compare synaptic density using distance from plaques and mouse genotype as the two variables. A t test was used to compare the percentage of 1C22 co-localizing at synapses close to plaques in APP and APP/APOEnull mice. One-way ANOVA test (or the nonparametric equivalent Kruskal–Wallis test) followed by a Holm Sidak’s or a Tukey’s multiple comparisons post hoc tests and t test (or the nonparametric equivalent Mann–Whitney test) were used to compare synaptic volume across genotypes far and close from plaques.

All the other results (stereology, Western blotting) were analyzed using GraphPad software. Normality was initially validated using the D’Agostino and Pearson omnibus normality test. Difference between each group was tested using a one-way ANOVA (in case of normality) or a Kruskal–Wallis test (normal distribution) followed by post hoc Dunn’s multiple comparison test. In case of comparisons between two groups only (APP/PSEN1 and APP/PSEN1/APOEnull), a conventional t test (normality) or a Mann–Whitney test (non-normal distribution) was performed. Data are presented as mean ± SD.

Supplementary Information

Supplementary Information is available at https://doi.org/10.26508/lsa.201900325.

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Author Contributions

E Hudry: conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, project administration, and writing—original draft, review, and editing.

J Klickstein: data curation, formal analysis, and writing—review and editing.

C Cannavo: data curation, formal analysis, and writing—original draft, review, and editing.

R Jackson: data curation, formal analysis, methodology, and writing—original draft, review, and editing.

A Muzikansky: formal analysis, methodology, and writing—original draft, review, and editing.

S Gandhi: data curation, formal analysis, and methodology.

D Urick: data curation and formal analysis.

T Sargent: data curation and formal analysis.

L Wrobleski: data curation and formal analysis.

AD Roe: data curation, formal analysis, and project administration.

SS Hou: formal analysis and methodology.

KV Kuchibhotla: supervision, methodology, and writing—original draft, review, and editing.

RA Betensky: formal analysis, supervision, methodology, and writing—original draft, review, and editing.

T Spires-Jones: conceptualization, supervision, funding acquisition, methodology, and writing—original draft, review, and editing.

BT Hyman: conceptualization, formal analysis, supervision, funding acquisition, and writing—original draft, review, and editing.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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