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Animal models of Alzheimer's disease and drug development

literature review

by

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Contents

Abbreviations

AD, Alzheimer’s Disease

CCD, Canine Cognitive Dysfunction

Aβ, Beta-Amyloid

APP, Amyloid Precursor Protein

PS-1, Presenilin 1

PS-2, Presenilin 2

ROS, Reactive Oxygen Species

FAD, Familial Alzheimer's Disease

SAD, Sporadic Alzheimer’s Disease

FLTD, Frontotemporal Lobar Degeneration

NFT, Neurofibrillary Tangle

MAPT, Microtubule Associated Protein Tau gene

3xTg, Triple Transgenic

PDAPP, Amyloid Precursor Protein Double Mutant

CAA, Cerebral Amyloid Angiopathy

NHP, Non-human Primate

SP, Senile Plaque

ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale

NGF, Nerve Growth Factor

CM, Conditioned Medium

CGNs, Cerebellar Granule Neurons

1. Introduction

In this literature review I will be examining the different data available on the similarities of Alzheimer's disease to cognitive dysfunction in animals. I will be looking at different animal models available and discussing the advantages as well as the disadvantages of each. The animal models I will look at are rodent, canine, feline and non-human primate models. Furthermore, I will be reviewing the current treatment options available for neurodegeneration and their application in veterinary medicine. As of 2023 the WHO reports more than 55 million people are living with dementia with Alzheimer's disease responsible for up to 70% of those cases [1]. Cognitive dysfunction in canines is predicted to occur in up to 45 million senior dogs across the USA and Europe [2] while in felines it is estimated to be up to 50% of cats older than 15 years of age show signs of the disease [3]. However, diagnosis can be difficult as it is hard to distinguish symptoms related to natural ageing and due to similarities in symptoms caused by other diseases. There are several common symptoms seen between Alzheimer's and cognitive dysfunction in animals including memory loss, confusion, anxiety, inability to solve problems, decreased social activity and mood changes. In small animals this may also manifest in house soiling, reduced response to vocal commands and a change in eating and drinking habits.

There are a number of drug treatments available in the treatment of cognitive dysfunction which we will explore. At present, the treatment differs slightly between Alzheimer's wherein drugs used aim to increase the chemical communication compared to veterinary medicine where cerebral vasodilators are used [4]. Animal models can offer a great insight into the factors contributing to cognitive decline as well as the long-term effects of medication used to treat cognitive dysfunction as they can be assessed in a much shorter time span [4].

2. Literature review

2.1. Pathophysiology

Cognitive Dysfunction syndrome is a progressive neurological disease present in ageing animals that shares similarities with Alzheimer's in humans. Both are characterised by a buildup of a protein called beta-amyloid which leads to neuronal degeneration. Beta- amyloid plaques emerge when amyloid precursor protein (APP) is not sufficiently degraded. APP is normally present in the brain as it supports neuron growth and maintenance. It is normally degraded by alpha-secretase and gamma secretase which does not lead to plaque formation. However, when it is instead degraded by beta-secretase and gamma secretase, it results in beta-amyloid peptides which tend to clump together forming a beta amyloid plaque extracellularly. These plaques then create issues by causing signal disruption, inflammation and amyloid angiopathy. Furthermore, beta-amyloid build up induces oxidative damage, promotes tau hyperphosphorylation and this results in toxic effects on synapses and mitochondria [48].

Continuously, changes inside the neuron also occur. A protein called tau which normally supports the neurons' structure can undergo abnormal changes when it becomes hyperphosphorylated causing them to clump together resulting in neurofibrillary tangles. These deposits are the result of the wrong folding of native proteins, forming after altered cleavage of the amyloid precursor protein (APP) [49]. This causes a major disruption to intracellular transport of nutrients as tau is not supporting the structure of the neurons as efficiently as it should. The loss of structural integrity along with loss of synapse connection furthers degenerative damage. As the disease progresses there are major physical changes that occur to the brain. The brain undergoes a process of shrinkage known as atrophy resulting in the gyri of the brain becoming narrower and the sulci as a result becoming wider. The ventricles also become larger.

2.2. Factors influencing the disease

Alzheimer's disease can be broken down into two categories. The first is sporadic or otherwise known as late onset Alzheimer's. As the name suggests, it occurs mostly in older patients 65 or older and is the most common form of Alzheimer’s disease. Genetic factors can play a role here as the E4 allele of apolipoprotein E can contribute to the disease. Apolipoprotein E plays a role in the breakdown of beta amyloid and the E4 allele yields poorer breakdown compared to its E2 counterpart therefore contributing to the development of Alzheimer’s as these patients are more likely to develop beta amyloid plaques. All cases of sporadic Alzheimer’s disease are accompanied by a characteristic pathological process, but this process is much longer than the clinically recognisable phase of the disease [50].

The second category is early onset Alzheimer’s disease otherwise known as familial Alzheimer's. In this case Alzheimer's can manifest more often before the age of 65 and even as early in people in their 30s to 40s. A dominant gene is inherited that results in the faster progression of the disease. Only 5% of all AD cases can be considered familial with an autosomal dominant inheritance due to mutations [51]. There are three genes associated with disease including presenilin 1 (PS-1) found on chromosome 14, presenilin 2 (PS-2) found on chromosome 1 or trisomy 21 associated with down syndrome. PS-1 and PS-2 are protein subunits of gamma-secretase responsible for APP degradation which, if insufficient, can lead to beta amyloid plaques. In trisomy 21, there is an increased risk of Alzheimer’s disease as the gene that codes for APP is located on chromosome 21. Therefore, as there is an extra chromosome 21 then the expression of APP can also be increased potentially resulting in an increased amyloid plaque buildup.

Regarding examples of cognitive dysfunction in the animal kingdom, it is a little harder to distinguish that from the signs resulting from the natural ageing process. The most applicable example available is Canine Cognitive Dysfunction. The pathophysiology of CCD is not as well established as Alzheimer’s disease although there is much evidence to support that they are very similar disorders. The causes of CCD are also less well known; however, it is believed that oxidative stress associated with ageing can contribute to the disease. Oxidative stress occurs when there is an imbalance between free radicals and the antioxidants

that neutralise them. Free radicals can be produced by cellular metabolism and inflammation. Free radicals cause damage via lipid peroxidation, protein oxidation and DNA damage. Neurons in the brain are particularly sensitive to this damage due to the brain's high metabolic rate and oxygen consumption. Furthermore, free radicals such as ROS can directly damage APP and its processing as well as enhancing beta amyloid aggregation.

It is suggested that there may be a correlation between breeds and the development of canine cognitive dysfunction. However, this correlation seems to be more associated with living to an older age which is more frequent in small dog breeds with one study suggesting age was found to be the most prominent risk factors of CCDS [2]. Furthermore sex, weight, reproductive state and dogs' housing were not significantly associated with the development of canine cognitive dysfunction [2].

2.3. Animal Models

2.3.1. Rodent Models

A. Transgenic Mice

Transgenic mice are genetically modified with genes from other species inserted in their genome. Transgenic mice can be created either by a genetic modification which is introduced on top of the existing genetic makeup or the homologous gene of interest is modified selectively in its normal chromosomal position; this process is called gene targeting [5]. In the study of Alzheimer's disease Chen et al state that in order to make an animal model a successful study for Alzheimer's disease that FAD-associated human genes must be introduced as rodents do not spontaneously develop AD due to APP sequence differences between rodents and humans [6]. Elder et al attest to the application of PDAPP (mice with overexpression of human APP with the Indiana mutation V717F) as they exhibited age dependent amyloid deposition as well as compact plaques with dense cores that were highly reminiscent of those seen in human AD [5]. Moreover, the similarities to Alzheimer's disease did not stop there as these mice demonstrated age-related learning defects and synapse loss [5].

As a result of the similar pathologies and symptoms displayed, transgenic mice seem to offer a promising model for the study of Alzheimer's disease. However, Elder et al

recognise the shortcomings of this model as the success of the transgenic mouse models has depended on the overexpression of the APP transgenes containing FAD mutations [5]. This causes a significant issue in research as Drummond explains that the vast majority of AD transgenic models have pathology that is dependent on the expression of FAD mutations however most AD clinical trials are conducted in SAD patients which has significant distinctions from FAD [7]. Although this research highlights that there is a gap between the form of Alzheimer's disease that can be produced in these mice and the form of Alzheimer's disease that most clinical trials are based on, this study produces significant findings using transgenic mice as they find plaque formation in the cortex and hippocampus as well as synaptic impairment resulting in decreased levels of synaptic markers such as synaptophysin manifesting in signs of cognitive impairment especially in spatial memory tasks [7].

