more likely to exhibit a phenomenon known as domain interaction due to the presence of arginine at residue 112 [5]. The location of this residue is thought to result in the side chain of the arginine at residue 61 (N-terminal domain) forming a salt bridge with the glutamate at residue 255 (C-terminal domain) [6]. While this model has been challenged [7,8], these alternative studies used a mutated version of the protein (F257A, W264R, V269A, L279Q, and V287E), and therefore may have limited physiological relevance, particularly in the absence of a direct comparison between apoE3 and apoE4. In summary, the structural differences between apoE3 and apoE4 are thought to be important for apoE4's association with AD pathophysiology, although the picture is far from clear.

The primary functional role of apoE in the brain is to transport cholesterol and other lipids, which are vital for multiple functions including synapse formation and repair. apoE is highly expressed in the liver and brain (with the primary brain source being astrocytes), and in the brain is associated with high-density lipoprotein-like lipoproteins [9]. apoE is secreted and loaded with lipids via the ATP-binding cassette transporter (ABCA1) to form lipidated lipoprotein particles. Endocytosis of these particles occurs via interaction with apoE receptors, namely low-density lipoprotein (LDL) receptor (primarily in glia) and LDL receptor-related protein 1 (primarily in neurons) [10]. Important to note is that despite its low expression in neurons at baseline, apoE can be significantly expressed in neurons in response to injury or stress [3].

While there are apparent structural differences between apoE isoforms, the differential impact on lipid metabolism and the relation to risk for AD remains an open question. Data from neuronal cultures support apoE4's acceptance of lipids being impaired in comparison with apoE2's acceptance in both astrocytes and neurons [11]. Mice expressing human apoE4 have altered cholesterol and lipid metabolism in the brain [12]. Notably, effects on lipid transport may have a particular impact on synaptogenesis and neurodevelopment, as discussed in a later section of this review, as well as the ability to repair membrane damage. In this case, apoE4-associated risk would represent a loss of protein function. Additionally, apoE is known to have significant impacts in the periphery, with apoE4 being associated with hyperlipidemia and heart disease [13]. How these peripheral effects may impact the risk association of apoE with AD is unknown, and it is important to note that there is little interaction between brain and peripheral cholesterol metabolism [14].

The cellular source of apoE is highly regulated; neuronal production of apoE appears to be mediated by signals from astrocytes [15]. Neuronal apoE is known to be upregulated in response to injury [16,17]. Postmortem human samples showed that apoE4 undergoes neuron-specific proteolysis [18], and that this is dramatically pronounced in comparison with apoE3 and apoE2. This effect is thought to be due to apoE4's tendency to exhibit domain interaction. Transgenic mice expressing human apoE4 demonstrate that apoE4 is cleaved by a putative chymotrypsin-like serine protease termed apoE cleaving enzyme (AECE) [19]. Although neurons take up apoE secreted by astrocytes (the normal physiological process), this apoE proteolysis occurs in the neuronal secretory pathway and not in an endocytic pathway, which seems to indicate neuronal-source specificity to this event [20]. The biochemical stability of apoE is dependent on C-terminal segments [21,22]. AECE-cleaved apoE4 missing C-terminus residues 272 to 299 (apoE4$^{\Delta 272-299}$) can translocate from the secretory pathway to the cytosol [23]. The LDL-receptor binding region (residues 136 to 150), which is rich in positively-charged amino acids arginine, lysine, and histidine, is required for escape in Neuro-2a mouse neuroblastoma cultures [23]. An enrichment in positively-charged amino acids is also seen in the protein-translocation domains of certain viral proteins, suggesting a similar mechanism for membrane penetration [23]. However, such a potential mechanism has not been tested directly on apoE cytoplasmic translocation.

