

Review

Transgenic Mouse Models as Tools for Understanding How Increased Cognitive and Physical Stimulation Can Improve Cognition in Alzheimer's Disease

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Abstract. Cognitive decline appears as a core feature of dementia, of which the most prevalent form, Alzheimer's disease (AD) affects more than 45 million people worldwide. There is no cure, and therapeutic options remain limited. A number of modifiable lifestyle factors have been identified that contribute to cognitive decline in dementia. Sedentary lifestyle has emerged as a major modifier and accordingly, boosting mental and physical activity may represent a method to prevent decline in dementia. Beneficial effects of increased physical activity on cognition have been reported in healthy adults, showing potential to harness exercise and cognitive stimulation as a therapy in dementia. 'Brain training' (cognitive stimulation) has also been investigated as an intervention protecting against cognitive decline with normal aging. Consequently, the utility of exercise regimes and/or cognitive stimulation to improve cognition in dementia in clinical populations has been a major area of study. However, these therapies are in their infancy and efficacy is unclear. Investigations utilising animal models, where dose and timing of treatment can be tightly controlled, have provided many mechanistic insights. Genetically engineered mouse models are powerful tools to investigate mechanisms underlying cognitive decline, and also how environmental manipulations can alter both cognitive outcomes and pathology. A myriad of effects following physical activity and housing in enriched environments have been reported in transgenic mice expressing Alzheimer's disease-associated mutations. In this review, we comprehensively evaluate all studies applying environmental enrichment and/or increased physical exercise to transgenic mouse models of Alzheimer's disease. It is unclear whether interventions must be applied before first onset of cognitive deficits to be effective. In order to determine the importance of timing of interventions, we specifically scrutinised studies exposing transgenic mice to exercise and environmental enrichment before and after first report of cognitive impairment. We discuss the strengths and weaknesses of these preclinical studies and suggest approaches for enhancing rigor and using mechanistic insights to inform future therapeutic interventions.

Keywords: Dementia, exercise, physical activity, environmental enrichment, 'brain training', cognitive stimulation, lifestyle factors, experience-dependent plasticity, enviromimetics

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LIFESTYLE FACTORS CONTRIBUTE TO ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a complex, multifactorial disease that affects an estimated 45 million people worldwide [1]. Despite over 100 years of research and great progress in understanding mechanisms of pathogenesis, no disease-modifying therapy has yet been approved for clinical treatment. Although the aetiology of AD is yet to be fully elucidated, a variety of risk factors, both genetic and environmental, have been reported to increase or decrease development and progression of the disease. It is estimated that 30% of cases are attributable to just seven modifiable risk factors - physical inactivity, smoking, depression, midlife hypertension, midlife obesity, diabetes and low education [2]. After accounting for non-independence between risk factors, around 20% of AD cases worldwide might be attributable to low education, and specifically in the US, 19% of cases due to lack of physical activity [2]. Targeting these environmental factors represents a potential way to reduce incidence of AD, and recent clinical trials employing such strategies reveal benefits. For example, the FINGER clinical trial showed that an intervention that improved diet, increased social activity, implemented an exercise regimen and cognitive training in a 'high-risk for dementia' group significantly increased neurological test battery scores compared with controls [3]. More specific interventions like exercise and computerised cognitive training (CCT) also show beneficial effects in healthy aging patients [4, 5] as well as those with mild cognitive impairment (MCI) or even AD [6, 7]. These early trials represent small cohorts, but before these regimes can be rolled out on larger scales, questions regarding timing, dose, and type of intervention must be answered. Although an excellent review of the effect of aerobic exercise in normal human subjects was published recently [8], the effects on exercise in MCI and AD are less well established. Another complication with moving an intervention from small to large-scale cohorts is the issue of adherence, which is a significant predictor of outcome [9]. Furthermore, it is difficult to determine whether a specific therapy is beneficial due to its effect on one or many potentially interacting risk factors (e.g., physical exercise improves depression and/or reduces vascular risk factors) or if it is unlikely to be effective in the presence of others (e.g., physical exercise and obesity or smoking). To answer these questions, investigations

utilising animal models, where dose and timing of treatment can be tightly controlled are warranted. In addition, these animal models will allow study into the molecular mechanisms underlying the beneficial effects of interventions, leading to novel treatment targets.

EXERCISE AND COGNITIVE STIMULATION INTERVENTIONS IN ALZHEIMER'S DISEASE ANIMAL MODELS

An approach to modelling AD in animals is to use transgenic mice expressing one or more mutations causative in familial AD (fAD). These mouse models recapitulate the first stages of disease; specifically amyloid beta ($A\beta$) accumulation and increased levels of hyperphosphorylated tau. However, they do not develop the tau neurofibrillary tangles that are seen in humans patients and are thus considered a model of a discrete component of pathology. Transgenic fAD mice also exhibit progressive cognitive decline as they age [10]. In humans, fAD mutations occur in either the amyloid precursor protein (APP) or the presenilin 1 genes (PS1). When expressed in animals they result in increased production of the $A\beta$ peptide. This peptide can exist in many states from monomer, oligomer, fibril or plaque. The rate of $A\beta$ accumulation, and the degree of and timing of cognitive decline in fAD mouse models is dependent on the promoter, number of fAD mutations expressed and if tau mutations are additionally introduced (See Supplementary Table 2 for the onset of cognitive symptoms in all mouse models reviewed here). Finally, background strain of the fAD mouse models can significantly interact with phenotype expressed. For example, C57/BL6 or DBA/2J backgrounds lead to higher epileptiform activity and seizures can modify severity of progression or can result in early death [11–13]. Specific features of fAD mouse models and their modifiers are reviewed in detail elsewhere [10, 14].

Exercise and cognitive stimulation interventions can be modelled in transgenic mice by increasing physical activity and by housing with environmental enrichment (EE). To model exercise in rodents, two regimes are predominantly utilised: 'forced' treadmill running (with gentle tail touches to encourage running), usually over a period of an hour each day, or 'voluntary' running wheels that are permanently

placed in the animal's cage for the duration of the regime. EE increases somatosensory, motor, social and cognitive stimulation and is considered a proxy for cognitive training interventions. The application of EE is simple, where the researcher modifies the environment of an animal to be more 'enriching' than the standard research environment of littermates [15]. Animals are housed with novel objects with varied surfaces to interact with, tunnels and ladders to climb through, material to chew and use for nest-building, and more space to roam in than their non-enriched controls. By its very nature, EE also increases the amount of exercise the animal is doing, and EE often includes running wheels as part of the enriched environment; in this review more than 75% of the studies include running wheels, and multiple of these studies specifically compare exercise and EE. It is difficult to disentangle the exact effects of EE from exercise without a physical exercise control. While not common, some EE paradigms add additional animals (possibly up to 10 animals per cage) to enhance social interaction [15], but the typical social aspect (i.e. 2–5 animals per cage) is present in all studies reviewed here. Dose of EE paradigms can be modified by restricting access to EE to short periods (i.e. 1 hour a day, 4 days a week), or animals can permanently live in the environment for weeks to months. Some EE paradigms include a 'super enrichment' component, where animals live in an EE cage but are placed in another, larger EE environment for short periods at a time (e.g. 1 hour a day, 3 days a week) [16].

In the context of Alzheimer's disease, the timing of the interventions is an important factor to consider. In the absence of a clear early diagnostic test for AD, interventions in the clinic are most commonly applied to patients after first report of cognitive symptoms. It is important to determine the efficacy of a lifestyle intervention if it is applied therapeutically, after first signs of cognitive impairment. Animal studies are able to determine this, with some applying pre-onset (preventative) or post-onset (therapeutic) of cognitive symptoms in fAD mice. While findings from exercise paradigms in mice are relatively easy to compare to exercise in patient populations, EE has no direct correlate in the clinic. In this systematic review, we examined all studies that applied exercise or EE to fAD mice in a preventative or therapeutic manner (for more information, see extended methods in supplementary materials). Due to differences in progression in fAD mouse models, classification of studies as preventative or therapeutic was determined by a consensus

of published reports of first cognitive impairment, unique to each model (see Supplementary Table 2). Overall findings of this review are summarised in Table 1.