Transgenic mice are also beneficial to research as they overcome the problem introduced by the lack of neurofibrillary tangles, a hallmark feature of Alzheimer's disease, which is lacking in wild-type mice. NFT's readily form in transgenic mice that express human tau containing mutations associated with FLTD which coincides with neurodegeneration, atrophy and motor deficits [7]. However, the necessity of these mutations for NFT development are not associated with AD in humans as well as the resulting significant motor deficits [7]. Furthermore, animal models that display the spontaneous expression of both plaques and tangles together are limited. Drummond explains that these models rely on the concurrent expression of mutated forms of APP, MAPT and occasionally also PSEN1 or PSEN2 to drive plaque and tangle formation in the same model [7]. The 3xTg mouse model has been widely used in AD studies and is considered the most complete transgenic model [7]. Chen et al concur as they state that the 3xTg model is widely used for the study of the development of tauopathy and amyloid pathology [6].

B. Knock in Rats:

The challenges presented by transgenic mice including the over-production of APP and lack of neuronal death resulting in an incomplete Alzheimer's disease pathology is largely overcome using the recently generated knock-in rat model wherein the sequence of for Aβ42 and the surrounding mutation sites of rat APP were substituted by human sequences [8]. In this way the rat model retains the endogenous levels of APP and its metabolites which better replicates the pathology followed in humans [8]. This new development is important as it

highlights the potential falsehoods that may be garnered from research gained from rodents that have been modified to overproduce APP. Knock in rats seem to better display the full pathology of AD as “the presence of abundant Aβ oligomers, plaques, their spatiotemporal distribution, and lack of Aβ deposit cerebellum in APP rats broadly resemble the amyloid pathology observed in human AD brains” [8]. Despite the rodent model shortcomings caused by their need for extensive manipulation to display AD pathology, the model is an important part of AD studies as they help to illuminate the different possible pathological presentations of AD patients.

2.3.2. Canine models

Parallels between canine cognitive dysfunction and Alzheimer’s disease have long been suggested with one study in 1995 noting that “one form of canine cognitive problem resembling human dementia has been characterised as retirement from participation with kennel mates, confusion upon presentation of simple routine tasks and deficits in conditioned learning” [9]. The comparison did not stop there as Ruehl et al cited pathology results focusing on plaque morphology and patterns of amyloid deposition which resembled early plaque formation in AD and this is illustrated by the following figure (1).

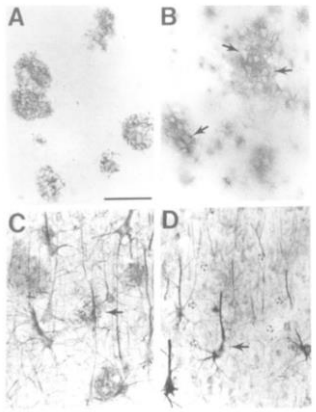

Figure 1. Comparison of plaque morphology and patterns in canines and humans [9].

(A) In the AD brain, β-amyloid immunohistochemistry following formic acid pretreatment reveals numerous senile plaques typically measuring less than 50 μm in diameter. (B) In the aged canine brain, β-amyloid immunoreactivity demonstrates extensive amyloid deposition surrounding neurons. Plaques within the canine brain are often greater than 100 μm in diameter. In C and D, β-amyloid immunocytochemistry has been followed by a different colores label for phosphorylated neurofilaments (SMI-311). (C) In a very early case of AD, many neurons can be detected within deposits of amyloid, although their morphology is sometimes abnormal (arrow). (D) In the aged canine brain, while neurons are often found within clouds of β-amyloid, some appear abnormal (arrow). A diffuse β-amyloid-positive plaque is outlined with open arrows.

More recent studies expand on the applicability of canine models in Alzheimer's research as they share a common environment (including diet) with humans as well as similar if not identical pharmacokinetics and similar dietary absorption of nutrients [10]. These similarities in environment and physiology seem to make canines a more suitable model compared to transgenic mice or knock-in rats whose environment differs greatly along with the absence of spontaneously occurring neurodegenerative disease. Continually, canines and humans have Aβ- containing lesions with identical amino acid sequence with deposition occurring earliest in the prefrontal cortex of the dog and later in temporal and occipital cortex, similar to previous reports in humans [10]. Another hallmark feature of Alzheimer's disease is the occurrence of cerebral amyloid angiopathy. Head confirms that vascular and perivascular abnormalities along with CAA pathology are frequently found in aged dogs, as well as that, the distribution of CAA in dog brains is similar to humans, with particular vulnerability in the occipital cortex [10]. Due to the many relevant comparisons seen between humans and canines regarding age associated cognitive dysfunction, Head concludes that the

aged dog may capture key features of human ageing, making them particularly useful for studies of therapeutics that can be translated into human clinical trials [10].

More recent research conducted has gone further than to just compare the two diseases and speculates that Canine Cognitive Dysfunction and Alzheimer's may be two facets of the same disease. Mihevc and Majdic (2019) note a number of common physiological changes such as neuronal loss and cortical atrophy that occurs in several brain regions including the cortex, hippocampus and parts of the limbic system in the cognitively impaired dogs, similarly to human brains affected by AD [11].

Table 1. Comparison of abnormalities present in Canine Cognitive Dysfunction (CCD) and Alzheimer's Disease (AD) [11].

Abnormality	**CCD**	**AD**
Cognitive decline	+	+
Brain atrophy	+	+
Neuronal damage and death	+	+
Aβ accumulation in brain parenchyma	+	+
Diffuse Aβn plaques	+	+
Dense-core Aβ plaques	-	+
Aβ accumulation in blood vessel walls (CAA)	+	+
Neurofibrillary tangles (NFTs) formation	-	+
Microglial dysfunction	+	+
Astrocyte dysfunction	+	+
Astroglial hypertrophy and hyperplasia	+	+
Oxidative brain damage	+	+
Mitochondrial dysfunction	+	+
Cholinergic dysfunction	+	+
Impaired neuronal glucose metabolism	+	+

However, there are some deviations between the disease presentation that is highlighted including the lack of neurofibrillary tangles. This is an important distinction from Alzheimer's as intraneuronal NFTs composed of hyperphosphorylated TAU and misfolded

insoluble TAU protein aggregates as well as extracellular Aβ inclusions are both present and necessary for the diagnosis of the disease [11]. Milhevc and Majdic (2019) do emphasise however that cytoplasmic deposits of phosphorylated TAU were detected in the prefrontal cortex, but no NFTs were observed [11]. Continually, there are some differences in the type of beta amyloid deposition that results in CAA as vascular Aβ deposition mainly consists of Aβ40 in humans, but both Aβ40 and Aβ42 were detected in dogs [11]. Furthermore, there are some differences regarding the possible origin of cognitive dysfunction as well as biomarkers of the disease as no mutations in specific genes have been reported in dogs with CCD so far and in dogs there are no biological markers that would allow accurate and early diagnosis of CCD [11]. The lack of biomarkers may either suggest that Canine Cognitive Dysfunction is not as comparable to Alzheimer's as we think or the biomarkers for neurodegeneration are species specific and in the case of canines have yet to be discovered.

In regard to the lack of NFTs found in canines, a more recent study delved into the significant difference between CCD and AD in terms of tauopathy [12]. NFTs emerge in Alzheimer's disease when a stabilising protein, normally present in the microtubules of neurons, called tau gets abnormally phosphorylated and aggregates forming NFTs. This process of phosphorylation mostly occurs at the serine amino acid at position 396 in the tau protein sequence. Thus, although NFTs have not commonly been observed in canines, a significantly higher S396+ p-tau immunoreactivity in the prefrontal cortex of CCD dogs has been observed and thus indicates that early stage tauopathy is present in CCD [12]. This is an important discovery as it may enforce the idea that CCD and Alzheimer's disease are heavily linked diseases as Abey et al. (2021) state that the regional distribution of this increased S396+ immunoreactivity is reminiscent of patterns described by Braak staging for human AD and that this may reflect a stage immediately prior to AT8+/argyrophilic NFT formation [12]. Furthermore, this study of tauopathy in CCD dogs offers a possible explanation for the lack of widespread observation of NFTs as "behavioural changes central to CCD such as spontaneous aggression and nocturnal disturbances are poorly tolerated in community dogs, with euthanasia typically considered within a short time frame following manifestation of these symptoms" [12]. Taking all of this into consideration, canine models prove to be an invaluable tool for AD studies as they not only recreate some of the key

features of the disease, but they give a better insight into the possible contributing factors to cognitive dysfunction as they often share a common environment and diet to humans.