Apolipoprotein E and tau pathology

apoE is known to have effects on tau pathology, which is a hallmark of AD, although the pathophysiology remains uncertain. Full-length apoE4 expressed in Neuro-2a cultures acts along with zinc to phosphorylate tau via the extracellular signal-regulated kinase pathway, and neuron-specific apoE4 expression in mice results in high levels of phosphorylated extracellular signal-regulated kinase and phosphorylated tau in the hippocampus [24]. apoE3 is effective at binding the microtubule-binding repeat region of tau, the region responsible for the formation of paired helical filaments; apoE4 does not bind this region and thus may not be as effective at limiting formation of neurofibrillary tangles [25].

apoE cleavage fragments (via the processes described in the previous section) have been shown to have effects on the cytoskeleton and intracellular trafficking. In Neuro-2a cells, apoE4$^{\Delta 272-299}$ expressed by transfection interacts with cytoskeletal proteins to form tangle-like structures containing phosphorylated tau [18]. Mice expressing neuronal apoE4$^{\Delta 272-299}$ at high levels develop neurofibrillary tangles and die at 2 to 4 months. At lower levels of expression, mice exhibit deficits in learning and memory at 6 to 7 months [19], and in humans preclinical alterations in cognition more strongly reflect the distribution of tau than of β-amyloid pathology [26,27]. Any damage to neurons due to apoE4-specific cleavage would reflect a toxic gain of function of the protein. apoE impact on tau has also been proposed to be one mechanism by which apoE4 has a significant

impact on neurogenesis and neuroenergetic processes, as discussed below.

Apolipoprotein E and neuroenergetics

apoE has been associated with cerebral energy metabolism via both β-amyloid-dependent and β-amyloid-independent mechanisms [28,29]. Brain imaging has provided significant insight into *APOE* associations with AD. Cognitively normal, late middle age (50 to 65 years old) *APOE* ε4 homozygotes exhibit significant reductions in glucose uptake (measured as the cerebral metabolic rate for glucose via fluorodeoxyglucose positron emission tomography) in the same parietal, prefrontal, and temporal regions found to exhibit changes in probable AD patients [30]. Additional studies found longitudinal declines in the cerebral metabolic rate for glucose [31] and gene dose effects [32]. Cognitively normal, 20-year-old to 39-year-old *APOE* ε4 carriers exhibit a significantly decreased cerebral metabolic rate for glucose in similar regions, in this case decades ahead of any apparent pathology or cognitive defects [33]. Recently, the *APOE* genotype was shown to modulate the cerebral metabolic rate for glucose in normal aging, with no contribution from fibrillar β-amyloid. Additional work investigating mitochondrial activity using cytochrome oxidase histochemistry to measure enzymatic Complex IV function found that young-adult *APOE* ε4 carriers display deficits in the superficial lamina of the cortex, specifically the posterior cingulate cortex [34]. Similar deficits are apparent in AD [35,36]. Changes in mitochondrial function and glucose uptake may therefore be an early indicator of AD-related risk and physiological alteration.

There is evidence that apoE4 has deleterious effects on neuroenergetics via interference with intracellular trafficking as well as through direct effects on mitochondrial function. The cytoskeleton plays an important role in the trafficking of mitochondria [37]. Human apoE4-expressing mice exhibit impairments in axonal transport and accumulate mitochondria in axonal dilations [38]. In PC12 cells, apoE4 can impair mitochondrial motility when compared with apoE3 [39]. Interestingly, small-molecule apoE structure correctors (reviewed in [40]) that alter apoE4's structure to be more similar to apoE3 have been shown to ameliorate this effect [37].

apoE4 has also been shown to directly impair mitochondrial function. apoE4 binds the alpha and beta subunits of the F1 portion of ATP synthase in liver [41], although the functional consequences of this are unclear. In Neuro-2a cultures expressing apoE4$^{\Delta 272-299}$, apoE4 fragments cause mitochondrial dysfunction that requires the lipid-binding region (residues 244 to 272) [23]. In further study using Neuro-2a, apoE4$^{\Delta 272-299}$ demonstrated the ability to bind ubiquinol cytochrome c reductase core protein 2 and cytochrome CI of Complex III and cytochrome c oxidase subunit 4 isoform 1 of Complex IV of the electron transport chain. This binding significantly reduced the respiratory function of both complexes [42]. apoE4-expressing Neuro-2a and mouse primary neuron cultures have reduced expression of subunits for all electron transport chain complexes [43]. Complex IV respiratory function is also significantly decreased. Proteomic analysis in mice expressing human apoE found that mitochondrially enriched fractions prepared from apoE4 and apoE3 mouse hippocampus differed in levels of proteins associated with mitochondrial function, oxidative stress response, and organelle transport [44]. In humans, postmortem tissue from the middle temporal gyrus of middle-aged *APOE* ε4 carriers displayed differential expression in 70 transcripts, 30 of which are involved in oxidative mitochondrial function, when compared with age-matched noncarriers [45]. Further work is needed to determine which of these potential insults exhibits primacy or whether all interact to knock down bioenergetic function.

