A lipid-leakage model for Alzheimer's Disease

Jonathan D'Arcy Rudge [1]

[1] School of Biological Sciences, University of Reading, Reading, Berkshire, United Kingdom

Corresponding Author: Jonathan Rudge [1]

Email address: jon_rudge@btinternet.com

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Abstract

This paper describes a potential new explanation for Alzheimer's disease (AD), referred to here as the lipid-leakage model. It proposes that AD is caused by the influx of lipids following the breakdown of the blood brain barrier (BBB).

The model argues that a principle role of the BBB is to protect the brain from external lipid access. When the BBB is damaged, it allows a mass influx of (mainly albumin-bound) free fatty acids (FFAs) and lipid-rich lipoproteins to the brain, which in turn causes neurodegeneration, amyloidosis, tau tangles and other AD characteristics.

The model also argues that, whilst β-amyloid causes neurodegeneration, as is widely argued, its principal role in the disease lies in damaging the BBB. It is the external lipids, entering as a consequence, that are the primary drivers of neurodegeneration in AD., especially FFAs, which induce oxidative stress, stimulate microglia-driven neuroinflammation, and inhibit neurogenesis. Simultaneously, the larger, more lipid-laden lipoproteins, characteristic of the external plasma but not the CNS, cause endosomal-lysosomal abnormalities, amyloidosis and the formation of tau tangles, all characteristic of AD. In most cases (certainly in late-onset, noninherited forms of the disease) amyloidosis and tau tangle formation are consequences of this external lipid invasion, and in many ways more symptomatic of the disease than causative.

In support of this, it is argued that the pattern of damage caused by the influx of FFAs into the brain is likely to resemble the neurodegeneration seen in alcohol-related brain damage (ARBD),

a disease that shows many similarities to AD, including the areas of the brain it affects. The fact that neurodegeneration is far more pronounced in AD than in ARBD most likely results from the greater heterogeneity of the lipid assault in AD compared with ethanol alone.

The lipid-leakage model, described here, arguably provides the first cohesive, multi-factorial explanation of AD that best accounts for all currently known major risk factors, and credibly explains all AD-associated pathologies, including those, such as endosomal-lysosomal dysfunction and excessive lipid droplet formation, that have been too readily overlooked by other accounts of this disease.

Keywords: *Lipids, Alzheimer's, alcohol-related brain damage, blood-brain barrier, β-amyloid, tau tangles, amyloidosis, neurodegeneration, neurogenesis, ethanol, anaesthesia*

A lipid-leakage model for Alzheimer's Disease

1 Introduction

Alzheimer's disease is a neurodegenerative disorder first described by the German physician Lois Alzheimer in 1907 (Stelzmann, Norman Schnitzlein & Reed Murtagh, 1995). It is a form of dementia characterised by the extensive death of brain cells and associated with widespread plaques and strongly staining fibrils.

Whilst these same characteristics, including the distinctive deposits now known as amyloid plaques and tau tangles, are individually seen in other forms of neurodegeneration, their occurrence together appears to be unique to AD. AD has emerged as the most common dementia, accounting for over half of all dementias, with an especially high prevalence amongst over-85 year-olds in the developed world (OECD, 2013). Yet, despite more than a century having elapsed since AD's first discovery, and, in spite of the extensive suffering and financial costs caused by the disease, only limited progress has been made in understanding its aetiology, with an effective treatment yet to be developed (Hardy, 2006; Castellani & Perry, 2012).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

This has not been for lack of trying. Amongst a number of promising explanations the cholinergic hypothesis, which emerged in the 1980s, sought to explain the disease in terms of reduced synthesis of acetylcholine (ACh) (Contestabile, 2011). But, whilst substantial evidence points to AD-associated deficits in the cholinergic projection system of the brain (Contestabile, 2011), animal studies indicate that cholinergic damage causes only moderate cognitive deficits (Parent & Baxter, 2004), and attempts to increase ACh levels with drugs, including acetylcholinesterase inhibitors, do not significantly slow disease progression (Frölich, 2002; Contestabile, 2011).

In the 1990s an alternative model emerged, the amyloid cascade hypothesis, which postulated that beta-amyloid (Aβ), a proteolytic product of amyloid precursor protein (APP), is the fundamental cause of the disease (Pimplikar, 2009). This is still the dominant model for explaining AD, backed by a substantial body of evidence, not least the fact that Aβ is the main component of amyloid plaques (Pimplikar, 2009). Moreover, in inherited forms of the disease, collectively referred to as familial AD (FAD), a number of genes related to normal APP processing have been found to be abnormal (Wu et al., 2012). Similarly, people with Down's syndrome (DS) who possess an extra copy of chromosome 21, on which APP resides, typically go on to develop a form of dementia largely indistinguishable from AD (Nieuwenhuis-Mark, 2009). Any model of AD needs to take into account these facts.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

However, the amyloid cascade hypothesis is not without problems of its own, not least the fact that a number of studies have shown a poor correlation between amyloid plaque distribution and disease progression (Terry et al., 1991; Bowman & Quinn, 2008; Pimplikar, 2009). In some instances high plaque levels are completely unassociated with dementia (Aizenstein H et al., 2008). And twenty years since the hypothesis was first raised, treatments aimed at preventing or eliminating amyloid plaques have yet to show any significant benefits in preventing dementia (Pimplikar, 2009; Sperling et al., 2011; Castellani & Perry, 2012).

Most studies of AD, proposing Aβ as the causative agent, assume that the Aβ found in cerebral plaques must originate within the brain. However, this has recently come into question, with doubts being raised as to whether cerebral production of Aβ is significantly elevated in individuals with non-inherited, late-onset forms of AD (LOAD) (Cummings et al., 1998; Takechi et al., 2010a).

This has led some researchers to propose that the Aβ deposits may originate from outside the brain (Deane et al., 2009; Takechi et al., 2010a). However, the size of the Aβ protein prevents it travelling across the BBB unaided (Deane et al., 2009). Thus, entry of the Aβ protein into the brain requires either that specific transporter proteins are available to carry it across, or that the

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

BBB is disrupted in some way. Whilst such transporters do exist there are also others that transport Aβ in the opposite direction (Deane et al., 2009) i.e. out of the brain, as well as alternative efflux mechanisms (Lam et al., 2001; Deane et al., 2009; Takechi et al., 2010a). Additionally, the brain appears to have more than adequate enzymatic mechanisms for eradicating excess Aβ arising from faulty transport (Iwata et al., 2000; Takechi et al., 2010a). Disruption of the BBB would thus seem to be a more plausible explanation for extravasation of Aβ into the brain.

In support of such an explanation, AD is associated with BBB disruption (Iadecola & Gorelick, 2003; Ujiie et al., 2003; Dickstein et al., 2006; Popescu et al., 2009; Kook et al., 2012). Evidence for this includes the fact that AD brains contain proteins that would normally be excluded by the BBB, most significantly apolipoprotein B, which is found in amyloid plaques along with Aβ (Namba, Tsuchiya & Ikeda, 1992; Takechi et al., 2009), as well as other large molecular-weight proteins such as albumin, fibrinogen and immunoglobulins (D'Andrea, 2003; Bowman & Quinn, 2008; Cortes-Canteli & Strickland, 2009; Ryu & McLarnon, 2009; Johnson et al., 2018). Also, AD brains stain for Evans Blue, which is normally substantially excluded by the BBB (Ujiie et al., 2003; Paul, Strickland & Melchor, 2007; Cortes-Canteli & Strickland, 2009).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Similarly, proteins such as S100B, normally only found in the CNS and considered a good marker of BBB disruption (Marchi et al., 2004), are present in systemic plasma in AD cases (Takechi et al., 2010b). Further evidence that BBB disruption may lead to AD also comes in the form of Chronic Traumatic Encephalopathy (CTE). This is a progressive degenerative condition, commonly affecting athletes and others with a history of brain trauma, which typically shows many similarities with AD (Stein, Alvarez & McKee, 2014). These include large-scale neuronal loss, severe memory deficits, extensive tau tangles and, frequently in advanced cases, diffuse amyloid plaques (Stein, Alvarez & McKee, 2014). Crucially, CTE appears to be strongly associated with BBB disruption (Chodobski, Zink & Szmydynger-Chodobska, 2011; Stein, Alvarez & McKee, 2014; Doherty et al., 2016; Johnson et al., 2018; Farrell et al., 2019). Finally, the many risk factors for LOAD include ApoE4 (Liu et al., 2013), hypertension (Kivipelto et al., 2002), diabetes (Goldbourt et al., 2004), smoking (Durazzo et al., 2014) and head injury (Gottlieb, 2000), all of which are associated with vascular damage (Salloway et al., 2002; Mazzone et al., 2010; Prasad et al., 2014; Alluri et al., 2015; Girouard, 2016).

There is also substantial experimental evidence of Aβ directly compromising the BBB (Jancsó et al., 1998; Farkas et al., 2003; Tai et al., 2010; Kook et al., 2012; Gosselet et al., 2013), in a number of ways. These include altering tight junction protein distribution and expression in brain endothelial cells (Ohtsuki et al., 2007; Tai et al., 2010; Hartz et al., 2012; Kook et al.,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

2012; Gosselet et al., 2013), increasing matrix metalloproteinase expression (Hartz et al., 2012), oxidative stress (Thomas et al., 1997), increasing apoptosis (Blanc et al., 1997; Fossati, Ghiso & Rostagno, 2012) and dysregulating calcium homoeostasis (Blanc et al., 1997; Kook et al., 2012). Finally, there is further indirect evidence that Aβ can damage the BBB, for example, in cases of cerebral amyloid angiopathy (CAA) (Carrano et al., 2011; Fossati, Ghiso & Rostagno, 2012; Hartz et al., 2012; Magaki et al., 2018).

The simplest interpretation of these findings is that Aβ has a dual role in AD progression, first disrupting the BBB, and then causing neurodegeneration by deposition in the brain. But, whilst there is abundant evidence that Aβ is toxic to the brain (Pimplikar, 2009), so are many of the other molecules that a disrupted BBB could be expected to let through [*such as?*]. If Aβ does play a major role in disrupting the BBB then any proposed model of AD must take into account what role the intact BBB plays in the human body, particularly with regard to the brain.

Unfortunately, nearly a century after the BBB was first discovered, its full role is still a matter of conjecture. What was considered to be a primary function, ensuring “immune privilege”, is now known to be far more limited and nuanced than once thought (Carson et al., 2006; Harris et al., 2014). Nevertheless, it would appear from its unique architecture that the BBB’s main purpose is to exclude certain cells and molecules from the brain. This architecture is found hardly

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

anywhere else in the human body and includes unusually strong tight junctions between endothelial cells, as well as a lack of endothelial fenestrations and endocytotic/transcytotic activity, a surrounding belt of basal lamina and large numbers of specialist cells such as pericytes and astrocytes (the latter attaching to the brain capillaries by so-called foot processes), and the presence of numerous efflux transporters (Rubin & Staddon, 1999; Dietschy & Turley, 2004; Abbott, Rönnbäck & Hansson, 2006; Carson et al., 2006).

Because of this architecture the BBB is known to substantially exclude lipids that remain bound to, or within, their normal transport partners (Jeske & Dietschy, 1980; Dietschy & Turley, 2004; Hamilton & Brunaldi, 2007; Zhang & Liu, 2015). Evidence (outlined in 2.4-2.5) suggests that unregulated external lipid influx, resulting from BBB compromise, or otherwise, will damage the brain. In the case of FFAs this will occur in at least three ways: (1) oxidative stress, lipid peroxidation and mitochondrial damage resulting from excess FFAs accumulation within neurons; (2) neuroinflammation; (3) disruption of neurogenesis, all characteristics that have been associated with AD (Markesbery, 1997; Hensley, 2010; Moreno-Jiménez et al., 2019). Other characteristics, such as endosomal-lysosomal pathway disruption, amyloidosis and tau tangle formation can also be explained by lipid influx in the form of external lipoproteins (2.6). These are rich in cholesterol, which has also been linked with AD (Simons et al., 2001; Wolozin, 2004; Xiong et al., 2008), particularly in connection with amyloidosis and tau tangles.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

In support of this, a recent study has reported the presence of lipids, including long-chained triglycerides, within fibrillar Aβ plaques (Kiskis et al., 2015), consistent with the evidence, previously alluded to, of the presence of apolipoprotein B within amyloid plaques.