2.3.3. Non-human primate models

As no other animal model is yet to offer the complete pathology of Alzheimer's disease, humans closest animal relatives, non-human primates (NHP), seem an obvious next step. Signs of neurodegeneration occur in non-human primates as they develop age-associated behavioural and brain abnormalities similar to those that occur in aged humans and to a greater extent, in individuals with Alzheimer's disease [13]. Upon neuropathological studies of NHP's brains, abnormalities such as degenerative changes in neurons, alterations in axons, the formation of abnormal neurites and the presence of amyloid accompany behavioural changes [13]. Haque and Levey concur as they argue that all NHPs studied to date develop Aβ pathology with advancing age, although the onset, distribution, and appearance of the lesions vary depending on the species and lifespan [14]. These findings are promising; however, it does not solidify non-human primates as an adequate model yet and further exploration as to whether they display the full complexity of Alzheimer's disease is needed.

In later studies Toledano et al. (2012) explore the comparisons between human and non-human primate brains more deeply. Although there are signs of neurodegeneration in other animal species the possibility that Alzheimer's is a specifically human problem has yet been ruled out as other species lack most of the higher functions displayed by humans [15]. However, Toledano et al. (2012) believe that we should take a different approach and consider that each separate species has its own type of higher cerebral functions, individuals of other species could therefore develop and suffer species-specific AD [15]. This theory might account for the lack of a complete pathology of Alzheimer's disease in non-human species.

Toledano et al. (2012) look at several different NHP species including Ring-tailed lemurs, Squirrel monkeys, Rhesus macaques and Chimpanzees. This complicates the study of neurodegeneration in NHPs as there is great variability in regard to how lesions are presented. Toledano et al. (2012) note that "the age at which amyloid neuropathy appears, the type of plaque, the location, and the association with other neuropathological signs all

vary considerably from one species to another, and between individuals belonging to the same species" [15]. One such example pertains to the shape of beta amyloid plaques found as "neuritic plaques, mainly in the parenchyma of the cerebral cortex, have been observed in the rhesus macaque: perivascular deposits are common in squirrel monkeys and diffuse plaques are generally observed in chimpanzees" [15]. Another complication presented which we have seen already in canine models is that intraneuronal neurofibrillary tangles are not generally observed in non-human primates [15]. As mentioned previously the lack of NFT's is a significant deviation from Alzheimer's disease as it is considered a hallmark feature of the disease required to make diagnosis in humans. However, later studies in NHPs manage to induce the appearance of NFTs with the use of oligomer injections. Forny-Germano et al. (2014) report that "cardinal features of AD pathology, including synapse loss, tau hyperphosphorylation, astrocyte and microglial activation were observed in regions of the macaque brain where Aβ oligomers were abundantly detected. Most importantly, oligomer injections induced AD type neurofibrillary tangle formation in the macaque brain" [16].

Furthermore, upon examination of the NFT's numerous thioflavin- S-positive neurons were found in the neocortex of macaques and the pattern of thioflavin-S-labelling throughout the macaque cortex resembled the pattern of tangles described in AD [16]. As the presence of spontaneous NFTs is scarcely found in non-human models of Alzheimer's disease, the induction of such NFT's is very significant as it more adequately represents the full picture of the disease. Although Forney-Germano et al. (2014) states that they developed a nonhuman primate model that accurately captures central pathological facets of human AD it is important to remember that the pathology observed has been induced acutely whereas the normal progression of Alzheimer's disease develops over several years [16]. Although the study deviates from the natural progression of Alzheimer's disease the " close similarities between the human and macaque brains in terms of overall architecture and functional networks, the macaque model of AD described here holds considerable potential for allowing detailed molecular mapping of pathology onto functional networks and its correlations with clinical outcomes in a context that would be much more readily translated to the human disease than with currently available rodent models" [16].

Even if NFTs are not modelled readily by NHPs there are still remarkable similarities between ageing human and NHP brains. Latimer et al. (2019) studied middle to old age

Vervet monkeys and found that histologically, amyloid plaques observed in the vervets, which develop spontaneously with advancing age, were remarkably similar to those seen in human AD (Figure 2) [17].

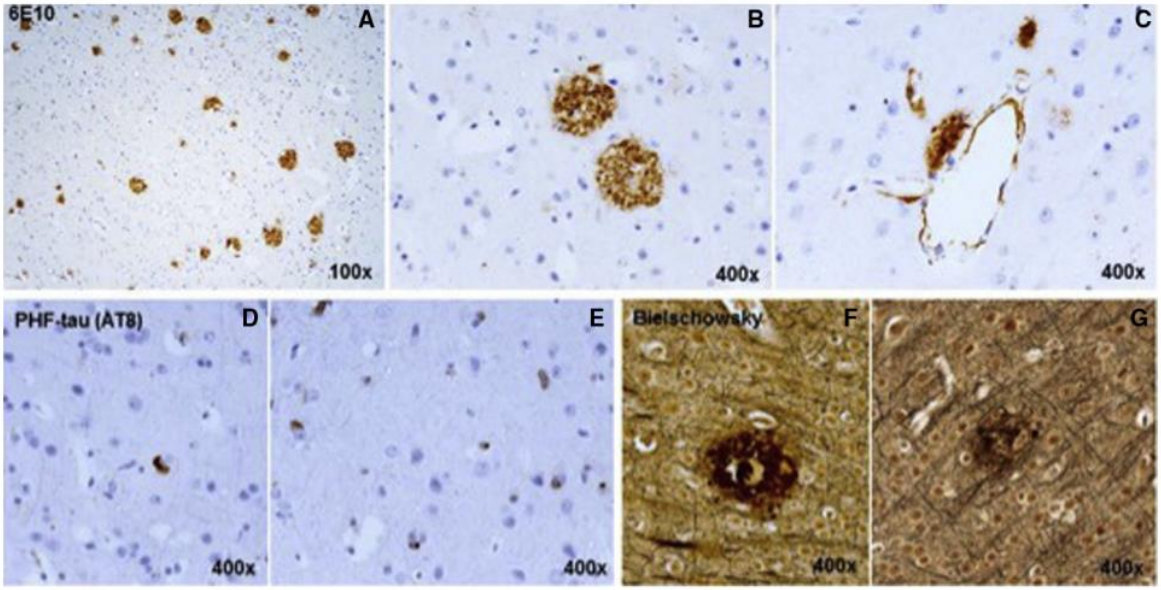

Figure 2. Comparison of plaque morphology in Vervet monkeys and humans. [17].
(A), (B) Aβ plaques were histologically similar to those seen in human AD. (C) Focal vascular wall Aβ was also noted. (D), (E) Granular cytoplasmic PHF- tau aggregates were present, but neurofibrillary tangles were rare. (F), (G) neuritic plaques were confirmed with Bielschowsky stain.

Continually, NHPs prove to be a potentially more useful model than canines as in this study with vervet monkeys they were able to use biomarkers that could aid in diagnosis of neurodegeneration. Latimer et al. (2019) found that as in humans, CSF Aβ42 levels correlated negatively with Aβ plaque density "and these results demonstrate the relative ease with which biomarker protocols that closely follow those used in humans for the pre-mortem evaluation of neurodegenerative disease can be implemented in an NHP" [17]. This is an important distinction from other models as biomarkers play an important role in diagnosis and treatment monitoring. Taking this into consideration along with the remarkable physiological similarities between humans and NHP's, they may serve as a valuable study for Alzheimer's treatment in the future.

