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ORIGINAL PAPER

Knockout of Amyloid β Protein Precursor (APP) Expression Alters Synaptogenesis, Neurite Branching and Axonal Morphology of Hippocampal Neurons

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Abstract

The function of the β-A4 amyloid protein precursor (APP) of Alzheimer's disease (AD) remains unclear. APP has a number of putative roles in neuronal differentiation, survival, synaptogenesis and cell adhesion. In this study, we examined the development of axons, dendrites and synapses in cultures of hippocampus neutrons derived from APP knockout (KO) mice. We report that loss of APP function reduces the branching of cultured hippocampal neurons, resulting in reduced synapse formation. Using a compartmentalised culture approach, we found reduced axonal outgrowth in cultured hippocampal neurons and we also identified abnormal growth characteristics of isolated hippocampal neuron axons. Although APP has previously been suggested to play an important role in promoting cell adhesion, we surprisingly found that APPKO hippocampal neurons adhered more strongly to a poly-L-lysine substrate and their neurites displayed an increased density of focal adhesion puncta. The findings suggest that the function of APP has an important role in both dendritic and axonal growth and that endogenous APP may regulate substrate adhesion of hippocampal neurons. The results may explain neuronal and synaptic morphological abnormalities in APPKO mice and the presence of abnormal APP expression in dystrophic neurites around amyloid deposits in AD.

Keywords Amyloid · APP · Neurite outgrowth · Axon pathfinding · Alzheimer's disease

Abbreviations

AICD	Amyloid intracellular domain
APP	Amyloid β protein precursor
DIV	Days in vitro
FAK	Focal adhesion kinase
KO	Knock-out
MAP2	Microtubule-associated protein 2

Introduction

The β-A4 amyloid protein precursor (APP) of Alzheimer's disease (AD) is a type I transmembrane protein. APP is the precursor of the β-amyloid protein (Aβ) [1–3], the main component of the extracellular amyloid plaques [4, 5] that are a pathological hallmark of AD [1, 4, 5]. APP has been proposed to have a role in a variety of developmental processes such as neuronal stem cell proliferation and differentiation, neuronal outgrowth and pathfinding, synaptogenesis, synaptic plasticity, cell adhesion and cell motility [2]. The APP gene is located on chromosome 21 which explains why individuals with Down syndrome develop AD-like pathology [6].

Full-length APP can be cleaved by several proteases [7]. Cleavage of APP by β-secretase and subsequent cleavage by γ-secretase yields Aβ. However, cleavage can also occur via the α-secretase pathway, which results in cleavage of the Aβ sequence. This pathway prevents Aβ production and results in formation of an extracellular secreted fragment (sAPPα) which may have trophic activity [2].

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The structure of APP suggests that it could act as a cell-surface receptor. However, the nature of any APP ligand remains unknown. The protein contains a large N-terminal extracellular region containing putative binding sites for extracellular matrix components [2, 8, 9], suggesting that the protein may have some role in cell-substrate adhesion. APP may act as a co-receptor with a binding partner [2] such as glypican-1 [10], neural cell adhesion molecule [11] or contactins [12]. The amyloid intracellular domain (AICD) is also likely to be important for APP function [2, 13]. The AICD has been shown to regulate gene transcription through its interaction with adaptor proteins. In many cases, these interactions involve a conserved YENPTY motif that is located in the AICD [14].

APP is highly expressed during neuronal development, suggesting that it has an important role in neurogenesis or maturation of the CNS. APP expression is typically highest early in development and then declines [15]. However expression of APP remains high during adulthood in stem cell populations in the hippocampus and olfactory system [16, 17], suggesting that APP is important for cell proliferation. APP expression is upregulated following injury to the CNS in response to neuronal damage. APP rapidly accumulates in the characteristic axonal swellings that form following injury [18, 19]. Indeed, the presence of APP accumulations in damaged axons is considered a hallmark feature of traumatic brain injury [20].

In vitro studies support the hypothesis that APP is required for normal proliferation and differentiation of neural stem cell progenitor cells [21, 22]. APPKO mice have reduced density of dendritic spines in the hippocampus, indicating that synaptogenesis is impaired in these animals [23]. Indeed, APPKO mice are susceptible to spontaneous and kainic-acid induced seizures [24] and demonstrate subtle cognitive deficits [25, 26]. It remains unclear how APP is required for a seemingly diverse range of roles, from cell adhesion, proliferation of neural stem cells, neurite outgrowth and synaptogenesis. Synaptogenesis is a complex event requiring coordination of axon outgrowth and formation of the synaptic bouton [27]. Although APP is clearly required for this to occur as demonstrated by synaptic deficits in APPKO models, it remains unknown how.

Despite the large number of studies that have implicated APP in neurite outgrowth and synaptogenesis, clear evidence linking APP with these phenomena has remained elusive. In the present study, showing that synaptogenesis is reduced in APPKO hippocampal neurons, our results demonstrate that this reduction in synaptogenesis in vitro is due to APP-associated defects in dendrite and axonal growth. Furthermore, we show that APPKO neurons have reduced adhesion and this defect in adhesion underlies the abnormal neurite growth that occurs in these APPKO hippocampal neurons.