Based on the above evidence, the lipid-leakage model argues that breakdown of the BBB, by Aβ or other means, and the subsequent influx of lipids, leads to lipid-driven neurodegeneration and dysfunction, including the long-term form known as Alzheimer's disease. According to this hypothesis, it is peripheral lipids, not Aβ, that primarily drive AD.

One reason for believing this is the similarity between the overall structural pattern of neurodegeneration seen in AD and that seen in ARBD, resulting from chronic exposure of the brain to ethanol. Ethanol passes relatively easily through the BBB and, for the reasons argued below, can be expected to have some of the same overall effects on the brain as exposure to one major class of lipids, FFAs, but without the amyloid plaques, tau tangles and endosomal-lysosomal abnormalities seen in AD. (See 2.4-2.5.)

This suggests that further study of ARBD may yield insights into the aetiology of AD. One area of potential overlap emerges from extensive evidence that the detrimental effects observed in the

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

brain from chronic alcohol exposure are the result not only of neurodegeneration but also of reduced levels of neurogenesis (Fadda & Rossetti, 1998; Nixon, 2006; Crews, 2008; Morris et al., 2009).

Recent studies also demonstrate that the neurodegenerative effects of chronic alcohol abuse may be reversible (Pfefferbaum et al., 1997a; Crews & Nixon, 2009), following the cessation of ethanol treatment. This could mean that if neuroinflammation and neurogenetic inhibition could be ameliorated then the neurodegenerative effects of AD may also be reversible, giving hope of finding effective treatments for the disease.

2 Evidence and explanation of the model

It follows from the above, that a full appreciation of the lipid-leakage model requires an understanding of the similarities between AD and ARBD.

2.1 Similarities between AD & ARBD

That AD and ARBD may share common elements in their aetiology is apparent from comparisons of brains of individuals with either disease, including direct visual comparisons (see

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Figure 1), and whole brain MRI scans (Figure 2), (Sullivan, Adron Harris & Pfefferbaum; Fox et al., 2001; Zahr, Kaufman & Harper, 2011; Teipel et al., 2015).

Image awaiting copyright owner's permission.

Figure 1. Visual comparisons of the brains of (A) normal elderly person; (B) a person with AD and (C) a chronic alcoholic. Source [*references?*].

Image awaiting copyright owner's permission.

Figure 2. Coronal plane MRI comparison between brains of (a) a normal person and (b) a typical AD case (Duara et al., 2008) and that of (c) a patient with alcohol-related brain damage ("Alcoholic dementia, MRI scan"). Outlined areas in (a) & (b) correspond to hippocampus (outlined in red); entorhinal cortex (blue) and perirhinal cortex (green). Source: [*references?*].

2.1.1 Brain shrinkage

Such scans typically reveal pronounced similarities between the two diseases in their pattern of neurodegeneration, including evidence of brain shrinkage (Pfefferbaum et al., 1992, 1997a; Kril & Halliday, 1999; Thompson et al., 2007; Hua et al., 2008; Paul et al., 2008; Spreng & Turner, 2013), loss of cortical folding (involving widening of sulci and thinning of gyri) (Harper & Kril,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

1985; de la Monte SM, 1988; Pfefferbaum et al., 1997a; Hua et al., 2008), enlargement of ventricles (de la Monte SM, 1988; Pfefferbaum et al., 1997a; Silbert et al., 2003; Hua et al., 2008; Nestor et al., 2008; Wobrock et al., 2009), (especially the lateral ventricles), together with shrinkage of the hippocampus and entorhinal cortex (Fadda & Rossetti, 1998; White, Matthews & Best, 2000; Beresford et al., 2006; Hua et al., 2008; Duara et al., 2008) and thinning of the corpus callosum (Harper & Kril, 1988; Pfefferbaum et al., 1996; Estruch et al., 1997; Teipel et al., 2002; Frederiksen et al., 2011; Preti et al., 2012).

On their own, such similarities could be dismissed as the effects of general brain shrinkage and other generalised damage. However, the similarities appear to run much deeper than this, with many of the same regions of the brain principally affected in both cases, especially early on in the disease process. In particular, both AD and ARBD appear to be substantially "frontal" diseases, as suggested by physiological, behavioural and sensory studies, in line with imaging studies of both diseases (Pfefferbaum et al., 1997b; Kril & Halliday, 1999; Harper, 2007; Hall et al., 2008; Grothe, Heinsen & Teipel, 2012; Schmitz et al., 2016).

2.1.2 Basal forebrain damage in AD and ARBD

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Measurements of brain volume reveal both diseases to be associated with significant shrinkage in the frontal region of the brain, particularly the prefrontal cortex and basal forebrain regions (Pfefferbaum et al., 1997a; Fadda & Rossetti, 1998; Moselhy, Georgiou & Kahn, 2001; Teipel et al., 2005; Hall et al., 2008; Grodin et al., 2013), including the cholinergic basal forebrain projection system (Arendt et al., 1989; Muir, 1997; Fadda & Rossetti, 1998; Teipel et al., 2005; Miki et al., 2014). This is backed up by studies in animal models, which suggest that chronic exposure of the brain to ethanol causes a specific pattern of degeneration, including a marked loss of cholinergic neurons, accompanied by a reduction in acetylcholine and choline acetyltransferase activity (Arendt et al., 1989; Floyd et al., 1997; Fadda & Rossetti, 1998; Mufson et al., 2003; Miki et al., 2014). Again, this is very similar to what is seen in AD (Muir, 1997; Baskin et al., 1999; Auld et al., 2002; Mufson et al., 2008), which is, indeed, why the cholinergic hypothesis was proposed in the 1980s (Contestabile, 2011).

Related behavioural evidence pointing towards frontal damage as a factor in both diseases includes personality changes (Bózzola, Gorelick & Freels, 1992; Chatterjee et al., 1992; Oscar-Berman et al., 1997; Moselhy, Georgiou & Kahn, 2001; Talassi et al., 2007; Echeburúa, De Medina & Aizpiri, 2007; Ball et al., 2010), disinhibition and impulsivity (Chen et al., 2007; Ball et al., 2008; Crews & Boettiger, 2009; Dick et al., 2010; Bidzan, Bidzan & Pąchalska, 2012; Finger et al., 2017), confabulation (Kern et al., 1992; Brun & Andersson, 2001; Tallberg &

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

222 Almkvist, 2001; Attali et al., 2009; Maurage et al., 2011; Rensen et al., 2015) and a noticeable
223 tendency towards perseverative behaviour. This last attribute is readily apparent in individuals
224 with AD (Bayles et al., 2004; Serna, Pigot & Rialle, 2007; Pekkala et al., 2008; Kaufman, 2015;
225 De Lucia, Grossi & Trojano, 2015), while studies in adult rats chronically exposed to ethanol
226 (but given a nutritionally adequate diet) point towards a similar pattern of behavioural and
227 neurological deficit (Obernier et al., 2002; Crews & Nixon, 2009) [*more references?*],
228 confirming findings in humans (Giancola, Peterson & Pihl, 1993; Oscar-Berman et al., 1997;
229 Fadda & Rossetti, 1998; Ratti et al., 2002; Oscar Berman, 2009). Possibly such behaviour
230 involves deficits in the dopamine system (McNamara & Albert, 2004; Campos-García Rojas et
231 al., 2015), principally centred in the frontal lobe, as well as of the cholinergic system
232 (McNamara & Albert, 2004). But certainly it is known that various forms of motor perseveration
233 and similar behavioural inertias are frequently associated with damage to the frontal lobes
234 (Luria, 1965; Stuss & Benson, 1984; Ridley, 1994; Munakata, Morton & Stedron, 2003).
235
236 There is also very strong experimental evidence suggesting that, from comparatively early on,
237 both AD and ARBD are associated with olfactory deficits (Ditraglia et al., 1991; Collins, Corso
238 & Neafsey, 1996; Mesholam RI et al., 1998; Christen-Zaech et al., 2003; Doty, 2005; Rupp et
239 al., 2006; Maurage et al., 2011; Velayudhan et al., 2013), although not always perceptible to
240 demented patients (Doty, Reyes & Gregor, 1987). These are also very likely to involve damage

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

241 to the basal forebrain, including the olfactory bulb (Ohm & Braak, 1987; Collins, Corso &
242 Neafsey, 1996; Obernier et al., 2002; Christen-Zaech et al., 2003; Rupp et al., 2006) and
243 cholinergic systems (Arendt et al., 1989; Mundiñano et al., 2013; Doty, 2013; D'Souza &
244 Vijayaraghavan, 2014), amongst others.
245
246 More generally, both forms of dementia are associated with deficits in executive functions (Rupp
247 et al., 2006; Duarte et al., 2006; Harper, 2007; Ball et al., 2008; Marshall et al., 2011; Houston et
248 al., 2014; Weiss et al., 2014), such as attentional and inhibitory control, working memory and
249 reasoning - i.e. those faculties which allow problem-solving, planning, self-control and the
250 attainment of goals. Clearly there are difficulties separating the immediate effects of drinking
251 alcohol from the long-term neurodegenerative effects of alcoholism, as well as questions as to
252 what degree executive function is under the control of the frontal region. Nevertheless, taken
253 collectively, the evidence presented here points to a strong involvement of the frontal lobe
254 degeneration in both ARBD and AD.
255

256 ### 2.1.3 Medial temporal lobe damage in AD and ARBD

257
258 As well as the basal forebrain, the medial temporal lobe is also found to be significantly
259 atrophied in both ARBD and AD (Bengochea & Gonzalo, 1990; Smith et al., 1992; Fadda &

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Rossetti, 1998; Korf et al., 2004; Duara et al., 2008; Vetreno, Hall & Savage, 2011). This is most obvious in the hippocampus but is also in immediately adjoining regions, such as the entorhinal cortex and perirhinal cortex (Squire, Amaral & Press, 1990; Jernigan et al., 1991; Ibáñez et al., 1995; Sullivan et al., 1995; Fadda & Rossetti, 1998; Juottonen et al., 1998; Traissard et al., 2006; Augustinack et al., 2013; Velayudhan et al., 2013; Hirni et al., 2016; Topiwala et al., 2017).

Given the well-established link between the hippocampus and memory formation (Riedel & Micheau, 2001), it is unsurprising, therefore, that AD is associated with anterograde amnesia (AA), including severe deficits in spatial memory (Sun et al., 2005; Cherrier et al., 2005; Hort et al., 2007; Vlček, 2011; Moodley et al., 2014; Zhu et al., 2017). However, such deficits in ARBD appear to be minor (Vetreno, Hall & Savage, 2011; Ridley, Draper & Withall, 2013), once one has discounted the temporary effects of acute ethanol intoxication (Boulouard et al., 2002) and (Wernicke-)Korsakoff Syndrome, resulting from vitamin B1 deficiency (Ridley, Draper & Withall, 2013). Certainly, permanent AA in alcoholics appears to be mainly associated with Korsakoff Syndrome (Parkin, 1991; Joyce, 1994; Vetreno, Hall & Savage, 2011; Fama, Pitel & Sullivan, 2012; Ridley, Draper & Withall, 2013), rather than from chronic exposure to alcohol itself. Moreover, chronic alcohol-associated AA appears to be reversible, unlike AA in Alzheimer's (Fein et al., 1990, 2006; Pfefferbaum et al., 1995, 1998; Parsons & Nixon, 1998;

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Ridley, Draper & Withall, 2013), and much of the damage appears to result immediately after cessation of drinking (Fadda & Rossetti, 1998; Vetreno, Hall & Savage, 2011).

Nevertheless, there is sufficient evidence in animal models to suggest that both acute and chronic alcohol exposure lead to pronounced deficits in spatial memory (Santín et al., 2000; Pires et al., 2005; Assunção et al., 2007; Cippitelli et al., 2010; García-Moreno & Cimadevilla, 2012), evidence that appears to be mirrored in humans (Bowden & McCarter, 1993; Beatty et al., 1997) [*more references?*]. Similarly, so-called "blackout" episodes, commonly associated with drinking large amounts of alcohol over short periods of time (Goodwin, Crane & Guze, 1969; White, 2003), are clearly largely defined by and associated with AA (White, 2003; Nelson et al., 2004; Perry et al., 2006), appearing to involve both the frontal lobe and hippocampal regions (White, 2003; Oscar-Berman et al., 2004; Alderazi & Brett, 2007; Vetreno, Hall & Savage, 2011; Wetherill, Schnyer & Fromme, 2012; Hermens & Lagopoulos, 2018) [*more references?*]. In particular, chronic alcoholism appears to act synergistically with the normal ageing process to exacerbate the memory and other cognitive deficits commonly resulting from the latter (Pfefferbaum et al., 1992; Kim et al., 2012; Guggenmos et al., 2017), [*more references?*].