2.3.4. Feline Models

In examining the different animal models available for the study of Alzheimer's disease, the lack of spontaneous NFT formation is a shared problem that arises for mice, dogs and NHP's. Felines are not as well researched in AD studies as the other animal models; however, they have been proven to be uniquely promising. Feline models appear to display a more complete Alzheimer's pathology as the aged domestic cats naturally accumulate Aβ oligomers, produce NFT, and moreover suffer hippocampal neuronal loss [18]. This may be a very significant development as spontaneous NFTs play a central role in AD pathology and have so far been lacking in other species. Moreover, the appearance of NFT's seems to develop in an earlier age comparatively to humans, making felines an attractive model for studying therapeutic intervention for AD [18]. The study postulates that the lack of SP formation is important for the early development of NFTs in cat brains [18].

Another important difference established is that parenchymal Aβ deposits in cat brains have no central core as seen in mature plaques of human AD [18]. Klug et al. (2020) expand on the similarities in feline models as they report that the intracellular Aβ oligomers were composed of hexamers and dodecomers and found in the same brain regions as NFTs with associated neuronal loss, similar to AD patients [19]. Furthermore, specifically in reference to NFTs they state that ultrastructurally, cat NFTs are similar to those in humans, consisting of some straight filaments but mostly paired twisted patterns of filaments [19]. This may mark out felines as a potentially superior translational and preclinical predictive power compared to pet dogs and nonhuman primates [19].

The study of NFT presence in feline brains however is not uniform. A more recent study suggests that cats produce similar tau isoforms to those seen in humans however, there is still controversy regarding the presence of NFT in the brains of elderly cats [20]. Sordo et al. (2021) argue that other feline studies have found no evidence of NFT formation or instead of finding NFTs, there is evidence of intracytoplasmic hyperphosphorylated immunolabeling, within neurons, which is believed to be an early stage of NFT, known as pre-tangles [20]. This may not be the only deviation in tau pathology as pre-tangles were primarily observed in the cortex of cats, suggesting that significant tau pathology starts in the cortical regions of the cat brain [20]. Although the presence of fully formed NFTs and their

origin is brought into question, Sordo et al. (2021) still emphasise considerable parallels with AD and felines with cognitive dysfunction as they noted with the development of extracellular Aβ deposits. It is noted that after accumulating in the cortical areas, Aβ pathology in cats progresses to the hippocampus and this same pattern has been described in the human brain during stage II of Aβ progression [20]. All things considered, felines prove to be a useful model for the study of AD as they better encapsulate tau pathology compared to other species, and this is worth further exploration.

2.4 Drug Development

2.4.1. Selegiline

Selegiline is the only drug approved by the FDA for the treatment of CCD. In dogs with CCD, there is a reduced amount of catecholamine neurotransmitters such as dopamine as dopamine metabolism is believed to accelerate in senior dogs because these animals have an increased accumulation of neuromelanin in their brains [21]. Catecholamines are normally catabolized by monoamine oxidase, so to directly increase the amount of dopamine available, MAO inhibitors, such as Selegiline, can be used as inhibition of MAO-B appears to enhance dopaminergic function [21]. Furthermore, selegiline can indirectly enhance the effects of dopamine through the inhibition of the catabolism of phenylethylamine (PE) which at pharmacologic levels can increase the release and decrease the reuptake of dopamine [21]. As well as increasing the amount of dopamine, Selegiline also increases levels of superoxide dismutase and catalase in the striata of dogs which may decrease free radical formation and subsequent damage to neurons [21].

In a study conducted on 641 dogs presenting with symptoms of CCD, dogs with disorientation, decreased interaction and loss of housetraining, had a similar response to treatment (77.5%, 76.4% and 73.5% reported as improved respectively) as they were given 0.5 to 1.0 mg/kg once daily for 60 days [21]. These results offer hope in the management of ageing dogs with CCD, but there are limitations of the drug, as shown by further results. CCD dogs suffering from sleep-wake cycle disruption had the lowest response for changes as 62.5% of the dogs reported as improved [21]. Due to this, it may be advisable to pair

Selegiline with another drug targeted at solving disrupted sleep-wake cycles. Continually, another lack of response was noted as animals older than 16 years showed the least amount of improvement [21]. As these dogs were older it may be logical to assume that they displayed the most extensive neurodegeneration and so were not as suitable for therapeutic measures. Selegiline also displayed a number of possible side effects and the most frequently reported clinical signs included diarrhoea (4.2%), anorexia (3.6%) and vomiting/salivation (3.4%) [21]. The study concluded that after 30 days of treatment, 80% of the dogs showed overall improvement in the clinical signs of CDS; after 60 days of treatment, 77% were reported as improved [21]. The encouraging results of the previous study have been echoed in others as 69 senior dogs displaying CCD symptoms responded to L-deprenyl therapy with improvement in every parameter by the first month [9]. The following parameters tested are displayed by the following table (2).

Table 2. The percentage of dogs affected by different CCD symptoms and their response to L-Deprenyl treatment [9].

Problem	**Mild (% of dogs affected)**	**Moderate**	**Severe**	**Total**	**Response to L-Deprenyl**
Housetraining	18	22	27	67	++
Interest in food	26	12	4	42	++
Activity, or attention to environment, including people or other animals	26	26	25	77	++
Awareness or orientation to surroundings	17	28	23	68	++
Ability to recognise familiar places, people or other animals	25	22	16	63	++
Ability to recognise or respond to commands or when called by name	17	19	44	80	++
Hearing	7	12	68	87	++
Climbing up or down stairs	22	31	25	78	++
Tolerance to being alone	19	16	9	44	++
Development of compulsive behaviour	25	32	12	69	+
Circling	13	10	6	29	++
Tremor or shaking	16	28	13	57	++
Wakes owner more at night and/or sleeps more in daytime	16	19	32	67	+
Inappropriate, persistent vocalisation	19	7	16	42	-
Increased stiffness or weakness	16	29	30	75	++

The study concluded that the dogs responded quite favourably to once-daily therapy with 0.5 mg/kg bw. L-Deprenyl [9]. This is in agreement with the FDA recommendation as they state that a dose of 0.5 to 1.0 mg/kg once daily, selegiline was both effective and safe in controlling the clinical signs associated with canine CDS [21]. However, a more recent analysis of the available studies in 2021 brings into question Selegiline's efficacy as most of these studies are based on owner responses to questionnaires rather than standardised comparative cognitive testing [22]. This is an issue as the owner's responses are subjective and may have been biased to the knowledge that their dogs were receiving treatment.

Furthermore, Huntingford (2021) postulates that the owner responses may not be based on true improvement in cognitive abilities but rather on the fact that selegiline increases brain catecholamines and this can produce nonspecific low-level hyperactivity [22].

Further studies in other models produce mixed results. In mice injected with Aβ to simulate AD pathology it was found that both acute and sub-chronic selegiline administration reverted Aβ25-35 peptide induced cognitive impairment in the object recognition task in male mice [23]. Pazini et al (2013) attributed this improvement to decreased MAO-B activity in the cerebral cortex as well as the hippocampus, perirhinal and remaining cerebral cortices [23]. As selegiline performed well in other models, further studies were conducted in AD patients. In an analysis of selegiline's efficacy in numerous human trials the drug had a positive effect on cognition as there was better mental alertness; short- and long-term memory; concentration; attention; self-care, verbal fluency; visuospatial abilities; capacity for processing, storage, and retrieval of information [24].

However, Tolbert et al (1996) highlight an issue present in a number of the studies conducted as these were open-label or single-blind trials, no claims of efficacy should be inferred from these studies [24]. However, they do cite some double-blind trials which "showed a positive response in the following areas: episodic memory and learning tasks, word fluency, digit span, long-term and spatial memory, total and delayed recall, picture copying tasks, verbal fluency and attention tasks" [24]. In a meta-analysis of the drug, Wilcock et al (2002) conclude that although selegiline produced positive results initially, they found that at later time points for assessment of cognition and functional ability, the outcome of treatment with selegiline was disappointing, and did not provide evidence of a clinically relevant effect [25]. Selegiline does not provide a uniform effect on cognition across different species and its applicability should be studied further.

