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Neuropathology, diagnosis, and potential treatment of feline cognitive dysfunction syndrome and its similarities to Alzheimer's disease

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Declaration

I declare that the work presented in this thesis has been composed solely by myself. This work has not been previously submitted, in whole or in part, for any other degree or professional qualification. All the work and publications presented in this thesis are my own work, except where indicated.

Lorena Sordo Sordo

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Abstract

Cognitive dysfunction syndrome (CDS) is a common age-related condition in domestic cats that is characterised by behavioural changes that ultimately lead to cognitive decline and dementia. The most common behavioural changes displayed by cats affected by CDS have been summarised under the acronym VISHDAAL and include excessive Vocalisation, especially at night, alterations in their Interaction with their owners (e.g., increased affection), alterations in their Sleep-wake cycles, House-soiling, spatial and/or temporal Disorientation, alterations in Activity levels, Anxiety, and/or Learning/memory deficits.

Cats with CDS develop neuropathologies that share many similitudes to those seen in the brains of humans with Alzheimer's disease (AD), which are the abnormal accumulation of amyloid-β (Aβ) forming senile plaques, and the presence of neurofibrillary tangles (NFT) formed by hyperphosphorylated tau deposits. In the present study, immunohistochemistry of seven different brain regions of cats of various ages, with and without CDS, revealed that cats accumulate intra and extracellular Aβ deposits, plus intranuclear and intracytoplasmic hyperphosphorylated tau deposits. The intracytoplasmic deposits of Aβ were mainly found in younger cats, whereas elderly cats accumulated large diffuse extracellular Aβ deposits. While Aβ senile plaques in people with AD have a dense core, the diffuse Aβ deposits in cats are believed to be an early stage of senile plaques.

In this study, intranuclear labelling for hyperphosphorylated tau was mainly found in the younger cats. In contrast, intranuclear labelling was rarely found in elderly cats, especially if pre-tangles were present. Furthermore, intracytoplasmic immunolabelling for hyperphosphorylated tau was mainly found in the brains of elderly cats. Due to its ultrastructural features, these deposits are considered to be pre-tangles, which are an early stage of the characteristic NFT seen in the human AD brain.

Diagnosing CDS can be challenging, especially as the diagnosis can only be achieved by ruling out all other potential causes for the behavioural changes displayed by the cats. Veterinarians can only diagnose CDS after performing thorough examinations of the cats, which can be a slow, time-consuming, and expensive task. Hence, diagnostic tools and/or methods that may facilitate prompt diagnosis are urgently needed.

Imaging techniques, such as magnetic resonance imaging (MRI), have recently emerged as potential diagnostic tools for assessing brain changes associated with ageing and AD in humans. These imaging techniques also have the potential to be effective *in vivo* diagnostic tools for assessing age-related brain changes and CDS in cats. In this study, age-related changes were assessed in different brain regions of cats of various ages, with and without CDS, by using MRI. Elderly cats in this study were shown to develop atrophy of the whole brain, the hippocampus, and the occipital lobe, plus enlargement of the ventricles. Most of these changes have also been described in elderly humans, especially in those with AD.

While there is currently no treatment available for CDS in cats, interventions that can be considered for its management include environmental enrichment, dietary supplementations, specific diets, and potentially, medication (licensed for the treatment of dogs with CDS). Telmisartan is an angiotensin receptor blocker and an activator of PPARγ that has shown to reduce neuroinflammation, provide neuroprotection, and to restore cognition in humans and rodents. In this study, a three-month double-blinded placebo-controlled trial was performed in cats with severe CDS. Even though no statistically significant differences were found between the treatment groups, further studies are needed to determine the true potential of telmisartan for the treatment of CDS in cats.

The present work furthers our understanding about the neuropathology of ageing and CDS in cats. Furthermore, it proposes the use of imaging techniques, especially of MRI, as a potential *in vivo* diagnostic tool for age-related changes that may lead to cognitive decline. Finally, it proposes the use

of telmisartan as a potential treatment to reduce the clinical signs of CDS in cats.

Lay summary

Cognitive dysfunction syndrome (CDS) is a common age-related condition in older cats that leads to cognitive impairment and dementia. Cats with CDS display certain behavioural changes, including increased vocalisation, especially at night, altered interactions with members of the family (e.g., cats being more affectionate and demanding more attention), altered sleep-wake cycles, toileting mistakes, disorientation, altered activity levels (i.e., aimless wandering), anxiety, and learning or memory deficits.

Cats with CDS develop brain changes that are similar to those seen in humans with Alzheimer's disease (AD). These changes include the abnormal accumulation of two proteins: amyloid-β (Aβ) and tau. In this study, Aβ and tau in cats was found to accumulate in a similar pattern and distribution to that seen in humans with AD, with Aβ deposits inside the cells (i.e., within the cytoplasm) and in the space around the cells showing a diffuse distribution pattern, and tau deposits inside the cells, within the cytoplasm and the nucleus.

The diagnosis of CDS can be challenging as it can only be made by exclusion, ruling out all other potential causes, making it a slow, time-consuming, and expensive task. For this reason, diagnostic tools that can help to gain a prompt diagnosis are urgently needed. Magnetic resonance imaging (MRI) has been shown to be an efficient diagnostic tool when looking for ageing brain changes in humans, particularly those with AD. Therefore, MRI also has the potential to be an effective diagnostic tool when looking for age-related brain changes in cats. Cats in the present study were shown to develop similar age-related brain changes to those seen in humans with AD, including shrinkage of the whole brain and the hippocampus (an area of the brain involved in making memories), plus enlargement of the structures that are filled with cerebrospinal fluid, such as the ventricles.

Even though some interventions can be offered to cats with CDS, such as environmental enrichment, specific diets, and supplements, to date, there is

no treatment available to help cats with CDS. This study proposed the use of the drug Telmisartan to reduce the clinical signs of CDS in cats, because it has been shown to have beneficial effects in the cognition of humans and rodents. However, the results in cats in the present study were inconclusive, and further studies will be needed to determine the true potential of telmisartan for the treatment of CDS in cats.

Publications

1. **Sordo L.** and Gunn-Moore D. (2021). Cognitive dysfunction in cats: update on neuropathological and behavioural changes plus clinical management. *Veterinary Record*, 1-12.

2. Černá, P., Gardiner H., **Sordo L.**, *et al.* (2020). Potential Causes of Increased Vocalisation in Elderly Cats with Cognitive Dysfunction Syndrome as Assessed by Their Owners. *Animals,* 10(6): 1092.

3. Miele, A., **Sordo, L.**, & Gunn-Moore, D. A. (2020). Feline Aging: Promoting Physiologic and Emotional Well-being. *Veterinary Clinics: Small Animal Practice*, *50*(4), 719-748.

4. **Sordo, L.**, Breheny C., Halls V., *et al.* (2020). Prevalence of disease and age-related behavioural changes in cats: past and present. *Veterinary sciences,* 7(3): 85.

5. Malbon A.J., **Sordo L.**, Paraschou G., *et al.* Preliminary investigation into the presence of neuropathological changes in the brains of aged donkeys indicates similarities to human ageing. In Preparation

6. **Sordo L.**, Ellis J., Angel C., *et al.* The effect of telmisartan on the clinical signs of cognitive dysfunction syndrome in the domestic cat. *Journal of Veterinary Pharmacology and Therapeutics.* In Preparation

7. **Sordo L.**, Dishong O., Marioni-Henry K., *et al.* Assessment of age-related brain atrophy in live cats and fixed cat brains with and without dementia using 1.5T MRI. *Journal of Veterinary Radiology and Ultrasound.* Under review

8. **Sordo L.**, Martini A.C., Houston F., *et al.* Neuropathology of ageing in cats and its similitudes to human Alzheimer's disease. *Frontiers of Aging*. Under review

Presentations and memberships

Invited speaker at the ARUK Outreach Conference: from cats to care, The University of St. Andrews, UK, October 2019. "*It's not only humans: feline cognitive dysfunction".*

Invited speaker at the Dementia and Multi-species Workshop, The University of West of Scotland, UK, May 2019. *"What about the animals?".*

Invited keynote speaker at the 7th Winter Seminar on Dementia, SINdem4Juniors in Bressanone, Italy, January 2019. *"Is Alzheimer's disease a uniquely human disorder?".*

Member of the *"Dementia: A Multi-Species Perspective"* network since September 2019.

Grants and awards

Successful grants and awards

Scottish Dementia Research Consortium and Brain Health Research: SDRC Early Career Researcher Resource Programme: £2,500 (2020).

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Unsuccessful grants and awards

Morris Animal Foundation: Neuropathology of cognitive dysfunction syndrome in cats and development of a potential diagnostic tool (2020) (with Danièlle Gunn-Moore).

Animal Welfare Foundation: Feline cognitive dysfunction: Potential biomarkers and treatment (2017) (Primary applicant, with Danièlle Gunn-Moore).

The Winn Feline Foundation: Feline cognitive dysfunction: Potential biomarkers and treatment (2017) (Primary applicant, with Danièlle Gunn-Moore).

Future work

Secured a post-doctoral position in the laboratory of Professor Elizabeth Head at The Department of Pathology and Laboratory Medicine at The University of California, Irvine where I will be studying the neuropathology and biomarkers of 1) cognitive dysfunction syndrome in dogs; 2) Alzheimer's disease in people with Down Syndrome; and 3) the role of neuroinflammation in feline cognitive dysfunction syndrome.

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List of Acronyms and Abbreviations

Symbols

αAPP	Alpha amyloid precursor protein
α-CTF	Alpha C-terminal fragment
βAPP	Beta amyloid precursor protein
β-CTF	Beta C-terminal fragment (see C99)
μl	Microlitre
μm	Micrometres

Numbers

0N	No inserts
1N	One insert
2N	Two inserts
3R	3-repeats tau isoform
4R	4-repeats tau isoform

A

A	Ampere
Aβ	Amyloid-beta or beta-amyloid
Aβ40	Aβ containing a 40 amino acids sequence
Aβ42	Aβ containing a 42 amino acids sequence
A_1	Total dorsal brain area
A_2	Total sagittal brain area
A_3	Total transversal brain area
AAFP	American Association of Feline Practitioners
AAHA	American Animal Hospital Association
ACE	Angiotensin converting enzyme
ACh	Acetylcholine
AD	Alzheimer’s disease
Ang II	Angiotensin II
Ang IV	Angiotensin IV

APA	Aminopeptidase A
APN	Aminopeptidase N
APP	Amyloid precursor protein
ARBs	Angiotensin II type I receptor blockers
AT_1	Angiotensin II type I receptor
AT_2	Angiotensin II type II receptor
AT_3	Angiotensin II type III receptor
AT_4	Angiotensin II type IV receptor
ATP	Adenosine 5'-triphosphate

B

b_2	Transversal brain height
BBB	Blood-brain barrier

C

C99	also known as β-C-terminal fragment (see β-CTF)
C	Centigrade
CDS	Cognitive dysfunction syndrome
Chrom. Nuc.	Chromatin-bound nuclear fraction
CI	Confidence interval
CSF	Cerebrospinal fluid
CT	Computed tomography
CTF	C-terminal fragment
Cytopl.	Cytoplasmic fraction
Cytosk.	Cytoskeleton fraction

D

DAB	3,3'-diaminobenzidine substrate
DISHAAL	Acronym to summarise the behavioural changes seen in dogs with CDS, which stands for Disorientation; alterations in Interactions between the pet and its owners or other pets; alterations in the Sleep-wake cycle; House-

	soiling; alterations in Activity levels; Anxiety; and Learning and memory deficits
DLH	Domestic longhair
DNMP	Delayed nonmatching to position test
DSH	Domestic shorthair
DTI	Diffusion Tensor Imaging

E

ECVIM	European College of Veterinary Internal Medicine

F

FeLV	Feline leukaemia virus
FIC	Feline idiopathic cystitis
FIV	Feline immunodeficiency virus
FROr	Frontal lobe ratio

G

GM	Grey matter

H

HEMr	Hemispheres ratio
HIPr	Hippocampal ratio

I

IgG	Immunoglobulin G
IHC	Immunohistochemistry
ITAr	Interthalamic adhesion ratio

K

kDa	Kilodalton

L

LDS	Lithium dodecyl sulphate
LPS	Lipopolysaccharide
LVr	Lateral ventricles ratio

M

MAP	Microtubule-associated protein
MCTs	Medium-chain triglycerides
MES	2-N-morpholino ethane sulfonic acid
mm^2	Squared millimetres
mM	Millimolar
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MTBD	Microtubule binding domain

N

NFT	Neurofibrillary tangle
Nm	Nanometre
Np	Number of (moderate) positives
Nsp	Number of strong positives
Ntotal	Total number (positives and negatives)
Nwp	Number of weak positives

O

OCCr	Occipital lobe ratio

P

pH	Potential of Hydrogen
PHF	Paired helical filaments
PBS	Phosphate-buffered saline
PO	*Per os* or oral administration
PMI	*Post-mortem* interval

PPARs	Peroxisome proliferator activated receptors
PPARγ	Peroxisome proliferator activated receptor gamma
PRD	Proline-rich domain
PVDF	Polyvinylidene fluoride

Q

Q24h	Once a day (every 24 hours)

R

RAS	Renin-angiotensin system
RD3	3-repeat isoform of tau protein
RD4	4-repeat isoform of tau protein
RDSVS	Royal (Dick) School of Veterinary Studies
RNA	Ribonucleic acid
ROS	Reactive oxygen species

S

SAMe	S-adenosyl-L-methionine
SD	Standard deviation
SE	Standard error
Sol. Nuc.	Soluble nuclear fraction

T

TE	Time to echo
TEMr	Temporal lobe ratio
TR	Repetition time

V

V	Volts
VERC	Veterinary Ethical Review Committee
VISHDAAL	Acronym to summarise the behavioural changes seen in cats with CDS, which stands for excessive Vocalisation;

	alterations in Interactions; changes in the Sleep-wake cycle; House-soiling; Disorientation; alterations in Activity levels; Anxiety; and Learning and memory deficits
W	
WM	White matter

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Chapter 1 Introduction

“In ancient times cats were worshipped as gods; they have not forgotten this”

-Terry Pratchett

Note:

Sections of the current chapter have already been published by the author:

Sordo L., and Gunn-Moore, D.A. Cognitive dysfunction in cats: Update on neuropathological and behavioural changes plus clinical management. *Veterinary Record,* 188(1), pp. 30-41.

Author's contribution to the paper:
The author performed all the review of the literature and wrote the paper.

Feline dementia (also known as cognitive dysfunction syndrome; CDS) is a common age-related condition that is becoming increasingly prevalent in veterinary practice. The PhD thesis furthers our understanding about this condition in cats, its neuropathology, diagnosis and treatment. Moreover, it highlights the similarities of this condition to human Alzheimer's disease (AD).

Thanks to advances in veterinary medicine, improvements in veterinary nutrition, and changes in the way we manage our pets (e.g., indoor living), the life expectancy of pet cats is increasing, with a reported median longevity of 14 years[1]. When classifying cats by age, they are considered to be 'mature' at 7 to 10 years of age, 'senior' at 12 to 14 years of age, and become 'geriatric' or 'super senior' at 15 years of age[2, 3]. The most common age-related neurological changes include deterioration of cognition (ultimately resulting in dementia), motor performance, and visual and auditory decline[4-6].

1.1 Behavioural changes in elderly cats

Since behavioural changes in aged pets often appear before other signs of illness, they can be useful early indicators of a decline in health and welfare. There are many potential causes of these behavioural changes, as diverse as CDS, hypertension, hyperthyroidism, pain (commonly associated with osteoarthritis), and separation anxiety, to name but a few, so it is important that our elderly cats get a full clinical examination, to determine the cause of their specific behavioural changes (see section on Diagnosis). It is essential to identify these behavioural changes early, in order to provide the most effective interventions. In a questionnaire-based study, 36% of owners of cats aged 7-10 years of age reported that their cats had developed age-related behavioural problems, including house-soiling and alterations in their activity levels, with the percentage of affected cats increasing to 88% in cats aged between 16-19 years[7]. Since some of these changes can be subtle or misinterpreted as 'normal ageing', cat owners often need assistance in identifying and reporting

them to their veterinary surgeon[8]. This is made worse when owners are reluctant to take their elderly cats to the veterinary clinic. Many owners and cats find clinic visits stressful (getting the cat into its basket, driving it to the practice, and coping with the consultation)[9]. In addition, many owners worry that little can be done to help improve their cat's quality of life, so euthanasia may be suggested[10]. Veterinarians need to be aware of these concerns and counsel owners about how best to reduce this stress (e.g., giving gabapentin or trazadone to the cat 90 minutes before travel is planned)[11, 12], and making sure that owners know there are many potential interventions that can help elderly cats to have a better quality of life.