Methods

Culture of Hippocampal Neurons

All animal experiments were approved by the University of Tasmania animal ethics committee (Permit Number A14987) in accordance with national and institutional guidelines for the care and use of animals. APPKO and the corresponding C57Bl/6 background strain (wild-type control) mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All solutions for cell culture were obtained from Life Technologies Australia (ThermoFisher Scientific, VIC, Australia) unless otherwise stated. Hippocampal neurons were prepared from hippocampi of embryonic day 15.5 (E15.5) mice, derived from time-mated pregnant female mice. Hippocampi were dissected from E15.5 brains, trypsinised (0.025% w/v) for 5 min at 37 °C. The tissue was washed once with neuron-plating medium (neurobasal supplemented with 10%, v/v, fetal bovine serum, 2%, v/v, B27 supplement, 4 mM Glutamax and 1%, v/v, penicillin/streptomycin) before dissociating the cells by trituration. Cells were plated onto poly-L-lysine-coated 13 mm^2 coverslips (Menzel Gläser, ThermoScientific) in 24-well culture trays or onto poly-L-lysine coated 6-well culture dishes (at a density of 3×10^4 or 1.5×10^5 cells per well, respectively) containing neuron-plating medium. Alternatively, cells were plated into microfluidic culture chambers prepared as previously described [28] at a density of 4×10^5 cells per chamber. Neurons were allowed to grow for 24 h in neuron plating medium (37 °C, 5% CO_2), before the medium was changed to neuron growth medium (plating medium lacking serum). For long-term cultures, the growth medium was refreshed after culturing for 8 days in vitro (DIV).

Cell Fixation and Immunocytochemistry

For immunocytochemical studies, cells were fixed at 14 DIV in 4% (w/v) paraformaldehyde. Cells were permeabilized using 0.1% (v/v) Triton-X-100 for 15 min at room temperature and blocked using 4% (v/v) goat serum in PBS for 1 h. Cells were incubated overnight at 4 °C in 2% (v/v) goat serum with one of the following primary antibodies: mouse anti-β-III-tubulin (1:2000, Promega), mouse anti-drebrin (1:100, Abcam), mouse anti-FAK (1:100, Merck Millipore), mouse anti-MAP2 (1:1000, Merck Millipore), guinea-pig anti-PSD95 (1:250, Synaptic Systems), rabbit anti-synaptophysin (1:500, Dako) and rabbit anti-tau (1:5000, Dako). AlexaFluor-conjugated secondary antibodies raised against the primary species were used to visualise proteins by fluorescence microscopy. All cultures

were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, 20 µg/ml) to identify cell nuclei.

Electrophoresis and Western Blotting

All stock solutions were purchased from ThermoFisher Scientific (VIC, Australia) unless otherwise stated. Cultured cells and neonatal hippocampi were lysed from 14 DIV neurons in 150 mM NaCl, 50 mM Tris-HCl pH 7.4, 1% (v/v) Nonidet P-40, 1% (w/v) sodium deoxycholate, 0.1% (w/v) sodium dodecyl sulphate (SDS) and Complete™ protease inhibitor cocktail (Roche Molecular Systems, NSW, Australia) (RIPA buffer). Proteins were separated on 4–12% SDS/bis-Tris BOLT™ gels at 200V and electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad, NSW, Australia) at 20 V for 1 h at 4 °C. Membranes were blocked by incubation for 20 min in 5% (w/v) non-fat milk powder in 50 mM Tris-buffered saline containing 0.05% (v/v) Tween 20, pH 8.0. Membranes were incubated with the following primary antibodies: guinea-pig anti-PSD95 (1:500, Synaptic Systems), rabbit anti-synaptophysin (1:1000, Dako), mouse anti-beta-actin (1:10,000, Sigma-Aldrich) diluted in blocking solution overnight at 4 °C. Membranes were washed and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Dako) for 2 h at room temperature, followed by detection using chemiluminescent HRP substrate (Merck-Millipore). Quantification of chemiluminescence was performed by image-capture analysis using ImageJ (version 1.5b, NIH, Washington).

Cell Adhesion Assay

Neurons were plated onto poly-L-lysine-coated 13 mm coverslips (Menzel Gläser) and incubated in initial plating medium for 15 min. Coverslips were washed with plating medium containing 10% v/v trypan blue to differentiate live and dead cells. Coverslips were lightly dried and images captured at low magnification from three non-overlapping regions across the coverslip. Cell adhesion was calculated as the number of live (trypan blue-excluding) cells per mm^2 from five separate neuron cultures within, using two coverslips from each culture. Data was collected from a minimum of 350 cells per culture per genotype.

Microscopy

Images for synaptic analysis were captured using an UltraView confocal microscope (Volocity software, Version 6.3, Perkin Elmer, MA, USA). z-Stacks (3 µm) were generated using the 60× objective with 0.5 µm steps and exported with z-planes merged. Additional images for neuronal morphology analysis were captured using the 20× objective at a single optical plane. Images were captured at semi-random locations across experimentally blinded coverslips avoiding excessively dense or sparse regions.