2.4.2. Nicergoline

Nicergoline affects the brain in a number of ways as it inhibits Ca^{2+}/calmodulin-dependent PDE1 and cGMP-stimulated PDE2 activity as well as non-competitively inhibits Ca^{2+}/Mg^{2+} dependent brain adenosine triphosphatase [26]. Continually it activates Na^{+}/K^{+} ATPase at low concentrations but inhibits at high concentrations and it acts as a potent α1A adrenergic

receptor antagonist [26]. Due to nicergoline's multiple effects it was thought that it would be useful for CCD as the drug induces vasodilation, increased acetylcholine release from the hippocampus and improvement in learning and memory [27]. However, when trialled against adrafinil and propentofylline in a group of aged dogs in a study in 2000, the results showed that adrafinil was the only 1 of the 3 drugs tested that was effective in enhancing behaviour (i.e., locomotion) of aged dogs [27]. This may be indicative that nicergoline may not be as suitable a drug for the treatment of CCD as hoped or that more extensive cognitive parameters need to be studied alongside its treatment. A more recent examination of the different treatment options available for CCD states that nicergoline, an α-adrenergic antagonist, improves cerebral blood flow and metabolism, but there are limited clinical studies on its efficacy and safety in canines [28].

Although nicergoline did not produce the desired results in canines, studies of the drug in mice with AD showed that it improves impaired neurogenesis and cognitive competence [29]. Nicergoline seemed to improve cognitive function in several ways as pathogenic Aβ-42 and –40 peptides and APP were downregulated as well as inhibited apoptosis in hippocampal cells [29]. Furthermore, the drug was seen to have an effect on decreasing inflammation along with oxidative stress as concentrations of IL-1, IL-6 and TNF-α were decreased and the expression levels of reactive oxygen species, superoxide dismutase and glutathione were downregulated [29]. Finally, Zang et al (2018) reported that insulin-like growth factor-binding protein 3 and vascular endothelial growth factor β protein levels were increased [29]. However, these promising results haven't been translated into human studies as the response has been more mixed. In a recent study, it was found that nicergoline resulted in a modest but significant improvement in AD symptoms on the ADAS-Cog at 6 months and produced no significant improvement at 12 months but rather slowed an increasingly precipitous decline at 12 months [26]. In another study with AD patients, it was found that acetylcholinesterase inhibitors plus nicergoline preserved cerebral blood flow to the left temporal pole and the middle cingulate gyrus but did not result in any significant difference in dementia severity [26]. Similar to the results in studies on the use of nicergoline in CCD dogs, it was inconclusive whether nicergoline is useful for the treatment of AD [26].

2.4.3. Propentofylline

Propentofylline is a drug licensed in the UK for treating canine cognitive dysfunction. It is a methyl xanthine derivative like caffeine that acts as a relatively potent and nonspecific cAMP/cGMP PDE inhibitor and adenosine reuptake inhibitor [26]. Furthermore, propentofylline has been shown to suppress Aβ plaque deposition, tau hyperphosphorylation, GSK3β activation and microglial ROS generation [26]. Propentofylline seems to target key areas in the pathophysiology of cognitive dysfunction. In a study conducted in 1989, Shinoda et al examined propentofylline's effect on nerve growth factor in cultured mouse astroglia cells. Examining propentofylline's effect on NGF is useful as NGF is part of the cholinergic system which is heavily affected in AD and it plays a critical role in neuron cell maintenance. Shinoda et al (1989) found that propentofylline clearly increased the NGF content in the CM and the maximum response was observed at 1.11 mM [30]. They also noted that above this concentration, propentofylline appeared to cause cell damage, for the increase was markedly less at 3.33mM and non-existent at 10 mM [30]. From this study propentofylline seems to show promise for cognitive dysfunction at a low dose.

Further studies in rodents produced results which seem to be in agreement as Goto et al. (1987) found that propentofylline improved the decreased learning ability of 12-month-old spontaneously hypersensitive rats [31]. They believed the drug had an anti-amnesic effect which was involved in the activation of the retrieval process of memory [31]. As such they concluded that propentofylline may be a therapeutic agent for disturbed learning and memory [31]. Another study in rodents induced with Alzheimer's specific pathology was conducted and found that propentofylline showed a tendency to improve the impairment of motor habituation caused by β-amyloid protein [32]. Continually, propentofylline attenuated the β-amyloid protein-induced impairment of spatial learning [32]. Interestingly, the results of this study appear to be similar to the previously discussed research on propentofylline's effect on NGF in mouse astroglia cells as they too noted a decreasing efficacy of the drug with increasing dose. Yamada et al. (1998) report that a low dose of propentofylline (10 mg/kg) was more effective than a high dose (25 mg/kg) in the passive avoidance test [32].

As propentofylline showed encouraging results in rodent models, it would be expected that this may also be the case in human trials, however the results are mixed and there are very few studies to examine. One study reported that propentofylline's benefits

persisted even after treatment cessation, suggesting a disease-modifying rather than a purely symptomatic improvement [26]. While another study of propentofylline use in dementia patients stated that propentofylline treatment resulted in significantly improved cognition at 3, 6, and 12 months [26]. As of now propentofylline is licensed for the treatment of dullness and lethargy in old dogs in a number of European countries as it is purported to inhibit platelet aggregation and thrombus formation, make the red cell more pliable and increase blood flow [33]. However, there is a lack of studies to confirm this and so more research is needed to discover the applicability of propentofylline for canine cognitive dysfunction.

2.4.4. Adrafinil

Adrafinil is a drug that has been more extensively tested in animals for its effect on cognition. Adrafinil's mode of action is not entirely understood; however it is generally linked to an agonistic effect on the noradrenergic system in the central nervous system, specifically to postsynaptic alpha-1 receptors [34]. It is thought that adrafinil also may have a possible inhibitory action on GABA release, increased glutamate release or an increase in cerebral metabolism as well as potentially indirectly affect dopamine levels through inhibition of GABAergic neurons [34]. Adrafinil's effects were studied in a number of animal models. In one early study, modafinil, the active metabolite of adrafinil, was studied by examining its effect on the behaviour of mice and monkeys. It was found that modafinil appears to produce a strong stimulating effect [35]. More precisely, the drug caused an increase in locomotor activity in mice and in nocturnal activity in monkeys [35]. However, there were some differences between the species as the dose range for mice ranged between 4 to 256 mg/kg bw. while for monkeys it was between 16 to 64 mg/kg bw.[35].

As Adrafinil appeared to be a potentially useful drug in augmenting cognition the drug was next tested in elderly beagles using discrimination tasks to evaluate the drug. Milgram et al. (1999) found that it produced significant improvement in learning, as indicated by a decrease in both errors and trials [36]. Milgram et al. (1999) postulate that this effect could be caused by changes in attention, motivation, vigilance, or memory and that it could also be effective in improving cognitive dysfunction in humans [36]. A similar study occurred simultaneously examining aged beagles but this time examining a number of specific behaviours including locomotion and sniffing. Siwak et al. (2000) discovered that

adrafinil produced a marked increase in locomotion and a more transient increase in sniffing [34]. However, they note that individual data revealed considerable intersubject variability, which was not obviously related to the dose level [34]. Furthermore, adrafinil's results show marked differences between canines and other species when results are compared to other studies, and they propose that these differences probably relate to species differences in metabolism [34].

Siwak et al. (2003) continue to study the effects of adrafinil in elderly beagles in a later study that focused on visuospatial function using a delayed nonmatching-to-position task. Although their previous study found that an increase of locomotion and sniffing might indicate that the drug was helpful in boosting cognition, in this study they found impairment rather than improvement [37]. Rather the drug showed to have the opposite effect of boosting cognition as a dose of 20 mg/kg disrupted performance of the DNMP task [37]. Siwak et al (2003) proposed that adrafinil impairs working memory on a DNMP test, which could be linked to disruptive effects on noradrenergic function in the prefrontal cortex and that these effects are actually consistent with an alpha-1 adrenergic mechanism of action [37]. To understand this effect more deeply Siwak et al. (2003) explain that alpha-1 stimulation can increase excitatory currents in apical dendrites thereby increasing background noise which can interfere with the signal transfer to prefrontal cortex cell bodies and as a result the prefrontal cortex can no longer inhibit processing of irrelevant information and working memory functions are impaired [37]. This study suggests that adrafinil's effect on cognition may be more complex as the drug selectively improves encoding, the acquisition of new information while disrupting working memory possibly through additional mechanisms [37]. From these studies we can conclude that Adrafinil does not affect all cognitive processes in the same way, and this should be taken into consideration before treatment.

As adrafinil showed some promising results in animal trials, the drug was marketed for elderly people struggling with cognition deficits. Lowe et al. (2021) report that adrafinil indicated improvements in vigilance, attention, memory, orientation, depression, fatigue, autonomy, and sociability [38]. However, adrafinil caused a number of issues. Lowe et al. (2021) report a number of side effects as one patient developed orofacial dyskinesia while another patient experienced elevated blood pressure [38]. Continually in another case, adrafinil was potentially causing irritability, muscle twitches, tachypsychia, and insomnia.