1.2 Behavioural changes in cats with CDS

Cognitive dysfunction syndrome (CDS) describes the age-related decline in cognitive abilities, characterised by certain behavioural changes, that cannot be attributed to any other medical condition[13, 14]. Although CDS in cats is a well-established condition, much of its pathophysiology has been extrapolated from findings in other species[15], mainly dogs with CDS and people with Alzheimer's disease (AD), so our understanding of CDS in cats is less detailed.

The prevalence of the signs of CDS varies by species and age: for pet cats, around 28% of cats between 11-14 years of age develop at least one behavioural problem related to CDS, with this increasing to 50% in cats over 15 years of age[14, 16].

The major signs of CDS in cats and dogs have previously been summarised by the acronym DISHAAL, which stands for: spatial or temporal Disorientation; alterations in Interactions between the pet and its owners or other pets; alterations in the Sleep-wake cycle; House-soiling; alterations in Activity levels; Anxiety; and Learning and memory deficits[8, 17]. While DISHAAL does recognise many of the major behavioural changes reported in cats with CDS,

it fails to emphasise the importance of excessive vocalisation, especially at night, which is often the most common finding, particularly in the oldest cats. In the prevalence study mentioned above[14], altered interactions with the family (especially increased attention seeking) was the most common behavioural change in cats aged from 11-14 years, while alterations in the activity levels and excessive vocalisation were the most common signs in cats aged over 15 years. A study of 100 cats with behavioural abnormalities (7-11 years old), found 36% of them vocalised excessively, especially at night, and 48% presented with house-soiling[8]. In another study of 100 cats, 61% of cats between 12-22 years of age vocalised excessively, with 31% vocalising mostly at night. In the same study, 27% of cats presented with house-soiling and 22% were disorientated[15]. Additional studies further emphasise the importance of increased vocalisation in cats with CDS, while also trying to determine the likely cause. In our most recent study of more than 850 cats of 11 years of age or more, almost 60% of cats were reported to vocalise excessively, particularly at night, and 50% were more affectionate towards their owners, demanding more attention[18] (Figure 1.1).

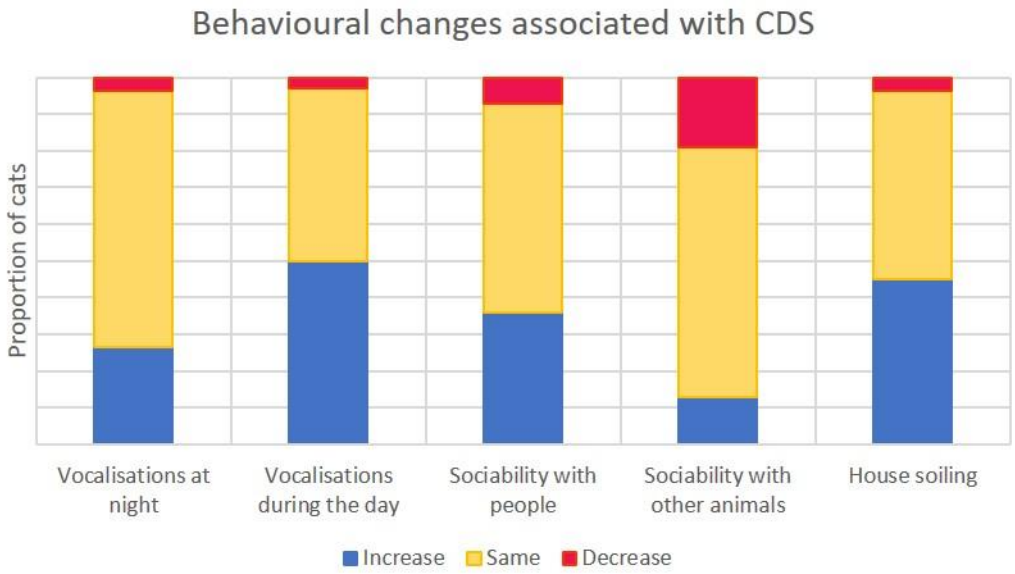

Figure 1.1 Behavioural changes associated with cognitive dysfunction syndrome (CDS). *In response to a questionnaire, cat owners reported whether each of the age-related behaviours increased, decreased, or remained the same, when compared to when their cats were younger. Graph taken from Sordo* et. al., *2020[18]*

In a detailed study of 37 cats with CDS where owners reported increased vocalisation, 67% vocalised more at night and 64% vocalised more during the day. When asked to suggest the likely main cause of their cats increased vocalisation, 41% suggested disorientation, 41% suggested attention seeking (i.e., wanting affection and attention from their owner) and 16% that their cat was looking for food (Figure 1.2). The owners also reported that 67.5% of cats were more affectionate[19]. These studies show that most of the behaviours detailed in the acronym DISHAAL can in themselves cause increased vocalisation; 'alteration in the Interactions between the pet and its owners', 'Anxiety' (e.g., causing attention seeking), 'spatial or temporal Disorientation' and 'deficits in Learning and memory' (e.g., causing a cat to forget that they have been fed), and 'alterations in the Sleep-wake cycle' likely to play a role in night crying. However, while these findings clearly support that increased vocalisation is part of CDS, the acronym DISHAAL does not recognise the importance of increased vocalisation within the acronym itself, with the V of Vocalisation being given prime position.

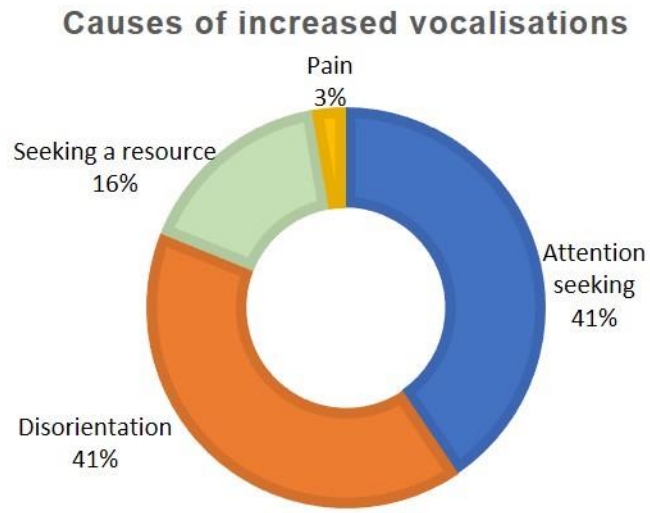

Figure 1.2 Main causes of increased vocalisations in cats with cognitive dysfunction syndrome (CDS), as reported by their owners. *Graph modified from Černá* et. al.*, 2020[19]*

Given that cats with CDS present differently to dogs with CDS, for whom the DISHAAL acronym was originally developed[8, 17], it was proposed that a different acronym was needed for cats. Hence, a new feline-focused acronym was created by the authors: VISHDAAL[20] describes the behaviours seen in cats with CDS, including excessive Vocalisation; alterations in Interactions (e.g., increased affection); changes in the Sleep-wake cycle; House-soiling (urination and/or defecation); Disorientation; alterations in Activity levels; Anxiety; and Learning and memory deficits. The behaviours included in this new acronym are more specific to cats and are listed in order of prevalence, as supported by the aforementioned studies.

1.3 Cognitive decline in cats with CDS

While cognition in humans is composed of multiple features such as learning, memory, executive function (the processes to manage resources in order to achieve a goal, for example planning, reasoning and problem solving), attention, language, psychomotor and spatial abilities, this is less well understood in pets, especially cats. There is limited evidence assessing cognitive decline in cats with CDS. Since changes in cognition may initially be subtle in pets affected by CDS, and their recognition may present a challenge for their owners, laboratory-based protocols have been developed to try to assess and measure cognitive function in cats[8, 14]. Testing cognition in cats is far more challenging than in dogs; they are motivated by different things, with few cats being motivated by food the way many dogs tend to be[8, 14].

A neuropsychological test, originally created for dogs to assess cognitive function, has been remodelled for use in cats. The cats were divided into three groups: 1) young adults aged 3.0-3.8 years; 2) mature cats aged 7.7-9.0 years; and 3) senior cats aged 10.5-15 years. The cats were put through a battery of cognitive tasks, including a T-maze (i.e., a behavioural test that assesses exploratory behaviour in a new environment) and matching tasks (also known

as delayed nonmatching to position memory task, which assesses short-term visuospatial memory). The results showed an age-dependent decline in both discrimination and reversal learning*, as well as significant differences between groups during the matching tasks, suggesting that the older the cat, the more severe the cognitive decline they may develop[21, 22]. *(Reversal learning is tested when the cat is first trained to remember under which shaped pot food is hidden (discrimination learning). Once the cat has learned this, the food is then hidden under a different-shaped pot, challenging the cat to reverse or inhibit what they previously learned during the discrimination phase).

1.4 Causes of CDS

The exact cause of CDS remains unknown; however, several alterations in the brain are believed to be involved in its development, including oxidative damage, vascular changes (see vascular pathology) and compromised cerebrovascular blood flow.

There is normally a balance between the production and removal of free radicals within the body such that oxidative damage is limited. However, factors such as stress, ageing or disease can alter this balance, reducing their elimination and promoting an excess of free radicals which may damage the brain[13]. This oxidative damage is believed to occur in cats, dogs and most, if not all other species[14, 23].

With age, the blood flow to and within the brain can be compromised by changes to the vessels themselves, systemic hypertension, heart disease, anaemia, alterations in blood viscosity and/or hypercoagulability, all of which exacerbate neuronal hypoxia[13, 14].

1.5 Changes in the brain with CDS

It is useful to compare changes seen in cats and humans. With ageing, the brains of cats and humans suffer several anatomical and physiological changes, ultimately associated with the development of dementia[24, 25]. These changes include gradual atrophy of the cerebral cortex and basal ganglia; region-specific neuronal loss; increased ventricular size; vascular and perivascular changes; lipofuscin accumulation; amyloid-β (Aβ) deposition and tau hyperphosphorylation, amongst others[14, 25, 26]. In old cats, brain atrophy, neuronal loss, increased ventricular size and widened sulci have all been described, although these changes are usually less pronounced than in people[15], and as yet, these changes have not been directly associated with cognitive dysfunction[14, 27, 28].

1.5.1 Brain atrophy and neuronal loss

Brain atrophy is one of the most important changes associated with ageing; the human brain is known to shrink at a rate of 2-5% per decade[29, 30]. The most affected brain region is the frontal lobe[31, 32], with more subtle changes affecting the temporal and parietal lobes[31, 32]. In AD, additional age-related brain changes have been identified, including enlargement of the ventricles and atrophy of the hippocampus[31-35]. Since these changes are initially subtle and can appear decades before a clinical diagnosis of AD can be made, they have been proposed to be early predictors of the disease[36-39].

Similar age-related brain changes are known to occur in the elderly cat brain, including brain atrophy, and enlargement of the ventricles[40]; however, these have not yet been associated to CDS. Further discussion on these changes and its similarities to human age-related brain changes and AD can be found in Chapter 3.

The cerebella of aged cats have a reduced number of Purkinje cells (the neurons located in the cortex of the cerebella that are involved in learning and play a vital role in controlling motor movement) and other neurons[27]. In cats, this neuronal loss is more evident within the molecular layer of the cerebellum in animals of 12-13 years of age, compared with 2-3 year old cats[27].

Initial changes in the caudate nucleus, which include neuronal loss and reduced numbers of synapses[41, 42], appear in cats as young as 6-7 years of age[5, 42]. However, these changes are typically more evident in cats over 10 years of age, when compared with cats between 1-3 years of age[43]. They are believed to cause impairments in motor function and the inability to habituate to repeated stimuli in older cats[44-46]; hence, elderly cats can be repeatedly scared by common noises.

The cholinergic system (which regulates attention and higher-order cognitive processing) and the locus coeruleus (which is the main site for norepinephrine/noradrenaline synthesis in the brain, and responsible for promoting arousal at times of stress) are both affected in humans with AD[47-49]. The loss of cholinergic neurons may directly impair cognition[48, 49]. Cats aged 15-18 years of age show myelin disruption, axonal degeneration, and a marked reduction in both the size of cholinergic neurons and the dendritic length in the locus coeruleus, when compared with cats aged 2-3 years of age[50]. These abnormalities, as well as other neuronal deficits, may cause the alterations in the sleep-wake cycle of affected cats[50, 51].

The hippocampus (an essential area for learning and memory) is also affected. Neuronal loss is seen in cats over 14 years of age, being most severe when both Aβ plaques and hyperphosphorylated tau deposits are present[52].

1.5.2 Neuropathological changes

In AD, the two main pathognomonic changes are the abnormal accumulation of Aβ aggregates forming senile plaques, and the abnormal accumulation of hyperphosphorylated tau forming neurofibrillary tangles[53].

1.5.2.1 Amyloid-β pathology

Amyloid-β (Aβ) derives from the amyloid precursor protein (APP), a glycoprotein of 695 amino acids, which is highly expressed in different human tissues, including the brain[53-55]. The APP protein is a transmembrane protein, which means that part of it is located in the membrane of the cell; however, it has a long N-terminus that reaches into the extracellular space, and a shorter C-terminus located intracellularly[53]. The Aβ fragments are derived from parts of the extracellular domain, close to the cell membrane, and the transmembrane region[53].

The APP protein is cleaved, at different sites, by three enzymes: alpha (α), beta (β), and gamma (γ)-secretases. Depending on the site it is cleaved, this process leads to one of two pathways: the non-amyloidogenic pathway, which does not generate Aβ; and the amyloidogenic pathway, in which Aβ is generated[56] (Figure 1.3).

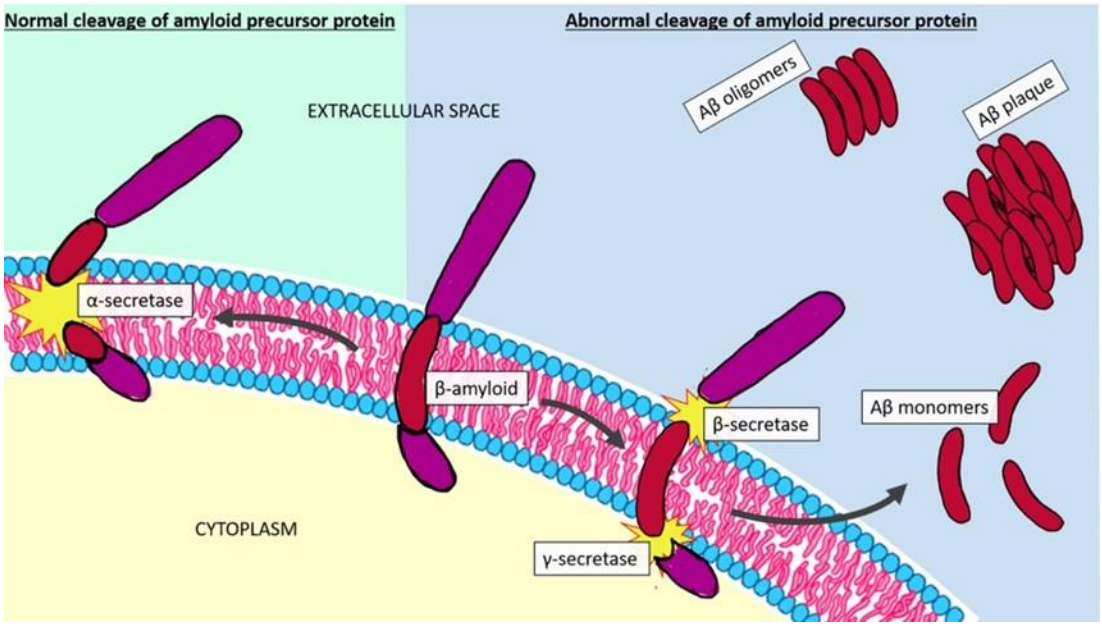

Figure 1.3 Cleavage of the amyloid precursor protein (APP). *Normal (non-amyloidogenic) and abnormal (amyloidogenic) cleavage of APP and formation of amyloid-β (Aβ). Image from Sordo and Gunn-Moore, 2020*[20]

The first cleavage on APP occurs between its extracellular and transmembrane portions by either α or β secretases. This cleavage generates an N-terminal soluble fragment (i.e., αAPP and βAPP, respectively) and a C-terminal fragment (CTF) attached to the membrane forming α-CTF and β-CTF; the latter is also known as C99[57].