apoE4 can induce endoplasmic reticulum stress in astrocytes [46], an effect that does not occur in neurons [47]. Interestingly, apoE4 trafficking is impaired in the endoplasmic reticulum and Golgi apparatus in Neuro-2a cultures, and small-molecule apoE structure correctors can rescue this impairment [47]. Unlike neurons, however, mouse primary astrocyte cultures expressing apoE4 do not exhibit changes in electron transport chain gene expression [43]. apoE4 may therefore alter neuronal metabolic function via different mechanisms, including direct mitochondrial impairment in the neurons themselves, and indirect effects via harm to astrocytes, which provide neurons with essential metabolic support [46]. apoE isoforms expressed in b12 cells display differing antioxidant ability in a manner correlated with disease risk (apoE2 > apoE3 > apoE4) [48]. In AD, oxidative stress is thought to be an early feature of pathophysiology [49,50]. Taken together, effects of apoE on neuroenergetics may represent a toxic gain of function leading to mitochondrial impairment, disrupted trafficking, or astrocyte damage and/or loss of antioxidant or other normal functions (see Figure 1).

Apolipoprotein E, neurodevelopment and synaptic plasticity

A number of brain imaging studies support a potential differential effect for *APOE* genotypes in neurodevelopment. In volumetric studies utilizing magnetic resonance imaging, young *APOE* ε4 carriers display thinner entorhinal cortices than noncarriers [51] and reduced hippocampal volumes compared with both noncarriers [52] and *APOE* ε2 carriers alone [53]. Additionally, magnetic resonance imaging studies in neonates have found volumetric reductions in several regions of *APOE* ε4 carriers [54]. However, the *APOE* ε4 carrier to noncarrier