The drug was discontinued as studies were unable to conclude that adrafinil provided benefit as well as safety data indicating known adverse effects [38].

2.4.5. Levetiracetam

Levetiracetam's mode of action is not fully understood however it appears to depend on its binding to the synaptic vesicle protein 2A and it seems that it reduces calcium release from intracellular stores [39]. Furthermore, it is postulated that levetiracetam might prevent excessive glutamate accumulation at the synaptic cleft and that this mechanism could counteract the pathogenic effects of Aβ, which may cause glutamate spillover at the synaptic cleft [39]. Levetiracetam (LEV) is traditionally used to treat epilepsy however Sanchez et al (2012) report that seizures are closely linked to Alzheimer's as AD patients have increased incidence of epileptic seizures, and the incidence is highest in patients with early-onset AD who overexpress human amyloid precursor protein [39]. Sanchez (2012) explored the idea that Levetiracetam could be used in the treatment of AD using hAPP mice. They also compared Levetiracetam's efficacy against other antiepileptic drugs.

Table 3. Acute effects of different antiepileptic drugs in hAPPJ20 mice [39].

FDA-approved antiepileptic drug	Acute injection dose (mg/kg)	Fraction of mice with >50% spike reduction	Change in spikes relative to baseline (%) *	Statistical Significance **	Effect
Ethosuximide	400	1/6	-0.8 (±18.9)	P=0.96	None
Gabapentin	100	0/4	-10.3 (±12.1)	P=0.45	None
Levetiracetam	200	7/7	-70.6 (±4.9)	P<0.0001	Suppression
Phenytoin	100	0/3	+183.2 (±48.7)	P=0.043	Exacerbation
Pregabalin	200	0/3	+87.7 (±14.6)	P=0.026	Exacerbation
Valproic acid	300	0/4	+20.3 (±15.4)	P=0.28	None
Vigabatrin	300	0/4	+0.8 (±15.6)	P=0.96	None

*Over a period of 6 h postinjection **One-sample *t* test compared to baseline

Levetiracetam stood out against other drugs tested as it caused a significant decrease in the number of spikes seen on EEG tests of the hAPP mice which have pathologically elevated levels of human Aβ in the brain and show neuronal network dysfunction, including frequent abnormal spiking activity and more intermittent epileptic seizures [39]. This led to significant developments as LEV treatment reversed the behavioural abnormalities in hAPP mice [39]. Continually LEV-treated hAPP mice learned better and faster than saline-treated hAPP mice and they experienced improved nonspatial learning and memory [39]. Sanchez et al (2012) did highlight that although levetiracetam seemed to be an effective treatment in cognitive deficits in the mice that it did not alter Aβ1-x and Aβ-42 levels or Aβ1-42/Aβ1-x ratios [39]. Another possible shortcoming of the drug is that in contrast to low-dose treatment, high-dose treatment did not reverse behavioural abnormalities [39].

Further studies in rat models assessing levetiracetam's effect on memory did not garner the same promising results. Zwierzynska et al. (2022) found that levetiracetam administered at an acute high (500 mg/kg) or low dose (100 mg/kg) did not alter spatial memory in rats using the Morris water maze test [40]. Furthermore, using the novel object recognition test the study found that levetiracetam did not alter short-term memory, but the drug disturbed the long-term recognition memory in rats [40]. The contrary results of this test may be because these rats were not modified to display AD pathology and so may not represent the full potential of the drug in this case. More recent studies of the drug in mice which display AD pathology have resulted in more encouraging results. Zheng et al. (2022)

found that “low levetiracetam concentration improves kainic acid – impaired learning and memory ability in APP23/microtubule-associated protein tau mice; however, a high concentration of levetiracetam did not induce similar results” [41]. Levetiracetam was shown to relieve AD symptoms in this study and improve the beta amyloid load.

In contrast to the earlier study with Sanchez et al. (2012) wherein they found no alteration in the Aβ levels, Zheng et al. (2022) found that a high levetiracetam concentration mitigates kainic acid- induced production and aggregation of Aβs by inhibiting the amyloidal procession of amyloid precursor protein as well as promoting the clearance of Aβ [41]. Additionally, the drug seems to influence tau levels also as levetiracetam dephosphorylates tau via GSK3α/β and CDK5 pathways [41]. Moreover, Levetiracetam inhibited the phosphorylation of tau by deactivating CDK5 and GSK3α/β [41]. Comparable to other studies of the drug, they found a difference in the effects of low and high doses of levetiracetam. Zheng et al. (2022) found that low concentrations of levetiracetam protect neurons from neuronal dystrophy and neuronal loss, whereas high concentrations induce apoptosis in neurons [41].

In another recent study of levetiracetam on cognitive dysfunction induced by diabetes mellitus in rats, it was found that the low and high dose of levetiracetam treatment could reduce the serum levels of IL-1β, IL-6 and TNF-α [42]. As neuroinflammation is part of AD it is an encouraging find as results indicate that levetiracetam can inhibit neuroinflammatory responses in several neurological diseases [42]. In this study Zhang et al. (2023) also found a difference in the outcome linked to changing the quantity however in contrast to the previous study they found that a high dose of levetiracetam was better than a low-dose in ameliorating the cognitive dysfunction and hippocampal damage [42]. Further exploration of the dose dependent effect of levetiracetam is required to understand the varying results as well as further testing in other species such as canines and felines with specific regard to cognitive dysfunction.

2.4.6. Memantine

In a study on mice using memantine which could bind to and antagonise NMDA receptors it was found that treatment significantly decreased the expression of total APP as well as enhanced the expression of α-secretase and Aβ-degrading enzyme neprilysin which accelerated the decomposition of Aβ and APP [43]. Memantine not only targeted Aβ protein as it consistently suppressed tau hyperphosphorylation, another important facet of AD pathology [43]. It made further improvements as the treated mice experienced a reversal of the depressed expression of synapse related proteins, including synaptophysin, PSD95, synapsin I, synapsin II and drebin [43]. It was also found that memantine prevents glutamate-induced excitotoxicity in CGNs as well as reverses the dysregulations of ERK and PI3K/Akt/GSK3β pathway induced by glutamate which helped to protect neurons [49]. Memantine not only proved to be an effective drug as it penetrates the blood-brain barrier to achieve effective therapeutic concentrations in the brain tissue but also a safe one as it has favourable PK and safety profiles displaying little adverse effects [43].

To investigate whether memantine is an effective treatment in other species, the drug was trialled in rhesus macaques using delayed matching to sample tasks. However, in contrast to the study in mice, Schneider et al. (2013) found that there were no statistically significant effects of memantine on DMTS performance at any of the doses studied [44]. A meta-analysis of the studies of memantine in human trials show similarly disappointing results. One study in dementia patients seems to be in agreement with the primate model as it was found that memantine did not affect mood, attention, immediate or delayed verbal or visuospatial memory [45]. Rather it did impair cortex excitability, eye blink conditioning and delayed object recognition [45]. Akin to this, another study in dementia patients found a dose-dependent increase in adverse events [45]. It was found that most adverse events being mild or moderate severity including a significantly higher incidence of dizziness, headache, constipation and somnolence [45].

In a systematic review of memantine for AD therapy Kishi et al. (2017) are in agreement with the previous study as they report a risk of dizziness, vertigo and somnolence with memantine monotherapy [46]. Kishi et al. (2017) found that there was some success with the drug when used in combination therapy as it ameliorated behavioural disturbances in patients with AD [46]. Therefore, it appeared that combination therapy with memantine

and donepezil was superior to ChEI monotherapy [46]. Further exploration of the drug and its applicability in dogs has been conducted. Although studies in canine cognitive dysfunction and memantine are lacking, the drug has been tested in other behavioural disorders in canines.

In one study looking at the use of memantine in canine compulsive disorders it was found that memantine is well tolerated by them at dose rates of up to 5 mg/kg bw. [47]. Schneider et al found that seven (64%) of the 11 dogs included in the analysis improved on the treatment consisting of behaviour modification [47]. However, like other trials with memantine some adverse effects were noted including ataxia and reduced body weight with food consumption unchanged or increased [47]. As this study provides some evidence in behaviour modification with the use of memantine it highlights the need for further studies in other behavioural disorders such as in canine cognitive dysfunction.