Determination of Synaptic Density

Synapses were identified by colocalisation of synaptophysin (presynapse) and PSD95 (postsynapse) along MAP2 positive dendrites. Individual pre- and post-synaptic structures (synaptophysin and PSD95 labelled images respectively) were identified using the ImageSURF plugin for ImageJ [29] which automatically sorts regions on the images as synapses or background pixels using random forest classification [30, 31]. The classifier was trained using a random selection of annotated synaptophysin and PSD95 labelled images. To identify synapses, synaptophysin and PSD95 labelled segmented images were overlaid using ImageJ and colocalised puncta superimposed on the corresponding MAP2 images (Fig. 1a). Un-branched sections of dendrite were randomly selected, measured, and colocalised synaptic puncta along each section were manually counted and expressed as the number of colocalised puncta per 100 µm.

Neurite Branching

Cultures, stained with MAP2 to identify dendrites, were imaged and the images traced using NeuronJ (Version 1.4.3) plugin for ImageJ [32]. Primary neurites arising from the neuronal soma were designated N1, with subsequent branches denoted N2, N3, etc. Tracing data were used to determine the length of individual branch sections, total neurite length per neuron, number of branch points at each level per neuron and the number of neurites per neuron by averaging values obtained from four separate cultures per genotype using a minimum of eight cells per culture.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software, version 6.05. For a simple comparison of two means assuming parametric distribution, data analysis was performed using unpaired Student's *t* test. For multiple comparisons, a one-way analysis of variance (ANOVA) with Tukey test, or a two-way ANOVA with Sidak test was employed. Frequency distribution data were analysed using Kolmogorov–Smirnov analysis. Statistical significance was assumed when the probability (*P*) of the null hypothesis was < 0.05. Data are presented as means ± standard error of the mean (SEM). Analysis of cultured cells was always performed blinded to genotype.