Non-amyloidogenic pathway

In this pathway, α-secretases cleave APP at the amino acid residues 15 to 17, breaking the portion of the protein where Aβ is located; hence, the released segment (i.e., soluble αAPP) does not contain complete or intact Aβ[56, 58].

Amyloidogenic pathway

In this pathway, APP is cleaved at two different sites releasing intact Aβ into the extracellular space, where it may accumulate[54, 58]. The first cleavage, by

β-secretase, occurs between residues 671 and 672 of the APP molecule and generates the N-terminus of Aβ (i.e., βAPP). The second cleavage, by γ-secretase, occurs at the C99 fragment, generating the C-terminus of Aβ[56].

This second cleavage can occur at one of multiple sites of the C99 fragment, resulting in various lengths of the Aβ peptide, ranging from 36 to 43 amino acid residues[56]. However, there are two species that are mostly found in the human brain and that are believed to be involved in AD[55]. These contain 40 amino acids (Aβ40) which is the most abundant and accumulates within blood vessels[54, 56]; and a second one with 42 amino acids (Aβ42), which accumulates and forms senile plaques[54].

After being released into the extracellular space, Aβ fragments form small clusters, known as oligomers; if several of these clusters aggregate, they form fibrils (assembly of Aβ oligomers that become insoluble and are less likely to degrade). Bundles of fibrils form β-sheets, which aggregate and form the characteristic senile plaques[54, 56].

1.5.2.1.1 Types of β-amyloid deposits

Different types of Aβ deposits have been identified, such as diffuse deposits and senile plaques[54].

Diffuse deposits

Immunohistochemical studies have shown that these deposits are areas of granular staining containing few to no Aβ fibrils. These deposits are commonly seen in the brains of healthy elderly individuals with no signs of cognitive impairment[53, 54]. It has been suggested that these diffuse deposits are an early stage of Aβ plaques[53, 54]. Since they contain few to no Aβ fibrils, diffuse plaques cannot be detected with congo red, thioflavin, and silver stains[53, 54].

Furthermore, diffuse deposits are not associated with dystrophic neurites (see senile plaques) and/or reactive glial cells[53, 54] (Figure 1.4A and B).

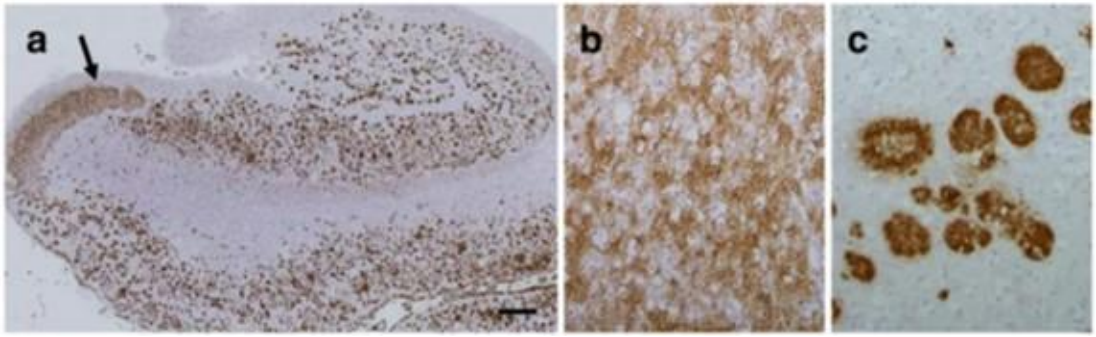

Figure 1.4 Amyloid-β (Aβ) deposition in the Alzheimer's disease (AD) brain. *In humans with AD, Aβ accumulates as a) and b), large diffuse Aβ deposits; and c) well-defined, dense core, Aβ senile plaques. Image adapted from Murray* et. al.*, 2018[59]. Scale bar 1000 μm in a and 50 μm in b and c*

Senile/neuritic plaques

Senile or neuritic plaques are Aβ deposits characterised by having a central core mainly composed of Aβ fibrils, amongst other proteins such as Apolipoprotein E[53, 54] (Figure 1.4C). Unlike diffuse deposits, senile plaques are surrounded by reactive microglia and astrocytes, plus abnormal neuronal processes (i.e., dendrites and/or axons) called dystrophic neurites[53, 54]. Moreover, dystrophic neurites may contain paired helical filaments (PHF), which may later form neurofibrillary tangles (NFT) (see below Tau pathology)[53].

Another type of senile plaque, called the "end-stage" plaque, has also been identified. These plaques share the same characteristics with the senile plaques; however, "end-stage" plaques are not surrounded by dystrophic neurites[54]. For this reason, these plaques are believed to be the remnants of senile plaques[54].

1.5.2.2 Progression of Aβ pathology in AD

Braak and Braak (1991) were the first in identify the progression of Aβ pathology in AD and found that Aβ deposits can vary in size and shape between individuals[60]. However, while there is variability at early stages of disease, they found that similar patterns of distribution are constant during end-stages of disease. Therefore, five stages of Aβ pathology were created[60, 61] (Figure 1.5).

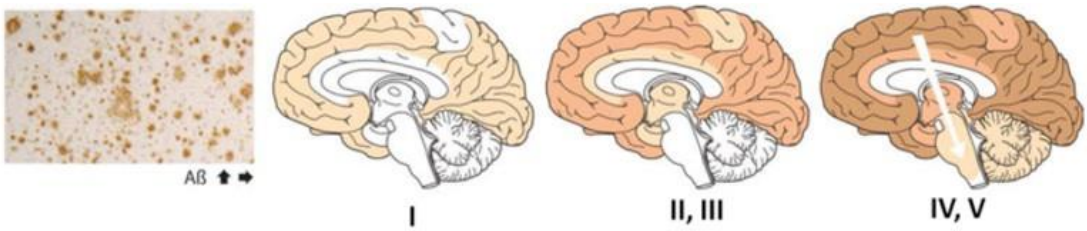

Figure 1.5 Progression of amyloid-β (Aβ) pathology in Alzheimer's disease (AD). *Aβ pathology begins in the cortical regions (stage I); as the disease progresses, Aβ pathology spreads to other regions such as the hippocampus, the amygdala, thalamus, and hypothalamus (stages II and III), until reaching the medulla oblongata, locus coeruleus, and cerebellum in later stages of disease (stages IV and V). Image modified from Jouanne* et al.*, 2017*[62]

Stage I

In stage I of Aβ pathology, low densities of Aβ diffuse deposits are mainly found in the cortex, particularly in the frontal, temporal, and occipital cortices[60, 61].

Stage II

In stage II, Aβ pathology increases in the cortical regions, and spreads into the white matter, Aβ deposits begin to be found in the entorhinal region, the hippocampus and the amygdala, amongst others[60, 61].

Stage III

In stage III, all cortex regions and the hippocampus are severely affected by Aβ. Furthermore, Aβ deposits aggregate and affect other structures, such as the thalamus and the hypothalamus, amongst others[60, 61].

Stage IV

In stage IV, Aβ pathology progresses to other regions, such as the substantia nigra and the medulla oblongata, amongst others[61].

Stage V

In stage V, Aβ pathology is found in the same regions affected by stages I-IV; however, the locus coeruleus and the cerebellum are also affected[61].

Cats over 10 years of age have been shown to have aggregates of Aβ[63-67]. However, these accumulations have a more diffuse distribution than those in humans[14, 52, 63-65, 67] (Figure 1.6). Similar to humans, Aβ deposits in the brains of cats are made predominantly of the peptide Aβ42[63, 64] and are almost only detectable using certain antibodies e.g., anti-Aβ17-24 (4G8) or anti-Aβ42[64]. However, since anti-Aβ antibodies in cats do not bind to amino acid residues 1-16 of Aβ (e.g., 6E10)[64], it is possible that Aβ in cats is formed by a cleaved Aβ protein (i.e., amino acid residues 17-42). The lack of Aβ40 in cats may be due to its high solubility and rapid turnover, which impedes its accumulation in the brain[63, 68, 69]. However, Aβ40 deposition has been reported within blood vessels in cats, associated with cerebral amyloid angiopathy, as is also seen in humans[65].

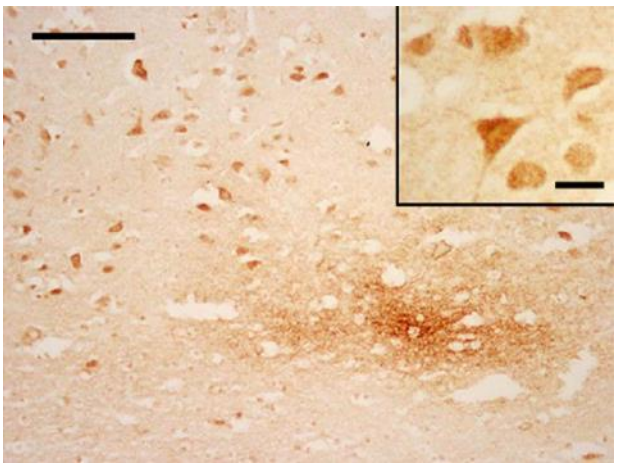

Figure 1.6 Amyloid-β (Aβ) deposition in the cat brain. *Extracellular diffuse Aβ immunolabeling (4G8 antibody) in the frontal cortex of a 13-year-old cat. Image taken from Gunn-Moore* et al.*, 2006[67]. Scale bars 100 μm and 25 μm*

While there are similarities between the Aβ plaque-like deposits seen in cats and those in humans, the Aβ plaque-like deposits in cats are less mature than those in humans with AD; the Aβ plaques in AD have dense cores, which are rarely seen in the other species[14, 63, 65]. The pattern and distribution of Aβ in aged cats are perhaps more similar to those seen in healthy, non-demented, aged human brains, rather than humans with AD[14, 63-66]. However, cats with Aβ deposits can display abnormal behaviours, such as confusion, excessive vocalisation and wandering[63, 67], although the severity of these behaviours, as yet, does not seem to be well correlated with the extent of Aβ deposition[64].

1.5.2.3 Tau pathology

The cytoskeleton is a complex network that occupies the cytoplasm of a cell. The main function of the cytoskeleton is to maintain the shape, structure and integrity of cells[70, 71]. It is composed of actin filaments, intermediate filaments, and microtubules[71]. Microtubules are composed of tubulin, and are the largest filaments in the cytoskeleton[71].

Maintenance and stability of microtubules is provided by microtubule-associated proteins (MAP)[70]. Tau is a MAP that is mainly located in the cytosol

and the axons of the neurons where it regulates tubulin stability[70, 72], maintaining the structure of the microtubules[54].

A total of six tau isoforms have been identified in the adult human brain, they result from alternative RNA splicing of exons 2, 3, and 10[73, 74]. Differences between isoforms rely on either the absence, or the presence of one or two 29 amino acid inserts in the N-terminus, which are encoded by exons 2 and 3[70, 74]; plus the presence of either three-repeats (3R) or four-repeats (4R) of 31-33 amino acids in the C-terminus, where the microtubule binding domain (MTBD) is located, which are encoded by exon 10[70, 74] (Figure 1.7).

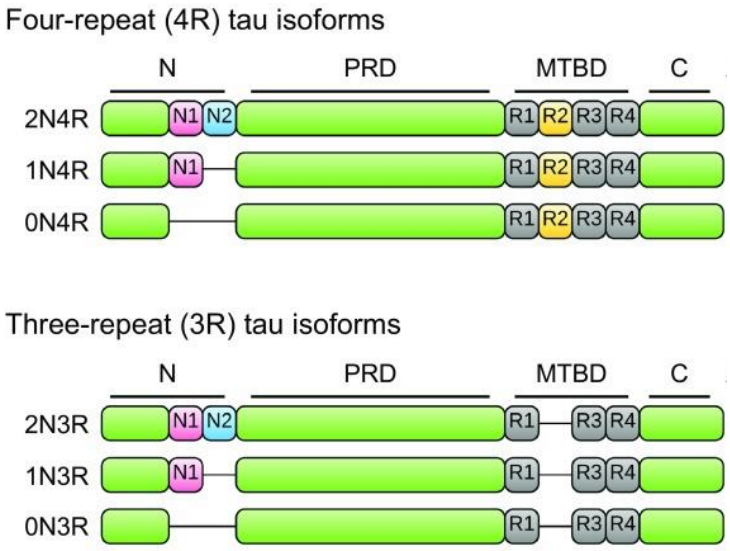

Figure 1.7 Tau isoforms in the human brain. *Tau isoforms result from alternative RNA splicing. Splicing of exons 2 and 3 in the N-terminus results in either the absence (0N) or presence of one (1N; exon 2) or two inserts (2N; exons 2 and 3). The splicing of exon 10, within the microtubule binding domain (MTBD) results in 3-repeat or 4-repeat tau isoforms. The central region of tau consists in a proline-rich domain (PRD). C-terminus remains the same in all six tau isoforms. Image modified from Guo* et al., *2017*[75]

Tau has a total of 85 phosphorylation sites, which are mainly located in the proline-rich domain (PRD) and the C-terminus[70, 73]. It is believed that tau phosphorylation regulates its capacity to bind and stabilise microtubules[70]; hence, abnormal phosphorylation affects its binding ability. While reduced phosphorylation or hypophosphorylation facilitates and gives tau a high affinity

to bind to microtubules; hyperphosphorylation, on the other hand, reduces its binding capacity, jeopardising microtubule stability[70].

When abnormally hyperphosphorylated, tau fragments (of one or more isoforms) tend to aggregate and form paired, twisted fibrils of approximately 10nm in diameter, known as paired helical filaments (PHF)[53, 54, 70]. When PHF accumulate in the dendrites and/or axons, they form dense networks of filaments called neuropil threads; and when they aggregate within the neuronal bodies, they are called neurofibrillary tangles (NFT)[53, 54, 70, 73] (Figure 1.8).

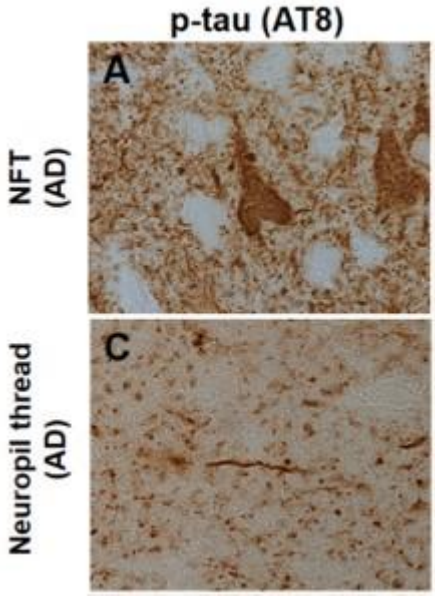

Figure 1.8 Hyperphosphorylated tau in the human brain. *Fragments of hyperphosphorylated tau accumulate and form paired helical filaments (PHF). When accumulated within the neuronal body, aggregates of PHF are known as neurofibrillary tangles (NFT); in contrast, if accumulation occurs in the axons and/or dendrites of neurons, they are known as neuropil threads. Image modified from Kurihara* et al., *2020[76]. Antibody AT8. Scale bars 20 μm*

Under normal conditions, all tau isoforms are highly expressed in human brain tissue[73]. They have also been found in people with AD; however, they are found in the hyperphosphorylated state[73].

1.5.2.3.1 Types of NFT

Pre-tangles

Pre-tangles are considered to be the earliest stage of NFT[77]. In these, hyperphosphorylated tau aggregates occupy the whole cell, including the axons[77] (Figure 1.9A). During this stage, neither the cell nor the neuronal processes show any significant shape alterations[77].

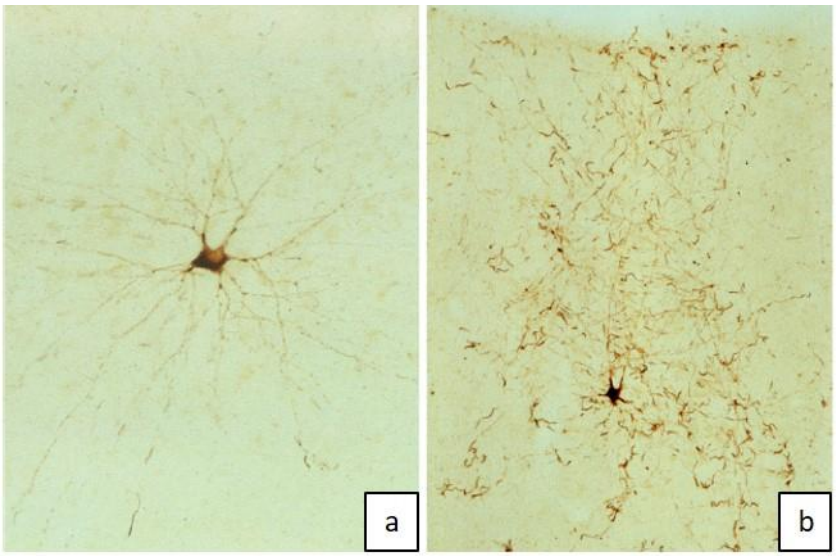

Figure 1.9 Types of neurofibrillary tangles (NFT) in the human brain. *Different types of NFT have been identified, including a) pre-tangles; and b) mature NFT. Image modified from Braak and Tredici (2010)[77]. Antibody AT8. Scale bar 100 μm*

Mature NFT

These are characterised as containing compact aggregates of hyperphosphorylated tau within their cytoplasm, which tend to displace the nucleus to the periphery of the soma[78] (Figure 1.9B).