3. Discussion

In the search for an appropriate animal model for AD research I found that transgenic mice were the most frequently used. As wild mice have not been shown to spontaneously develop AD pathology, genetic modification involving the insertion of human AD genes into the genome of mice has proved to be an effective method of study. Transgenic mice display both the typical AD pathology such as Aβ plaques as well as the resulting behavioural changes such as observed learning deficits. This makes transgenic mice an important area of research in understanding AD pathology, however there are some challenges with this model. To create the transgenic mice model, it is necessary to use genes which cause the familial type of Alzheimer's disease otherwise known as early-onset Alzheimer's. However, this type of Alzheimer's disease accounts for a much smaller proportion of the AD displayed by the human population. Rather, the other type, spontaneous or otherwise known as late onset Alzheimer's accounts for most cases of AD. Therefore, this is the type of Alzheimer's disease that is focused on in drug trials. Due to this, there is an obvious discrepancy between the AD that can be displayed by transgenic mice and the AD that is studied in the context of clinical trials.

Therefore, the use of transgenic mice in the case of AD seems misdirected as they cannot encapsulate the form of disease that we are most interested in. Furthermore, unlike other available models, mice do not share a common environment with human beings. As Alzheimer's disease is a multifactorial issue wherein predisposing factors heavily influence the disease progression, this is an important digression. Continually, during my comparative study of the different models it was evident that the results gained from drug trials in mice did not translate well into other models. This difference was most notable when results were compared with human trials. Mice are however a favourable model due to their short lifespan. This is a particularly useful tool in the study of chronic disease such as AD as researchers can recreate the chronic progression of the disease in a much shorter timeframe.

Although transgenic mice models have dominated AD research thus far, in more recent times other models including canines are being explored. Canines prove to be a superior choice of model for AD for a number of reasons. Firstly, aged canine models naturally display facets of AD pathology such Aβ plaques and can suffer from canine

cognitive dysfunction, a disease comparable to AD in humans. Moreover, canine models can often share the same environment and diet as humans which may contribute to the development of cognitive dysfunction. Furthermore, canines are physiologically more similar to humans than rodents as they share nearly identical pharmacokinetics making them a better model for drug trials. Canines also display cerebral amyloid angiopathy, a frequent complication AD. However, like transgenic mice, canines do not regularly produce NFTs, but research has discovered that early stage tauopathy does occur in canine models which may come immediately prior to the production of NFTs. Furthermore, on account of the various behavioural changes displayed by dogs with canine cognitive dysfunction such as unrest, house soiling and confusion, many dogs may be euthanized by their owners before the development of extensive AD like pathology. Whether Alzheimer's disease is very similar to canine cognitive dysfunction, or they are facets of the same disease is yet to be determined but due to the evident parallels between the two, more rigorous research should be conducted. However, the canine model has several disadvantages, as in the research I studied, much of the results were obtained from the owners and this had the potential to introduce significant bias and inaccuracy.

In search of a more complete model of AD pathology, non-human primates seem to be a promising candidate as they are our close relatives. It is true that they are similar in that they display behavioural changes in old age caused by amyloid build-up and degenerative changes in the neurons. However, the NHP model poses significant disadvantages as there is great variation of the disease between different species. Comparing species, it is evident that there is a difference in Aβ distribution, location and the age at which an animal is affected. There is even a significant lack of uniformity in disease presentation between individuals of the same species. Continually, as with rodent and canine models the lack of spontaneous NFT's is a considerable digression from AD pathology in humans. Although it was found later that NFTs could be induced in NHPs treated with Aβ oligomer injections. Another issue in the use of the NHP model is the lack of a complete AD pathology which may be caused by the lack of higher cerebral functions displayed uniquely by human brains. Instead, some studies propose that we view AD as species specific. However, this is potentially a significant progression in our understanding of the disease and of how it should be studied. Furthermore, NHPs prove to be a more useful model than canines due to the presence of biomarkers making

diagnosis of cognitive dysfunction more accurate. A more accurate diagnosis allows for a more accurate interpretation of therapy outcomes. Although NHPs still do not offer a complete picture of Alzheimer's, the model has contributed to highly significant insights in our understanding of the disease.

A model that has been studied less frequently in AD research but one that may prove to be superior to its counterparts is the feline model. Like other models, felines display naturally accumulating Aβ and consequent neurodegeneration. The feline model diverges from all others as it displays spontaneous NFT formation, making this model superior to rodents, canines and non-human primates. These NFTs seem to develop at an earlier age in cats compared to humans which highlights the applicability of the feline model in AD research. Moreover, felines are similar in that the Aβ oligomers are composed of the same hexamer and dodecamer shapes as humans as well as similarly shaped NFTs. However, some studies have argued that felines do not actually display the formation of NFTs but instead show the production of pre-tangles. In any case felines are an advantageous model as they show more extensive tau pathology compared to any other model thus far. Although, there are some differences between feline and human cognitive dysfunction. The feline has a disadvantage as they lack the central core which is present in plaques in human brains as well as the production of senile plaques. Therefore, felines may not offer an entirely complete model of AD pathology, but it does encapsulate the essential parts. Despite the advantages of the feline model, however, it is the least studied model in AD research compared to the others discussed. All of the models examined have brought to light different aspects of the disease and increased our understanding.

After looking at the different models used in AD research, I then examined the different drugs that have been tested in the treatment of cognitive dysfunction in the discussed models. Firstly, I will be discussing selegiline, a MAO inhibitor, which increases the amount of catecholamines such as dopamine in the brain. In mice the drug is shown to be effective at ameliorating cognitive deficits caused by Aβ build-up as demonstrated by results in object recognition tasks. The drug's advantages appear to perform even better in dogs with canine cognitive dysfunction as it increases sociability as well as decreasing signs of confusion and house-soiling. However, the drug has its limitations as it did not show improvement in dogs over the age of 16 and it failed to improve disrupted sleep-wake cycles. It may be the case

that the drug did not improve signs of cognitive dysfunction in the eldest dogs as the Aβ infiltration was too extensive and so earlier intervention should be considered. Due to the continued sleep-wake cycle disruption despite the use of selegiline, it is worth considering combining selegiline with melatonin to target this. Furthermore, another issue with selegiline was reported as it caused some gastrointestinal side effects in a small proportion of the tested population. Taking this into consideration, it might be worth pairing selegiline with other drugs such as maropitant, metronidazole and probiotics to counteract this. Another disadvantage found in the research was the extent of selegiline's efficacy as much of the research is based on the owner's perception of their pet's behaviour which is liable to be subjective and inaccurate. Furthermore, it is unclear whether selegiline made real cognitive improvements or if it simply caused an ambiguous increase in activity due to boosted catecholamines. Similarly, in human research the drug faced the same challenges as there was a lack of double-blind trials and although selegiline showed promise with initial treatment, as time went on the drug failed to continue to improve cognitive deficits.

Nicergoline is an ergot derivative which affects cognitive function through alpha-adrenergic antagonism, cholinergic modulation, antioxidant effect and cerebral vasodilation. An advantage of the drug was found in mice as the drug improved cognition as APP was downregulated and inflammatory markers that contributed to oxidative stress also decreased while growth factors increased. However, these results did not translate well into other models. In canines with CCD, it seemed to improve cerebral blood flow. However, research indicated that the drug failed to improve cognition and behaviour. Continually in human trials there was a lack of any significant development in cognition in dementia patients. Although nicergoline has numerous effects on the brain it seems to fall short in the treatment of Alzheimer's disease and CCD. This is a major disadvantage of the drug as its promising results in mice could not be applied in other models. Although it appears to be incompatible with Aβ induced cognitive dysfunction, due to its positive effect on cerebral perfusion the drug is favoured in cases of vascular dementia.

Propentofylline is a drug licensed in the UK for treating CCD as it has been shown to target the two main problematic proteins in cognitive dysfunction, beta-amyloid and tau. Studies in rodents show encouraging results as it seemed that propentofylline increased learning and memory. During rodent research it was discovered that these improvements

correlated with a lower dose of the drug. Another potential advantage of propentofylline is its potential in disease-modification as it was found that behavioural improvements persisted beyond the end of therapy. However, this drug is majorly disadvantaged by the lack of available research to support these claims. As well as a lack of volume in the research conducted on the drug there is a lack of variation as it has not been tested across numerous species.