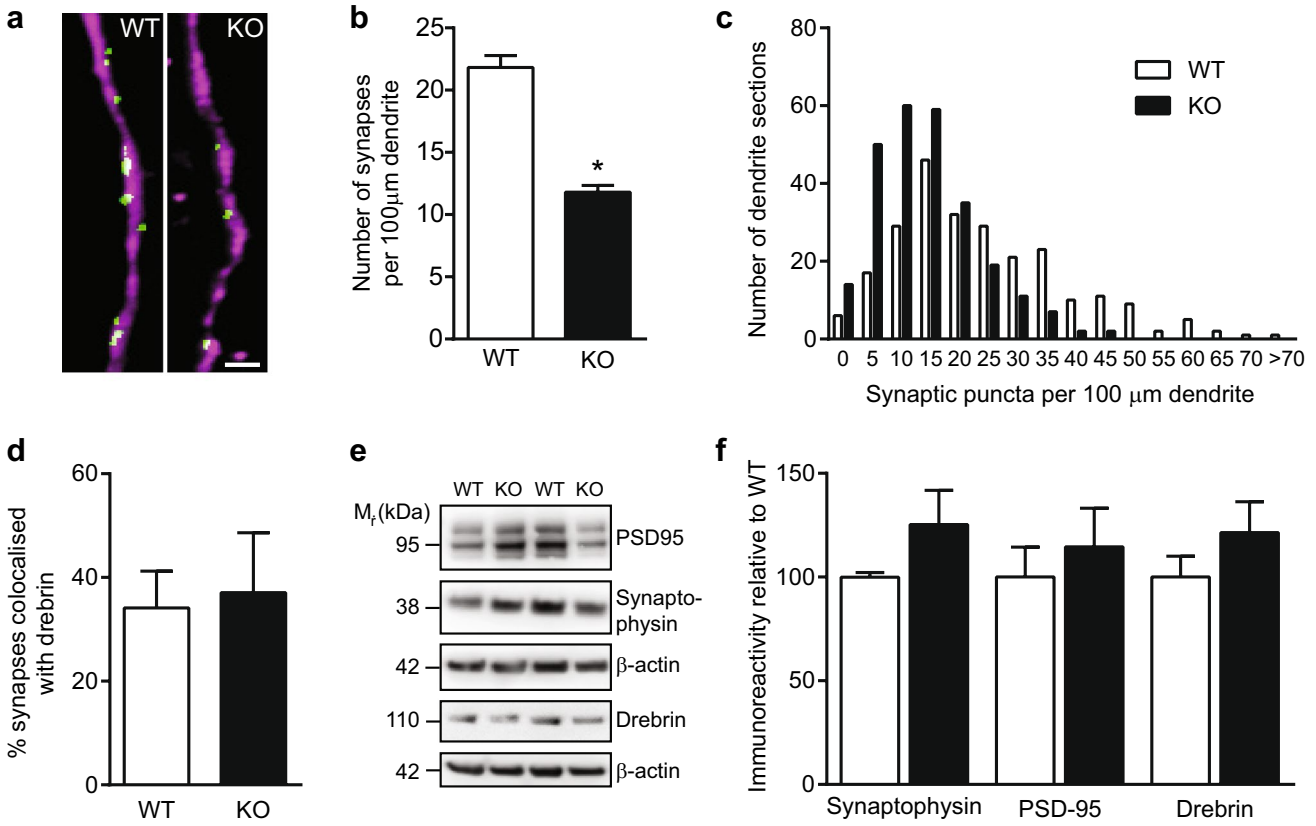

Fig. 1 The density of synaptic puncta is reduced in cultured APPKO hippocampal neurons. **a** Synapses were identified by colocalisation of PSD95 and synaptophysin (green puncta), along MAP2 (purple) positive dendrites. **b** Synapse density was significantly ($P<0.05$) reduced on dendrites of APPKO hippocampal neurons compared with wild-type controls. **c** Kolmogorov–Smirnov analysis of the distribution of synaptic puncta reveals synapse density is significantly ($P<0.0001$) reduced along APPKO dendrites. **d** There was no significant ($P>0.05$) difference in the proportion of synaptic puncta that were colocalised with the actin-binding protein drebrin. **e** Western blotting of PSD95, synaptophysin and drebrin detected no significant ($P>0.05$) difference in the levels of the synaptic proteins between APPKO and wild-type hippocampal cultures **f**. Data shown as mean ± SEM. Asterisk denotes significance. Scale bar = 5 µm

Results

Synaptic Density is Reduced in Cultured APPKO Hippocampal Neurons

As some studies have provided circumstantial evidence that APP plays a role in synapse formation [23], we examined the possibility that knocking out APP expression may disrupt synaptogenesis in cultures of hippocampal neurons. Hippocampal neuron cultures were prepared from APPKO mice and from wild-type controls. After 14 DIV, the density of synaptic puncta in the cultures was assessed by colocalisation of pre- and post-synaptic markers along MAP2 positive neurites. The number of synaptic puncta per 100 µm of dendrite was found to be significantly ($P<0.0001$, Kolmogorov–Smirnov probability analysis) reduced in the APPKO neuronal cultures compared with the wild-type controls (Fig. 1a–c).

To determine whether the reduction in synapses in the APPKO mice was associated with a decrease in synapse stability, we examined whether the synaptic localisation of the actin-binding protein drebrin, a marker for synaptic stabilisation [33], was altered in APPKO cultures. Synapses were co-labelled with drebrin and analysed as above. There was no significant ($P>0.05$) difference in the proportion of synapses that were colocalised with drebrin (Fig. 1d). We also analysed cell culture lysates by western blotting to determine whether the reduced synapse density in the APPKO cultures was associated with reduced levels of the synaptic marker proteins synaptophysin, PSD95 or drebrin. However, no significant ($P>0.05$) differences in the levels of synaptophysin, PSD95 or drebrin in the APPKO cultures compared with the background strain cultures were found (Fig. 1e, f).

Dendritic Branching is Reduced in APPKO Neurons

The results suggested that the lower number of synapses in the cultures was not due to a loss of a specific subtype of synapses. For this reason, we examined whether the reduction in synaptic density might be due to reduced neurite outgrowth and/or dendritic branching in APPKO cultures. Early neurite outgrowth was measured along β-III-tubulin-positive neurites after 3 DIV. There was no significant ($P > 0.05$) difference in the total length of neurites at 3 DIV between APPKO and wild-type neurons (Fig. 2b). Similarly, there was no significant ($P > 0.05$) difference in neurite extension in additional cultures analysed at 8 DIV (not shown).

However, differences in neurite outgrowth were seen in older cultures. By 14 DIV, cultured hippocampal neurons typically extend long, highly branched neurites radiating from the soma [34]. Dendrite tracing and branch-order allocation was performed on MAP2 positive neurites to determine whether branching was reduced in the APPKO cultures compared with the wild-type neurons. There was a significant ($P < 0.05$) reduction in the overall length of MAP2 positive neurites in APPKO cultures compared with wild-type neurons (Fig. 2c). This result was found to be due to a reduction in the number of N4, N5, N6, N7 and N10 branches in the APPKO neurons compared with wild-types ($P < 0.05$) (Fig. 2d). Despite the alteration in dendrite branching, there was no significant ($P > 0.05$) difference in the average length of dendritic segments between APPKO and wild-type neurons, with dendrites from both genotypes branching on average every 40 µm (Fig. 2e).

Abnormal Distal Axon Outgrowth in APPKO Neurons

To determine whether axonal outgrowth was altered in APPKO neurons, cells were cultured in microfluidic chamber devices designed to allow axonal extension through microgrooves into a second culture chamber without somal or dendritic component [28, 35]. Neurons were plated in the microfluidic chamber dishes and then neuronal growth was monitored daily to assess distal axon protrusion into the distal compartment. After 8 DIV, the neurites were stained for MAP2 (to identify dendrites) and tau (axons and dendrites).