CAN ENRICHMENT AND EXERCISE IMPROVE COGNITION IN AD MOUSE MODELS?

Overwhelming evidence supports the beneficial effects of enrichment and exercise on memory in fAD mice. Can these investigations shed light on when these interventions need to be applied, and to what degree? When exercise interventions are applied before the first reported cognitive impairment, they predominantly rescue performance on the Morris water maze (MWM) test of spatial long-term memory [17–28], excluding one study in APP23 mice [29]. Lack of beneficial effect was described on a non-water version of the MWM, the Barnes maze [30, 31]. Exercise studies also suggest improvements in short-term spatial memory in the Y-maze [31–33]. However, other researchers have shown no change [34, 35]. The effects of EE interventions applied before onset of cognitive decline mirror those seen with exercise. EE reproducibly improves performance in MWM in a diverse range of transgenic models [29, 36–46], with one exception [47]. Improvements were also seen in short-term memory tasks including the Y-maze [40], and novel object recognition [37, 42, 45, 48, 49]. Interventions applied after first report of cognitive impairment also positively impact on the phenotype of transgenic fAD mouse models. The majority of studies utilising exercise as a therapy in transgenic mice report improvements in MWM [18, 20, 23, 25, 26, 50–60], Barnes maze [61], and Y-maze [56]. Despite these reports, a number of studies scrutinising effects of late disease exercise also showed no change in MWM performance from control [57, 62–64]. One study comparing voluntary and forced exercise paradigms in separate cohorts of Tg2576 mice reported improvements in short-term memory only in mice allocated to the voluntary exercise [65]. Forced running has been shown to impair memory and induce a stress response in mice and thus may not adequately translate to the clinic where exercise regimes are voluntary [66, 67]. EE applied after first report of cognitive impairment has been shown to rescue long-term spatial memory [48, 68, 69], but not reliably [45, 70]. While there are

Table 1
Effects of Exercise and EE on fAD models

Category	Environmental Enrichment		Exercise	
	Preventative studies	Therapeutic studies	Preventative studies	Therapeutic studies
Cognitive behavioural changes	Spatial memory	↑ [38, 42-44, 46, 49, 88, 134, 138-140, 178] =[46, 134, 135, 139, 141]	↑ [48, 68, 70] =[45, 70]	↑ [17-25, 25, 27, 35] =[29-31, 35]
	Short-term memory	↑ [37, 42, 49, 105]	↑ [45, 92] =[92]	↑ [31-33, 65] =[34, 35, 65]
Working memory		↑ [36, 42] =[47, 90]	↑ [70]	↑ [56] =[63]
	Exploratory/ affective behaviour			↑ [58] =[23, 63]
Fear conditioning/ passive avoidance		↑ [85] =[35]	↓ [19, 20, 30, 35] ↑ [33, 80] =[23, 80]	↓ [61, 62] =[23]
	Aβ in hippocampus	↑ [38, 39, 89] =[29, 41-43, 45, 47, 90, 105]	↑ [69] =[45, 68, 69, 92, 94, 95]	=[18, 22, 23, 30, 31, 65] ↓ [17, 21, 25, 32, 79, 80]
Aβ in cortex		↓ [36, 37, 45, 86, 87] =[29, 41-43, 47]	↓ [95, 145] =[68, 69, 92, 95]	↓ [17, 21, 100] =[18, 22, 30, 31, 65]
	Aβ in brain*	↓ [36, 37, 86, 87] ↑ [99] =[39]	↓ [93] =[45, 94]	↓ [18, 51, 54, 62] ↓ [61]
Aβ40		↓ [45, 93] ↑ [38, 89]	↓ [94, 95] =[93]	↑ [179]= [18, 23, 57, 61] ↓ [51, 62, 83, 84]
		=[37, 42-44, 47, 86, 91, 105] ↓ [85, 87, 88, 93]		

Aβ42	↑ [38, 89] =[37, 41-44, 47, 86, 91, 105] ↓ [85, 87, 88, 93] =[29, 37, 39, 42, 86-88, 105]	↑ [69] =[93] ↓ [94, 95] ↑ [95]	= [17, 18, 30, 65, 100]. ↓ [17, 23, 80, 81, 100].	= [23, 57, 61] ↓ [18, 50-53, 55, 62, 84]
APP			↑ [17] =[18, 100] ↓ [29, 79] ↑ [17, 79] =[17, 18, 100] ↓ [79, 81] ↑ [80, 81] =[100] ↓ [81]	= [18, 51] ↑ [51, 84] =[61, 100] ↓ [51, 54, 84] ↑ [51] =[61] ↓ [51, 54]
Aβ processing	↑ [41, 87, 93] =[37, 47, 93, 105]			
Aβ trafficking	↑ [87, 91, 93] =[37, 41, 91, 93]			
Tau	↓ [43, 88]		= [19-21, 101] ↓ [18, 21]	= [52, 61, 64] ↓ [18, 62]
Neuronal factors	↑ [37, 42, 96] =[29]	↑ [92] =[92, 136] ↑ [95, 167]	↑ [20, 80] = [20, 23, 29] ↓ [27, 29] ↑ [21, 32] =[29, 32] ↑ [23] =[32]	↑ [18, 52, 53, 55, 57, 59, 84] =[23, 64] ↑ [64, 82] =[64] ↑ [23, 82] =[64] ↓ [54]
Neurogenesis (BrdU+)	↑ [44, 88, 139-142] =[29, 133]			
Neurogenesis (inferred through markers of proliferation)	↑ [42, 43, 134, 135, 138] =[90, 105]			
Neural plasticity	↑ [36, 48, 88, 105]	↑ Synapse density [48, 92, 137] = Synaptic proteins [92]	↑ [18, 25, 80, 101] =[17, 27] ↓ [25, 101]	↑ [18, 25, 26, 61] =[61, 65] ↓ [25]

(Continued)

Table 1
(Continued)

Category	Environmental Enrichment		Exercise
	Preventative studies	Therapeutic studies	
Apoptosis	↓ [105, 145]	↓ [146]	↑ [52, 55, 59] =[53, 54, 59] ↓ [51–55]
Neuronal survival	↑ [42, 44, 139] =[90, 105]		
Astrocytosis	↑ [140, 172] =[37, 86, 138, 139]	↓ [94]	
Microgliosis	↑ [46, 86, 93] =[37, 105, 133]	= [79] ↓ [22, 23]	= [83] ↓ [51, 61]
Cytokines	↓ [161] =[36]	↓ [146]	= [56] ↓ [54, 83]
Oxidative stress	↓ [145]	↑ [19, 20, 33, 81] = [19] ↓ [19, 33]	= [57, 61–63] ↓ [26, 55, 57, 62, 63]
Energy metabolism		↓ [19, 146]	↓ [50, 52, 53, 55, 62] ↑ [50, 53, 55]
Corticosterone (EE)	= [36, 43, 85, 99]	= [69]	

A total of 119 peer-reviewed, published scientific articles met the study inclusion criteria (Supplementary Figure 1). From these articles, 33 studies investigated exercise before the age of first cognitive deficit as a preventative measure and 39 studies included a preventative EE cohort. 24 studies applied exercise after the time of first cognitive deficit and thus as a therapy and 13 studies applied EE therapeutically. A diverse range of outcomes were measured following EE or exercise including: changes in cognition (and other behaviour), amyloid and associated markers, tau, neuronal integrity/survival, neuroinflammation and oxidative stress and markers of stress. Due to inconsistencies in quantification methods, outcome measures were not examined statistically but were categorised as increased, no change, or decreased, consistent with article reporting. ↑ arrows indicate studies that find increases in those variables, = symbols indicate studies that found no change while ↓ arrows indicate decreases in those variables. *Brain in this context indicates any brain area that was not the cortex or hippocampus.

some inconsistencies, there is no evidence to support negative effects of these interventions, only a lack of effect in a small number of studies.