Adrafinil works by increasing a number of neurotransmitters such as dopamine, norepinephrine and histamine while inhibiting GABA. It appears to have a stimulatory effect on mice and monkeys as seen by increased locomotion. Similarly in elderly beagles it seems to have several advantageous effects including improved learning as well as increased locomotion and sniffing although there was some individual variability. However, a major disadvantage of the drug was found in another study as it showed to impair rather than improve working memory. Adrafinil's effect on cognition is complex because although it appears to improve learning, it can have a negative impact on working memory. This is a major issue when considering its applicability for AD as it does not have a uniform positive response on different cognitive functions. As adrafinil produced favourable results in canines the drug was tested further in elderly people. The drug did not recreate the same positive response in humans as it was associated with a number of undesirable side effects including orofacial dyskinesia, elevated blood pressure, muscle twitches and insomnia. Although adrafinil was reported to improve cognition its significant side effects are a major disadvantage.

Levetiracetam is a drug traditionally used for the treatment of epilepsy, however it is being examined for its applicability in cognitive dysfunction as there is an increased incidence of seizures in AD. Its mode of action involves binding the synaptic vesicle protein 2A and thereby modulating neurotransmitter release. Advantages of the drug were found when it was tested in hAPP mice as it was found that levetiracetam reduced the number of spikes seen on EEG tests as well as the reversal of behavioural abnormalities resulting in improved learning. However, a disadvantage of the drug was that it did not cause a reduction in the levels of Aβ load. Another issue arose in the lack of uniformity of dose dependent effects. It was found in one study with mice that a low dose more readily produced desirable results compared to a high dose. However, in other similar studies with mice it was found

instead that the high dose inhibited APP collection and promoted clearance in contrast to the previous study. Further studies in rats produce contrasting results as it was found the drug disturbed long-term recognition memory however these rats were not altered to display AD pathology and so it may not be representative of the drug's potential in this case. Another significant disadvantage of levetiracetam is that its efficacy in cognitive dysfunction in canines, NHPs and humans is not well documented. Therefore, there is a lack of evidence solidifying this treatment as a viable option for cognitive dysfunction.

Memantine is an NMDA antagonist which has been shown to decrease the expression of APP and accelerate its decomposition in mice models. In mice memantine proved to be an effective drug with little adverse effects. However, as with the other drugs studied, memantine's promising results in mice failed to translate to other models which is a significant disadvantage. When trialled in NHPs there was no significant improvement in cognition. The lack of significant results in NHPs is echoed in some studies of human trials where no improvement in attention or memory was observed. Furthermore, another disadvantage was the occurrence of adverse effects such as dizziness, headaches and somnolence in humans. This disadvantage also appeared in canine studies as its use was associated with ataxia and reduced body weight. A further disadvantage of the drug is the lack of research conducted specifically with Canine Cognitive Dysfunction.

In summary, Selegiline showed clear improvement in canines although there were limitations associated such as the lack of response in dogs over 16 years and the drug's lack of efficacy in restoring disturbed sleep-wake cycles. However, the drug did face scepticism as many of the studies conducted lacked standardisation and objectivity. Nicergoline failed to show improvement in non-mice models. While adrafinil's adverse effects make it unfavourable in the treatment of cognitive dysfunction. As well as that adrafinil was found to actually impair rather than improve some areas of cognition. Propentofylline levetiracetam and memantine are all hindered by a lack of research specific to their effects on cognition.

4. Conclusion

All of the models examined including rodents, canines, felines and NHPs offered different insights into the pathophysiology of AD. However, each of these models also presented their own challenges as they deviated from the complete AD pathology. Furthermore, there is a discrepancy between the most frequently used model in AD research and the most applicable model available. Modified rodents appear to be the most widely used model. However, after an analysis of the studies conducted thus far, cats appear to be the most successful model available for their unique similarity to humans regarding tau pathology. The research on feline models is still sparse and warrants further attention.

There are very few drugs which are licensed for the treatment of cognitive dysfunction in animals and the research conducted on the drugs discussed is scarce. The research available is often limited by its method and the medications discussed often are not tested across the different AD animal models. Once again this is most emphasised in the case of feline models wherein they appear to be the least tested model in drug trials despite potentially being the most applicable. There is also a lack of uniformity across the cognitive parameters tested in different studies. Often the research fails to examine an adequate sample of cognitive parameters which limits the understanding of the drug's effect. Furthermore, much of the research examined relied on subjective results obtained by the owner’s perception of their pet.

In conclusion, there is yet a complete animal model which encapsulates the full spectrum of AD pathology although there is some emerging promise. Drug trials in animal models lack uniformity in the species being used, the parameters being tested, and consequently the results. The parallels between Alzheimer’s disease and cognitive dysfunction in animals are striking and understanding this is imperative to further discovery.

5. Summary

I looked at various animal models in the search of a complete pathology of Alzheimer's disease. In this study I evaluated rodents, canines, felines and non-human primates. I found that each model resembled different aspects of AD however, no model captured the complete picture. To confirm the occurrence of AD the presence of the two hallmark features of the disease, Aβ plaques and neurofibrillary tangles have to occur together. I found that rodents did not display these pathological changes naturally but rather they had to be genetically modified to do so. Canines proved to be a more suitable model in comparison as they display similar signs of neurodegeneration and cognitive dysfunction as they age. However, as they lack the presence of neurofibrillary tangles, they diverged from the complete picture of AD. Non-human primates faced the same challenge although both NHPs and canines show evidence of early stage tauopathy. I then looked at feline models which seemed to provide the most promising representation of AD as evidence of tangles was found in cats suffering with cognitive dysfunction although this fact is in dispute as some argue that these structures should be considered as pre-tangles.

I then continued my research in looking for a suitable drug for the treatment of cognitive dysfunction in animals and humans. The drugs studied include selegiline, nicergoline, propentofylline, adrafinil, levetiracetam and memantine. I found that there were two problems common in the research of each drug which included a lack of diversity in the species used in drug trials, this is most apparent with the feline model as it was consistently excluded, and a lack of standardised testing of sufficient cognitive parameters making the comparison of the different therapies challenging. Furthermore, the means by which much of the studies were conducted, especially in canines and felines, relied on the subjective interpretation of their owners. Adrafinil appeared to be a potentially effective treatment for CCD and AD although there was insufficient double-blind testing to confirm this. Nicergoline has been studied in canines, but the research is scarce and the results are not encouraging. Propentofylline is a licensed drug for the treatment of CCD, but the results are conflicting and again there is significant lack of research conducted on the drug. Adrafinil has a more robust history of research in comparison to some other drugs as its effect on rodents, NHPs, canines and humans has been recorded. However, its effect on different

cognitive parameters is not uniform and it has several adverse effects associated with its use. Levetiracetam has been studied in rodents and humans offering some hope in reducing the number of pathological processes that occur in cognitive dysfunction, however more extensive research is required to determine its efficacy. Memantine was studied in rodents, NHPs, canines and humans. In rodents and canines, it appears to achieve behaviour modification, but this does not translate into research conducted with NHPs and humans. I conclude that more extensive research should be conducted on the different pharmaceutical remedies available for cognitive dysfunction which include more suitable models such as the feline model.

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Thesis progress report for veterinary students

Name of student: Erica Gosnell……

Neptun code of the student:…...U4LAAY.....

Name and title of the supervisor: ..Dr Orsolya Palócz……

Department of Pharmacology and Toxicology……

Thesis title: Animal models of Alzheimer's disease and drug development literature review
……

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024	02	05	Consultation on structure	
2.	2024	03	07	Introduction	
3.	2024	03	28	Suitable models	
4.	2024	04	15	Progress of the disease	
5.	2024	05	21	Evaluation of the work	

Grade achieved at the end of the first semester: ……5 (excellent)……..

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024	09	30	Available medicines and usage	
2.	2024	10	03	Advantages, disadvantages of the models and medicines	
3.	2024	10	22	Review of the text	
4.	2024	11	04	Review of the whole work	
5.	2024	11	12	Final review	

Grade achieved at the end of the second semester: …..excellent (5)……..

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

……
signature of the supervisor

Signature of the student: ……

Signature of the secretary of the department: ……..……

Date of handing the thesis in…… 26/11/24 ……..…...