Whatever the reason, the similarities between AD and ARBD listed above would seem to provide the most obvious reason why heavy drinking appears to be associated with a higher risk

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

297 of developing Alzheimer's and other dementias (Anttila et al., 2004; Järvenpää et al., 2005; Kim
298 et al., 2012; Schwarzinger et al., 2018; Sabia et al., 2018) [*more references?*]. The fact that
299 people with the ApoE4 allele appear to have a much greater risk of developing dementia as a
300 result of drinking ethanol (including even light-to-moderate drinking), compared with non-
301 carriers of the allele (Anttila et al., 2004; Kim et al., 2012; Downer, Zanjani & Fardo, 2014)
302 [*more references?*], would seem only to add further weight to this association.
303

304 2.1.4 Summary of similarities between AD and ARBD

305
306 In summary AD and ARBD show a strikingly similar pattern of neurological damage,
307 particularly evident in the basal forebrain and hippocampal region of the medial temporal region,
308 accompanied by marked degeneration in the cholinergic projection system. In keeping with this
309 pattern of damage both AD and ARBD sufferers show deficits in executive function, olfaction
310 and anterograde memory (especially spatial memory) formation and a tendency towards
311 perseverative behaviour.
312
313 Taken together, these similarities would seem more than sufficient to warrant further
314 investigation. Yet it is hard to explain the mechanism by which long-term exposure of the brain
315 to two such different molecules, ethanol and Aβ, vastly different in size and sharing no obvious

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

316 chemical or physical properties in common, should lead to such a similarly distinctive pattern of
317 damage. Rather, it suggests that AD could be caused by molecules whose effects are likely to be
318 more similar to those of ethanol. One such candidate is FFAs which, for reasons discussed later,
319 share some crucial properties of ethanol and other aliphatic 1-alcohols (including fatty alcohols).
320 However, in order to appreciate how FFAs can become a major driver of AD, one must first
321 understand the differences between lipid metabolism either side of the BBB.
322

323 2.2 Differences between lipid metabolism on either side of the BBB

324

325 Whatever the exact biological role of the BBB may be, it is clear that many aspects of lipid
326 metabolism and transport greatly differ either side of it. This is most apparent in the case of fatty
327 acids (FAs) and cholesterol.
328

329 2.2.1 Fatty acid metabolism

330

331 For efficient transport within plasma, the vast majority of FAs, being highly hydrophobic, must
332 travel within lipoproteins or must be bound to the protein serum albumin to improve solubility
333 (Vance & Vance, 2008; van der Vusse, 2009) [*more references?*].

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

334

335 Immediately after eating, dietary FAs, bound to glycerol as triacylglycerol esters (TAGs) and
336 transported within the class of lipoproteins known as chylomicrons, constitute a major
337 proportion of the plasma transport pool (Vance & Vance, 2008; Rang, 2012). At the same time,
338 high blood glucose levels associated with satiety lead to hepatic neogenesis of FAs and glycerol,
339 with the resulting TAGs being transported in the blood within Very Low Density Lipoproteins
340 (VLDLs) (Vance & Vance, 2008; Rang, 2012). During subsequent plasma transport most of the
341 TAGs within chylomicrons and VLDLs are taken up by tissues, principally adipocytes and
342 muscle cells [*references?*].

343

344 The chylomicrons and VLDLs are relatively large (typically within a range of 30-80nm and 100-
345 1000nm, respectively (Vance & Vance, 2008; Rang, 2012)) and lipid-rich by virtue of their
346 association with ApoB isoforms. ApoB is synthesised only in the liver and in enterocytes, and
347 thus is normally unavailable to the CNS (Young, 1990; Vance & Vance, 2008). Such
348 lipoprotein-mediated FA transport appears to allow only very restricted access to the postnatal
349 brain across the BBB, given its architecture, mentioned earlier (Beffert et al., 1998; Björkhem &
350 Meaney, 2004; Elliott, Weickert & Garner, 2010; Orth & Bellosta, 2012), with only much
351 smaller (?), less lipid-rich (?) high-density lipoproteins (HDL) appearing to cross the BBB in
352 any quantity (Wang & Eckel, 2014).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

353

354 During the fasting state, adipocytes release stored FFAs directly back into the bloodstream, with
355 the majority being subsequently bound to serum albumin (Vance & Vance, 2008; van der Vusse,
356 2009). Because serum albumin is created almost exclusively in the liver (Ballmer, 2001; van der
357 Vusse, 2009; Schiff, Maddrey & Sorrell, 2011) and cannot pass readily through the BBB (Nag,
358 2003; Banks, 2006, 2008), it has until recently been assumed that albumin-bound FFAs must
359 also be largely excluded, in the same way as lipoprotein-associated FFAs. The reason for this
360 conclusion comes not just from the structural properties of the BBB mentioned above, but also
361 from the widespread expression within BBB endothelial cells of efflux pumps, such as P-
362 glycoprotein, which have hydrophobic molecules amongst their principal ligands (Rubin &
363 Staddon, 1999). This would seem to suggest that even unbound FFAs (either those unloaded
364 from albumin or never loaded in the first place) would tend to be pumped back out of the brain
365 in the same way that all large lipophilic molecules tend to be (Roninson, 1992).

366

367 Together, such features would appear to provide an obvious reason why, almost uniquely
368 amongst organs, the brain does not rely on the external supply of FAs (especially in albumin-
369 bound form) as a major energy source (Schönfeld & Reiser, 2013). This is despite the fact that
370 the brain has a high energy turnover, and other organs with high energy needs, such as the heart
371 and kidney, preferentially oxidise FAs (Johnson et al., 1990; Schönfeld & Reiser, 2013).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Instead, the liver must first transform plasma FFAs into much smaller ketone bodies, which, having been transported through the BBB, are used as an energy source by the brain [*references?*].

However, it has become clear in recent years that the BBB does not exclude FFAs from the brain (Schönfeld & Reiser, 2013; Panov et al., 2014) [*more references?*] and the most likely reason is for why the brain does not use them extensively for its energy needs is that they would prove toxic to neurons (Schönfeld & Reiser, 2013; Ioannou et al., 2019) [*more references?*]. (Another possible reason is that the rate of ATP generation from FAs is slower than from glucose and ketone bodies, meaning that FAs may not be able to yield ATP fast enough for rapidly firing neurons, especially under conditions of sustained activity (Schönfeld & Reiser, 2013).)

Recent evidence suggests a key role for astrocytes in protecting neurons from FA-mediated lipotoxicity. It appears that they do this in at least two ways. Firstly, they internalise medium-chain-length FAs, breaking them down by β-oxidation and secreting a proportion as ketone bodies, or the much shorter chain-length FA butyrate, both of them much less toxic to neurons (Edmond et al., 1987; Ebert, Haller & Walton, 2003; Schönfeld & Reiser, 2013; Plötz et al., 2017; Sonnay et al., 2019). Secondly, they directly take up excess FFAs from hyperactive neurons, preventing oxidative stress and other forms of lipotoxic damage, as well as preventing

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

391 accumulation of lipid droplets in the neuronal cytoplasm (Unger et al., 2010; Nguyen et al.,
392 2017; Ioannou et al., 2019).
393
394 This second mechanism appears to involve neuronal exocytosis of ApoE-containing lipoprotein-
395 like lipid particles, and subsequent endocytosis by astrocytes into lipid droplets (Ioannou et al.,
396 2019). Furthermore, neurons that express the ApoE4 allele appear not to secrete FAs as
397 efficiently as wild-type ApoE, resulting in the greater lipid peroxidation and other forms of
398 lipotoxic damage mentioned above (Ioannou et al., 2019).
399
400 Collectively, then, astrocytes appear to protect neurons by importing FAs from neurons and from
401 the immediate external interstitial fluid, and then either utilising them for generating ATP or
402 ketone bodies/butyrate (both as a result of β-oxidation), or else storing them within lipid droplets
403 (as TAGs) for future use. Except perhaps in times when other energy sources are not available,
404 astrocytes appear to export most of the ketone bodies and butyrate for neuronal usage, relying on
405 FFAs for much of their own energy needs.
406
407 As a consequence, astrocytic energy metabolism appears to be mainly aerobic (Farmer,
408 Kluemper & Johnson, 2019), whereas neuronal energy metabolism (which primarily uses
409 glucose, lactate, ketone bodies or butyrate) appears to be mainly anaerobic, protecting the latter

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

410 from oxidative stress, including lipotoxicity (?)(Schönfeld & Reiser, 2013). This may explain
411 why neurons are reported to have relatively poor antioxidative defences, certainly compared to
412 astrocytes (Bolaños et al., 1995; Schönfeld & Reiser, 2013), despite, at first sight, being more
413 obviously at risk from oxidative damage.
414
415 Such an explanation appears to account for why FFAs are not used for neuronal energy
416 metabolism, despite seemingly being available in substantial quantity for this purpose. *[*
417 *Similarly, it may explain why, despite neurons accounting for as much as 80% of energy*
418 *expenditure (?), FFA-mediated energy metabolism appears to account for only 20% of overall*
419 *energy metabolism.]*
420
421 But this still leaves a number of important questions unresolved. Most importantly, what
422 happens to the FFAs, once they cross the BBB, given that albumin transport is no longer
423 available to them (Roheim et al., 1979) [*more references?*]? And how are they transported? In
424 the absence of any obvious alternatives to albumin in the CNS, some form of lipoprotein-
425 mediated transport seems the most obvious alternative, mirroring the situation in the plasma
426 compartment outside the CNS. However, there are important differences between lipoprotein
427 transport in the CNS and lipoprotein transport in the plasma compartment.
428

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

In contrast to what is seen in plasma, as described above, the principal apolipoproteins expressed in the CNS (including Apo E, D and J (Danik et al., 1999; Elliott, Weickert & Garner, 2010)) associate into lipoprotein particles that are relatively small (typically less than 20nm) and lipid poor, containing modest amounts of lipids (Roheim et al., 1979; Ladu et al., 2000; Vance & Vance, 2008) [*more references?*]. Such CNS lipoprotein particles tend to resemble High-Density Lipoproteins (HDL) (Roheim et al., 1979; Ladu et al., 2000; Elliott, Weickert & Garner, 2010; Rang, 2012) much more than the larger ApoB-associated lipoproteins that predominate outside the CNS.

Furthermore, astrocytes are known to be a principal source of many of these CNS-originating apolipoproteins, particularly Apo E and J (Ladu et al., 2000; Mahley, Weisgraber & Huang, 2006; Elliott, Weickert & Garner, 2010), and lipoproteins have been isolated from the conditioned medium of astrocytic cultures (Danik et al., 1999). The fact that astrocytic foot processes are estimated to cover as much as 99% of the brain surface of capillaries (Johanson, 1980; Pardridge, 2005; Wilhelm et al., 2016), would seem to provide an obvious route of entry for FFAs that have managed to detach from their albumin transport partners and pass through the BBB. They can then be assembled into HDL-like lipoproteins within the astrocyte body and secreted into the interstitial fluid of the brain compartment, for onward transport and uptake by neurons and glial cells (Farmer, Kluemper & Johnson, 2019).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

From the above description, it would appear that FA transport and metabolism in the CNS is very different from that seen in the rest of the body. In particular, there appears to be little, if any, non-lipoprotein FA transport in the CNS and, on average, CNS lipoproteins are much smaller than their plasma equivalents. In many respects, FA transport seems more tightly controlled in the brain compartment than outside it. Certainly, it is hard to see how such differences would be possible without a substantially intact BBB.