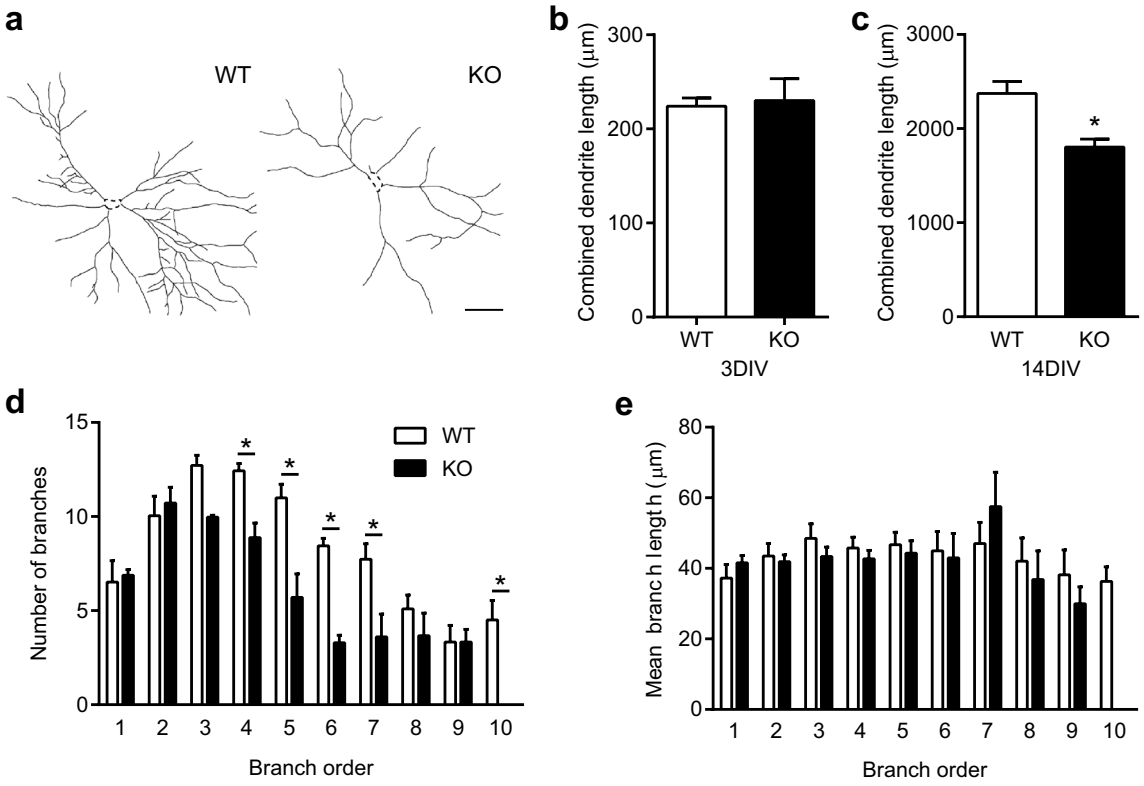

Fig. 2 APPKO hippocampal neurons have reduced dendritic branching compared with wild-type neurons. **a** Dendrite arborisation was assessed by tracing MAP2 positive neurites (dendrites) using the NeuronJ plugin for ImageJ. **b** Total dendrite length was not significantly ($P > 0.05$) altered in 3 DIV neurons, however total dendrite length was significantly ($P < 0.05$) reduced in 14 DIV APPKO hippocampal neurons compared with wild-type neurons (**c**). **d** There was a significant ($P < 0.05$) reduction in the higher-order dendritic branches (N4, 5, 6, 7, 10) in APPKO neurons. **e** Mean dendrite section length was not altered between genotypes. Data shown as mean ± SEM. Asterisk denotes significance. Scale bar = 50 µm

Axons were defined as those processes that were tau-positive and MAP2-negative. The staining pattern confirmed that only axons were present in the distal axon compartment (Fig. 3c), consistent with previous findings [28, 35, 36].

There was a significant reduction in the number of APPKO axons that protruded into the distal compartment at 6 DIV (0.45 ± 0.14 axons per microchannel) and 7 days (0.95 ± 0.24 axons per microchannel) compared with wild-type distal axons (1.27 ± 0.17 axons per microchannel at 6 days; 1.75 ± 0.24 axons per microchannel at 7 days; $P < 0.0001$) (Fig. 3a). Similarly, there was a significant reduction in the length of the APPKO distal axons protruding from the microfluidic channels at 6 days (129.0 ± 47.4 µm) and 7 days (284.0 ± 98.2 µm) compared with wild-type distal axons (502.0 ± 89.0 µm at 6 days; 698.5 ± 117.2 µm at 7 days; $P < 0.0001$) (Fig. 3b).

An initial examination of the cultures revealed that the distal axons of APPKO neurons were more curved than the distal axons of wild-type neurons. To quantify this difference, axonal curvature was calculated from the ratio of the total distal axon length and the straight-line length. Both measurements began at the point where each axon exited the microchannel and ended with the growth cone terminus (Fig. 3d). There was a significant increase in the ratio of total/straight path length in APPKO distal axons (1.48 ± 0.05) compared with wild-type axons (1.12 ± 0.04; $P < 0.01$) (Fig. 3e) indicating that distal axon curvature was significantly increased in APPKO neurons.