Unlike the pre-tangles, mature NFT have not been shown to aggregate within the axons; however, neuropil threads may still be present[77], with the affected dendrites becoming shorter, and they may detach from the soma[77].

Depending on their location within the cell, NFT can be classified as *globose*, if they remained within the cell body, occupying most of the soma[77]; or as *flame type*, if they fill the soma and spread in to the proximal dendrites[77] (Figure 1.10A).

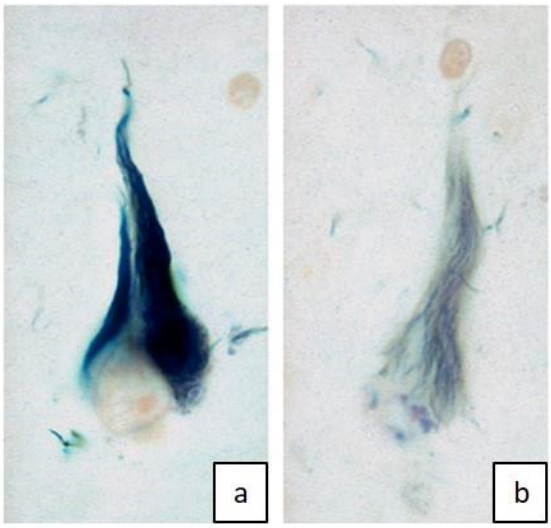

Figure 1.10 Types of neurofibrillary tangles (NFT) in the human brain. *Different types of NFT have been identified, including a) flame type NFT; and b) ghost tangles. Image modified from Braak and Tredici (2010)[77]. Antibody AT8. Scale bar 100 μm*

Ghost tangles

These are thought to be the remnants of mature NFT after the host neuron dies; however, the NFT stays as a visible extraneuronal aggregate[77]. As a result, ghost tangles do not have a nucleus[77] (Figure 1.10B).

1.5.2.4 Progression of tau pathology in AD

Braak and Braak identified the progression of tau pathology in AD and created six stages. Stages I and II are known as “Transentorhinal stages”; Stages III and IV are known as “Limbic Stages”; and Stages V and VI are known as “Cortical stages”[60] (Figure 1.11).

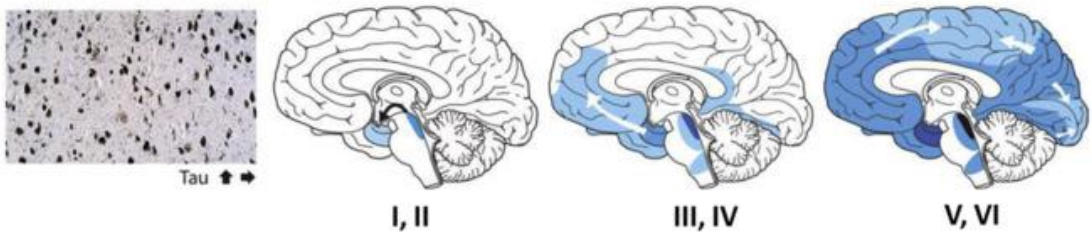

Figure 1.11 Progression of tau pathology in Alzheimer's disease (AD). *Tau pathology begins in the transentorhinal region (stage I); as the disease progresses, tau pathology spreads to the entorhinal cortex and hippocampus (stages II and III); then to the cortical regions (stages IV-VI) in later stages of disease. Image modified from Jouanne* et al.*, 2017*[62]

Stage I

In stage I, a modest number of NFT are confined to the transentorhinal region of the brain, which is located between the entorhinal cortex and the temporal cortex[60].

Stage II

Stage II is an aggravation of stage I. In stage II, several NFT are found in the transentorhinal cortex. In addition, moderate tau pathology appears in the entorhinal cortex and in the hippocampus[60].

Stage III

In Stage III, tau pathology severely affects both the transentorhinal and the entorhinal regions, where ghost tangles can be seen for the first time. Moderate number of NFT are found in the hippocampus and a few may start to develop in the cortex[60].

Stage IV

In Stage IV, both the transentorhinal and entorhinal regions are severely affected, with a large number of ghost tangles. In addition, several NFT are found in the hippocampus, and moderate numbers can be found in the cortex and the amygdala[60].

Stage V

In Stage V, severe pathology and very large numbers of ghost tangles are found in both the transentorhinal and entorhinal regions. Severe pathology is also observed in the hippocampus, all cortex regions, and the amygdala[60].

Stage VI

In Stage VI, the changes seen in Stage V are even more pronounced[60].

Interestingly, severe tau pathology has been correlated with large deposits of Aβ pathology; in contrast, severe Aβ pathology does not necessarily correlate with a large number of NFT[60]. Furthermore, the presence and location of NFT, plus the progression of tau pathology has been correlated with both the clinical symptoms of AD and cognitive decline[54, 60, 70, 79].

Finally, tau pathology or "tauopathies" are not exclusive of AD and have been linked to other diseases including, but not limited to Down Syndrome, Parkinson's disease and its related dementia, plus other forms of dementia, such as frontotemporal dementia[54, 70].

Cats produce a number of different tau isoforms similar to those in humans with AD[52, 64, 67]. While some studies found no evidence of neurofibrillary tangles (NFT) in the brains of old cats[65, 80], others found occasional hyperphosphorylated tau deposits, which are an early stage of NFT (i.e., pre-tangles)[52, 64, 67] (Figure 1.12). As there is only evidence of hyperphosphorylated tau in a small number of cats so far, an association between NFT and CDS cannot yet be established[14, 52, 67]. Interestingly, intracytoplasmic hyperphosphorylated tau is also found in kittens during early postnatal development[81] and as a result of ischaemia and seizures[64, 67].

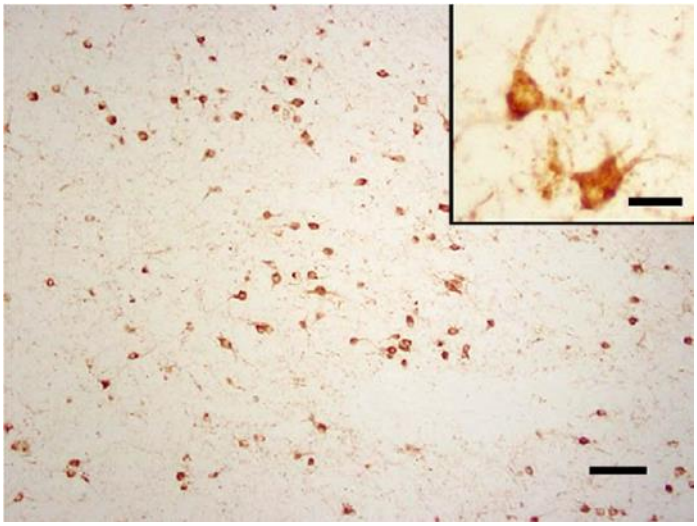

Figure 1.12 Hyperphosphorylated tau deposits in the cat brain. *Positive immunolabelling of hyperphosphorylated tau protein (AT8 antibody) in the anterior cerebrum of a 14-year-old cat. Image taken from Gunn-Moore* et al.*, 2006[67]. Scale bars 100 μm and 25 μm*

Further discussion on the neuropathology of feline ageing and CDS, plus its similarities with AD are discussed in Chapter 2 of this thesis.

1.5.2.5 Vascular pathology

Several vascular and perivascular changes have been associated with neuropathological ageing and CDS in cats. These include micro-haemorrhages, infarcts, a non-lipid variety of arteriosclerosis, and the accumulation of β-amyloid in plaques and sometimes around blood vessels in the brain[13, 14, 63].

Cerebral amyloid angiopathy occurs when Aβ accumulates around the meninges and blood vessels[82, 83]. It is believed to be a major cause of vascular dysfunction and cognitive decline in humans[84-86]; however, it can also be found in the brains of healthy, non-demented people, suggesting that this pathology is not specific to AD[82]. In cats, there is controversy regarding the existence of cerebral amyloid angiopathy. Some studies have found this in aged cat brains[3, 63-65, 67], while some have not[52].

1.6 Diagnosis

The diagnosis of CDS is challenging, especially as the clinical signs involve behavioural changes, which can be nebulous and complicated to investigate. Before making a diagnosis of CDS it is necessary to rule out other potential causes of the behavioural changes[8, 14, 15]. These include pain, hypertension, chronic, endocrine and infectious diseases (Table 1.1).

Table 1.1 Potential causes for behavioural changes. *Diagnosis of cognitive dysfunction syndrome (CDS) can only be made by ruling out other potential causes of the behavioural changes*

Potential causes for behavioural changes
• Hypertension • Pain (e.g., due to osteoarthritis or other musculoskeletal problems, gastrointestinal, pancreatic or dental disease, etc.; pain may be a more insidious cause of behavioural changes in cats than previously considered[87-89], for this reason, an analgesia trial may be needed to determine whether or not it is playing a role in the behavioural alterations) • Chronic diseases (e.g., liver or kidney failure) • Endocrine disorders (e.g., hyperthyroidism – which can cause many of the same behavioural changes as those seen with CDS – or diabetes mellitus) • Infectious diseases (e.g., toxoplasmosis, feline immunodeficiency virus [FIV], feline leukaemia virus [FeLV], urinary tract infections) • True behavioural problems (e.g., separation anxiety) • Neoplasia (e.g., meningioma) • Inflammatory diseases

Of note, some of these conditions may exacerbate the clinical signs of CDS, such as pain, hyperthyroidism, hypertension, and chronic kidney disease, and since elderly cats are more likely to have a number of concurrent interacting conditions, this can complicate both diagnosis and treatment[90].

Veterinary surgeons have to conduct a complete evaluation of the cat, assessing its history for medical and behavioural problems, performing a full physical examination (including determining systemic blood pressure), undertaking blood and urine analysis (as needed), and pursuing further diagnostics in many cases (Figure 1.13). Only then can the veterinary surgeon identify any underlying cause(s) for the behavioural signs. Elderly cats frequently have a number of concomitant interacting conditions, so it can be difficult to establish exactly what role CDS may be playing. However, it is necessary to identify all contributing disorders in order to allow for accurate intervention[8]. Veterinarians need to educate owners on how to recognise and monitor changes in their cat's behaviour, and to report not only any further changes in these behaviours, but also any changes in their cat's body weight, food/water consumption, and urine/faeces production[90].

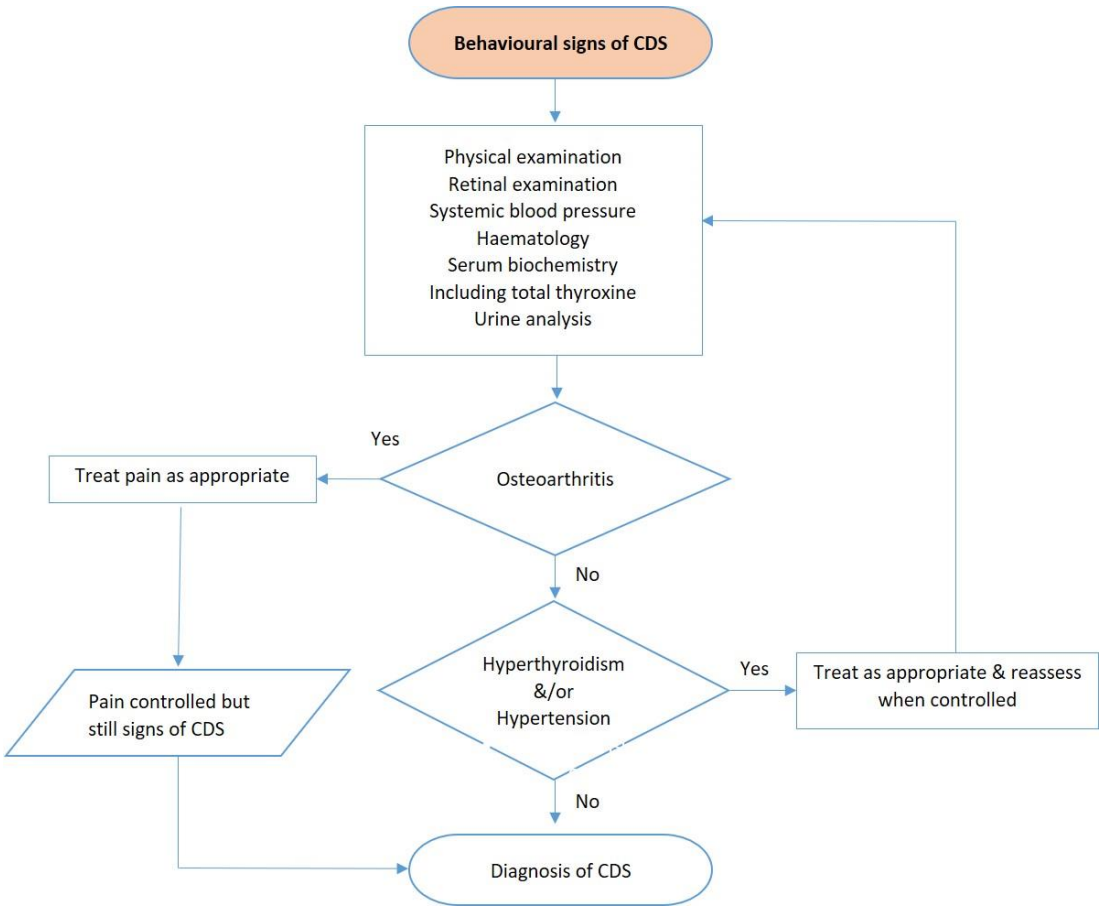

Figure 1.13 Diagnosis of cognitive dysfunction syndrome (CDS). *Veterinarians have to undertake a complete examination of the cat and rule out other potential causes of behavioural changes. Diagram modified from Černá* et al.*, 2020*[19]

It is clear that diagnosing CDS can be a slow and time-consuming task. Moreover, making an early diagnosis is critical, especially with elderly patients, in order to provide prompt interventions that will improve the quality of life and welfare of the affected animals.

Advanced imaging techniques, such as magnetic resonance (MR) imaging and computed tomography (CT) have been shown to be effective diagnostic tools for age-related brain changes and AD in humans[33, 34], especially as some of these changes (i.e., enlargement of the ventricles and hippocampal atrophy) can be detected decades before making a diagnosis[91]. Furthermore, MR imaging has proven to be an efficient method to assess the progression of brain changes in AD[92-95]. It is therefore possible that imaging techniques could

aid in the diagnosis of age-related brain changes and CDS in cats. The use of MR imaging for the assessment of brain atrophy and other age-related brain changes in cats can be found in Chapter 3.

1.7 Management

Although CDS cannot be cured, appropriate management can reduce its clinical impact, and improve the cats' quality of life. Since it is stressful when a loved cat becomes disoriented and confused – and increased vocalisation at night (i.e., night-crying) can cause broken nights – improving the cat's quality of life can also help the owner, and preserve the cat-owner bond. Potential interventions include environmental enrichment/modification, dietary supplementations, specific diets and medication. Interventions need to be tailored to each individual cat, and its specific behavioural changes as suggested by the authors' recent study[19] (Figures 1.14 and 1.15), as well as any concomitant illnesses. Unfortunately, as yet, there is little information to indicate which interventions are most likely to help in specific cats, leading to trial and error, which is not ideal in cats that are easily frustrated. More work is urgently needed in this area.