2.2.2 Cholesterol metabolism

Numerous studies have shown that, except in very early foetal development, almost all cholesterol in the CNS is of local origin, relying on endogenous de novo biosynthesis rather than external, lipoprotein-mediated provision (Dietschy & Turley, 2004; Björkhem & Meaney, 2004; Elliott, Weickert & Garner, 2010; Orth & Bellosta, 2012). This appears to be true for a wide range of animals, including birds and mammals, with much of cholesterol production for neuronal consumption being delegated to local astrocytes (Pfrieger, 2003; Dietschy & Turley, 2004; Elliott, Weickert & Garner, 2010).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Moreover, cholesterol turnover in the mature CNS is very low, typically only around 5% of the turnover seen in the rest of the body (Dietschy & Turley, 2004; Björkhem & Meaney, 2004; Orth & Bellosta, 2012). A major reason for this is that a large proportion of such cholesterol remains locked up within the insulating myelin sheath that permanently encases the axons of many neurons, particularly within the white matter of the brain (Zhang & Liu, 2015). Much of this myelination takes place early in organismal development (Deoni et al., 2012).

In the rest of the body (and thus on the other side of the BBB) a large proportion of cholesterol is either of dietary origin or else the result of neogenesis in the liver (Vance & Vance, 2008; Rang, 2012). From there much of it is transported in the same large, lipid-rich, ApoB-containing lipoproteins (i.e. chylomicrons and VLDLs) that also transport dietary and liver-derived FAs (Young, 1990; Vance & Vance, 2008; Rang, 2012). Thus, for reasons of size (along with the other reasons explained above), much cholesterol of non-CNS origin is unable to cross the BBB (Kay et al., 2003; Björkhem & Meaney, 2004; Elliott, Weickert & Garner, 2010; Orth & Bellosta, 2012).

By contrast, within the brain and wider CNS, cholesterol is transported within the same HDL-like lipoproteins described in the previous section. As explained, such lipoproteins tend to be

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

small, compared to many of their plasma counterparts, typically containing only modest amounts of cholesterol and other lipids (Vance & Vance, 2008).

2.2.3 Overall differences in lipid transport either side of the BBB

Certainly, from birth onwards (Saunders et al., 1999), the BBB separates two compartments with very different lipid systems (Pardridge & Mietus, 1980; Dietschy & Turley, 2004). Compared to the rest of the body the mature CNS compartment is distinguished by a much lower circulation of lipids, with apparently restricted external lipid supplementation and a set of lipoproteins that are noticeably smaller and less lipid-rich [*references?*]. Much of this difference can be accounted for by the BBB, and by the fact that ApoB is not produced in the brain.

Given that this distinction appears to have first emerged comparatively early in vertebrate evolution (Bundgaard & Abbott, 2008) [*more references?*], it seems plausible that serious disruption to the BBB will have lipid-related consequences. This can be inferred from the fact that the mature brain compartment has evolved for so long to function in an environment low in circulating lipids compared with the rest of the body. And, given the relative volumes of the two compartments, it seems likely the brain will be the most vulnerable to lipid incursion if they are no longer separated by the BBB.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

503

504 2.3 The causes of BBB disruption in the lipid-leakage model

505

506 Clearly, an explanation of how the BBB becomes disrupted in AD is central to the lipid-leakage
507 model. It is generally established that the BBB slowly degrades with age (Farrall & Wardlaw,
508 2009; Popescu et al., 2009), providing a simple reason, according to the model, why LOAD
509 incidence is also closely correlated with age. But any model with such disruption at its centre
510 needs to account for the many inherited and non-inherited risk factors that accelerate the onset of
511 AD.

512

513 In FAD this can accounted for by Aβ, which, as explained earlier, is known to impair BBB
514 integrity (Thomas et al., 1997; Su et al., 1999; Marco & Skaper, 2006; Takechi et al., 2010a),
515 especially in association with the ApoE4 genotype (Alonzo et al., 1998) [*more references?*].
516 Numerous studies show that ApoE protects the BBB, with its absence leading to progressive
517 BBB leakage, in excess of what is seen as a result of normal ageing (Mulder et al., 2001; Methia
518 et al., 2001; Hafezi-Moghadam, Thomas & Wagner, 2007). Compared to the other ApoE
519 isoforms, however, ApoE4 is associated with impaired BBB function, particularly involving
520 tight junctions, whose integrity is critical to the BBB's capacity to exclude a wide range of
521 molecules (Salloway et al., 2002; Nishitsuji et al., 2011; Bell et al., 2012).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

However, recent studies have suggested that Aβ has an important function as a regulatory apolipoprotein, being highly expressed in both the liver and small intestine, and associated with triglyceride-rich lipoproteins of similar origin (Galloway et al., 2007; Mamo et al., 2008; Takechi et al., 2010a). In absorptive enterocytes, Aβ is seen to collocate with $ApoB_{48}$, forming chylomicrons, with enterocytic levels of Aβ and plasma levels of Aβ-associated chylomicrons both increasing in response to a diet high in saturated fats (Galloway et al., 2007; Pallebage-Gamarallage et al., 2010).

In a standard transgenic mouse model of AD in which Aβ is overproduced, disease progression and onset were seen to be strongly correlated with rates of secretion into the blood of TAG-rich, Aβ-associated lipoproteins, and with their subsequent plasma levels (Takechi et al., 2010a). Such overproduction, whether resulting from dietary causes or from direct Aβ over-expression, leads to BBB disruption (Mamo et al., 2008; Takechi et al., 2010a; Pallebage-Gamarallage et al., 2010).

This helps explain, amongst other things, why amyloid plaques in human brains show immunoreactivity for ApoB, similar to that seen in the brains of AD mouse models (Namba, Tsuchiya & Ikeda, 1992; Takechi et al., 2010a). For the reasons stated earlier, such ApoB

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

deposition is only possible if the BBB has been disrupted in some way, as well as being consistent with the premise that invading, lipid-rich, lipoproteins are primary actors in endosomal pathology (as described in 2.6.2) and amyloid plaque formation.

This suggests that the aetiology of both familial and late-onset forms of AD could be linked through excess levels of TAG-rich chylomicrons. In the former case this would primarily result from over-production of Aβ, whilst in the latter case it would primarily result from dietary causes. This in turn would lead, in both cases, to BBB disruption (which can be exacerbated by other factors, as explained above) and to the characteristic neurodegenerative effects outlined below. However, evidence for such chylomicron excess as a general characteristic of AD is limited at present and is not a requirement of the model.

2.4 AD-relevant consequences of lipid influx to the brain

2.4.1 Oxidative stress

In recent years a considerable body of evidence has accumulated that suggests that AD-affected brains are subject to high levels of oxidative stress (Markesbery, 1997; Huang, Zhang & Chen, 2016). This evidence includes increased protein and DNA oxidation (Smith et al., 1991; Mecocci, MacGarvey & Beal, 1994; Markesbery, 1997; Korolainen et al., 2002; Santos et al.,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

559 2012), as well as an increase in lipid peroxidation (Subbarao, Richardson & Ang, 1990; Bradley-
560 Whitman & Lovell, 2015), together with various associated peroxidation biomarkers (Lovell et
561 al., 1997; Bradley-Whitman & Lovell, 2015). Such lipid peroxidation may account for an
562 observed decrease in the levels of polyunsaturated FAs, which appear to be more vulnerable to
563 such peroxidation (Markesbery, 1997; Conquer et al., 2000; Tsaluchidu et al., 2008; Fotuhi,
564 Mohassel & Yaffe, 2009; Dyall, 2010; Huang, Zhang & Chen, 2016). Other indications of
565 oxidative stress in AD-affected brains include raised levels of advanced glycation end-products,
566 that is to say proteins or lipids that have become glycated (Smith et al., 1994; Markesbery, 1997;
567 Sasaki et al., 1998; Drenth et al., 2017).
568
569 Perhaps not surprisingly, there has been much focus on the role of Aβ and amyloid plaques as
570 principal drivers of this oxidative stress in AD (Markesbery, 1997; Huang, Zhang & Chen,
571 2016). Certainly, there is substantial evidence to suggest that both Aβ and its precursor APP
572 contain high affinity binding sites for metal such as copper, zinc and iron, with amyloid plaques
573 seen to be highly enriched with these metals, some of which are redox-active (Barnham et al.,
574 2003; Huang et al., 2004; Smith, Cappai & Barnham, 2007; Strozyk et al., 2009; Liu et al.,
575 2019). And subsequent findings have led many researchers to propose a positive feedback
576 mechanism whereby Aβ amyloidosis and metal-induced oxidative stress reinforce each other,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

577 thus contributing strongly to AD-associated neuropathology (Huang et al., 2004; Smith, Cappai
578 & Barnham, 2007; Strozyk et al., 2009; Faller, 2009).
579
580 However, despite more than 20 years of research into this relationship, there are still many
581 questions that remain unresolved, not least concerning the respective roles of copper and zinc
582 (Cuajungco & Fagét, 2003; Atrián-Blasco, Conte-Daban & Hureau, 2017; Drew, 2017).
583 Furthermore, there is, as yet, no convincing evidence that therapeutic metal chelation has any
584 substantial impact, if at all, in slowing down AD progression, leading some to question the
585 relevance of such metal-induced oxidative stress to AD (Drew, 2017; Liu et al., 2019).
586
587 But there are many other ways in which AD might lead to oxidative stress, without requiring the
588 involvement of metals. In particular, neuroinflammation triggered by the presence of Aβ,
589 provides a straightforward reason why oxidative stress should increase with AD progression,
590 given the well-established link between neuroinflammation and increased levels of reactive
591 oxygen and nitrogen species (Agostinho, Cunha & Oliveira, 2010; Dyall, 2010; González-Reyes
592 et al., 2017). This is addressed in more detail in the next section.
593
594 As explained later, a key prediction of the lipid-leakage model is that an increase in Aβ
595 production will occur as a direct consequence of lipid invasion from outside the brain.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Therefore, oxidative stress, as a consequence of Aβ-driven neuroinflammation, can be easily accounted for by the model. And, as explained below, FA invasion may drive neuroinflammation more directly, acting on same pathways that drive ethanol-induced neuroinflammation. Thus, there are good reasons for believing that FA-driven neuroinflammation alone is sufficient to account for

However, the description of FA metabolism in section 2.2.1 above suggests another, even more direct, way in which the lipid-leakage model can account for oxidative stress in AD. Substantial damage to the BBB will mean that the brain is exposed to albumin-bound FFAs and, larger, more lipid-rich lipoproteins, originating from the external plasma compartment.

As a consequence, it may be that astrocytes are no longer able to protect neurons from excessive FA accumulation, leading to lipid peroxidation and other forms of oxidative stress. Certainly, there is much evidence to suggest that lipid homoeostasis becomes badly disrupted in AD (Foley, 2010; Di Paolo & Kim, 2011; Farmer, Kluemper & Johnson, 2019). Indeed, in the earliest reports of the disease, by Alois Alzheimer and colleagues, there are numerous references to various intracellular lipid inclusions and other lipid-related abnormalities within the brain of affected subjects (Stelzmann, Norman Schnitzlein & Reed Murtagh, 1995; Di Paolo & Kim, 2011).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

615

616 Given that normal lipid homoeostasis appears to be critical to preventing excessive oxidative
617 stress within the brain, as described earlier, it can easily be appreciated how breakdown of the
618 BBB, as predicted by the lipid-leakage model, might lead to appreciable increases in such stress.

619

620 ### 2.4.2 Neuroinflammation

621

622 Extensive research has established that neuroinflammation is an important cause of ethanol-
623 induced neurodegeneration (Syapin & Hickey, 2006; Blanco & Guerri, 2007; Crews, 2008;
624 Crews & Nixon, 2009) and that microglia are central agents of such inflammation (Syapin &
625 Hickey, 2006; Crews, 2008; Zhao et al., 2013; Walter & Crews, 2017) [*more references?*]. This
626 central role is perhaps unsurprising, given that the "immune-privileged" status conferred on the
627 brain by the BBB leaves microglia as the primary immune cell (Kaur et al., 2010; Yang et al.,
628 2010), a role not seen as a rule in macrophages in the rest of the body. Their ability to perform
629 this role seems to depend in large part on being abnormally sensitive to a wide range of ligands
630 (Gehrmann, Matsumoto & Kreutzberg, 1995; Dissing-Olesen et al., 2007; Yang et al., 2010), and
631 this, in turn, helps to explain why chronic ethanol, largely unobstructed by the BBB, causes such
632 extensive inflammatory damage to the brain over time (Fadda & Rossetti, 1998) [*more*
633 *references?*]. Additionally, the mechanism through which this occurs suggests that FAs,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

provided they could pass through the BBB in quantity, would have similar inflammatory effects, since both are known to powerfully activate the same critical receptor.