IS THE DOSE OR TIMING OF INTERVENTION IMPORTANT FOR COGNITIVE IMPROVEMENT?

The inconsistency of these results could be explained by length of intervention, differences in methodology (of both intervention and test), mouse model, and timing of intervention. One study examined the differences in timing and length of EE interventions in the APP/PS1 mouse model [48]. Animals were housed in EE conditions from 3 and 6 months of age and cognition was assessed using the novel object recognition task and Barnes maze [48]. They showed that both interventions, regardless of the time when they were applied or the length of exposure, improved performance of the novel object recognition task, but only the version of the task testing short-term memory.

Differences in methodology are critical when assessing cognition in animal models. Furthermore, improvements in methods to assess cognition are needed. A comprehensive review of 49 studies testing cognition in the same fAD mouse model found that while significant variation between the tasks existed, some tasks were more reliable in terms of reproducibility [71]. Conflicting results in this field are common, with some investigators finding deficit at one age and others finding no change in the same strain-matched animal model [72]. Translational touchscreen cognitive testing offers robust and reproducible protocols, that provide more sensitive methods to detect subtle impairments and also cognitive changes [73]. While no study to date has investigated the effect of EE on an AD mouse model in touchscreens, changes in WT cognitive performance following EE have been described [74, 75], thus providing promise of more reproducible and translatable methods of assessment of the beneficial effects of exercise and EE interventions.

Given the limitations in sensitivity, reproducibility and translatability of cognitive testing in fAD models, it is best to assess improvements in cognition in conjunction with molecular changes in the brain. Increased knowledge of molecular and cellular mechanisms may facilitate new approaches, including enviromimetics, novel therapeutics that mimic or enhance the beneficial effects of cognitive stimulation

and physical activity [76]. As such, in the next few sections we investigate which cellular markers correlate best with improved cognition following exercise or EE in fAD mouse models.

THE EFFECT OF LIFESTYLE INTERVENTIONS ON AMYLOID AND TAU ARE NOT CONSISTENT

Both amyloid (A β) and tau are well-validated biomarkers of Alzheimer's disease and as such, reduction of A β and/or tau levels are a prime target of therapeutics [77]. Accordingly, researchers hypothesise that if a therapy is to be effective in human AD, that it must lead to a decline in amyloid and phosphorylated tau pathology in fAD mice administered the treatment. Do exercise or EE interventions lead to reduction in amyloid or tau pathology and most importantly, does this also correlate with improved cognitive performance?

Overall, when enrichment and exercise were applied either prior or after the first reported cognitive impairment in fAD mouse models, reductions in amyloid pathology were not seen consistently. Amyloid pathology can be measured in many ways, with studies investigating number and/or volume of A β plaques, indicative of the amount of amyloid present, and/or the levels of certain amyloid peptides, A β 40 & A β 42. A β 42 appears to be the pathogenic, more toxic form of A β , so an increase in this (often accompanied by a relative decrease in A β 40, or an increase in the A β 42:40 ratio) suggests that a higher level of neurodegeneration is occurring. Furthermore, soluble and insoluble forms of the peptide are scrutinised as measures of pathology, with soluble A β appearing to be a more toxic form [78]. In studies applying exercise prior to first cognitive impairment, reductions in amyloid pathology in the cortex and hippocampus were seen, in either plaques and/or insoluble/soluble A β 40 and/or A β 42 [17, 21, 24, 25, 32, 33, 79–81]. However, counter to this, many reports of exercise having minimal effect on A β have also been made [17, 18, 22–24, 29–31, 65]. Inexplicably, amyloid precursor protein (APP), whose proteolysis generates beta amyloid, was found to be decreased [29, 79] unchanged [18, 24], or increased [17] in fAD mice exposed to increased exercise. When fAD mice were exposed to exercise later in the disease, little change in number of plaques were seen [23, 25, 54, 56, 57, 64, 82, 83] except in two studies [18, 61]. However, reductions in the toxic A β 42 form were

regularly reported in fAD mice following a late exercise intervention [18, 50, 51, 53–55, 62, 63, 84]. Yet this reduction was not seen consistently with a few studies reporting no change [23, 57, 61, 83]. This could indicate that A β 42 levels are reduced due to decreased production, rather than through other mechanisms such as increased clearance or conversion to other amyloid states. Despite some conflicting reports, exercise, regardless of timing of intervention, generally reduced amyloid pathology. When fAD mice were housed in enriched environments, distinct effects on amyloid were even more intractable. EE applied early, prior to cognitive decline in fAD mice, was reported to decrease [36, 37, 85–88] and increase [38, 39, 89] amyloid markers and also result in no change in pathology [29, 40–44, 47, 90, 91]. When EE was applied late, after the first report of cognitive impairment, amyloid pathology in the cortex was unaffected [45, 68, 69, 92–95]. A small number of studies report reductions in A β 40 and 42 [94, 95] and plaques in the hippocampus [95]; however, it still appears that the positive effect of EE on cognition is not mediated by amyloid.

Comparisons of exercise and EE-induced changes on amyloid pathology between studies is difficult due to methodological differences in not only measurement of markers but also between dose and timing of the lifestyle intervention. However, even if a similar EE paradigm is applied to the same model, the APP/PS1 mouse, for approximately the same amount of time, studies have shown A β increases [38, 89], no changes [40] and decreases [87, 88, 96]. Studies scrutinising different timing of intervention within the same model and importantly same laboratory, are helpful to shed light on how these factors change the ability of exercise or EE to elicit cognitive benefits along with changes in pathology. One study applying 1 month of forced exercise to APP/PS1 mice at both 7 months and 24 months of age, showed reduced A β 40/A β 42 levels, but no difference in plaques in younger mice while the older animals had no change in amyloid pathology at all [23]. Another group that exposed APP/PS1 mice to 5 months of forced exercise at 3 and 12 months did not observe this specific effect on younger mice; in fact, in this study, exercise reduced amyloid plaques and A β 40/A β 42 levels in both groups [25]. A beneficial effect of early EE was seen in a study housing Tg2576 mice in EE for 10 weeks from 3, 5 and 10 months of age. Only the 3 month EE cohort had reduced A β load in the forebrain and hippocampus of mice and rescued MWM impairment [45] but intriguingly, regardless of the

age of exposure, EE was able to improve short-term memory impairments, suggesting a sensitivity of this form of cognition to EE. Intuitively it does make sense that interventions applied early have the most potential to halt pathology, however another study highlighted that interpretations are not always this simple. This group housed TgCRND8 mice in EE from 1–3 months and 3–5 months and reported that while both exposures reduced A β , they did so in different ways [97]. Early exposure to EE reduced the number of amyloid plaques, but these plaques were much larger in size, and reduced the A β 42:A β 40 ratio, seemingly through increased clearance. Meanwhile, mice exposed to EE after the development of cognitive deficits would have occurred had more A β plaques, but they were smaller in size, and this effect did not appear to be due to increased clearance [97], highlighting the potential differential effects and mechanisms EE may cause when applied at different stages of pathology. Limited numbers of studies incorporating multiple experimental groups to investigate timing make it difficult to conclude if early application of interventions is essential.