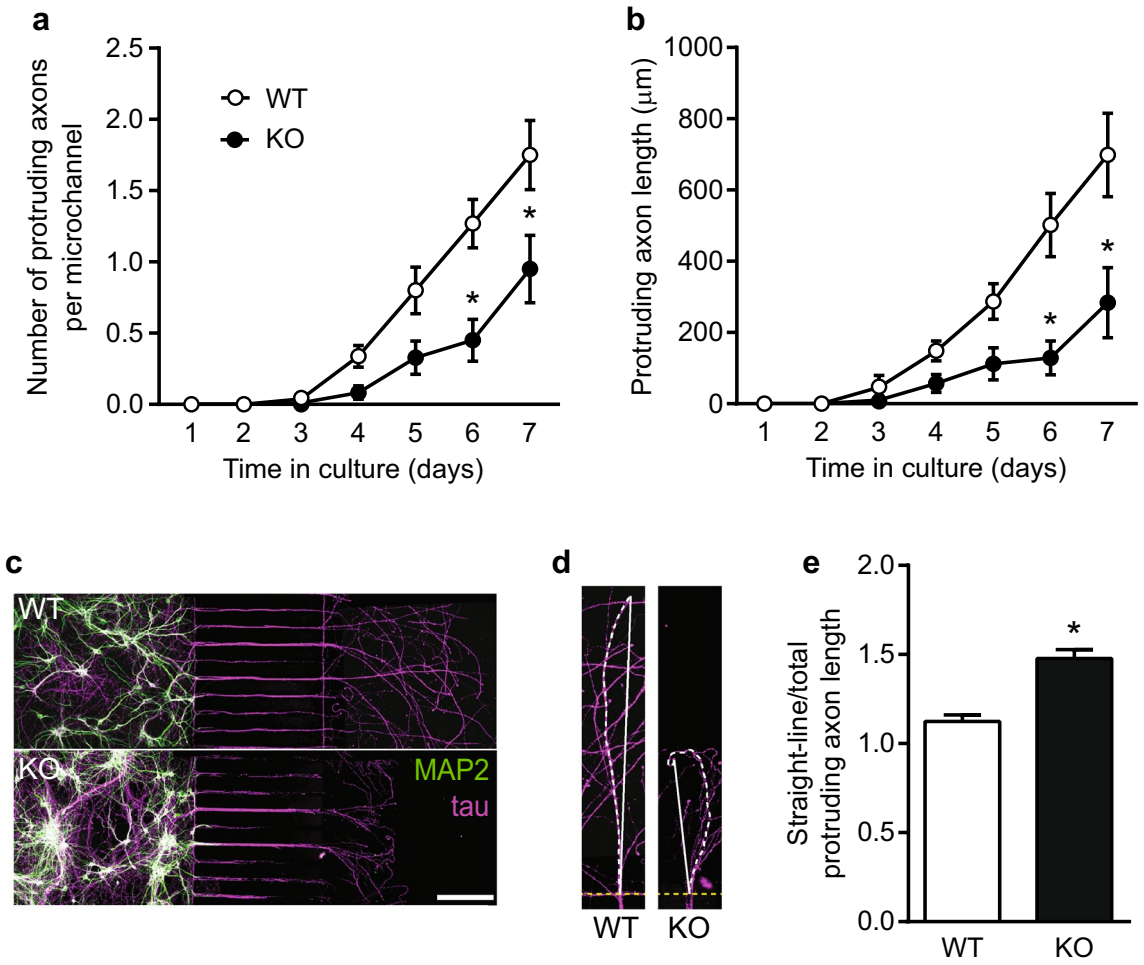

Fig. 3 Distal axon outgrowth is reduced in APPKO hippocampal neurons. **a** APPKO neurons show a significant ($P < 0.05$) reduction in protruding distal axons per microchannel into the distal compartment of microfluidic chambers at 6 and 7 DIV. **b** There was also a significant ($P < 0.05$) reduction in mean distal axon length in APPKO cultures at 6 and 7 DIV compared with wild-type axons. **c** Immunocytochemical staining of axons revealed that MAP2-positive dendrites (green) were restricted to the somatodendritic compartment (left-side), and that only tau-positive axons (purple) extended through the microchannels into the distal compartment (right-side). **d** Higher magnification of the image in (**c**) shows how axon curvature was measured by generating a ratio of straight (solid line) to total axon (dashed line) length. Yellow dashed line indicates location of microchannels. **e** There was a significant ($P < 0.05$) increase in the ratio of straight line/total length in APPKO distal axons. Data shown as mean ± SEM. Asterisk denotes significance. Scale bar = 200 µm

APPKO Axon Growth is Mediated by Altered Cell Adhesion

It is well known that cell adhesion molecules play a major role in axon pathfinding [37–39]. Therefore, to investigate whether abnormal axonal curvature in the APPKO neuron cultures was due to altered cell adhesion, cell adhesion was assessed at time of plating. Cell adhesion was significantly ($P < 0.05$) increased in APPKO neurons compared with wild-type cells (Fig. 4a).