Ethanol activation of microglia (Crews, 2008), is accompanied by upregulation of the transcription factor NF-κB (Zou & Crews, 2010; Alfonso-Loeches et al., 2010) and other macromolecules known to be involved in inflammation and in the immune response. The evidence suggests that toll-like receptors, particularly TLR4, a receptor that binds bacterial lipopolysaccharide (LPS), appear to be central to such activation and the subsequent neuroinflammation (Alfonso-Loeches et al., 2010; Fernandez-Lizarbe, Montesinos & Guerri, 2013).

If TLR4 is central to ethanol-induced neuroinflammation then there seems every reason to think that FFAs entering the brain would have similar neuroinflammatory effects. Saturated (but not, apparently, unsaturated) FAs are known to activate TLR4 in macrophages, leading in turn to activation of NF-κB and the other pro-inflammatory molecules referred to earlier (Chait & Kim, 2010; Wang et al., 2012). And TLR4 activation in adipocytes by saturated FAs (and perhaps by some unsaturated FAs) is an essential step in lipid-induced diabetes mellitus (Shi et al., 2006; Chait & Kim, 2010), which is now thought to be substantially inflammatory in nature [*references?*]. In support of this, knockdown or ablation of TLR4 has been shown to inhibit both

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

FFA-induced and ethanol-induced inflammation (Shi et al., 2006; Alfonso-Loeches et al., 2010; Wang et al., 2012) [*more references?*] .

Given how responsive microglia are to pathological stimuli (Kreutzberg, 1996; Rock et al., 2004; Rangaraju et al., 2015; Lenz & Nelson, 2018), one could reasonably expect activation by both ethanol and FFAs to result in far more vigorous inflammatory activity than seen in other parts of the body. And, whilst the relative affinities of ethanol and FFAs for TLR4 have yet to be determined, the fact that saturated fatty acyl groups are known to be crucial to TLR4 recognition of LPS (TLR4's principal pathogenic ligand) (Hwang, 2001) suggests that FFAs should have a substantially higher affinity than ethanol for TLR4. Thus the relatively low levels of FFAs seen in plasma (generally agreed to fall within an average range of 0.3-0.6 mM [*references?*]) should be sufficient to generate a steady level of neuroinflammation, following major BBB insult, especially if they are accompanied by pathogen-associated LPS, as seen in ethanol-induced liver injury (Nagy, 2003). Thus it may be this, rather than TLR4 stimulation by amyloid (Walter et al., 2007), that is the primary driver of microglial-based neuroinflammation in LOAD.

2.4.3 Inhibition of neurogenesis

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Ethanol-induced neuroinflammation has also been linked to inhibition of neurogenesis (Crews & Nixon, 2009) [*more references?*], with many studies suggesting that such neurogenetic deficits are almost as important a factor as neuroinflammation in ethanol-mediated brain degeneration (Crews, 2008) [*more references?*] . Here too, TLR4 is likely to have a prime inhibitory role (Barak, Feldman & Okun, 2014) [*more references?*], diminishing proliferation of adult neuronal progenitor cells (NPCs) and restricting neuronal differentiation from NPCs. Such inhibition would obviously be most apparent in the main neurogenic niches, i.e. the subgranular and subventricular zones, which provide new interneurons to (respectively) the hippocampus and the olfactory bulb [*references?*]. This could explain the deficiencies in learning and olfaction common to both AD and ARBD.

Furthermore, current evidence indicates that the overall level of neurodegeneration is determined almost as much by the relentlessness of the ethanol assault as by the concentrations involved (Nixon & Crews, 2002; Crews, 2008; Crews & Nixon, 2009). Thus, one can reasonably infer that constant exposure of the brain to plasma levels of FFAs is likely to overwhelm the brain's capacity to recover, especially in the elderly. Such a conclusion is further supported by evidence that inhibition of neurogenesis, by both ethanol and FFAs, does not need to rely on the TLR4 receptor alone, and may, in fact, depend more on GABAergic effects, as explained in the next section.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

690

691 ## 2.5 GABAergic effects

692

693 Recent research has indicated a possible role for the inhibitory neurotransmitter gamma-
694 aminobutyric acid (GABA) in the development of AD (Rissman & Mobley, 2011; Wu et al.,
695 2014; Jo et al., 2014), with a number of possible mechanisms being suggested. One such
696 mechanism, GABA-induced tonic inhibition within the hippocampus, provides an obvious
697 explanation of why AD is characteristically associated with AA. However, the proposed source
698 of this excess GABA within hippocampal-resident reactive astrocytes, does not have much
699 support in the literature, either for AD or ARBD.

700

701 The lipid-leakage model provides an alternative mechanism, extending beyond tonic inhibition,
702 and accounting for the coexistence of AA in AD and ARBD, as well as other similarities,
703 including similar patterns of neurodegeneration within two major neurogenic niches, the SGZ
704 and SVZ. Underlying this common mechanism is the proven affinity of ethanol, and likely
705 affinity of FFAs, for GABAA receptors (GABAARs), as well as the recently-discovered role of
706 high-affinity extrasynaptic GABAARs in both tonic inhibition and anaesthesia-associated
707 amnesia.

708

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

709 In the 1950s onward, Samson and Dahl and other groups showed that injection of FFAs induced
710 light anaesthesia in a range of mammals (Samson Jr, Dahl & Dahl, 1956; White & Samson,
711 1956; Matsuzaki & Takagi, 1967; McCandless, 1985). Anaesthetic potency increases (up to an
712 undetermined cut-off) with FFA chain length (and thus hydrophobicity), in line with Meyer-
713 Overton (Samson Jr, Dahl & Dahl, 1956; White & Samson, 1956; Dahl, 1968; Perlman &
714 Goldstein, 1984), falling within the low millimolar range (expressed both as moles per litre and
715 moles per kilogram of body weight) and showing similar potencies to structurally comparable 1-
716 alcohols (including ethanol) (Alifimoff, Firestone & Miller, 1989), as well as to alkanes (Hau,
717 Connell & Richardson, 2002) and aldehydes (Deneer, Seinen & Hermens, 1988).
718
719 Given the general correlation between hydrophobicity and anaesthetic potency first described by
720 Meyer-Overton (Evers & Crowder, 2009), it would perhaps be surprising if fatty acids did not
721 show similar anaesthetic potencies to structurally very similar fatty alcohols (Ueda & Suzuki,
722 1998; Matsuki et al., 1999; Frangopol & Mihailescu, 2001; Evers & Crowder, 2009), nor, given
723 the established anaesthetic properties of various steroids (Kappas & Palmer, 1963; Belelli &
724 Lambert, 2005), should it be a surprise that other lipids might display similar properties.
725
726 The immediate significance of lipids' anaesthetic properties to dementia lies in the fact that, at
727 concentrations well below those needed for clinical anaesthesia, the vast majority of anaesthetic

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

agents are known to cause AA (Orser, 2007; Bonin & Orser, 2008; Evers & Crowder, 2009). Such low-level anesthesia-induced AA is now known to involve extrasynaptic GABAARs (Orser, 2007; Bonin & Orser, 2008) whose subunit composition (including either α5 or δ subunits) gives them sufficient sensitivity to respond to low levels of ambient GABA (Brickley & Mody, 2012). It is the resulting low-level inhibitory currents, termed "tonic inhibition", which is associated with AA (Cheng et al., 2006; Nutt et al., 2007; Sikka, Beaman & Street, 2015). (By contrast lower-affinity synaptic GABAARs, with different subunit compositions, respond only to the higher concentrations of GABA released within their associated synapses, with the resulting phasic inhibition causing the other anaesthetic effects (Evers & Crowder, 2009) [*more references?*], including analgesia, immobility and unconsciousness.) In support of this, pharmacological and genetic knockdown of extrasynaptic α5- and δ-containing $GABA_ARs$ in mice has been shown to improve performance on learning and memory tasks (Collinson et al., 2002; Shen et al., 2010; Clarkson et al., 2010), possibly by lowering the threshold for long-term potentiation (Liu et al., 2010; Martin et al., 2010; Whissell et al., 2013).

The reason for all this is that GABAARs have associated ion channels, which become permeable to chloride (and, to a lesser extent, HCO_3) ions, in response to GABA ligation (Li & Xu, 2008) [*more references?*]. Upon such activation, chloride ions flow through these GABAAR channels in a direction determined by their electrochemical gradient. Since mature neurons maintain an

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

747 excess of chloride ions externally, the normal response to GABA binding is therefore for these
748 negative ions to flow in through the GABAAR channels, increasing the negative membrane
749 potential and thereby hyperpolarising (i.e. inhibiting) the affected neuron (Kaila, 1994; Li & Xu,
750 2008). Tonic inhibition is just the extrasynaptic form of this (Petrini et al., 2004; Jia et al.,
751 2005). The majority of anaesthetic agents (including those that are only weakly anaesthetic, such
752 as ethanol) are known to enhance this GABA binding, acting as positive allosteric modulators
753 (Orser et al., 1998; Krasowski, 2003). Accordingly, they tend to inhibit normal activity in
754 mature neurons of the CNS (Orser et al., 1998; Krasowski & Harrison, 1999; MacIver, 2014).
755
756 However, recent research has shown that the same high-affinity extrasynaptic GABAARs that
757 mediate tonic inhibition in mature neurons (Yeung et al., 2003; Brickley & Mody, 2012) also
758 play a significant role in neurogenesis and neuronal plasticity (Liu et al., 2005; Bordey, 2007).
759 In support of this, pharmacological and genetic suppression of tonic GABA inhibition, including
760 by down-regulation of extrasynaptic GABAAR activity, is associated with marked
761 improvements in functional recovery after stroke (Clarkson et al., 2010; Paik & Yang, 2014).
762 This is in agreement with findings that suggest that increased GABA tonic inhibitory currents, in
763 the days after stroke, hinder recovery (Clarkson et al., 2010; Clarkson, 2012).
764

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

765 Since the extrasynaptic GABAARs containing the δ-subunit are known to be especially sensitive
766 to positive modulation by ethanol (Wei, Faria & Mody, 2004; Meera et al., 2010) this may
767 explain alcohol-mediated neurodegeneration seen in ARBD. As explained earlier, disruption of
768 neurogenesis appears to be critical to the neurodegenerative effects of ethanol upon the brain.
769 Specifically, chronic exposure of the brain to ethanol is characterised from comparatively early
770 on by erosion of the hippocampal region (Nixon & Crews, 2002; Crews, 2008), loss of
771 interneurons (the primary product of neurogenesis (Mandyam, 2013)), AA (White et al., 2004;
772 Sanday et al., 2013) and olfactory deficits (Ditraglia et al., 1991; Collins, Corso & Neafsey,
773 1996).
774
775 An obvious explanation for these findings is inhibition of neurogenesis in the SGZ and SVZ,
776 given that the former supplies interneurons to other hippocampal regions (Eriksson et al., 1998)
777 [*more references?*], whilst the latter is known to replenish the olfactory bulb interneurons via the
778 rostral migratory stream (Lim & Alvarez-Buylla, 2016) [*more references?*]. Since much
779 evidence suggests that FFAs have similar, if not higher, anaesthetic potency levels to ethanol
780 (Samson Jr, Dahl & Dahl, 1956; Walker et al., 1970; Pringle, Brown & Miller, 1981; Wong et
781 al., 1997; Ueda & Suzuki, 1998; Frangopol & Mihailescu, 2001), implying a similar affinity for
782 GABAARs, it may well be that chronic exposure of the brain to excess FFAs over many years

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

783 will have similar results. This would provide an explanation of, why AD and ARBD share these
784 hallmark effects on the brain.
785
786 A complicating factor here is that, in immature neurons, the chloride gradient is reported to be in
787 the reverse direction to that of their mature counterparts (Ben-Ari & Holmes, 2005; Li & Xu,
788 2008). That is to say, chloride ions are held internally in excess of their external levels. If so,
789 GABA binding to GABAARs could reasonably be expected to activate such precursor neurons
790 and, by extension, one would expect anaesthetic agents (and other positive modulators) to
791 overactivate them. A further consideration is that such precursor cells initially exhibit few
792 synapses, with most GABAARs having a subunit composition typical of extrasynaptic
793 GABAARs in mature neurons (Henschel, Gipson & Bordey, 2008; Song et al., 2012; Pallotto &
794 Deprez, 2014), with synapses only tending to emerge later as the neuronal precursors mature and
795 become integrated (synaptically and otherwise) with the existing network (Ge et al., 2007; Ben-
796 Ari et al., 2007; Ming & Song, 2011). So GABAARs in these cells tend to have a high affinity
797 for ambient GABA, and one would expect the dominant response to GABA stimulation to be
798 tonic activation (Ming & Song, 2011; Song et al., 2012). So, if ethanol (and, as we are arguing
799 here, by extension, FFAs) abnormally enhance this effect, one should expect to see overgrowth
800 rather than erosion in adult neurogenic regions. Why is this not so?
801

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

One mechanism that might explain such neurogenetic deficits in the SGZ and SVZ, is GABA-mediated feedback inhibition. Recent discoveries suggest that non-synaptic paracrine GABA signalling provides information on population size to control proliferation and migration of neural progenitor cells in the SVZ (Liu et al., 2005; Bordey, 2007; Ge et al., 2007; Pallotto & Deprez, 2014). Specifically, adult SVZ neuroblasts synthesise and release GABA, which acts on GABAARs in neural stem cells, inhibiting NSC division and thus effectively applying a brake on neurogenesis. In confirmation of this, removal of neuroblasts is seen to release this brake.