Another factor influencing the potential of an intervention to mitigate amyloid pathology is dose. Two studies using female TgCRND8 mice have reported that two or five but not one month of voluntary exercise decreased amyloid pathology [24, 32]. This effect however, was not replicated when other groups examined the effects of 2.5 and 4 months of voluntary exercise in TgCRND8 mice [31, 98], where amyloid measures were found to be unchanged. Could differences in the degree of exercise influence the level of change to pathology? Higher intensity regimes have shown a greater potential to decrease amyloid than low intensity regimes in Tg2576 mice [81]. It is also possible to modify EE paradigms to increase the degree of enrichment experienced. One group examined the effect of two different doses of the environmental enrichment on amyloid pathology in the APP/PS1 mouse model [69]. Mice were housed in standard EE cages, with regular cycling of objects for novelty or in the same EE cage but also placed in larger exploration chambers daily. While no changes in A β were seen in the EE-cage only group, mice receiving additional opportunities for exploration counter-intuitively showed increased plaques in the hippocampus (but not cortex) and A β 42 in the cortex (but not hippocampus). The increase in amyloid pathology was correlated with elevated levels of corticosterone, leading the authors to conclude that stress was likely a factor in this adverse effect. Inter-

estingly, all other studies that assessed corticosterone levels following EE showed no differences [36, 43, 85, 99]. Thus, while it would seem logical to conclude increased doses of exercise or EE would lead to increasing attenuations of pathology, that is not what the data indicate.

When amyloid levels are reduced, what mechanisms are at play? A few studies have investigated A β kinetics in and out of the brain, processing, generation and degradation to try to answer this question. Increased clearance of amyloid in fAD mice following preventative exercise and EE interventions may occur, as changes in A β trafficking proteins LRP1, TTR and RAGE have been seen [41, 80, 81, 91, 93], although this is not consistent [17, 24, 99]. When investigating A β generation and degradation, both preventative exercise and EE studies indicate that the enzymes involved are unchanged [24, 37, 40, 61, 100], although a few studies do report both decreased and increased levels [17, 87, 93]. Thus, it appears that when exercise or EE reduced amyloid levels, it may have been through increased trafficking; however, the small number of studies and inconsistent findings make this difficult to conclude.

IT TAKES TAU TO TANGLE, BUT DO LIFESTYLE INTERVENTIONS AFFECT TAU PATHOLOGY?

Unlike amyloid, tau protein changes after lifestyle interventions have not been robustly investigated in fAD models – only 9 exercise and 3 EE studies have assessed hyperphosphorylated tau at all. Preventative exercise studies that measure phosphorylated tau show mixed reports. One study saw decreases of phosphorylated-tau proteins in the hippocampus and cortex in 3xTG-AD [18] while another study noted decreases of the At8 tau epitope in the cortex but not the hippocampus of APP/PS1 mice [21]. However, 3 other studies saw no change in phosphorylated tau [26, 62, 101]. Similar effects were seen following exposure to therapeutic exercise, post cognitive decline, with some reports of decreased phosphorylated tau in fAD mice [18, 52, 62] and some, unchanged [61, 64]. Reductions in tau phosphorylation have been seen consistently following exposure to early EE [43, 88, 96]. One of these studies showed that this reduction correlated with improved cognition in Tg2576 mice, in the absence of change in A β [43]. Thus, EE seemed to consistently downregulate tau hyperphosphorylation, while exercise applied

both preventatively or therapeutically was shown to have mixed effects.

The limited number of studies investigating tau in this context are surprising, as tau pathology correlates more strongly than amyloid to neuronal loss and cognitive decline in patients [102, 103]. Furthermore, recent studies indicate certain phosphorylation sites on tau protein might be viable biomarkers for Alzheimer's disease [104]. The phosphorylation sites investigated in these studies are variable however, and replication is warranted. More attention should be directed to measuring tau following exercise or EE interventions.

IS PATHOLOGY PREDICTIVE OF COGNITIVE IMPROVEMENT FOLLOWING LIFESTYLE INTERVENTIONS?

Varied reports of the ability of lifestyle interventions to reduce amyloid and tau pathology make it difficult to examine how pathology may be predictive of cognitive improvement. Are reductions in amyloid and tau consistently seen with improvements in cognitive performance following lifestyle interventions in fAD mice? In the hippocampus, there were equal numbers of reports of concurrent improvement in cognition and reductions in A β following exercise [17, 18, 21, 25, 32, 52, 54, 61, 62] as there were reports of improved cognition and no change in pathology [18, 22, 23, 25, 30, 31, 56, 57, 65, 83]. This is also the case for studies applying preventative EE, with evidence that amyloid is predictive of improved cognition [36, 37, 45] and also not [29, 41–43, 45, 68, 92, 105]. Curiously, in two EE studies where amyloid levels were seen to increase, cognition was still rescued by the EE intervention [38, 39]. Lack of amyloid reduction with improvement in cognition extends to other areas of the brain, where the number of studies that show improved cognition with [17, 18, 21, 24, 36, 37, 45, 51, 54, 61, 62] or without [18, 22, 29–31, 41–43, 45, 65, 68, 92] decreases in amyloid are equivalent; again with one EE study showing increases in A β and improved cognition [39]. The only possible exception is in studies utilising therapeutic exercise interventions, where all report reductions in amyloid pathology and improved behaviour [18, 51, 54, 61, 62]. When broken down to A β 40 and/or A β 42 levels, a similar trend is seen, where equal if not more studies find no change in A β 40/42 levels but see improved cognition in EE [37, 41–44, 105] and exercise [17, 18, 23, 30, 57, 61, 65] compared to studies that see

decreased levels of A β peptides in exercise [17, 23, 24, 51, 62, 83, 84] and EE [85]. Increased levels of A β peptides and improved cognition are also seen in two EE studies [38, 92]. Thus, reductions in amyloid levels are not accurately predicting improved cognitive function.

Somewhat predictably then, changes in A β processing and trafficking do not line up with improved cognition either. No consistent trend was evident in studies that find improved cognitive performance following exercise, with increased [17], unchanged [18, 24, 51] and decreased APP [29] reported. A similar lack of trend was seen when scrutinising A β processing and/or trafficking following exercise (increased [17, 51], remain unchanged [17, 18, 61, 100] and decreased [51, 54]). This lack of trend is seen despite reports of improved behavioural performance consistently across all of these studies. When EE interventions are applied, APP was reported unchanged in all studies that also show improved cognition [29, 37, 39, 42, 105]. Similarly, unchanged trafficking and processing of A β was seen in fAD mice following EE, despite improvements in cognition [37, 41, 105]. One exception is a study reporting increased A β processing along with improvements in the MWM and RAWM in PS1/PDAPP mice exposed to EE [41]. Only a handful of studies investigate phosphorylated tau in animals that have been subjected to cognitive testing after lifestyle interventions, thus clear correlations are hard to see. Following exercise interventions, the majority of studies reporting improved behaviour report that tau phosphorylation is either unchanged [19–21, 52, 61] or decreased [18, 21, 62]. Only one EE study investigated both tau phosphorylation and behaviour and reported decreases in tau phosphorylation along with improved cognition [43]. While there is limited focus on how tau may relate to cognitive improvement in fAD mice, like amyloid, tau measures do not seem to be predictive of improved cognition following lifestyle interventions.

Is this unexpected? Amyloid and tau pathology have not been seen to be reliably predictive of cognition and disease in the clinic. While studies have shown that the presence of high A β is highly predictive of the progression of patients from MCI to AD, some individuals with A β + status do not transition to AD [106]. Not only this, but clinical trials with A β antibodies that show reduction in amyloid have not translated to improved cognitive outcomes in the clinic [107]. These observations indicate that A β is not the only factor involved in disease progression

and in particular, in modulating cognitive decline. Conversely, it has been reported that the pattern of tau pathology correlates well with cognitive decline in AD patients [102, 103], so one would expect tau pathology to line up more accurately – again, the small number of studies and varied phosphorylation sites examined makes this difficult to confirm.