As focal adhesion kinase is a major marker of neurite adhesion, the number of FAK puncta is an index of axonal and dendritic adhesion. Therefore, the density of focal adhesion kinase (FAK) puncta was measured along the length of dendrites and axons at 14 DIV (Fig. 4b, c). There was a significant increase in the number of FAK positive puncta along proximal (< 100 µm of soma) sections of tau-positive axons in APPKO cultures compared with wild-type neurons ($P < 0.05$) (Fig. 4d). There was no difference in the density of FAK puncta in distal axon segments (> 100 µm of the soma) ($P > 0.05$) (Fig. 4e). Analysis of dendritic FAK puncta revealed a significant ($P < 0.05$) increase in puncta density along APPKO dendrites when compared with wild-type neurons (Fig. 4f).

Discussion

Although a large number of studies have provided evidence that APP has a role in promoting nerve growth and development, the evidence for this role has been mostly circumstantial, and the precise function of APP in regulating neuronal growth and development has been obscure [1, 2]. In this study, we show that KO of APP results in morphological and developmental abnormalities in cultured hippocampal neurons. We found that both dendritic and axonal growth abnormalities in APPKO hippocampal neurons suggesting that APP plays an important role in the growth of both dendrites

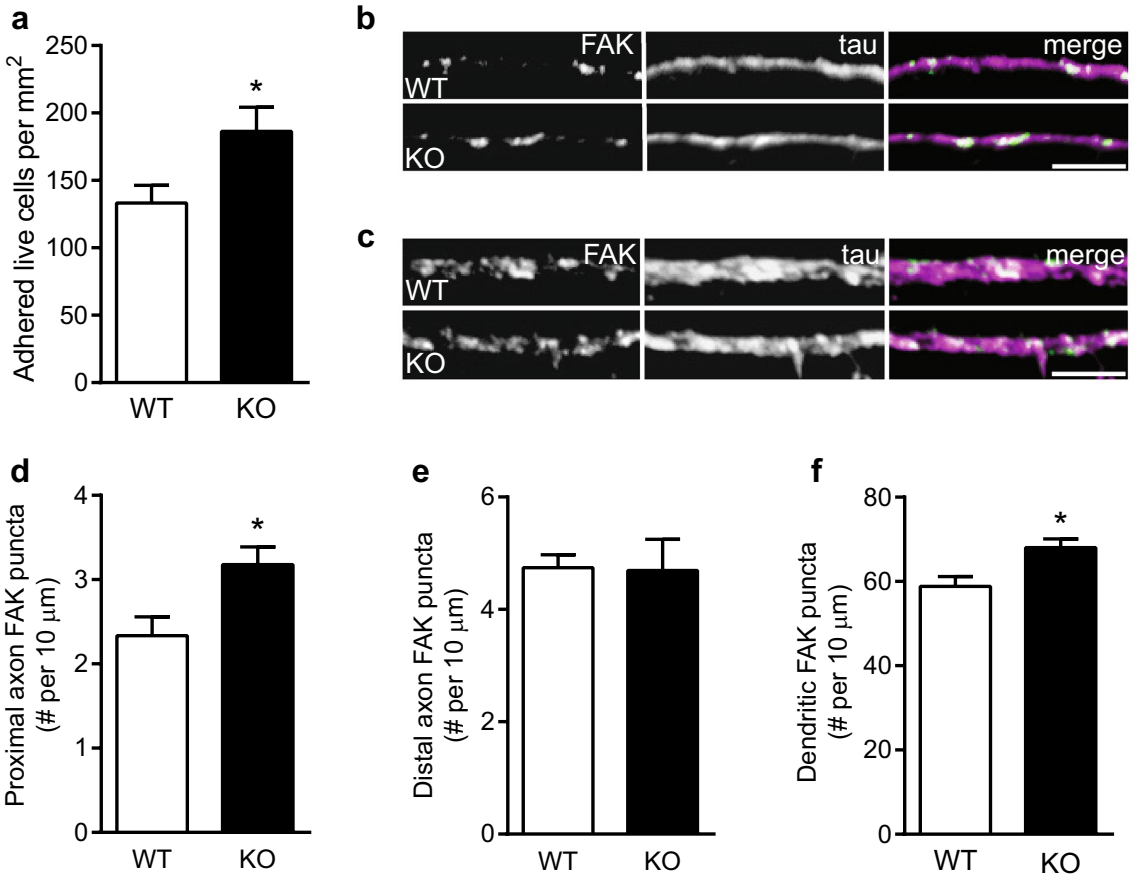

Fig. 4 Cellular adhesion and neurite focal adhesion puncta are increased in APPKO hippocampal neurons. **a** Cellular adhesion to poly-L-lysine coated plates 15 min post-plating was significantly ($P < 0.05$) increased in APPKO neurons compared with wild-type neurons. Representative focal adhesion kinase (FAK) and tau staining for identification of FAK puncta along axons (**b**) and dendrites (**c**). **d** The density of FAK puncta was significantly ($P < 0.05$) increased along proximal axon segments, however there was no change in the frequency of FAK puncta along distal axon segments (**e**). **f** FAK puncta were significantly ($P < 0.05$) increased along dendrites in APPKO neurons compared with wild-type neurons. Data shown as mean ± SEM. Asterisk denotes significance. Scale bar = 5 µm