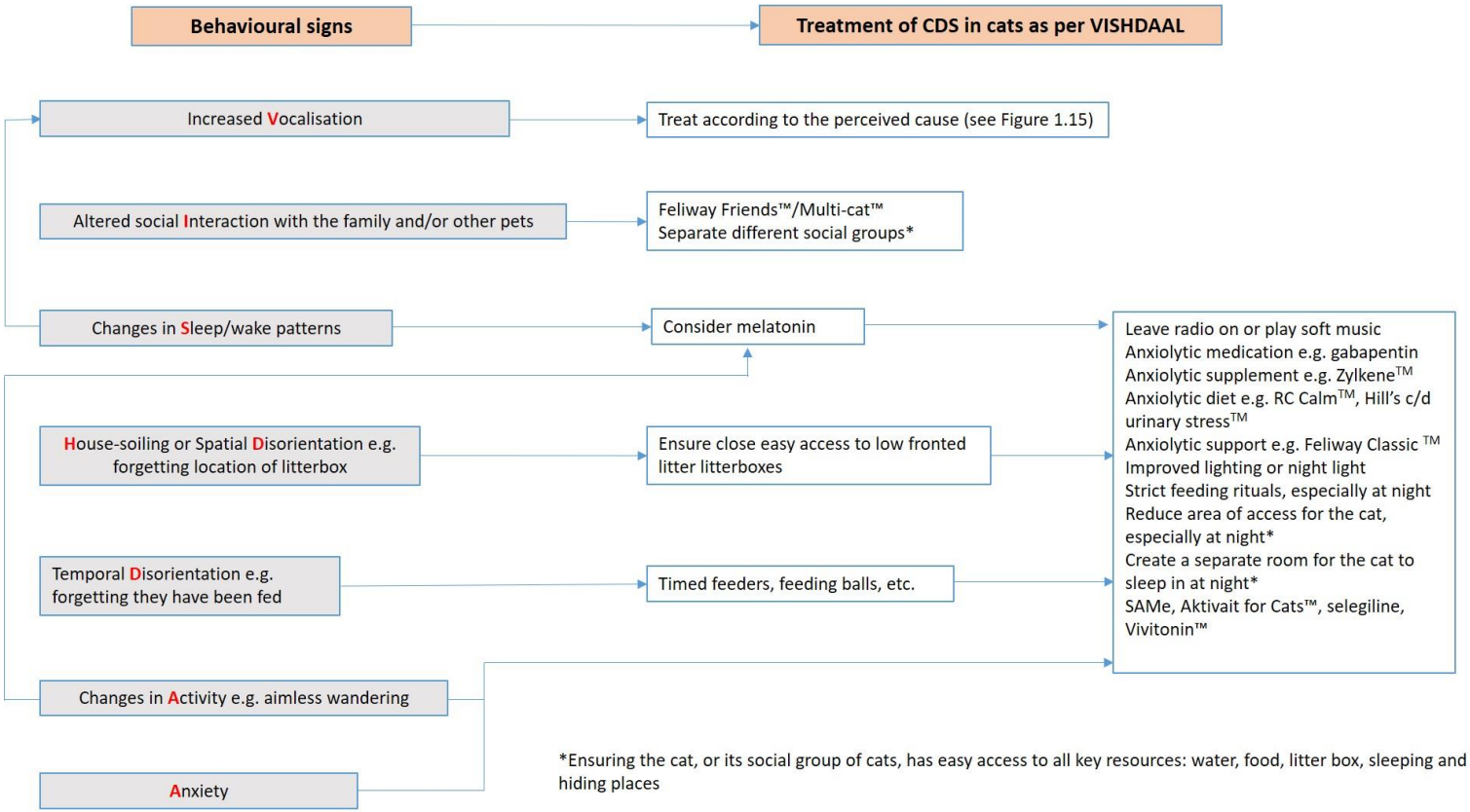

Figure 1.14 Treatment of cognitive dysfunction syndrome (CDS). *Treatment of CDS targeting the behavioural changes displayed by the cat. Diagram taken from Sordo* et. al., *2020*[20]

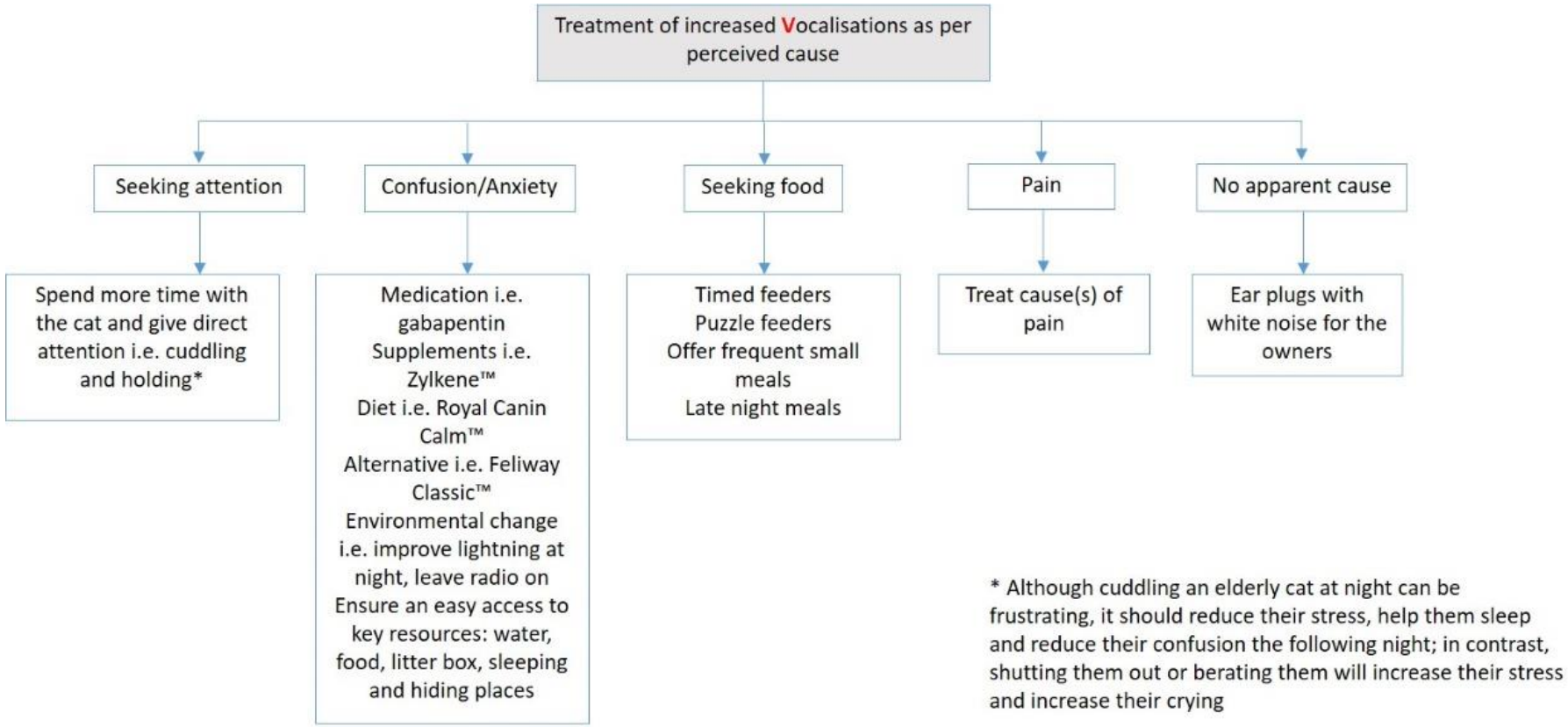

Figure 1.15 Treatment of increased vocalisation as per perceived cause. *Diagram modified from Černá* et. al., *2020*[19]

1.7.1 Environmental enrichment/modification

Environmental enrichment provides mental stimulation and increases activity levels; it enhances the growth and survival of neurons, and improves cognition[14, 96]. Environmental enrichment can be synergistic with antioxidant-enriched diets.

Poor environmental stimulation increases the risk of developing CDS in later life; it can also cause frustration in cats that have already developed CDS, aggravating it[97]. Environmental enrichment is recommended for all young cats, especially if they have no access outside. Environmental enrichment includes activities and objects that promote play, exploration, hunting, climbing, and perching behaviours, and includes novel ways to obtain food or treats[8].

However, in older cats with significant to severe CDS, environmental changes can potentially have a negative effect, leading to confusion. These animals cannot cope with changes (e.g., environment, daily routine, diet) and become stressed, thus, exacerbating CDS (e.g., anorexia, hiding, house-soiling)[17, 98, 99]. It is the best for these cats to keep changes to a minimum or to reduce the size of their environment to minimise stress, while ensuring that their key resources are all within easy reach (i.e., food, water, litter box, resting/sleeping places, and escape routes and/or a safe place to hide)[97]. Where changes have to be made, for example, where they may improve the cat's quality of life, they need to be made slowly; reassuring the cat frequently, to ensure it does not become too stressed[20]. Since osteoarthritis is very common in older cats[87], these resources need to be on all floors of the home that the cat can access; raised food bowls can help, as can low fronted litterboxes, etc.[10]. The application of synthetic feline pheromone diffusers may help to reduce anxiety (Feliway Classic™; Ceva Animal Health Ltd., Zenifel™; Virbac UK) and reduce conflict with other cats (Feliway Friends™; Ceva Animal Health Ltd.)[100].

Environmental needs will differ for each cat. Cats that cry for food may be helped by introducing an automatic feeder timed to give many small meals throughout the night, scatter feeding, forage feeding, or being given a feeding ball at night[20]. Cats which cry for their owner's attention may need positive affirmation and reassurance (often at night). Anecdotally evidence showed that while cuddling an elderly cat at night can be frustrating, it will reduce their stress, helping them sleep and so reduce their confusion the following night; in contrast, shutting them out or shouting at them will increase their stress, and so increase their crying. Cats which cry when they wake up confused and disorientated may be helped by having a night-light, leaving a radio on to play soft music or a speech radio station, plugging in synthetic pheromone dispensers (e.g., Feliway Classic™), or reducing the area the cat has access to so it cannot get lost so easily[19]. Ultimately, the cat may need to have a room of its own to sleep in, with all of its key resources, including a warm comfortable bed (e.g., a heat pad under a snuggle bed plus a blanket)[90, 101]. A strict night-time routine with a warm meal, warm bedding and a cuddle before shutting the door at night may help them to settle. Adding a 'kitty camera' can help to reduce owner stress as they can then check on the cat, without waking it up[20].

1.7.2 Dietary supplements

S-adenosyl-L-methionine (SAMe) (NoviSAMe™; generic SAMe) helps to maintain the fluidity of cell membranes and enhances the production of the antioxidant glutathione[102], and when given to elderly cats, there was an improvement in cognitive tests, including object discrimination and reversal learning; the effects were most evident in cats with mild CDS, suggesting that it is more likely to help early in disease[103].

While proprietary dietary supplements, such as Senilife™ (CEVA Animal Health)[8, 104], and Aktivait™ (VetPlus)[105], have been shown to reduce signs of CDS in dogs, there is little evidence of their efficacy in cats. Senilife™ contains

gingko biloba (a natural ingredient that has been proposed to improve memory and cogniton)[106], antioxidants such as D-alpha-tocopherol, vitamins B6 and E, and resveratrol (a natural compound that has antioxidant, antiinflammatory, cardioprotective, and antitumor properties)[107], amongst other components. Although it is labelled for use in cats, no trials have yet been performed in this species.

Aktivait™ contains omega-3, fish oils, and antioxidants such as vitamins E and C, L-carnitine, alpha-lipoic acid, and phosphotidylserine, amongst other compounds. It is important to highlight that products developed for dogs are not always safe for cats, for example, Aktivait™ as it contains alpha-lipoic acid, and antioxidant which is toxic in cats[108]. A feline-safe version of Aktivait™ has been commercially released; however, trials still need to determine its efficacy.

Complementary remedies such as melatonin, plug-in pheromones[8, 15] (Feliway Classic™ and/or Friends™ aka Multicat™ Ceva Animal Health Ltd.), suntheanine (Pet Remedy™ and/or Anxitane™ Virbac), milk protein hydrolysate (Zylkene™ Intervet Schering Plough)[109], essential oils (e.g., lavender)[8, 15, 17] and amino acid/herbal combinations (e.g., Help My Pet – Nerves™, VetPartners Limited), may help in re-establishing the sleep-wake cycles and reducing anxiety.

1.7.3 Specific diets

Diets high in fruits, vegetables, nuts and whole grains, as well as vitamins C, E and B_{12}, may potentially improve cognition and delay the development of dementia in humans; deficiency in these vitamins is a risk factor for strokes, brain ageing and dementia[110]. Diets enriched with antioxidants are known to decrease oxidative damage, whilst other compounds, such as alpha-lipoic acid, L-carnitine and omega-3 fatty acids, may have a positive effect in the

management of dementia as they enhance the health of the cell membranes and mitochondrial function[111].

Developing diets to reduce the signs of CDS in cats has proven to be challenging. Some of the compounds used in diets designed for dogs with CDS, for example alpha-lipoic acid, are toxic in cats[108], and the effect of diets enriched with medium-chain triglycerides (MCTs) is as yet unclear, as MCTs are unpalatable to cats[97]. Nevertheless, it is believed that MTC consumption may improve feline metabolism[112].

While no specific diet has been designed for cats with CDS, some diets have shown positive effects. Commercial diets containing fish oils, antioxidants and other supplements (Feline Mature Adult 7+™ Hill's Pet Nutrition); and others containing antioxidants, essential fatty acids and dried chicory root (Nestlé Purina Pro Plan Age 7+™ Nestlé Purina Pet Care) have been associated with increased longevity compared with non-supplemented diets[113, 114]. Another study by Nestlé Purina showed that the brain function of middle-aged and older cats improved significantly when fed a diet supplemented with fish oils, antioxidants, arginine, and vitamin B[115]. A separate study demonstrated that activity levels in aged cats increased when fed with a diet containing tocopherols, vitamin C, beta-carotene, and L-carnitine, amongst other ingredients[116]. Finally, a two-month long questionnaire-based study showed that CDS signs improved in around 70% of the cats that were fed a diet containing antioxidants, essential fatty acids, chondroprotectants, L-carnitine, and lysine, designed to help osteoarthritis (Prescription Diet Feline j/d™ Hill's Pet Nutrition) (Hill's Pet Nutrition, data on file, 2008).

Diets supplemented with milk protein hydrolysate and additional L-tryptophan (i.e., α-casozepine) have been shown to reduce anxiety (Royal Canin Calm™ Royal Canin; Hill's Urinary Support™ Hill's Pet Nutrition)[117]. [118, 119]

1.7.4 Drug treatments

Selegiline (Selgian™ Ceva Animal Health Ltd.; Anipryl™ Zoetis) and propentofylline (Vivitonin™ MSD Animal Health) are licensed in various countries to treat the clinical signs of CDS in dogs[120-123]. Selegiline has undergone many studies, although all in dogs[120, 121, 123]. It is an inhibitor of monoamine oxidase B, which has a neuroprotective effect as it reduces the production of free radicals and stimulates the production of enzymes that eliminate these free radicals. Propentofylline has neuroprotective properties and increases the blood flow to the heart and brain[8]. Despite these drugs having been shown to have positive effects in dogs with CDS[124, 125], they are not licensed for use in cats; however, positive effects have been anecdotally reported.

Anxiety and altered sleeping patterns are common features in pets with CDS. Antidepressants/anxiolytics, such as fluoxetine (i.e., Prozac™), have been used to treat the signs of CDS in pet cats[97]. Other anxiolytics, such as trazodone, gabapentin, benzodiazepines (e.g., alprazolam, diazepam, clonazepam, and lorazepam), or buspirone can be considered[8, 97]. However, diazepam in tablet form is not recommended as it may cause acute fulminant liver failure in cats[126], and care should be taken when combining drugs, such as selegiline, fluoxetine, gabapentin and others that may affect the concentration of serotonin as they could induce serotonin syndrome, seen as pyrexia, agitation, increased reflexes, tremor, sweating, dilated pupils, and diarrhoea. Melatonin may help to restore the sleep cycles.

Given that cats with CDS have reduced numbers of cholinergic neurons[50], drugs that enhance cholinergic transmission might improve the signs of CDS, such as cholinesterase inhibitor donepezil (Aricept™; Pfizer)[127], which is used in people with AD[128]; however, these have not been tested in cats.