Another potential explanation of the A β levels failing to predict both cognitive decline and the beneficial effect of lifestyle interventions is that, historically, the focus has been on measuring plaques, not other potentially more toxic states of A β . There is evidence to suggest that the oligomeric form of A β is the most toxic, leading to synapse loss [108], neuroinflammation and further propagation of oligomers (reviewed in [109]). Not only this, but oligomer load correlates with neuronal loss in an APP^{sw}-tau^{viv} mouse model while plaque load does not [110]. It may be a reduction or sequestering of the oligomeric toxic form of amyloid that is occurring in exercise and EE, however more studies are needed to test this theory.

Alternatively, the positive effects on exercise and EE may not be related to A β or tau at all. EE and exercise interventions have well-documented induction of neuro- and synapto-genesis, as well as upregulation of synaptic proteins and neurotrophic factors (for review see [111]). Could the positive effects of lifestyle interventions on cognition seen in AD models be mediated via these changes?

ARE THE EFFECTS OF EXERCISE AND EE MEDIATED VIA NEURONAL CHANGES?

Both exercise and EE consistently elicit positive effects on neurophysiology in wild-type (WT) rodents on molecular, synaptic and cellular levels, which we summarise here. Brain-derived neurotrophic factor (BDNF) is a neurotrophic protein required for neuronal development, survival and learning and memory processes [112]. Exercise and EE both increase hippocampal BDNF in concordance with their effects on increasing cognitive performance [113–115]. Simultaneously, EE increases neocortical and hippocampal synapses [116–119], synaptic proteins such as PSD-95 and synaptophysin [120], increases dendritic branching and synapse size [119, 121–123] and the rate of turnover of cytoskeletal proteins in synapses [124]. EE and exercise both also elevate adult hippocampal neurogenesis [125–127], although the effect is more pronounced with exercise [128–130]. EE also reduces

spontaneous apoptosis in the dentate gyrus of the hippocampus [131, 132]. While it appears that fAD mice undergo similar brain changes to WT mice, some studies show that these mouse models might be resistant to the beneficial effects of exercise [29, 32, 63] and EE (preventative: [90, 96, 133–135], therapeutic: [69, 136, 137]) or show an attenuated response to EE [44, 138–140].

Specifically, studies applying interventions in fAD mice prior to first report of cognitive impairment show enhanced neurogenesis in the hippocampus. Increases have been seen following both exercise [21, 23, 32] and EE [44, 88, 133, 139–142]. Other studies applying EE as a preventative intervention using indirect measures of neurogenesis have shown similar increases [40, 42, 43, 90, 134, 135, 138]. Two groups using EE interventions did not observe enhanced neurogenesis [29, 133]. While the majority of evidence points to increases in neurogenesis, some studies applying preventative EE have investigated these adult-born dentate gyrus neurons and did not find comparable survival and level of maturity to their wild-type counterparts [44, 133, 139, 140]. Neurogenesis is not as commonly investigated in studies utilising interventions post-cognitive impairment, nonetheless there have been reports increases markers of proliferation, BrdU+ [26, 64, 95] and Ki-67+ [82], following exercise and EE. When more closely scrutinised, evidence does not exist to suggest that these newly proliferated cells mature [54]. Some studies have attempted to address this by comparing the effect of exercise and EE on neurogenesis within the same model. In this study, EE was found to fully rescue levels of BrdU+ cells to WT levels at 6 months in APP23 mice, while exercising mice only show a modest increase [95]. In this same study, by 18 months, mice exposed to exercise or EE both showed higher numbers of BrdU+ and BrdU+/NeuN+ cells, despite overall decreases in new neurons compared to 6 months of age. Evidence supporting the generation of new neurons exists for both exercise and EE interventions, however these neurons are less likely to mature or survive in fAD models. It may be that highly plastic immature neurons are contributing to improvement in cognitive function in fAD mice, as newborn neurons at different maturation stages make distinct contributions to learning and memory [143]. Previous studies have shown that exercise is the main neurogenic factor following EE [144]. In this systematic review, over 75% of studies scrutinised used a running wheel in their EE paradigm. However, evidence from work comparing exercise and EE with-

out a running wheel [29] suggests that the increased area and novelty of EE induced significantly more exercise compared to standard-housed animals, thus contributing to neurogenesis.

In general, both exercise and EE interventions have been shown to also slow the progression of neuronal death in fAD mouse models. Following exposure to preventative EE, decreased apoptotic cells [40, 46], reductions in levels of pro-apoptotic factors and increased pro-survival markers have been observed in fAD mice [40, 41, 142, 145]. A similar trend has been seen for exercise interventions applied before cognitive decline [146]. Exercise applied as a therapeutic intervention has also been shown to prevent apoptosis, as reflected by decreased pro-apoptotic markers BAX, TUNEL+ and Caspase-3 [51–55]. The anti-apoptotic marker Bcl-2 was also found to be increased in two studies [52, 55]. Adding further to the evidence of decreased cell death, the ratio of Bcl-2/BAX was found to be increased [54, 59]. There is a report in conflict with the trend of decreased apoptosis, including lack of change in anti-apoptotic marker Bcl-2 after late exercise [53]. Another way that cell apoptosis is activated is by release of cytochrome c from the mitochondria into cytosol and one study has reported decreased levels of this protein in mitochondria and increase in cytosol, suggesting that apoptosis was activated in fAD mice following exposure to exercise late in disease [51]. Despite some conflicting reports, on the whole, a positive relationship between lifestyle interventions and reduction in apoptosis in fAD models has been reported, indicating slowed neurodegeneration. It is important to note however, that most fAD models do not undergo the same amount of neuronal death as human AD patients, but do show reductions in synapse quality and number (reviewed in [14]).

Improvements in synaptic properties and function have been consistently seen in fAD mice after preventative exercise [18, 101] and EE [36, 48, 88, 105]. Increases in dendritic complexity have been observed in fAD mice exposed to early exercise [80]. Increases in functional properties of synapse, as measured by decreased long-term depression (LTD) and increased long-term potentiation (LTP) have been described in two studies applying early exercise interventions [25, 26]. Late exercise may be as effective as early interventions. One report observes increases in PSD-95 and synaptophysin protein in the hippocampus and cortex of fAD mice after late exercise [18], however another showed no changes in synaptophysin or other markers of synaptic plasticity (GAP43, ARC)

[61]. Another group have reported enhanced synaptic plasticity through increased LTP and decreased LTD in APP/PS1 mice [27]. In agreement with this, increases in the complexity of apical dendrites and soma, and binding affinity have also been reported [27, 61]. Early application of EE interventions also enhances synaptic properties. Specifically, increases in synapse proteins, postsynaptic density (PSD) and synaptophysin have been reported [36, 48, 88, 105], with one exception [96]. Increased LTP was also seen following EE in one study [88], as well as upregulation of transforming growth factor beta-2 (TGFB2) and synaptosomal-associated protein 23 [41], while other markers of synaptic plasticity, including the ERK pathway [96] and calmodulin levels [41, 96], were unchanged. Increases in synapse density [48, 92, 137] were described when EE interventions were applied late in disease progression. In conflict with preventative EE studies, dendritic complexity and synaptophysin levels were found to be unchanged [92]. Overall, fAD animals do seem to upregulate synaptic proteins following exercise or EE, but more generalised benefits on synaptic properties and function are seen with preventative or early application of interventions.