The specific details of this appear to have been provided by a study of neurogenesis in postnatal rat striatum (Nguyen et al., 2003). Here, the growth factor EGF was seen to decrease GABA production and release in PSA-NCAM+ neural precursor cells, leading to their proliferation. A number of experiments suggested that GABA was indeed acting on GABAARs in an autocrine/paracrine mechanism to prevent cell proliferation by inhibiting cell cycle progression. Application of GABAAR antagonists inhibited proliferation, whereas positive allosteric modulators decreased it. As with other immature neuronal cell lineages, GABA-mediated GABAAR activation elicited inward currents (indicating outward flows of negatively-charged chloride ions), leading to tonic inhibition of the mitogen-activated protein kinase cascade and an increase of intracellular calcium levels (Nguyen et al., 2003).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

This agrees with the findings of the Liu study, which showed that, at least in GFAP-expressing neural progenitor cells in the SVZ, GABAAR activation limits progression through the cell cycle (Liu et al., 2005). It also suggests that, at least in the SVZ, adult neurogenesis is regulated by the same mechanisms that govern embryonic neurogenesis, where, for instance, GABA is seen to direct neuroblast migration, stimulating random mobility by promoting elevation of cytosolic Ca2+ levels (Barker et al., 1998; Ge et al., 2007), similar to what is seen in adult neurogenesis (LoTurco et al., 1995). While some related studies have shown that such effects appear to promote neuronal fate selection (Tozuka et al., 2005), the overall impression is that GABA stimulation also seems to limit proliferation (Barker et al., 1998; Nguyen et al., 2003). However, more recently, doubts have been raised about whether such tonic GABA-mediated depolarisation is sufficient to open voltage-gated calcium channels enough to permit substantial increases in intracellular calcium in the way proposed, requiring other explanations (Bordey, 2007).

An alternative explanation is that an epigenetic mechanism, involving histone H2AX phosphorylation following sustained GABAAR activation by GABA, inhibits DNA synthesis and cell cycle progression, and therefore proliferation of adult neural stem cells (Fernando et al., 2011). It is not clear that this mechanism also applies to SGZ neurogenesis but, if so, it could explain why GABAergic stimulation is similarly associated with quiescence of adult precursor cells in this niche (Duveau et al., 2011; Song et al., 2012; Pallotto & Deprez, 2014).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

But it may be that such involved explanations are not necessary, as recent research has brought into question the prevailing orthodoxy concerning GABA activation of immature neurons (Valeeva et al., 2016; Zilberter, 2016), concluding that, overall, GABA action on the neonatal brain is inhibitory. If this proves correct, and is found to be true also for adult neurogenic regions, then ethanol-induced deficits in neurogenesis can be simply explained as a result of excess inhibition.

Either way, assuming ethanol inhibition of neurogenesis in the SVZ and SGZ is mediated by GABAARs, then FFAs are likely to have a similar effect. This is because a number of studies point towards GABAARs as the most likely target and mediator of FFA's limited anaesthetic properties, not least the well-established anaesthetic effects (alluded to earlier) of structurally similar n-alkanes, n-alcohols and n-aldehydes. Furthermore, as with FFAs, anaesthetic potency increases with chain length but only up to a certain "cut off" length (Alifimoff, Firestone & Miller, 1989; Chiou et al., 1990; Wick et al., 1998; Frangopol & Mihailescu, 2001; Hau, Connell & Richardson, 2002; Lugli, Yost & Kindler, 2009)). This, together with direct evidence that the n-alcohols act on GABAARs (Wick et al., 1998; Davies, 2003), as does the endogenous, FA, anaesthetic oleamide (Lees et al., 1998; Laws et al., 2001; Coyne et al., 2002), suggests a common binding site. More direct evidence for this comes from the observed antagonising

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

effects of long-chain FFAs on GABAAR-mediated anaesthesia by volatile anaesthetics (Hanada, Tatara & Iwao, 2004; Yamakura, 2004), along with other evidence of direct interactions between FFAs and GABAARs (Koenig & Martin, 1992; Witt & Nielsen, 1994; Zhang & Xiong, 2009).

Taken together, a strong body of evidence points to the likelihood that FFAs, entering the brain through a damaged BBB (and therefore much in excess of their normal levels), will, if maintained over the long-term, tend to seriously disrupt neurogenesis by acting on GABAARs. Given the presence of major sites of neurogenesis in the SGZ and SGZ, this will principally manifest itself in anterograde amnesia and olfactory deficits. The first of these is of course the primary behavioural abnormality seen in AD, whilst the second has been argued to be another common (if less obvious) outcome. But, as described above, these are also seen in ARBD, driven by excess exposure to ethanol, which is known to act on GABAARs, accounting for the similarities between AD and ARBD detailed above.

2.6 AD-specific consequences of brain exposure to external lipids

If the above account explains many of the similarities seen between AD and ARBD, it does not explain why, unlike ARBD, AD is characterised by profuse plaques and tangles. The lipid-leakage model of AD explains this by the fact that the BBB has to be disrupted for fatty acids to

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

878 substantially enter the brain, unlike in ARBD, where ethanol can pass through the BBB
879 relatively unhindered [*references?*]. Consequently, in AD the brain is also exposed to other
880 molecules from which it is normally protected, including lipoproteins, which are much larger
881 and more lipid-laden than those normally found within the CNS compartment.
882
883 There is good reason to think that such lipoproteins may account for the amyloid plaques that
884 characterize AD. It has been known for some time that excess cholesterol is associated with
885 increased amyloidogenesis.
886

887 2.6.1 The role of excess cholesterol in amyloidogenesis

888
889 Cholesterol may have a role in increasing proteolytic production of amyloidogenic Aβ from
890 APP, as opposed to production of alternative non-amyloidogenic fragments (Bodovitz & Klein,
891 1996; Xiong et al., 2008; Nicholson & Ferreira, 2010). This appears to result from the influence
892 of cholesterol stimulation on an amyloidogenic pathway involving β- and γ-secretases (two
893 proteases involved in APP proteolysis) (Xiong et al., 2008), as well as on a non-amyloidogenic
894 pathway involving α-secretase (Kojro et al., 2001) (Figure 3.). Increasing the levels of
895 cholesterol stimulates the amyloidogenic pathway, at the same time inhibiting the non-
896 amyloidogenic pathway (Wolozin, 2004; Xiong et al., 2008). In contrast, cholesterol depletion,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

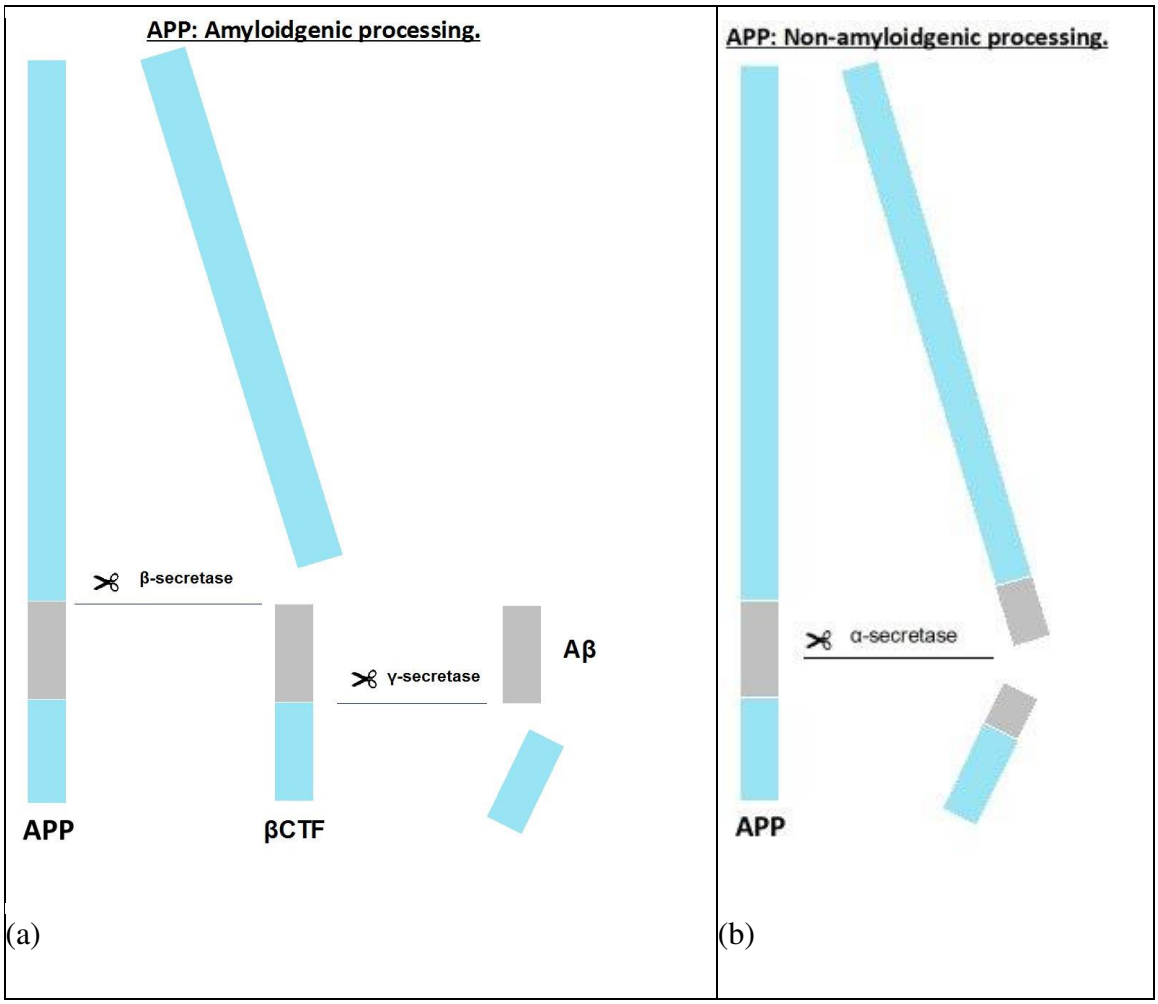
by various processes, inhibits the amyloidogenic pathway and enhances non-amyloidogenic processing, resulting in lower levels of Aβ (Simons et al., 1998; Kojro et al., 2001) [*more references?*].

Amyloidogenic processing appears to be initiated within cholesterol-rich lipid rafts (Ehehalt et al., 2003; Rushworth & Hooper, 2011; Nixon, 2017) (especially in early endosomes (Arriagada et al., 2007; Nixon, 2017)), whilst non-amyloidogenic processing occurs in the main phospholipid-rich region of the neuronal plasma membrane (Xiong et al., 2008; Grimm et al., 2013). This suggests that an important part of cholesterol's influence on amyloidogenic processing may be a consequence of its essential role as a major constituent of these lipid rafts, a conclusion that is well-supported in the literature (Ehehalt et al., 2003; Vetrivel et al., 2004; Nixon, 2017) [*more references?*].