It has been suggested that by blocking the specific receptor AT_1, the renin-angiotensin system (RAS) might be inhibited. Angiotensin receptor blockers (e.g., sartans) are drugs that inhibit the RAS and are commonly used for the treatment of cardiovascular and metabolic disorders (e.g., diabetes and kidney disease)[129, 130]; furthermore, it has been proposed that the antiinflammatory properties of angiotensin receptor blockers may also improve cognition[131, 132]. Telmisartan (Semintra™; Boehringer Ingelheim) blocks the AT_1 receptor of RAS and partially activates the peroxisome proliferator-activated receptor gamma (PPARγ), which regulates neurological disease by preventing inflammation and reducing the accumulation of Aβ plaques in the brain[133-135]. In rodents, telmisartan has been shown to: 1) inhibit inflammation, therefore reducing brain injury after the induction of ischemia[135]; 2) improve post-stroke effects[136]; 3) reduce the death and injury of neurons after glutamate-induced toxicity, which is an important neurotransmitter that, if released in excess, causes neurotoxicity, neuronal death and apoptosis[137]; 4) protect against oxidative damage caused by glucose administration[138]; 5) protect against nutrient deprivation-induced neuronal death[139]; 6) restore cognitive functions after chronic stress-induced cognitive impairment[140]; and 7) prevent cognitive decline[134, 141]. In addition, there are two ongoing studies aimed to assess the efficacy of telmisartan in treating human patients with AD[142]. The effect of telmisartan (Semintra™; Boehringer Ingelheim) on the clinical signs of CDS in cats is discussed on Chapter 4.In summary, ever more elderly cats are being recognised with behavioural changes suggestive of CDS, including increased vocalisation and house-soiling, amongst other signs. However, CDS can be challenging to diagnose; it is a diagnosis of exclusion and many other medical disorders can cause similar behavioural changes. It is essential that veterinary surgeons undertake a full assessment of affected cats and educate owners on how to recognise the clinical signs of CDS. Although CDS cannot be cured, there are several interventions that can help to improve the health, welfare and quality of life of the affected cats. The brains of cats with CDS show neuropathological changes similar to those found in the brains of humans with

AD; these similarities suggest that the domestic cat could be a natural model for AD.

1.8 Hypotheses, aims and objectives

In the present study, it was hypothesised that age-related neuropathological and neuroanatomical changes in cats would show common features with those seen in human AD; furthermore, by better our understanding of these changes, early diagnosis and effective interventions/management of CDS can be provided that will improve the quality of life and welfare of the affected cats.

The present work assessed neuropathological age-related changes in cats with and without CDS (see Chapter 2); furthermore it proposes the use of advanced imaging techniques, such as MR imaging as an *in vivo* tool for the assessement of age-related brain changes that could be considered early signs of CDS (see Chapter 3); and finally, this work proposes the drug telmisartan (Semintra™, Boehringer Ingelheim) as a potential treatment to reduce the clinical signs of severe CDS in cats (see Chapter 4).

The present study had three aims:

1. The first aim of the study was to assess the presence and distribution of Aβ and tau pathology in the brains of cats of various ages, with and without CDS. It was hypothesised that elderly cats would have larger Aβ deposits and higher numbers of hyperphosphorylated tau deposits than younger cats. It was also hypothesised that these changes would be more evident in cats with CDS. Finally, it was hypothesised that these neuropathological changes will show similar features to those seen in the human AD brain.

2. The second aim of this study was to ascertain whether there is age-related atrophy of the whole brain and/or specific brain structures, including the lobes, hippocampus, and the interthalamic adhesion, plus enlargement of the ventricles in cats by undertaking measurements of 1.5T MR images. It was hypothesised that elderly cats would have a marked atrophy of the whole brain and some of the different brain structures assessed (i.e., lobes

and hippocampus). Furthermore, it was also hypothesised that elderly cats, particularly those with diagnosis of CDS, would have larger lateral ventricles and smaller interthalamic adhesions than younger cats. Finally, it was hypothesised that these age-related neuroanatomical changes will share similarities to those seen in human AD.

3. The third aim of this study was to determine the effects of telmisartan (Semintra™, Boehringer Ingelheim) on the clinical signs of CDS in cats at its licensed dose (at the time this study commenced) of 1 mg/kg PO q24h, given for three months. It was hypothesized that the clinical signs of CDS would be significantly reduced in the cats receiving telmisartan (Semintra™, Boehringer Ingelheim).

Chapter 2 Neuropathology of ageing and cognitive dysfunction syndrome in cats

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“What greater gift than the love of a cat?”

-Charles Dickens

Note:

Parts of this chapter have been submitted for publication to the Journal Frontiers of Aging to their special issue “Rising stars in comparative biology of aging” and is currently under review:

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Author’s contribution to the paper:
The author carried out all the experimental work and statistical analyses, as well as writing the paper.

2.1 Introduction

Ageing produces several changes in the brain, including brain atrophy, neuronal loss, and vascular changes, that may ultimately lead to dementia[31, 32, 35, 143, 144]. While these age-related changes have been reported to occur in the brains of people with Alzheimer's disease (AD), the abnormal deposition of amyloid-β senile plaques and neurofibrillary tangles (NFT) of hyperphosphorylated tau proteins are thought to play a key role in the development of AD, and are considered to be the major pathognomonic neuropathologies[53]. Interestingly, similar neurodegenerative changes (i.e., brain atrophy, neuronal loss, vascular changes, and abnormal protein deposition) also occur in the brains of elderly cats[64, 67].

2.1.1 Amyloid-β pathology

Amyloid-β (Aβ) is generated in the amyloidogenic pathway after β- and γ-secretases cleave the amyloid precursor protein (APP) (See Chapter 1). Since these cleavages occur at different sites, various lengths of Aβ can be formed, with Aβ40 and Aβ42 being the most commonly found forms in human AD. While Aβ40 is the most abundant and tends to accumulate within blood vessels; Aβ42 tends to accumulate in the extracellular space and form the characteristic senile plaques found in human AD[53, 54].

In humans with AD, Aβ pathology is believed to start in the cortex (i.e., Stage I); with a later progression to the white matter and hippocampus (i.e., Stage II); and finally, in late stage of the disease, it reaches the thalamus, hypothalamus and cerebellum (i.e., Stages III-V)[60].

2.1.2 Tau pathology

Tau is a microtubule-associated protein located in the cytosol of the cells that stabilises microtubules[70, 72]. When abnormally hyperphosphorylated, tau aggregates, forming paired helical filaments (PHF) that may later accumulate forming neurofibrillary tangles (NFT)[53, 54, 70, 73] (See Chapter 1).

In humans with AD, tau pathology starts in the transentorhinal regions (i.e., Stages I and II); it later progresses to the hippocampus and begins affecting the cortex regions (i.e., Stages III and IV); and finally, at later stages of disease, it reaches all cortex regions (Stages V and VI)[60].

2.1.3 Amyloid-β pathology in cats

Diffuse Aβ accumulation has been previously reported in the brains of cats over 10 years of age[14, 63-67]. Since these deposits do not have dense cores and share similarities to the diffuse deposits seen in humans, it has been suggested that cats' Aβ deposits are less mature than the characteristic AD senile plaques[14, 63, 65, 145, 146]. Furthermore, unlike human Aβ, deposits in cats are predominantly formed by the Aβ42 peptide[63, 64] and are only detectable using certain antibodies, such as anti-Aβ17-24 (4G8) or the conformation specific Aβ1-42[64]. It is believed that the lack of Aβ40 in cats may be due to its higher solubility, which also promotes rapid clearance; hence, impeding its accumulation in the brain[63, 68, 69]. However, the Aβ40 peptide has been found occasionally within blood vessels in cats, where it is associated with cerebral amyloid angiopathy[65]. It has been suggested that both the pattern and distribution of Aβ in cats are more similar to those found in the elderly, non-demented human brains, rather than people with AD[14, 63-66]. Nevertheless, elderly cats with Aβ deposits are known to display behavioural abnormalities, such as excessive vocalisation, wandering, and confusion[63, 67]; however, the

severity of these behaviours and/or clinical signs does not necessarily correlate with the extent of Aβ deposition[54, 60, 64, 147].

2.1.4 Tau pathology in cats

Cats have been shown to produce similar tau isoforms to those seen in humans[52, 64, 67]; however, there is still controversy regarding the presence of NFT in the brains of elderly cats. While some research suggest that there is no evidence of NFT in cats[65, 80], others have reported intracytoplasmic hyperphosphorylated immunolabelling, which is believed to be an early stage of NFT, also known as pre-tangles[52, 64, 67].

Of note, most of the available evidence on the neuropathological changes associated with ageing in cats has been obtained from elderly cats with no diagnosis of CDS[52, 63, 65, 66]. Only two papers have assessed these changes in aged cats displaying behavioural and/or neurological abnormalities[64, 67]. One study assessed neuropathological changes in six elderly cats (aged from 16 to 20 years old) plus two young cats (< 4 years old). Five of the elderly cats displayed between one and five signs of CDS; however, these cats also had concomitant illnesses, such as anaemia, seizures, and intestinal bowel disease[64]. Hence, it is possible that some of the apparent signs of CDS displayed by these cats were actually caused by their concomitant diseases, instead than CDS itself. A separate study assessed 19 cats aged from 16 weeks to 14 years old, with 17 of these cats displaying signs of neurological dysfunction[67]. While the exact neurological disorders and/or signs were not described in this study, it is possible that undisclosed systemic disorders were the cause of the neuropathological changes.

The aim of the present study was to assess the presence and distribution of Aβ and tau pathology in the brains of cats of various ages, with and without CDS. It was hypothesised that elderly cats would have larger and more widely

distributed extracellular Aβ deposits, and higher numbers and more widely dispersed hyperphosphorylated tau deposits, than younger cats. It was also hypothesised that these changes would be more evident in cats with CDS.

2.2 Materials and Methods

2.2.1 Sample collection

The brains of cats of different ages were collected *post-mortem* through routine necropsy submissions. Some of these cats were patients at The Royal (Dick) School of Veterinary Studies (RDSVS) and others came from a shelter in Scotland. Most of the cats were affected by old age and/or chronic disease and were euthanised after veterinary assessments revealed that no further treatment could have improved their health. A small number of cats were euthanised after being diagnosed with behavioural problems (i.e., untreatable aggression) that would impede their rehoming. Of note, none of the cats displayed any signs of neurological disease. In all cases the owners or shelter carers gave written consent to donate the cats' bodies for research purposes.

Some of the cats used in this study were diagnosed with CDS before euthanasia. Diagnosis of CDS was determined as detailed in Chapter 4.

After collection, brains were fixed by immersion in 10% buffered formalin for no less than one week.

After fixation, transversal sections of the brain were taken from the rostral cortex (Figure 2.1A); parietal cortex, including hippocampus (Figure 2.1B); occipital cortex, including the entorhinal cortex (Figure 2.1C); the locus coeruleus and the cerebellum (Figure 2.1D).

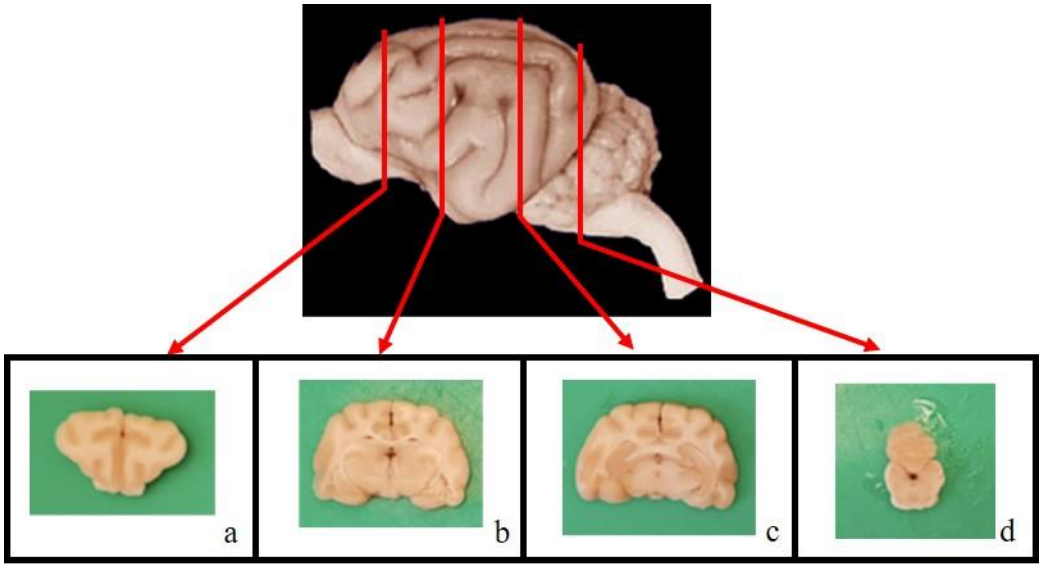

Figure 2.1 Sectioning of the cat brains. *Transversal sections of the brain were taken from a) the rostral cortex; b) the parietal cortex, including the hippocampus; c) the occipital cortex, including the entorhinal cortex; and d) the locus coeruleus and the cerebellum*

Sections were processed overnight in hot paraffin (Leica Tissue Processor ASP300S; Leica Biosystems™) and blocks were made. Consecutive slices of 6 μm were taken from the blocks by using a microtome (Microtome HM325; Leica Biosystems™) and mounted on positively charged glass slides (Polysine®, VWR™).

2.2.2 Grouping of cats

Since the exact age of some of the cats was less certain, particularly in those cats coming from the shelter, age groups were created following the American Association of Feline Practitioners (AAFP) and the American Animal Hospital Association (AAHA) life stages guidelines (Table 2.1)[148]. In addition, this allowed the assessment of neuropathological changes at the different life stages and to determine whether there were differences between them.

Table 2.1 Grouping of cats. *Cats were grouped into different categories according to their age following the American Association of Feline Practitioners (AAFP) and the American Animal Hospital Association (AAHA) life stages guidelines*

Category	Age	Number of cats	CDS
Kitten	0 to 6 months	n = 0	No
Junior	7 months to 2 years	n = 0	No
Prime	3 to 6 years	n = 6	No
Mature	7 to 10 years	n = 24	No
Senior	11 to 14 years	n = 9	Yes (n = 1)
Super Senior (Geriatric)	15 years and over	n = 16	Yes (n = 9)

2.2.3 Immunohistochemistry (IHC)

Consecutive 6 μm sections were taken from the rostral lobes, parietal lobes (including the hippocampus), and occipital lobes (including the entorhinal cortex); the cerebellum and locus coeruleus were also sectioned. After dewaxing and rehydrating sections, antigen retrieval pre-treatment was performed by immersing slides in 90% formic acid for six hours[149] for antibody 4G8, and heat incubation (120 °C for 40 minutes) in Sodium Citrate Buffer (10 mM, pH 6) for the rest of the antibodies. All antibodies were incubated in 3% hydrogen peroxide in 10% methanol for 30 minutes to block endogenous peroxidase activity. Sections were incubated overnight at room temperature in each of the primary antibodies: two anti-β-amyloid and six anti-tau antibodies, listed in Table 2.2. Bound antibody was detected by incubating slides in biotinylated goat anti-mouse/rabbit antibodies (1:500; ThermoFisher Scientific™) for one hour, followed by incubation in ABC peroxidase (1:100; ThermoFisher Scientific™) for 30 minutes. Slides were visualised with 3,3'-diaminobenzidine substrate (1:100; DAB; Sigma-Aldrich™) under light

microscope (Nikon™ Brightfield). Negative controls were used by omitting the primary antibodies.

Table 2.2 Primary antibodies used for the detection of amyloid-β and tau proteins

Clone	Antigen	Dilution	Source
Amyloid-β			
4G8	Anti-β-amyloid 17-24 and APP	1:1500	Biolegend™; San Diego, CA
mOC64	Anti-β-amyloid 1-42	1:6000	Abcam™; Cambridge, UK
Total tau and tau isoforms			
8E6/C11	Anti-tau 3-repeat isoform (RD3)	1:1000	Sigma-Aldrich™; Missouri, USA
1E1/A6	Anti-tau 4-repeat isoform (RD4)	1:1000	Sigma-Aldrich™; Missouri, USA
Tau46	Anti-tau 404-421	1:1000	Biolegend™; San Diego, CA
Hyperphosphorylated tau			
AT8	Phospho-PHF-tau pSer202+Thr205	1:200	ThermoFisher Scientific™; Massachusetts, USA
A15091A	Purified anti-Tau phospho (Ser 262)	1:1000	Biolegend™; San Diego, CA
AT100	Phospho-PHF-tau pThr212+Ser214	1:1000	ThermoFisher Scientific™; Massachusetts, USA

In addition, Congo red and thioflavin-S staining were performed for Aβ detection; plus, Silver-Gallyas for neurofibrillary tangles.

2.2.4 Validation of intranuclear hyperphosphorylated tau immunolabelling

2.2.4.1 Isotype controls

To validate that intranuclear immunolabelling was not caused by an artifact or non-specific staining, IHC was performed following the above protocol and using antibodies with the same isotypes for AT8 (mouse IgG isotype control; ThermoFisher Scientific™), AT100 (mouse IgG1 kappa isotype control; ThermoFisher Scientific™), and A15091A antibodies (mouse IgG2b kappa isotype control; Biolegend™) as negative controls. Isotype controls are antibodies that have the same class and type of the primary antibody, but do not bind to the target.