Neurotrophins enhance synaptic growth and function and are commonly measured post exercise and EE interventions, with robust increases in WT animals [113–115]. However, the effects of exercise on brain-derived neurotrophic factor (BDNF) and its pathway are inconsistent, with decreased [25, 29] increased [20, 80] and unchanged [23, 29] levels all reported. In addition, it is difficult to gather much information about the effects on other neurotrophins as only a small number of studies look at neurotrophins other than BDNF. One study found TrkB (the receptor for BDNF and associated neurotrophins (NT), NT-3 and NT-4) increased [80], and another found it to be unchanged [20]. Another group report unchanged levels of vascular endothelial growth factor (VEGF), nerve-growth factor (NGF) and NT-3 in the hippocampus; conversely, they saw decreased levels of NGF and BDNF in the cortex [29]. There are more consistent reports of increases in BDNF following late application of exercise in fAD mice, with increased BDNF observed in all [18, 52, 53, 55, 57, 59, 84] but two [23, 64] studies. The only other factor investigated following late exercise interventions is NGF, which was seen to increase [52]. Following EE interventions applied early in disease progression, BDNF was consistently seen to be upregulated [29,

37, 42, 47, 69, 96] except in one study [142]. Less consistently, other neurotrophins were reported as increased (insulin growth factor 1 (IGF-1) [96], NT-3 [29]) but also unchanged (VEGF [29, 47, 142], NGF [29, 47, 96], insulin growth factor 1 (IGF-1) [29, 142] and NT-3 [142]). One study has compared exercise and EE interventions applied early in disease progression and showed increased BDNF and NT-3 (but not IGF-1, VEGF, FGF2, NGF and APP) following EE only [29]. In this study, exercise, while reducing APP (amyloid processing protein), had no effect on growth factors in the hippocampus (BDNF and NT-3, IGF-1, VEGF, and NGF). Paradoxically, decreases in growth factors were seen in the cortex [29].

Some measures of neuronal health are reliably (on the most part) predictive of cognitive improvements in AD models, however timing of intervention seems to enhance ability to elicit beneficial effects. All studies, excepting one [90], investigating neurogenesis and cognitive performance following exercise and EE show increases in both [17, 21, 32, 42–44, 49, 105, 142]. Decreased apoptosis is also predictive of improved cognition [52, 55, 105]. Increases in synaptic function were mostly predictive of improved cognition for exercise [18, 21, 23, 25, 27] and EE [36, 40, 41, 49, 142], specifically when these interventions were applied early. A small number of studies applying exercise and EE therapeutically show variable correlations, potentially indicating a crucial window for exposure for these interventions in order to elicit beneficial effects. In one study applying exercise late to 3xTG-AD mice, increases in proliferative marker BrdU+ were seen, this occurred in the absence of cognitive improvement [64]. Exposing APP/PS1 or APP+PS1 mice to EE later in disease progression resulted in congruent improvement in cognition and increases in markers of synaptic plasticity [36, 40]. In contrast to neurogenesis and synaptic plasticity, neurotrophins do not reliably predict improved cognitive performance [23, 27, 29, 37, 42, 47, 48, 92, 142], with only one report showing positive correlation [20]. It thus seems that the positive effects of interventions on neurogenesis and synaptic plasticity are relatively good predictors of increased performance in fAD mice in a variety of tasks. However, given that these interventions have a myriad of effects on other aspects of the CNS, including glia, it is unlikely that these brain changes are exclusively responsible for cognitive improvement in fAD mice.

DO EXERCISE AND ENVIRONMENTAL ENRICHMENT INTERVENTIONS ALTER INFLAMMATION IN AD?

An additional hallmark of AD is neuroinflammation [147]. Exercise and EE interventions have diverse effects on the glial cells that help to coordinate the response to neuroinflammation in the brain, microglia and astrocytes. In WT mice, exercise has been shown to increase microglia numbers throughout the brain [133, 148–150]. These microglia were observed to be ramified and show no signs of hypertrophic or macrophagic transformation [148, 149]. The ramified morphology denotes a maintenance function and could be crucial for maintaining adult hippocampal neurogenesis. On the other hand, EE has been shown to alter numbers [148, 151–154], and markers of astrocytes [151, 155–157]. Increases in ramification and stellate appearance of astrocytes has been shown following EE, which has been related to increased hippocampal synaptic density [158]. The interpretation of the consequence of these changes post-exposure to intervention in WT mice is not easy, as baseline levels of neuroinflammation are low in these animals. Thus, it is likely the effects of exercise and EE interventions will be very different to those in a compromised, disease model like the fAD mice.

The key role of microglia in AD has been made increasingly clear with various unbiased genetic screens uncovering many AD risk genes that are expressed highly or exclusively in microglia. Genes involved in phagocytosis and sequestering of amyloid beta like CD33 and TREM2, when mutated, increase risk of disease (reviewed in [159]). However, the role of microglia is complex. Depending on the function of the microglia, they can be both beneficial and pathogenic in AD [160]. In the following studies, microglial numbers are quantified but the function of the cells is rarely investigated, with increased microgliosis being taken as proxy for activation leading to increased inflammatory markers. Considering the differential role microglia can play in AD depending on phenotype, future studies should concentrate on not just defining number but also function of the microglia following lifestyle interventions.

When investigating microglia in both preventative and therapeutic exercise, the microglia marker ionised calcium-binding adapter molecule 1 (Iba-1) expression decreases across the brain [22, 23, 51, 61] except in one study [83]. This is interesting

considering that in WT mice, exercise increases microglia numbers. This conflict indicates that microglia in an AD brain may react differently to exercise compared with a WT control brain. The effect of EE on microglia is inconsistent, with markers (Iba-1, F4/80 or Cd11b) shown to be upregulated [46, 86, 93], downregulated [161] or unchanged [37, 40, 133] following preventative EE. In one study, EE-induced microglia appeared to be more phagocytic and exhibited decreased ROS-mediating markers in A β seeded 5xFAD mice [46]. This decrease indicated reduced oxidative stress and increased clearance of debris including A β . This study also showed improvements in memory along with changes in microglial activation, suggesting that phagocytosis and ROS-mediated amyloid clearance may underlie EE-induced memory improvement [46]. A few studies scrutinised markers of inflammatory processes (such as pro-inflammatory cytokines) and have shown that they were reduced following exercise [54, 56, 61, 83] and EE [86, 105, 142, 145]. Some variability exists when applying exercise interventions with a number of studies showed no change in inflammation [36, 56, 83]. There are no therapeutic EE studies that investigate microglia. The effect of EE and exercise on microglia and inflammation are not consistent, but overall the studies seemed to indicate reduced or at least unchanged inflammation following these interventions.

Astrocytosis is an abnormal increase in the number of astrocytes in response to injury and is typically quantified using glial fibrillary acidic protein (GFAP) in fAD mouse models. GFAP levels have been shown to be unchanged in standard housed fAD mouse models [37, 86, 139, 140], however this marker has some flaws and may not be the best representation of astrocytosis (for further information, see [162–164]). The two preventative exercise studies that investigated astrocytosis through the GFAP marker both found it to have decreased after exercise in the cortex and hippocampus [21, 79], possibly indicating a decrease in astrocyte reactivity or number. There are no therapeutic exercise studies that investigate astroglial cells. Early EE interventions have the opposite effect to preventative exercise, resulting in an increase in astrocyte number or astroglial cells following exposure [140, 142]. This is in line with the effects of EE in WT animals [148, 151–154]. Other groups have found no differences in astroglial cells in fAD mice following early EE [133, 139]. But this conflict in findings may be due to differences

in length of EE intervention. Specifically, lack of effect of EE on astroglialogenesis was seen in the same lab, where the protocol was applied intermittently (3 hours/day) for one month [133, 139]. Longer (2 and 4 months respectively) and permanent EE resulted in positive changes to astroglialogenesis [140, 142]. The first three of these studies are in PS1 variants, while the last is in TgCRND8 mice. These findings potentially indicate that a threshold dose of EE is needed to increase astroglialogenesis. An additional therapeutic EE study prevented an increased astrocyte branching and decreased cell volume in PDAPP-J20 mice (except for astrocytes proximal to plaques) [94].