and axons. Dendrites of APPKO hippocampal neurons were found to be less highly branched than normal neurons. A defect in axonal growth was also observed. The axons of APPKO neurons failed to grow through microfluidic chambers as readily as normal axons, and those axons that did grow through exhibited abnormal morphology. The defect in both dendritic and axonal growth is likely to explain that the number of synapses in APPKO cultures was reduced compared to wild-type neuron cultures.

Previous studies from our group [2, 8, 15, 40, 41] and others [42–45] also support the view that APP may be involved in the regulation of both axonal and dendritic outgrowth. In the olfactory system, APP expression correlates well with the growth of olfactory receptor axons and with the growth of dendrites from mitral cells [16]. Studies by Milward et al. [44] have shown that secreted APP may mediate nerve growth factor associated neurite outgrowth from PC12 cells and a number of subsequent studies have shown that secreted APP may act as a growth factor-like molecule to promote neurite outgrowth [2, 8, 10, 15, 40, 42, 43, 46, 47].

The idea that APP has a role in neurite outgrowth and synaptogenesis is also supported by the observation that APPKO mice and APP transgenic mice show some abnormalities. APPKO mice have smaller brains than normal mice and show reactive gliosis and impaired locomotor activity [48]. Recent studies on APPKO mice reveal reduced dendritic arbourisation in the dentate gyrus and reduced survival of newly born neurons [41]. Although it has been reported previously that mouse cortical neurons lacking APP show normal neurite outgrowth [49], it is likely that this failure previously to detect a difference in neurite outgrowth between APPKO mice and wild-type mice was due to the subtlety of the defect. In our experiments, it was only after analysis of the pattern of dendrite branching or after cultured neurons were allowed to grow in microfluidic chamber devices that the underlying defects in outgrowth were revealed. Previous studies investigating APP KO in cultured hippocampal neurons have reported reduced neurite arbourisation [45], suggesting that hippocampal neurons may be more vulnerable to the effects of APP KO than cortical neurons. Mice expressing the human APP transgene also exhibit some brain abnormalities [50, 51], although in these cases the causes of the abnormalities are unclear as it is difficult to determine whether the neuritic abnormalities are due to the transgene or to Aβ production which is a natural consequence of human APP overexpression.

The role for APP during neuronal development is not limited to neurons. Glial cells also express APP isoforms [52] and similarly secrete APP cleavage products [53]. The addition of astrocyte conditioned medium containing soluble products of APP cleavage has been shown to rescue some of the neuronal growth defects arising from APP KO [45], suggesting that astrocyte-derived APP products are important for neuronal growth. In our model, we used a culture paradigm with minimal astrocytic intrusion to show the effects of APP KO in neurons, however small numbers of astrocytes within the WT cultures may have enhanced neuronal growth. Conditioned medium transfer experiments between APP KO and WT cultures did not yield any significant differences in neuronal morphology (not shown), which suggests that the defects in neuronal growth as reported in this study are primarily driven by neuronal expression of APP.

While the precise mechanism by which APP influences neurite outgrowth is unclear, our data support the view that this defect may be due to an effect on cellular adhesion. Cellular adhesion to the substrate (poly-L-lysine) was increased in APPKO cultures and this increase in adhesion was associated with an increase in the number of focal adhesion puncta on distal axons and dendrites. This result was somewhat surprising, given that other studies, including our own [54, 55] have suggested that APP may be important for promoting cell adhesion. The idea that APP may promote cell adhesion was first suggested by Breen et al. [56]. This idea is consistent with the presence of extracellular matrix protein binding sites on APP [57]. Other studies support this view, although most of the evidence for an adhesive function for APP is circumstantial. It is possible that the increase in cell adhesion observed in APPKO neurons could be caused by a compensatory overexpression of other cell adhesion proteins following APPKO. Alternatively, the result may be explained by the fact that APP has multiple functions, perhaps exerting a pro-adhesive action on distant targets and an autocrine-like anti-adhesive action at the site of expression. Only further studies will be able to clarify this issue.

The significance of APP's physiological function for AD is unclear. APP is highly expressed in dystrophic neurites around amyloid plaques [58–62] and this increased expression may provide an explanation for the increased production of Aβ in the vicinity of amyloid plaques [63]. As amyloid deposits are known to damage synapses [64], it is tempting to speculate that the increased levels of APP in dystrophic neurites may be a consequence of this synaptic damage and that the APP upregulation is part of an attempted repair mechanism [47, 65–67]. While it is generally thought that APP expression promotes neurorepair mechanisms, it is also possible that the upregulation of APP around amyloid deposits could be part of the problem. Again, only further research will resolve this issue.

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