Certainly, some studies indicate that brain cholesterol levels may be raised in AD, compared to non-demented, brains (Xiong et al., 2008) [*more references?*], although not all studies concur [*references?*]. That cholesterol may be directly associated with amyloid plaque formation is supported by brain imaging studies, which show Aβ collocated with cholesterol within amyloid deposits in AD human brain samples (Xiong et al., 2008) [*more references?*].

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

916



917

918 **Figure 3. (a) Amyloidogenic and (b) non-amyloidogenic processing of APP.**

919

920

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

2.6.2 The role of excess cholesterol in endosomal-lysosomal pathway abnormality

Indirect evidence of raised brain cholesterol levels as a causal factor in AD comes from studies of human AD brains (Cataldo et al., 2000) [*more references?*]. Such brains show abnormalities in the endosomal-lysosomal system compared to normal brains, together with neurofibrillary (tau) tangles [*references?*]. Such endosomal pathway overactivity and compartmental enlargement appears to be an early marker in AD, especially in pyramidal neurons (which are known to be vulnerable in AD [*references?*]), and in endothelial cells [*references?*].

Interestingly, a very similar pathology is also seen in mouse and other models of DS (Cataldo et al., 2000, 2008; Arriagada et al., 2007) [*more references?*]. However, at least in the case of one mouse model, such pathology was seen to emerge only following lipoprotein-mediated cholesterol treatment (Arriagada et al., 2007), suggesting that cholesterol is a crucial causal factor.

Further support for this comes from a number of studies in in Niemann-Pick disease type C (NPC), a neurological disorder characterised by faulty cholesterol transport and by tau tangles (Saito et al., 2002), and in which endosomal-lysosomal pathology is also observed (Frolov et al., 2001). Such studies, whilst often contradictory in their results, collectively point to various

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

failings in cholesterol uptake, transport and recycling, and in abnormal endosomal-lysosomal pathway behaviour. Such reported failings include excessive uptake of exogenous LDL-derived cholesterol (Liscum & Faust, 1987), excessive synthesis of endogenous cholesterol (Liscum & Faust, 1987), enlarged early endosomes (Jin et al., 2004; Nixon, 2004), accumulation of unesterified cholesterol in late endosomes and lysosomes (Nixon, 2004; Sobo et al., 2007), defective post-lysosomal cholesterol transport (Roff et al., 1991) and redistribution of lysosomal hydrolases to early endosomes (Jin et al., 2004).

Yet such reports commonly claim that other aspects of cholesterol internalisation (and endosomal-lysosomal pathway behaviour) appear to be normal, particularly in the case of initial cholesterol uptake and early endosome behaviour (Nixon, 2004). However, a very similar phenotype is observed in a Chinese hamster ovary (CHO) cell mutant, which has a normal copy of NPC1 (the late endosome/lysosome-residing protein most commonly associated with NPC disease (Nixon, 2004)) , and of the HE/NPC2 protein (also associated with NPC, although less commonly) yet still exhibits NPC-like pathology (Frolov et al., 2001). In this mutant late sterol trafficking is reported to be normal despite obvious cholesterol accumulation in late endosomes/ lysosomes (Frolov et al., 2001). Instead, cholesterol build-up occurs as a result of much-increased LDL-R binding, probably leading to cholesterol uptake being in excess of the normal capacity of the cell to dispose of it (Frolov et al., 2001). Evidence in support of this conclusion

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

959 includes the finding that LDL starvation of this mutant resulted in the disappearance of the
960 cholesterol-laden aberrant late endosome compartment (characteristic also of NPC) that had
961 previously been observed, only for this compartment to reappear with the restoration of LDL
962 feeding (Frolov et al., 2001).
963
964 More generally, another study, using a human fibroblast model, appears to provide further
965 evidence for this conclusion. It found endosomal-lysosomal pathology in a number of inherited
966 sphingolipid-storage disorders (Puri et al., 1999). In almost all cases such pathology showed
967 strong similarities with that seen in NPC, with a marked reduction in the accumulation of both
968 cholesterol and a representative sphingolipid within the Golgi complex, accompanied by their
969 increased accumulation within many punctate cytoplasmic structures that also appeared to be
970 associated with the NPC1 protein (Puri et al., 1999).
971
972 The authors conclude that the observed pathology most likely results from a build-up of
973 cholesterol (which is known to associate with high affinity to sphingolipids (Brown, 1998;
974 Lönnfors et al., 2011)) within endosomes and lysosomes, since the reported pathology was seen
975 to disappear following cholesterol depletion, being replaced with normal endosomal-lysosomal
976 behaviour (Puri et al., 1999). However the same pathology could also be induced in normal cells
977 by application of excess external cholesterol in the form of low-density lipoprotein (LDL) (Puri

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

978 et al., 1999), similar to what is described for the CHO mutant mentioned above (Frolov et al.,
979 2001), and in line with another study linking raised levels of plasma membrane cholesterol with
980 correspondingly enlarged early endosomes in hippocampal neurons (Cossec et al., 2010).
981
982 As stated earlier, LDL is not normally seen in the brain (since it requires apolipoprotein B) and
983 tends to be both larger in size and more cholesterol-rich than the HDL-like lipoproteins typically
984 seen there (Danik et al., 1999; Vance & Vance, 2008). This suggests that externally-sourced
985 cholesterol, supplied in excess of normal brain levels, may be a causal factor of AD-related
986 endosomal abnormalities and of amyloidosis, at least in the late-onset form.
987
988 In further support of this hypothesis, inhibition of CYP46A1 (a protein indirectly responsible for
989 cholesterol clearance from the brain through the BBB (Lütjohann et al., 1996; Lund, Guileyardo
990 & Russell, 1999)) in mouse hippocampal neurons has been shown to lead to accumulation of
991 neuronal cholesterol. This, in turn, is associated with a distinctive AD-like pathology, including
992 marked changes in endosomes (increasing both in size and number), Aβ peptide production, tau
993 phosphorylation, endoplasmic reticulum stress and apoptosis, and eventually hippocampal
994 atrophy and cognitive impairment (Djelti et al., 2015; Ayciriex et al., 2017).
995

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

996 It has been argued earlier that the presence of a BBB has resulted in the brain (and the rest of the
997 CNS) evolving to have a different lipid system to the rest of the body, one characterised by a
998 much lower lipid turnover, and smaller, less lipid-dense lipoproteins. If so, it should therefore
999 not be unexpected that substantial damage to the BBB, leading to long-term exposure to a
1000 systemic lipid system characterised by high lipid turnover and larger, more lipid-dense
1001 lipoproteins, will result in neurons and other brain cells becoming overloaded and displaying the
1002 kind of abnormalities described above.
1003

1004 2.6.3 The role of the β-secretase-induced C-terminal fragment (βCTF)

1005
1006 Certainly, this interpretation fits in well with the evidence presented above, given that cellular
1007 LDL-cholesterol uptake is known to be dependent on the endosomal-lysosomal pathway, by way
1008 of receptors possibly bound within lipid rafts (Vance & Vance, 2008; Sun et al., 2010; Pompey
1009 et al., 2013; Nixon, 2017). Furthermore, APP seems to be central to endosomal-lysosomal
1010 pathology, as the latter can be induced by APP over-expression, or by the C-terminal fragment
1011 that remains after β–secretase cleavage of APP (Jiang et al., 2010; Nixon, 2017) [*more*
1012 *references?*], but prior to γ–secretase cleavage (Fig. 3).
1013

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Such cleavage is known to take place in early endosomes (Cataldo et al., 2000; Arriagada et al., 2007) and appears crucial to pathology, since inhibition of β–secretase (or the substitution of APP by constructs lacking β–secretase cleavage sites) restores normal endosomal-lysosomal behaviour (Jiang et al., 2010) [*more references?*]. Furthermore, treatments that increase levels of Aβ without increasing levels of βCTF do not result in endosomal-lysosomal pathology (Jiang et al., 2010), in line with other evidence that the endosomal abnormalities seen in a mouse model of DS do not appear to be associated with abnormally high levels of Aβ (Salehi et al., 2006; Choi et al., 2009). Meanwhile, inhibition of γ-secretase, which increases levels of βCTF at the expense of Aβ, induces endosome-lysosomal pathology in previously normal fibroblasts (Jiang et al., 2010).

The underlying reason for this appears to be that βCTF recruits the adaptor protein APPL1 (adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain, and leucine zipper motif) to Rab5 complexes on endosomes (Miaczynska et al., 2004; Zhu et al., 2007; Nixon, 2017). This stabilises the monomeric GTPase protein Rab5 in its GTP-bound, activated form, and therefore amplifies the Rab5 signalling associated with early endosomes (Gorvel et al., 1991; Grbovic et al., 2003; Mishra et al., 2010), leading in turn to the enlarged endosomes seen in both AD and DS (Kim et al., 2016; Nixon, 2017).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

(More on cholesterol? ApoE4?)

Thus, taken collectively, the evidence appears to explain the endosomal-lysosomal pathology seen in DS dementia, and in many forms of AD, by two related mechanisms.

In the case of DS dementia, and early-onset forms of AD resulting from APP mutations, the pathology is likely to be the product of βCTF over-expression. In the case of LOAD, over-supply of cholesterol, originating from outside the brain, results in preferential up-regulation of β-secretase (Xiong et al., 2008), leading to the same result. Amyloidosis inevitably follows in both cases, no doubt enhanced by the substantial presence of Aβ in enterocytic- and hepatic-derived lipoproteins (see 2.3). Tau tangles presumably result from amyloidosis or from a failure of cholesterol transport, by a similar mechanism to that seen in NPC.

3 Discussion

In the preceding text, evidence has been presented to support a lipid-leakage model of AD progression. This states that, in the majority of cases, if not all, AD is primarily driven by the influx of lipids of systemic non-CNS origin, following the breakdown of the BBB. From a general perspective, this emphasis on a mechanical, rather than a purely biochemical failure,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

would seem to provide a much better explanation of why AD is as prevalent as it is, in contrast to current models. In particular, such mechanical failure also provides a more straightforward explanation of why ageing is the primary risk factor for AD.

However, as has been shown above, many specific aspects of AD can also be said to support such a model. These include indirect evidence of BBB damage from the presence, in AD cases, of non-CNS proteins inside the brain, and of CNS proteins outside it. In particular, evidence of the presence of the systemic apolipoprotein ApoB, together with long-chain triglycerides, within Aβ plaques strongly suggests that, in AD, the BBB is failing to separate the highly distinctive lipid systems of the CNS and systemic non-CNS compartments in the normal way. Moreover, included amongst the non-CNS proteins mentioned earlier, are plasma proteins such as albumin, fibrinogen and immunoglobulins that are, like Apoβ100, exclusively synthesised in the liver (or, like, Apoβ48, in other non-CNS organs). Again, like Apoβ, they are of high molecular weight, meaning that they cannot readily pass through the BBB in normal circumstances.

Further support for the lipid-leakage model arises from the likelihood that the BBB will be compromised by many of the risk factors associated with AD. As well as ageing, these include brain trauma, diabetes, ApoE4 and Aβ. Similarly, CTE, a condition showing many similarities to

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

AD, has been associated with clear evidence of BBB disruption. Finally, there is clear evidence that Aβ directly disrupts the BBB, something most obviously apparent in the case of CAA.

Why should lipid influx from outside the CNS matter so much? As explained in some detail above, there are major differences in the two lipid systems either side of the BBB. In particular, and most relevantly to AD, lipoproteins on the non-CNS side are larger and more lipid-rich than on the CNS side, thanks in large part to the presence of ApoB. Similarly, unlike on the CNS side, there is extensive transport of FFAs. Reasons for this include the absence of large FA-storing adipocytes and of albumin synthesis in the CNS, as well as the presence of the BBB itself.