2.2.4.2 Dephosphorylation of the tau protein

To validate that the intranuclear labelling observed was caused by the hyperphosphorylated state of the protein, instead of the non-phosphorylated one, the tau protein needed to be dephosphorylated. To achieve this, IHC was performed by following the protocol described above, for the same hyperphosphorylated tau antibodies (i.e., AT8, AT100, and A15091A), separately; however, to dephosphorylate the protein, slides were incubated in alkaline phosphatase (FastAP Thermosensitive Alkaline Phosphatase; ThermoFisher Scientific™) following manufacturers' protocol at 37 °C for one hour, followed by three 5-minutes washes in fresh bovine serum albumin, before adding the primary antibodies.

2.2.4.3 Protein extraction

Extracts from the cytoplasm, the cytoskeleton, the soluble nuclear, and the chromatin bound nuclear fractions were obtained from frozen cat brain tissue (a 10-year-old cat) by using the Subcellular Protein Fractionation Kit for Tissues (ThermoFisher Scientific™) following manufacturer's protocol.

2.2.4.4 Western blot

Extracts from the different fractions (15 μl) obtained with the Subcellular Protein Fractionation Kit for Tissues (ThermoFisher Scientific™) were prepared in a buffer containing NuPAGE™ LDS Sample Buffer (4x) and NuPAGE™ MES running buffer (10x) and were loaded into a 12 well NuPAGE™ pre-cast gel, 4-12% bis-tris. The gel ran at 50 V for 20 minutes, followed by 120 V for 20 minutes, and 200 V for 40 minutes. The proteins were then transferred onto an Immobilon-FL PVDF membrane (Millipore™) using a semi-dry electroblotting unit (Trans-Blot® SD semi-dry transfer cell; Bio-Rad™), run at 0.06 A and 18 V for one hour. The PVDF membrane was transferred into LICOR blocking buffer (Licor Biosciences™) for one hour. Primary antibodies were added (AT8, 1:1000; and Tau46, 1:5000), and blots were incubated at 4 °C on a roller overnight. Goat anti-mouse antibodies were used as secondary antibodies (IRdye 680RD; Licor Biosciences™). Analysis was performed using LICOR odyssey (700 nm wavelength).

2.2.5 Quantification of protein deposition

Initially, all of the slides were visually inspected by light microscopy; this included the slides from the rostral, parietal, and occipital regions, plus the cerebellum, that had been obtained after performing IHC with all the nine antibodies.

For the automated quantification of protein deposition, only the slides stained against mOC64 for amyloid-β, and AT8 and AT100 for hyperphosphorylated tau, from all brain regions, were scanned using a Leica Aperio Versa 200 scanner (Leica Biosystems™).

Images were transferred to the Aperio eSlide Manager® v12.4.0.5043 and visualised with the Aperio ImageScope® v12.3.3.5048. Annotation boxes of 600 μm of width, height and length were manually placed at random on each

of the slides; in total five annotation boxes were created for grey matter, and five for white matter; plus, three boxes for hippocampus, and three for cerebellum (Figure 2.2). Fewer boxes were created for the hippocampus and cerebellum as the size of the tissue sections available from these two regions varied between cats.

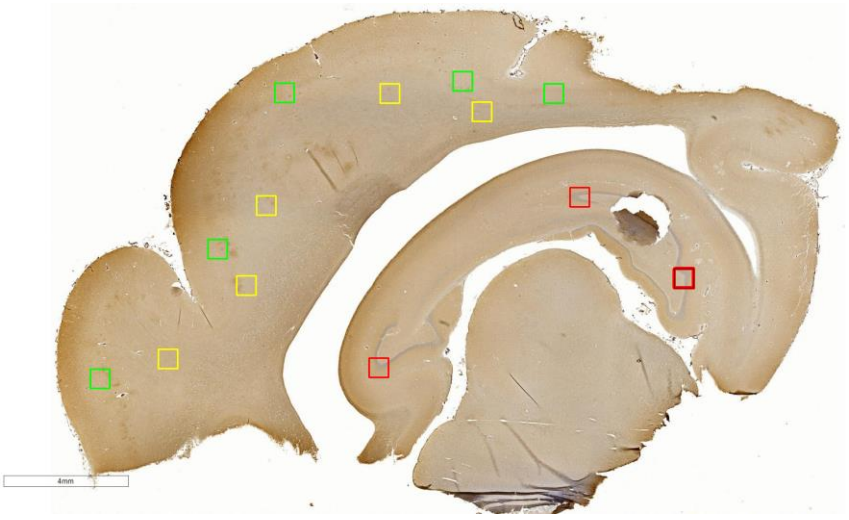

Figure 2.2 Example of annotation boxes on the parietal cortex of a cat brain. *The green boxes correspond to the grey matter; yellow boxes correspond to the white matter; and red boxes correspond to the hippocampus*

To analyse the annotation boxes and quantify protein deposition, three different algorithms were created by the author. Two versions of the Positive Pixel Count v9 algorithm were created by developing different parameters; one to detect the positive immunolabelling (i.e., load and burden; see below) of amyloid-β and another to detect positive immunolabelling (i.e., load and burden; see below) of hyperphosphorylated tau (Figure 2.3).

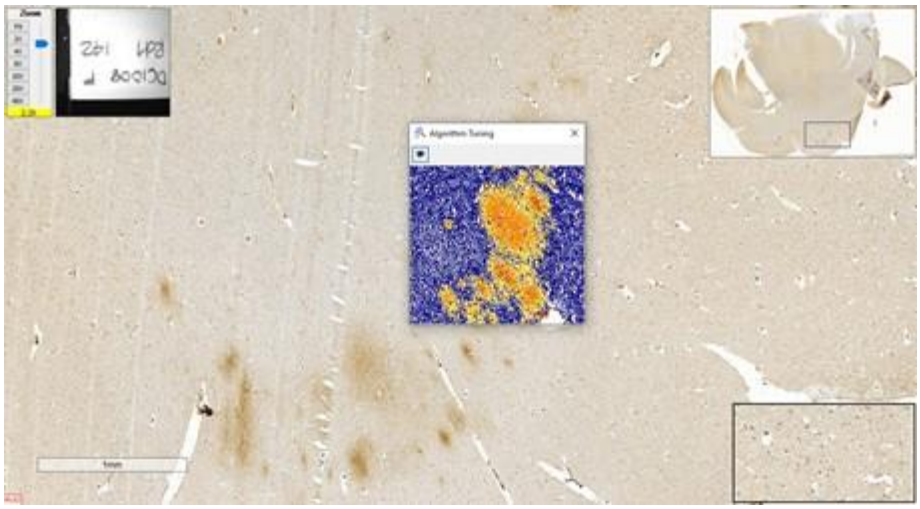

Figure 2.3 Positive Pixel algorithm. *Example of the Positive Pixel algorithm created to detect positive immunolabelling for amyloid-β (mOC64 antibody) in the parietal cortex of a 10-year-old cat. Positive immunolabelling is highlighted in warm colours. Of these, red represents strong staining; orange a moderate staining; and yellow a weak staining. Blue represents no staining*

A third algorithm was created by using the Nuclear Algorithm v9 to detect and quantify intranuclear immunolabelling for hyperphosphorylated tau (Figure 2.4).

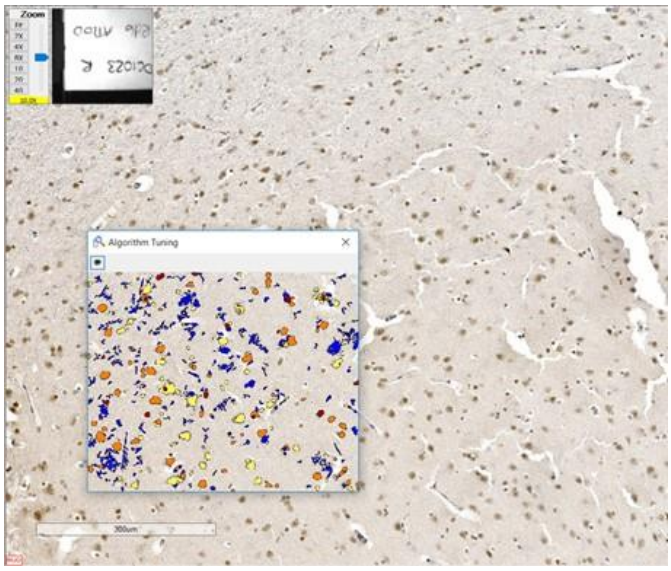

Figure 2.4 Nuclear algorithm. *Example of the Nuclear algorithm created to detect positive intranuclear immunolabelling for hyperphosphorylated tau (AT100 antibody) in the rostral cortex of a 16-year-old cat. Positive immunolabelling is highlighted in warm colours. Of these, red represents strong staining; orange a moderate staining; and yellow a weak staining. Blue represents no staining*

Data from the Positive Pixel Count v9 algorithms were exported to an excel file, where the load (i.e., percentage of total positives) and burden (i.e., the proportion of the total area assessed that contains positive labelling) of both Aβ and hyperphosphorylated tau were calculated, by using the following equations:

$$Load\ of\ protein\ (\%) = \left(\frac{Nwp+Np+Nsp}{Ntotal}\right) x\ 100 \qquad \textit{Equation 2.1}$$

$$Burden\ of\ protein = \frac{\frac{Nwp+Np+Nsp}{Total\ area}}{10{,}000}\ \textit{pixels/mm}^2 \qquad \textit{Equation 2.2}$$

where,

Nwp = Number of weak positives

Np = Number of (moderate) positives

Nsp = Number of strong positives

Ntotal = Number of total positives and negatives

Total area was of 1.8 mm^2 for cortical regions and 1.08 mm^2 for cerebellum and hippocampus. These were calculated by multiplying two dimensions of the annotation boxes (i.e., 600 μm x 600 μm), multiplying the result by the number of annotation boxes created for each region (i.e., multiply by five for cortical regions and by three for hippocampus and cerebellum) and converting the result to mm^2 (1 mm^2 = 1'000,000 μm^2).

Data obtained from the Nuclear Algorithm v9, including the total percentage of nuclei stained and the degree of staining (i.e., 1 +, 2 +, and 3 +) were exported to an excel sheet and used for statistical analyses.

2.2.6 Ethical approval

Ethical approval was obtained through the Veterinary Ethical Review Committee from the RDSVS, The University of Edinburgh (VERC 50.17 and 30.20).

2.2.7 Data analysis

Data were analysed using Minitab® v19.2020.1 statistical software for Windows.

Anderson-Darling tests were performed to assess normality. Non-normal data were transformed using Box-Cox transformation or Johnson transformation. Bartlett tests for equal variances with 95% confidence level, assuming normal distribution were performed. Non-parametric tests were performed in the variables in which transformation failed.

To determine whether there were differences in the positive immunolabelling of the proteins (mOC64 for Aβ, and AT8 and AT100 for hyperphosphorylated tau) in the different age groups, one-way ANOVA assuming equal variances (if applicable) with two-sided 95% confidence interval and a significance level of $\alpha = 0.05$ were performed. To determine differences between groups, Tukey tests and Games-Howell *post-hoc* tests were performed in those structures that showed statistically significant differences in the one-way ANOVA. In addition, non-parametric Kruskal-Wallis tests were performed to assess differences in non-normal data.

To determine whether there were differences in the positive immunolabelling of the proteins between grey and white matter, 2-sample t-tests with a 95% confidence level and a hypothesized difference of zero, assuming equal

variances were performed. Non-parametric Mann-Whitney tests were performed to assess differences in non-normal data.

To determine whether there were differences in the positive immunolabelling of the proteins between cats with and without CDS, 2-sample t-tests were performed as described above. Since cats with CDS were in the Senior and Super Senior groups, these cats were compared against the cats without CDS in these two same groups.

Finally, chi-square tests were performed to determine whether there was an association between cognitive status (i.e., cats with and without CDS) and the presence of pre-tangles.

2.3 Results

2.3.1 Demographics

A total of 55 cat brains were collected following submission for routine necropsy. The majority of cats were non-pedigree cats (98.2%; n = 54) and only one cat was a pedigree breed (a Bengal cat). Half of the cats (50.9%; n = 28) were females; of these, two thirds (67.9%; n = 19) were neutered, and a third (32.1%; n = 9) were intact. A third of the cats (32.7%; n = 18) were males; most of them (88.9%; n = 16) were neutered and only 11.1% (n = 2) were intact. The sex of 16.4% (n = 9) of the cats was unknown, mainly because this was not recorded by the pathologists performing the brain extractions which had occurred several years ago. Ages ranged between two and 24 years. After being grouped by age, six cats were in the Prime group; 24 in the Mature group; nine in the Senior group; and 16 in the Super Senior group. Ten cats had a confirmed diagnosis of CDS, nine of these cats were in the Super Senior group and one was in the Senior group. There were no cats in the Kitten and Junior groups (See table 2.1 in Material and Methods).

2.3.2 Amyloid-β pathology

Cats of all ages were shown to have Aβ pathology (i.e., 4G8 and mOC64 antibodies). Elderly cats had more extracellular Aβ deposition than younger cats. In contrast, younger cats had more immunolabelling for intracytoplasmic Aβ than older cats.

In this section, the results from the intracytoplasmic Aβ deposits with both 4G8 and mOC64 antibodies will be presented first, followed by the results from the extracellular Aβ deposits.

Intracytoplasmic labelling of Aβ was found in a number of cats. To determine whether this represented Aβ or normal APP, dyes that bind specifically to amyloid (i.e., Congo red and thioflavin-S) were used. While Congo red staining was negative, thioflavin-S demonstrated intracytoplasmic punctuated labelling (Figure 2.5).

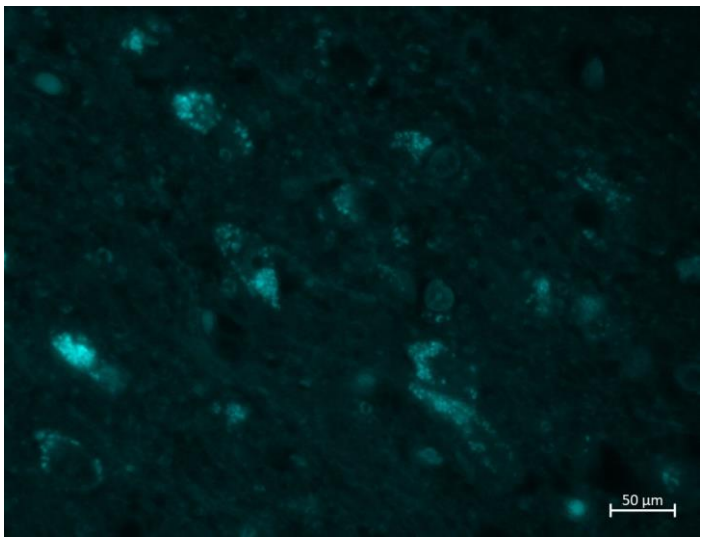

Figure 2.5 Confirmation of intracytoplasmic amyloid-β (Aβ). *The presence of punctuated intracytoplasmic Aβ labelling was confirmed by using Thioflavin-S staining*

Intracytoplasmic immunolabelling was found in the younger cats, using 4G8 antibody, and the percentage of cats with intracytoplasmic immunolabelling decreased with age, particularly in the cortex areas (Figure 2.6A). All cats in the Prime group showed intracytoplasmic immunolabelling in the occipital and parietal cortex, while this was evident in 85% of cats in the Super Senior group. Similarly, 83% of the cats in the Prime group had intracytoplasmic immunolabelling in the rostral cortex, compared to 77% of cats in the Super Senior group.

In contrast, the opposite was found in the hippocampus, locus coeruleus, and cerebellum. In these regions, the percentage of cats with intracytoplasmic immunolabelling increased with age (67% in Prime group vs 85% in Super

Senior group in the hippocampus; 83% in Prime group vs 92% in Super Senior group in both the locus coeruleus and cerebellum).