With regards to cognitive performance, studies describing effects of interventions on astrocyte and microglia are too inconsistent to draw conclusions [21, 37, 47, 49, 105, 142]. However, it seems decreased cytokines corresponded relatively well to improved cognition following preventative EE [40, 49, 142] except for one study [36]. In summary, the effects of lifestyle interactions on glial cells are unclear. Studies applying EE seem to indicate some astroglialogenesis, upregulation of GFAP and increased ramification, similar to phenomena seen in WT animals, while microglia may be driven to become more phagocytic. Exercise studies indicate that both GFAP and Iba-1 are reduced following it. Inflammation appears to decrease in mice exposed to both exercise and EE; however the small number of studies makes this difficult to conclude this with confidence.

LIFESTYLE INTERVENTIONS DO NOT CONSISTENTLY REDUCE OXIDATIVE STRESS

Oxidative stress may also be modulated by exercise and EE interventions. Measures of oxidative stress are consistently reduced after a preventative exercise regime, through increased antioxidant factors (superoxide dismutase (SOD), 70 kilodalton heat shock proteins, (HSP-70) and Glutathione (GSH) [19, 20, 26, 33, 81]) and decreased pro-oxidant lipoperoxidative and reactive oxygen species factors [19, 33], except in one study [26]. Oxidative stress is also modulated following therapeutic exercise interventions, but with reduced consistency compared to preventative exercise interventions. In some studies, decreased oxidative stress has been reported via measurement of lipoperoxidation [26, 57, 62, 63]. However, both increases and decreases in antioxi-

dant factors SOD and HSP-70 have been reported, making conclusion regarding the level of oxidative stress difficult [52, 53, 62, 63]. Further complicating interpretation of the effects of exercise on oxidative stress in fAD mice, an equivalent number of studies found no change [26, 52, 53, 55, 59]. Following therapeutic exercise, there was no strong evidence of effects on GSH or catalase [53, 55, 57, 62]. However the PI3K/Akt signalling pathway and corresponding factors involved in reduction of oxidative stress were also seen to increase [57, 59]. While the majority of studies exposing fAD mice to exercise early in disease progression show reductions in oxidative stress, inconsistencies exist, preventing clear interpretations. Furthermore, the limited focus on oxidative stress when interventions were applied late in disease progression add to this ambiguity. While decreases in oxidative stress following both preventative and therapeutic exercise have been shown to correlate with improvements in cognition in mice [19, 33, 55, 61] this is not the case in all studies [62, 63] adding to the uncertainty.

Following exposure to preventative EE, opposing effects on oxidative stress have been described [93, 145]. In one study comparing the effects of EE applied early (1–3 months) and late (3–5 months) and culling at 5 months in TgCRND8 mice, preventative EE was not shown to have an effect, while therapeutic EE was reported to decrease oxidative stress through reductions in nitro-tyrosine and increases in SOD1 levels [93]. The fact that only the late intervention reduced oxidative stress is interesting, as the same group, who applied EE from 1–5 months in TgCRND8 mice, show reduction of nitro-tyrosine, protein carbonylation and JNK2/3, a relevant mitogen-activated protein kinase pathway [145]. It seems likely that EE can directly reduce oxidative stress, but not prevent it, hence there being no effect of EE when the paradigm is applied early in disease progression. The limited data, lack of consistency in which of these markers are utilised, and the direction of effect all make it difficult to draw clear conclusions regarding the beneficial effects of exercise and EE on oxidative stress in fAD mice.

EXERCISE ENHANCES ENERGY METABOLISM IN AD MICE

Exclusive to exercise are measures of energy metabolism. Preventative exercise alters glucose metabolism [17], reduces white adipose tissue [19]

and improves glucose tolerance [146] in fAD models, while therapeutic exercise decreases levels of total cholesterol, glucose and insulin, triglycerides, white adipose tissue and low-density lipoprotein cholesterol [50, 52, 53, 55, 62] while increasing high-density lipoprotein cholesterol [50, 55]; all indicative of a healthier animal. Glucose transporter 1 receptors within the brain are increased [53] and corticosterone decreased [52]. It is widely established that one of the strongest benefits of exercise is improved cardiovascular fitness and health and this lends strength to the argument that it is also vital for the energy metabolism in the brain.

Exercise studies that have studied energy metabolism have also generally seen a concomitant improvement in behavioural tests [19, 50, 52, 53, 55], except for one study [62]. Patients who suffer from imbalances in insulin and glucose, such as those with diabetes, are also at an increased risk for cognitive impairment and memory loss [165]. Therefore, through exercise, a consistent decrease seen in energy metabolism may more convincingly be involved in increasing cognitive performance. Further studies should investigate the mechanism of how insulin, cholesterol and glucose could directly impact on memory as this is currently an area receiving little attention. Finally, measures of energy metabolism indicate that exercise animals are, unsurprisingly, healthier than controls. Overall, this indicates that exercise and EE have widespread non-neuronal effects, meriting further investigation.

OVERALL SUMMARY

Overall, it appears that EE tends to have a larger effect than exercise on synaptic plasticity, neurogenesis and cognition. Considering the paradigm of EE can stimulate both physical and cognitive functions, this is not surprising. What is interesting is that these two interventions can cause quite disparate effects - for example, in one study EE increased neurotrophins while exercise decreased them [95], and EE reduced microglial numbers with no change in size, while exercise increased the microglial size but did not change the number [161]. The few studies that investigate EE later in disease progression in mice, seem to indicate that it is not as useful at this timepoint. These studies suggest that in order to reap the maximum benefits of EE, it must be applied early in disease, before plaques develop. Conversely, studies applying exercise provide a contrasting picture, with

effects being more inconsistent when the intervention was used in a preventative way while more reliable when applied therapeutically. This may indicate that in early life, before plaques develop, both cognitive and physical exercise are important to bolster neuro- and synapto- genesis. After plaque development, it may be that the physical exercise aspect is most effective. This is a hypothesis that has not been thoroughly tested as no studies have yet looked at both neurogenesis/neurotrophic factors/synaptic changes as well as behavioural changes following therapeutic EE. Furthermore, as mentioned in the introduction, most EE studies will result in increased exercise (with and without inclusion of running wheels), so the effect of EE alone is difficult to parse.

FINDING A SIGNAL IN THE NOISE: STANDARDISATION IS REQUIRED IN EVERY SENSE

From all the studies applying exercise and EE interventions to fAD mouse models captured in this review, the only relatively consistent finding is that they can improve performance on memory tasks. The inconsistency of both methods and also the outcome measures used for almost every aspect of lifestyle interventions in preclinical fAD models has made clear conclusions difficult and thus we recommend that standardisation be attempted at a number of different levels.

IMPROVEMENTS IN STUDY DESIGN

In this systematic review, we describe a distinct lack of consistency between pathological and molecular markers and the cognitive changes reported in fAD mice. One interpretation of this is that there is no correlation. Another is that we are not powered well to draw clear conclusions. Many studies investigate the effect of exercise and/or EE interventions in fAD mice without assessment of cognition. Given that the major hallmark of AD is cognitive decline we believe that the absence of cognitive assessment is an oversight. How can a change in pathological or molecular marker be interpreted as beneficial if no information about the effect on cognition can be made? We recommend that studies applying lifestyle interventions include the assessment of cognition as their main indication that the intervention is having an effect and then secondary to this, scrutinise the underlying neurobiological mechanisms. Another issue with study

design in both the exercise and EE literature is the lack of WT controls. The absence of WT intervention groups in the following exercise [19, 58, 146, 166] and EE intervention studies [36, 47, 49, 87, 142, 167] makes interpretation of the intervention very difficult. In some studies, no WT controls were included at all, including those involving exercise [64, 65] or EE [29, 43, 47, 68, 86, 91, 93, 105, 141, 145, 168] interventions. Given the inherent variability between laboratories, different background strains and with differences in methodologies in animal studies [169], the inclusion of WT control groups is essential to ensure baselines have not shifted and lead to false interpretation of effects. In particular, confounding environmental factors like stress can unmask or mask phenotypes in fAD mouse models, making control groups essential [170]. In addition to appropriate controls, maximal rigor and reproducibility of findings will be achieved with best-case practices in sampling, experimental design, procedures and statistical analysis [171].