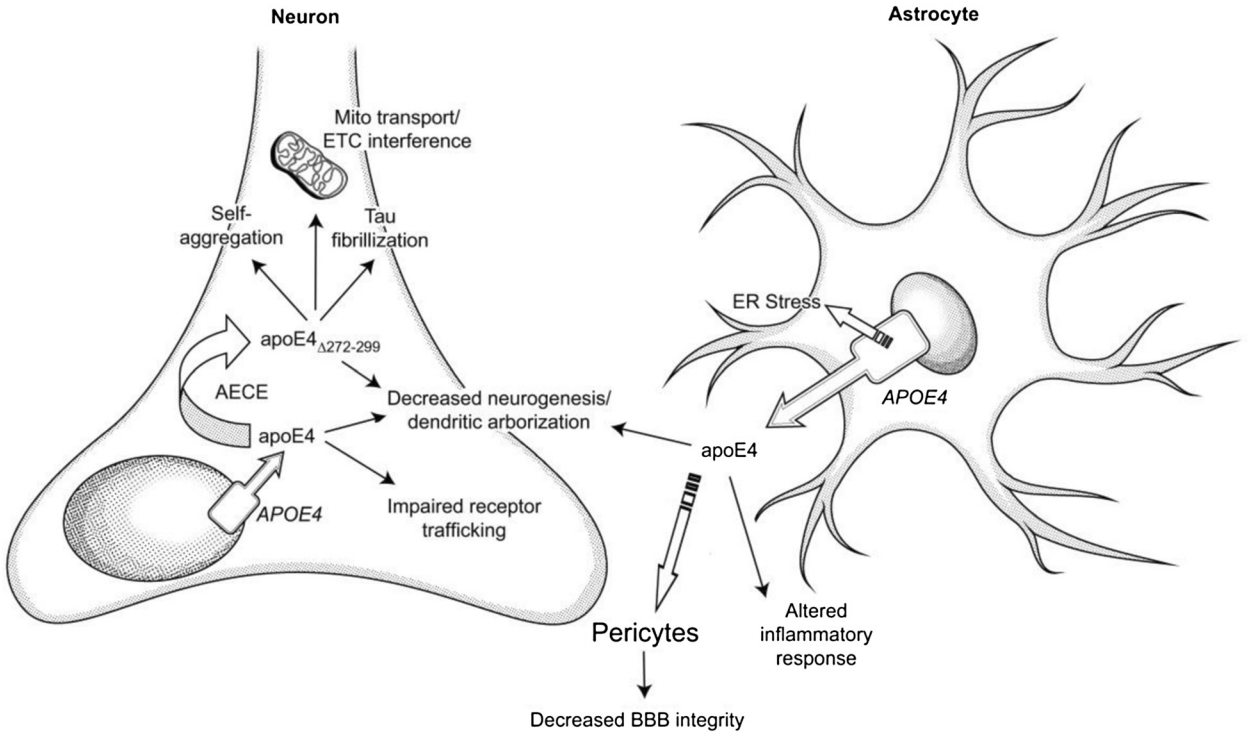

Figure 1 Effects of cell-type specific expression and aberrant processing of apolipoprotein E4 in the brain. Astrocyte-secreted apolipoprotein E (apoE) is internalized by neurons, but apoE4 expression in astrocytes has been associated with endoplasmic reticulum (ER) stress that may impair astrocyte function. Astrocyte-secreted apoE4 has also been associated with decreased blood–brain barrier (BBB) integrity via signaling in pericytes, decreased synapse development, and alterations in inflammatory response. A smaller proportion of brain apoE expression is attributed to neurons: apoE4 expressed in neurons (but not that internalized from astrocytes) is highly susceptible to cleavage by apoE cleaving enzyme (AECE) in the secretory pathway, becoming AECE-cleaved apoE4 missing C-terminus residues 272 to 299 (apoE$^{\Delta 272-299}$). This cleavage product is capable of escaping the secretory pathway, self-aggregating in the cytosol, increasing tau fibrillization, interfering with mitochondrial function and motility, and impairing neurogenesis. In neurons, full-length apoE4 has been associated with decreased dendritic arborization and impairment of receptor trafficking. ETC, electron transport chain.

difference in hippocampal volumes has not been found consistently [55].

The cellular and molecular mechanisms of these differences are unclear, but there are several interesting lines of research. Mice expressing human apoE4 display reduced neurogenesis, which is also apparent in apoE knockout mice [56]. apoE4 fragmentation and tau phosphorylation are also associated with decreased maturation of GABAergic neurons in primary cultures [56]. apoE4 mice also show declines in GABAergic neurons with age that are associated with deficits in learning and memory [57]. These effects are also found (to a more severe degree) in mice expressing neuronal apoE4$^{\Delta 272-299}$ [57]. Mouse studies have also shown that dendritic arborization is decreased in apoE4-expressing mice, compared with apoE2 or apoE3 knockin mice [56,58-60]. There is also evidence that neurite outgrowth is limited by apoE4, in comparison with apoE2 or apoE3 [61-66]. Microtubule depolymerization associated with apoE–tau interaction is thought to be important to apoE4 effects on neurite outgrowth [64]. apoE has also been shown to be involved in synaptogenesis via its cholesterol transport abilities [67] and plays a role in maintaining the neural progenitor pool in the adult dentate gyrus [68].

A number of mouse behavior studies have examined the effects of apoE4 on learning and memory, although outcomes have been inconsistent [69-72]. On the cellular level, apoE4 mice display deficits in excitatory synaptic transmission [60]. Interestingly, apoE4 is also believed to sequester apolipoprotein E receptor 2, leading to impairment of reelin signaling and synaptic plasticity, which is not seen with apoE3 and apoE2 [73]. In humans, *APOE* ε4 homozygotes and *APOE* ε4 carriers exhibit declines in memory (as assessed by neuropsychological testing) earlier than *APOE* ε4 noncarriers, and before age 60, while remaining free of clinically significant memory loss [27,74-76]. Van der Flier and colleagues reviewed an extensive literature and proposed that *APOE* ε4 carriers have a more typical amnestic syndrome with greater hippocampal atrophy and an older age of onset while non-ε4-related AD was more likely to manifest as dysexecutive, aphasic, apraxic, and visual variant syndromes with less