But why should these differences matter? It is argued here that, whatever the original physiological function of the BBB might have been, it has allowed the CNS (and the brain in particular) to evolve in ways that make it highly vulnerable to lipid incursion from the non-CNS compartment. In particular, it is predicted that exposure to the higher cholesterol content of the more lipid-rich lipoproteins from outside the CNS will lead to cholesterol overload in neurons and other CNS-specific cell types. This in turn will result in endosomal-lysosomal pathology, tau tangles and excessive formation of Aβ, similar to what is seen in AD.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

In support of this hypothesis, similar endosomal-lysosomal pathology is seen in NPC, a disease characterised by faulty cholesterol transport, resulting in the accumulation of unesterified cholesterol in late endosomes and the formation of tau tangles. Likewise, excess cholesterol has been shown to increase amyloidogenesis by stimulating amyloidogenic processing of APP at the expense of the non-amyloidogenic pathway, resulting in increased levels of Aβ. During this amyloidogenic processing, high levels of the intermediate βCTF fragment are produced, which have been shown to trigger endosomal-lysosomal abnormalities similar to those observed in early AD progression. (Presumably, the reason Aβ levels are much lower in NPC than in AD is because cholesterol buildup tends to affect late endosomes in the former disease, rather than early endosomes where Aβ is produced.)

But cholesterol is not the whole story here. Breakdown of the BBB also exposes the brain to higher levels of FFAs. It is argued here that such exposure will lead to neuroinflammation, as a result of these FFAs stimulating microglia by binding to TLR4 and other microglial receptors, similar to how FFAs activate macrophages outside the CNS and to how ethanol triggers microglial-mediated neuroinflammation.

This may help explain why the overall structural pattern of damage to the brain inflicted by long-term alcohol abuse so strongly resembles that seen in AD, and why there are similar behavioural

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

deficits. In particular, frontal regions of the brain (especially the prefrontal cortex and basal forebrain) suffer significant shrinkage in both ARBD and AD, helping to explain why both diseases are associated with deficits both in olfaction and in executive functions requiring attentional and inhibitory control, reasoning, problem-solving, the setting of goals and of planning. Similarly, both ARBD and AD are associated with shrinkage of the medial temporal lobes, including pronounced atrophy of the hippocampus and entorhinal cortex, resulting in the anterograde amnesia so characteristic of AD, along with more specific deficits in spatial memory.

However, it is hard to explain how such similarities might occur as a result of neuroinflammation alone. Studies have shown that inhibition of neurogenesis plays almost as important a role in ARBD, which would better explain why the principal areas of brain atrophy in ARBD and AD, the frontal and medial temporal regions, also host two of the principal neurogenic niches of the brain, the subventricular and subgranular zones. These provide new cells for the prefrontal cortex and the hippocampus, respectively. It is argued here that the principal mechanism by which ethanol inhibits such neurogenesis, involving extrasynaptic GABAARs, means that such regions are also likely to be similarly affected by long-term exposure to other molecules with weakly anaesthetic properties, including FFAs. Whilst the mechanism by which such inhibition occurs appears to be complex, and may well involve other receptors and pathways, these shared

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

properties, and the shared mechanism seen in most forms of anaesthesia [*references?*], suggest that long-term neurodegeneration will result in both cases.

Whilst this aspect of the lipid-leakage model might be considered to be its most speculative, it may help to explain why general anaesthesia is also considered a potential risk factor for AD (and dementia in general) amongst elderly patients (Bohnen et al., 1994; Eckenhoff et al., 2004; Xie & Tanzi, 2006; Vanderweyde et al., 2010; Fodale et al., 2010; Papon et al., 2011; Chen et al., 2014), as well as being associated with marked deterioration in those already affected with AD (Bone & Rosen, 2000; Xie et al., 2007; Planel et al., 2007; Papon et al., 2011). However, such an association is still a matter of dispute (Needham, Webb & Bryden, 2017), and a number of studies suggest that, where it does occur, anaesthesia-related deterioration is accompanied by increases in Aβ synthesis and oligomerisation, and by tau hyperphosphorylation (Eckenhoff et al., 2004; Xie & Tanzi, 2006; Xie et al., 2007; Planel et al., 2007; Fodale et al., 2010; Papon et al., 2011). If so, this tends to rule out any GABA-related mechanism.

But these are not the only reasons for suspecting a link with GABAARs. Ever since the first practical anaesthetic agents were discovered in the middle of 19th century (Robinson & Toledo, 2012), and later shown (independently) by Hans Horst Meyer and Charles Ernest Overton to display a remarkable correlation between potency and hydrophobicity (Lugli, Yost & Kindler,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

2009) [*more references?*], there has been considerable interest in their mechanism of action. Following the findings of Franks and Lieb in the 1980s this interest has focused on hydrophobic sites on membrane proteins, particularly those of the Cys-loop ligand-gated ion channel superfamily, which includes inhibitory GABAARs and glycine receptors, as well as the excitatory acetylcholine and 5-HT3 serotonin receptors [*references?*].

In terms of the obvious therapeutic endpoints of anaesthesia, including coma and analgesia, the findings of such research are not likely to have any relevance either to AD or ARBD. But the role of extrasynaptic GABAARs in anaesthesia-mediated anterograde amnesia clearly does, given the importance of such amnesia in ARBD and, particularly, in AD. This is especially the case now that research has shown that the same high-affinity extrasynaptic GABAARs that have been shown to play a critical role in such amnesia, also play a critical role in neurogenesis. Given that the hippocampal region is a principal region of such neurogenesis [*references?*] and is also known to be central to the formation of new memories (as well as being heavily degraded in both ARBD and AD), it is readily apparent how chronic exposure to ethanol, with its weakly anaesthetic properties, is able to cause progressive deterioration of this region.

But this same mechanism also appears to explain why FFAs, with similar low anaesthetic potencies, are largely excluded from the brain by the BBB. This despite FFAs being highly

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

energy-rich molecules and despite the brain being one of the most highly energy-consuming organs of the body. However, one explains the requirement for the BBB to in some way protect the brain from damage from external sources, it is not clear that FFAs could not be transported across it in the way many other macromolecules, including ketone bodies, are. They could thus provide the brain with a much-needed additional energy source. Indeed, the transporter ABCB1 (also known as P-glycoprotein 1 or multidrug resistance protein 1) is already known to transport lipids, including FFAs, across the BBB in the reverse direction (Gonçalves, Gregório & Martel, 2011) and its decreased expression has been associated with increased AD risk (van Assema & van Berckel, 2016). Therefore, there seems little reason why the BBB could not have evolved a similar transporter in the reverse direction. That the BBB has not evolved such transporters, it is argued here, is because FFAs, at levels commonly seen in the rest of the body, would be inimical to the normal working of the brain. As would be the case if more cholesterol-rich lipoproteins could gain access to the brain, for the reasons discussed above.

It is been shown how breakdown of the BBB, by allowing such lipid invasion, is predicted to result in the anterograde amnesia, amyloid plaques and tau tangles, so characteristic of AD, as well as endosomal-lysosomal pathology and neuroinflammation. However, in pointing to GABAARs as major agents of AD progression, the lipid-leakage model may also help to explain the severe disruptions of the normal "body clock" commonly seen in patients with AD.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

Although the neurological mechanism behind this biological clock is yet to be fully elucidated, it is generally agreed that, in vertebrates, the neurons of the suprachiasmatic nucleus (SCN) provide a central role (Ehlen & Paul, 2009)[*other references?*]. Furthermore, within the SCN it is clear that GABAARs play a critical role, including in their extrasynaptic form (Ehlen & Paul, 2009; McElroy et al., 2009; Hu et al., 2016; McNeill, Walton & Albers, 2018)[*other references?*], with some estimates suggesting that over 90% of SCN neurons express and respond to GABA (McNeill, Walton & Albers, 2018). A number of studies have shown that ethanol modulates circadian clock regulation (Prosser, Mangrum & Glass, 2008; Ruby et al., 2009; Brager et al., 2011; Prosser & Glass, 2015), including by its action at low concentrations on extrasynaptic GABAARs (McElroy et al., 2009). Given that the lipid-leakage model already proposes that FFAs inhibit neurogenesis by acting at low concentrations on extrasynaptic GABAARs to disrupt their normal behaviour, there is therefore a good reason to believe that FFAs might also be disrupting normal circadian rhythms by a very similar mechanism.

Of course, given that disruption of the body clock in AD is primarily inferred from behavioural abnormalities, particularly in regard to sleep patterns, it may be that what is being observed is merely a secondary consequence of amnesia and the general loss of self-control associated with AD. However, given that such sleep disturbances seem to be apparent very early in AD progression (Macedo, Balouch & Tabet, 2017), when amnesia and other AD-associated deficits

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

are only beginning to be noticeable, it seems likely that what is being seen has a physiological as well as a purely psychological basis.

An obvious challenge with the lipid-leakage model is how it explains FAD. In the vast majority of cases (Wu et al., 2012; Lanoiselée et al., 2017) these result from mutations in Aβ-related genes, primarily in presenilin-1 (PSEN1), but also in APP and presenilin-2 (PSEN2). As shown in Figure 3, APP is the precursor protein from which Aβ is cleft, as a result of the amyloidogenic pathway, whilst PSEN1 and PSEN2 provide catalytic components of the γ-secretase (Lanoiselée et al., 2017), responsible for the final step in such Aβ formation. Similarly, as stated earlier, an additional copy of the APP gene, such as is seen in Down's Syndrome, is associated with a much-increased risk of developing early-onset AD. This would appear to strongly suggest that it is amyloidogenesis rather than lipid-leakage that causes AD. However, it should be remembered that the lipid-leakage model assigns an important role for Aβ in BBB disintegration, a role well-supported by the literature. Also, as stated earlier, experimental results have shown that Aβ has a role as a regulatory apolipoprotein, with raised levels of Aβ being associated with increased secretion of lipid-rich lipoproteins, including chylomicrons. Taken together, it can be seen how overexpression of Aβ, as seen in FAD, will result in lipid invasion the same way as it does in LOAD. Similarly, because ApoE has been shown to protect the BBB against damage, with

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

ApoE4 associated with BBB impairment, it can be seen how the lipid-leakage model can perfectly adequately account for ApoE genotype as an important risk factor for AD.

Moreover, because it explains LOAD as a consequence of all forms of BBB damage, rather than just as a result of amyloidogenesis, the model arguably provides a better explanation than the amyloid hypothesis for why LOAD is so much more common than FAD. Ultimately, anything that substantially damages the BBB, including simple wear and tear, is likely to result in AD. For this reason, attempting to treat AD by inhibiting amyloidogenesis alone is unlikely to be an effective treatment. By the time AD is diagnosed, even in the case of FAD, it is likely that the BBB damage will be too advanced to benefit much from such inhibition.

Rather, the model predicts that effective treatment will need to have several goals, including protecting the BBB from further damage (and, if possible, reversing any damage that has already occurred), reducing levels of FFAs entering the brain (by other means), inhibiting neuroinflammation and preventing inhibition of neurogenesis.

Finally, it can be argued that the explanation of LOAD provided by the model is more consistent with the majority of highly prevalent pathologies in the elderly. Excluding cancer, which is really a multitude of pathologies with often very different genetic and biochemical origins, some

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

form of mechanical failure would seem to be central to them all. In particular, stroke and heart disease are known to be associated with rupture of blood vessels. For this reason, the lipid-leakage model, in placing failure of the BBB at the heart of LOAD aetiology, would seem to sit more comfortably than alternative explanations with our current understanding of other common devastating diseases of the elderly.

4 Conclusion

This all points to a much more complex explanation of AD progression, in which Aβ and tau tangles are only two of the more visible factors, in many ways as much symptomatic as causative. Indeed, rather than attempting to treat AD by reducing the extent of amyloid plaques and tau tangles, the model clearly suggests that treatment would be greatly more efficacious if it were to focus on more "upstream" factors. This most obviously includes treatments to repair and prevent further damage to the BBB, and to reduce levels of invading FFAs and lipid-rich lipoproteins within the brain. The model also suggests that treatments to reduce FFA-mediated neuroinflammation and inhibition of neurogenesis would also be efficacious. Certainly, treatments focused on specific aspects of AD pathology have yet to show meaningful efficacy. It is argued here that this is because they have all been based on models of AD that are too

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

simplistic, resulting in treatments that are too narrowly-focused and missing the most efficacious targets. By contrast, the lipid-leakage model shows AD to be a much more complex disease, explaining why it is associated with so many distinct brain pathologies. Whilst this implies that effective treatment may prove more challenging than once hoped, the better understanding of the disease provided by the model will surely greatly improve the chances of discovering such treatments.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019

5 Bibliography

Automatic citation updates are disabled. To see the bibliography, click Refresh in the Zotero tab.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27811v3 | CC BY 4.0 Open Access | rec: 18 Aug 2019, publ: 18 Aug 2019