WHAT MARKER BEST CORRELATES WITH EXERCISE AND/OR EE IMPROVEMENTS TO COGNITION?

In this review, we show that amyloid does not appear to be a useful measure of effectiveness of exercise or EE, in particular showing little correlation with cognitive improvement in fAD mouse models. Oligomeric forms of amyloid may correlate more closely with changes in cognition and advancement of methods have enabled this marker to be used more frequently in recent years. Perhaps there is a better molecular marker to explain intervention-induced benefits to cognition in mouse models? Increased neurogenesis is seen consistently following exposure to exercise and EE interventions and correlate with changes in cognition. Should neurogenesis be a primary measure of efficacy of the intervention? This question does not have a simple solution. While many studies scrutinise neurogenesis, most studies neglect synaptic proteins, neurotrophic factors and neuronal cell death and survival, making interpretation of the effect of interventions on neurons difficult to fully interpret. In particular, the few studies showing that new neurons may not survive in fAD models raise the question of how these new neurons could help ameliorate cognitive symptoms in fAD mice.

Astroglialogenesis was also shown to be increased, but overall glial changes have only been looked at in

a rudimentary way, and results are conflicting; it is unclear if astrocytes and microglia numbers and/or reactivity increases [46, 47, 86, 88, 93, 142, 172], decreases [105, 161] or remains unchanged [37, 86, 105, 133, 139, 140] and regardless of numbers, it is unclear what they are doing functionally, which is especially important as both these cell types have been shown to be beneficial and pathogenic in AD [160, 162, 164].

VARIATION IN METHODS OF INTERVENTIONS INTRODUCES NOISE

As important as standardising the molecular correlates are the standardisation of exercise and EE regimes. There is a lack of consensus across exercise studies as to how animals are trained over the course of a forced regime. Half the studies investigated ran their animals at a constant speed throughout the regime [27, 33, 50, 52–54, 57, 59, 166], five studies increased either speed or duration gradually over the days that mice run [22, 51, 63, 80, 173], and four increased intensity within the session while keeping a constant duration per session throughout the regime [18, 23, 25, 79]. An unconventional case uses a constant speed at a 10% incline which no other studies used [81]. A recent meta-analysis indicated that aerobic exercise alone of moderate intensity for approximately 45 minutes per day, three times a week, resulted in modestly improved cognitive function over other aerobic or combination training regimes in MCI/AD patients [6]. Given that clinical recommendations include increasing the overall amount of exercise over a week, this could be similarly applied to animals and divided accordingly so that animals run at a constant speed throughout the regime. Thus, comparisons between the literature would be much more transparent and could inform the field better.

There are also a wide range of EE protocols - some cages include running wheels, cage size is variable, number of animals is variable and some animals are left in the cages 24/7, while others are only placed in it for a short time per day. As variation in enrichment protocols may account for some of the variance in outcomes observed in this review, it is imperative that authors provide detailed information on their housing protocols. While the field is not moving towards standardised enrichment cages, detailing the age that enrichment commences, duration of enrichment, size of the cage, number of animals per cage, the nature

of the objects in the cage and their turnover frequency and inclusion of bedding material should be the minimum standard of information provided [174]. Furthermore, future meta-analyses and systematic comparison studies will be required to determine which specific aspects of EE protocols induce the greatest benefits (on specified parameters) in specific preclinical models, including fAD mice.

CONCLUSION

Exercise and EE both can have a wide range of effects on the AD brain, but overall we have found that the only relatively consistent finding is that they can both improve performance on spatial memory tasks, specifically the MWM. This finding mimics what is seen clinically – individuals that are physically and cognitively active have a later onset of disease [7, 175]. The beneficial effects of these interventions in the AD brain are not clear however. Reductions in amyloid pathology are only observed in approximately half the studies examined, and regardless, these measures do not correlate well with improved cognition regardless of timing, or the type of intervention. Thus, it seems amyloid is not the mediator in lifestyle interventions, and it is important to start looking elsewhere. Tau changes may be more promising, however there are simply not enough studies to in this area, and much more research is required.

Increased neurogenesis, synaptic plasticity and neurotrophic factors are seen consistently in preventative EE (with trends noted in therapeutic studies, but again, too few studies to be sure) and correlate well with improved cognition - they are good candidates for the mediation of cognitive deficits. However, the effects of exercise (both preventatively and therapeutically) on these measures are inconsistent, with about half the studies showing no changes in neurogenesis, synaptic plasticity and neurotrophic factors, and some of these studies that show no changes still have increased cognitive performance. After all studies were drawn together for this systematic review, a further study by Choi et al. [176] showed that the positive effect of therapeutic exercise on cognition in 5xFAD mice was only mimicked if both neurogenesis and BDNF were elevated; neurogenesis alone was not sufficient. However, there may be other mediators of improved cognition. Oxidative stress is shown to be decreased, unchanged and increased in a range of therapeutic and preventive exercise studies, as is energy metabolism. Another possible mediator

is neuroinflammation - namely, decreases in astrogliosis, microgliosis and pro-inflammatory cytokines as these seem to be relatively consistently decreased by exercise. The effect of EE on these measures is more complicated, and further studies should concentrate on the function of these microglia and astrocytes rather than just more or less reactivity/numbers.

While lifestyle interventions may be complicated with respect to their molecular and cellular effects, they are the most effective and very affordable changes that can be introduced clinically to benefit those developing or living with dementia [177]. A big issue with exercise and computerised cognitive training in the clinic is adherence [9]. However it is much easier to do 10 minutes of moderate exercise than an hour of intense weight lifting, and thus understanding the effect of dosing in lifestyle interventions is of utmost importance. Easier again would be a treatment that could help induce the brain changes we see following exercise and EE; but to do this effectively, we must first understand which changes are the most crucial.

We understand that throughout this review, we have been drawing potential correlations with individual factors and subsequent performance in behavioural tests. We acknowledge the complexity associated with these preclinical studies and that a combination of improvements over various domains may collectively result in improvements in cognitive ability. However, it appears that reductions in amyloid levels are not the driver of any positive benefits; rather, it seems enhanced cellular plasticity (adult neurogenesis and/or synaptic plasticity) are much better predictors of cognitive performance in these animals. It may be that these interventions do not actually improve the pathology of AD; rather they provide increased brain and cognitive reserve for the animal to cope with the negative AD-driven pathology (for review on this idea, see [70]). The original hypothesis that decreasing amyloid pathology would lead to improved cognition is not supported by the overall weight of evidence. The combination of mechanisms discussed in this review has a more convincing relationship with cognitive benefits.

Ultimately, the goal of this preclinical research is to deliver approaches to prevent, treat and eventually cure AD, and other forms of dementia. Whilst clinical interventions involving cognitive stimulation, exercise and other experience-dependent factors, are ongoing, the preclinical studies can also deliver mechanistic insights. Increased knowledge of molecular and cellular mechanisms may facilitate new

approaches, including enviromimetics, novel therapeutics which mimic or enhance the beneficial effects of cognitive stimulation and physical activity [76].

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <http://dx.doi.org/10.3233/BPL-180076>.

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