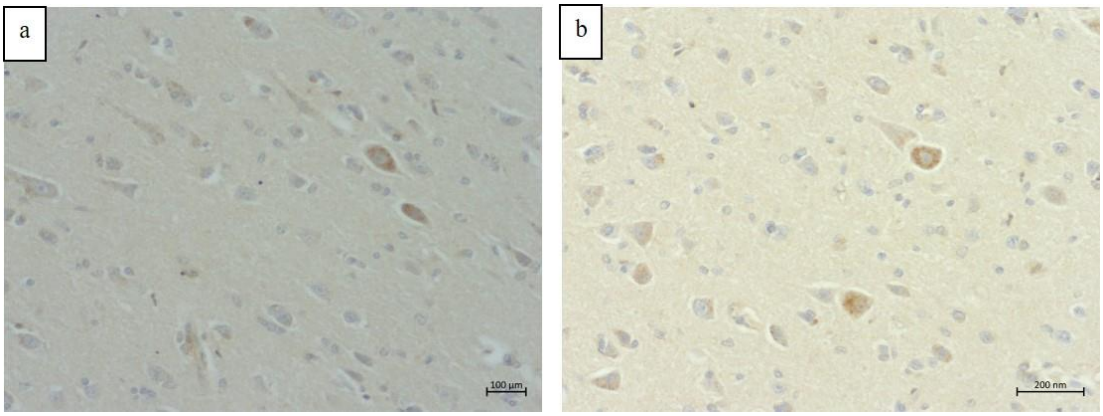

Figure 2.6 Intracytoplasmic amyloid-β (Aβ) deposition in the cat brain. *Intracytoplasmic immunolabelling of Aβ in a) the parietal cortex of a 13-year-old cat (4G8 antibody); and b) the occipital cortex of a 13-years-old cat (mOC64 antibody)*

Positive intracytoplasmic immunolabelling was similar with the mOC64 antibody. Immunolabelling was present in most of the cats from the Prime group and the percentage of cats with positive labelling decreased with age in the cortex regions (rostral cortex: 100% of cats in Prime group vs 46% of cats in Super Senior group; and occipital cortex: 83% vs 62%) (Figure 2.6B). Only half of the cats (50%) in the Prime group had intracytoplasmic immunolabelling in the parietal cortex, this increased to 80% of the cats in the Senior group and decreased back to 54% of cats in the Super Senior group. As seen with the 4G8 antibody, the proportion of cats with intracytoplasmic immunolabelling of the hippocampus and locus coeruleus increased with age, with none of the cats from the Prime group showing labelling in neither the hippocampus nor the locus coeruleus, to 38% and 8% of cats, in the Super Senior group, respectively.

Most of the extracellular Aβ in cats had a diffuse pattern which, in some cases, formed patches (Figure 2.7). However, one single cat had a plaque-like formation (Figure 2.8).

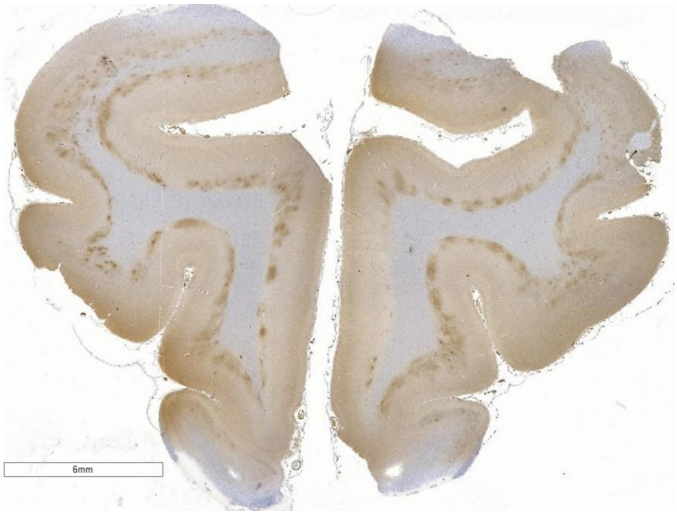

Figure 2.7 Pattern of amyloid-β (Aβ) deposition in the cat brain. *Aβ deposits showed a diffuse pattern which, in some cases, formed patches as seen in this image from the rostral cortex of a 12-year-old cat stained with mOC64 antibody. Scale bar 6mm*

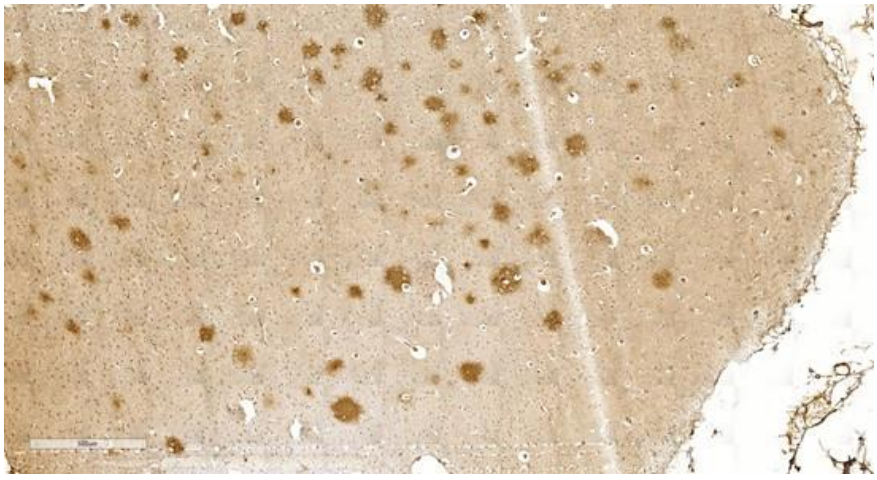

Figure 2.8 Amyloid-β (Aβ) plaque-like formation in the cat brain. *Plaque-like structures were found in the parietal cortex of a 16-year-old cat stained with mOC64 antibody. Scale bar 500 μm*

Diffuse extracellular Aβ deposition was mostly found in the cortex regions using the 4G8 antibody. There was an increase with age in both the severity of pathology and the numbers of cats with extracellular Aβ. Between 30-50% of the cats in the Prime group had extracellular β-amyloid deposition, primarily in the rostral cortex (50% of the cats), followed by the parietal and occipital cortex (33% each). In contrast, 60% of the cats in the Super Senior group had extracellular Aβ deposition, predominantly in the rostral and occipital cortex (62% of cats, each) followed by the parietal cortex (54%). Extracellular deposition of Aβ in the hippocampus and cerebellum appeared with age, from none of the cats in the Prime group, to 8% of the cats in the Super Senior group.

Similarly, a higher percentage of cats from the Super Senior group had diffuse extracellular Aβ deposits in most of the brain regions using the mOC64 antibody, when compared to the cats in the Prime group (rostral cortex: 77% of cats in the Super Senior group vs 50% of cats in the Prime group; parietal cortex: 85% vs 67%; occipital cortex: 85% vs 67%; hippocampus 38% vs none; and locus coeruleus: 8% vs none).

The extent and intensity of extracellular Aβ immunolabelling was greater with mOC64 antibody (Figures 2.9A and B) than with 4G8 (Figure 2.9C).

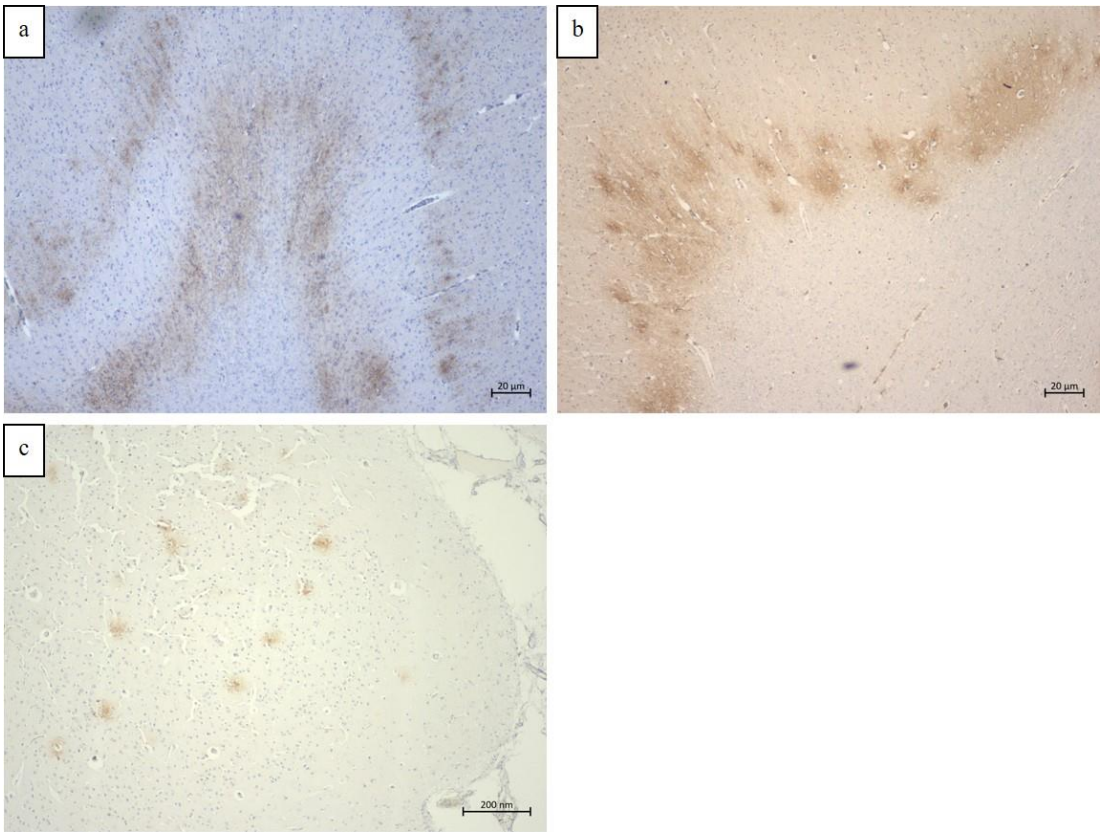

Figure 2.9 Extracellular deposition of amyloid-β (Aβ) in the cat brain. *Diffuse extracellular deposits of Aβ in a) the parietal cortex of a 12-year-old cat (mOC64 antibody); b) the parietal cortex of a 10-year-old cat (mOC64 antibody); and c) the parietal cortex of a 16-year-old cat (4G8 antibody)*

2.3.2.1 The effect of age in Aβ pathology

One-way ANOVA were performed to determine whether age has an effect on Aβ pathology (mOC64 antibody) and to assess whether there were differences between the age groups.

A statistically significant effect of age on positive Aβ labelling (i.e., burden) with mOC64 antibody was found in the rostral cortex ($F_3 = 4.55$, $p = 0.005$) (Table 2.3). Tukey tests demonstrated a significant difference were between the Prime and Mature groups (Difference of means = 10.24; SE of difference = 2.80; 95% CI [2.94, 17.54]; t = 3.66; p = 0.002) and between the Prime and Super Senior groups (Difference of means = 7.93; SE of difference = 2.97;

95% CI [0.17, 15.69]; t = 2.67; p = 0.043) (Figure 2.10). This showed that the Prime group had the lowest positive immunolabelling, when compared to the other age groups. The complete list of means with standard deviations and 95% CI can be found on Appendix 2.1.

Table 2.3 Positive amyloid-β (Aβ) immunolabelling in the different age groups. *One-way ANOVA was performed to determine whether there were differences in the positive Aβ labelling with mOC64 antibody between the age groups*

	F value	DF	p value
Rostral cortex			
% Load	0.98	3	0.405
Burden	4.55	3	**0.005**
Parietal cortex			
% Load	0.57	3	0.637
Burden	2.26	3	0.086
Occipital cortex			
% Load	0.98	3	0.406
Burden	0.97	3	0.412
Hippocampus			
% Load	0.19	3	0.901
Burden	0.57	3	0.638
Cerebellum			
% Load	-	-	-
Burden	0.41	3	0.746

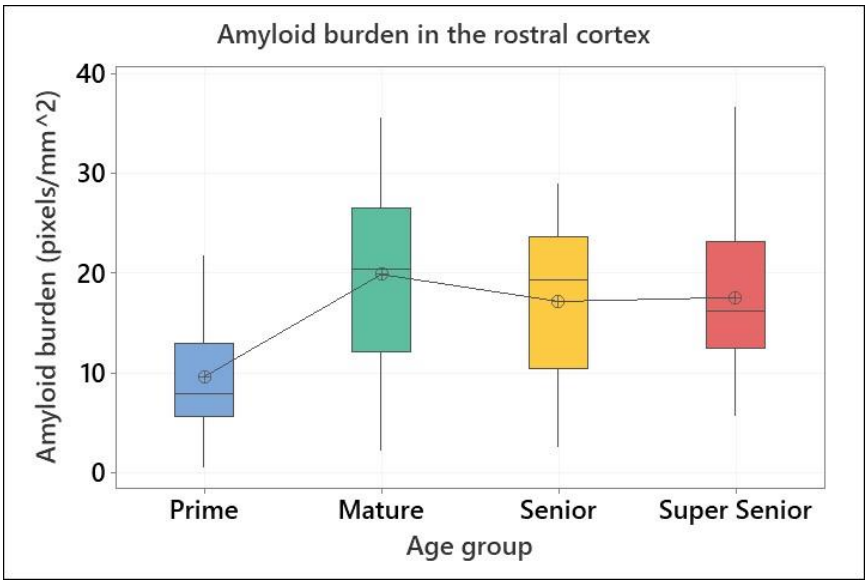

Figure 2.10 Amyloid-β (Aβ) burden in the rostral cortex of the different age groups. *Tukey tests showed statistically significant differences in the burden of Aβ (mOC64 antibody) between the Prime and Mature groups (p = 0.002); and between the Prime and Super Senior groups (p = 0.043)*

Furthermore, when comparing the effect of age between the different brain regions, the cerebellum was the brain region with the lowest load of β-amyloid when compared to the other brain regions ($F_4 = 4.58$, $p = 0.001$).

2.3.2.2 Differences in Aβ pathology between grey and white matter

Two-samples t-tests were performed to determine whether there were differences in the Aβ pathology between grey and white matter.

Statistically significant differences in the positive Aβ labelling (i.e., load and burden; mOC64 antibody) were found in all the cortex regions between grey and white matter, with the greatest immunolabelling being present in the grey matter (Table 2.4). The complete list of means with standard deviations and standard errors of the means can be found in Appendix 2.2.

Table 2.4 Positive amyloid-β (Aβ) immunolabelling in grey and white matter. *Two-sample t-tests were performed to determine whether there were differences in the positive Aβ labelling (mOC64 antibody) between grey and white matter*

	T value	DF	p value
Rostral cortex			
% Load	5.46	104	**<0.001**
Burden	5.31	104	**<0.001**
Parietal cortex			
% Load	3.89	104	**<0.001**
Burden	3.98	104	**<0.001**
Occipital cortex			
% Load	4.69	100	**<0.001**
Burden	4.59	100	**<0.001**

2.3.2.3 Differences in Aβ pathology between cats with and without CDS

Two-samples t-tests were performed to determine whether there were differences in the Aβ pathology between cats with and without CDS.

No statistically significant differences were found in the positive Aβ labelling (mOC64 antibody) between cats with and without CDS (Table 2.5). The complete list of means with standard deviations and standard errors of the means can be found in Appendix 2.2.

Table 2.5 Positive immunolabelling in cats with and without cognitive dysfunction syndrome (CDS). *Two-sample t-tests were performed to determine whether there were differences in the positive amyloid-β labelling (mOC64 antibody) between cats with and without CDS*

	T value	DF	p value
Rostral cortex			
% Load	0.14	44	0.889
Burden	0.58	44	0.567
Parietal cortex			
% Load	-0.79	44	0.433
Burden	-0.47	44	0.644
Occipital cortex			
% Load	-0.21	40	0.835
Burden	0.96	40	0.344
Hippocampus			
% Load	-1.91	16	0.075
Burden	-1.20	16	0.249
Cerebellum			
% Load	-0.02	19	0.984
Burden	0.52	19	0.606

2.3.3 Tau pathology

2.3.3.1 Non-phosphorylated tau and tau isoforms

Intranuclear and intracytoplasmic immunolabelling of non-phosphorylated tau (i.e., Tau46 antibody) was found in cats of all ages and in all brain regions. Furthermore, intracytoplasmic immunolabelling of the two isoforms of tau: 3- and 4-repeat (i.e., 8E6/C11 and 1E1/A6 antibodies, respectively) were also found in cats of all ages and in all brain regions.

The percentage of cats with intranuclear immunolabelling for non-phosphorylated tau (Tau46 antibody) increased with age (rostral cortex: 17% of the cats in the Prime group vs 38% of cats in the Super Senior group; parietal cortex: 33% vs 46%; occipital cortex: 33% vs 38%; locus coeruleus: none vs 15%; and cerebellum: none vs 23%).

The proportion of cats showing intracytoplasmic immunolabelling for non-phosphorylated tau (Tau46 antibody) remained the same in the parietal and occipital regions, with all of the cats showing positive labelling. In contrast, the percentage of cats with positive immunolabelling increased slightly with age in some regions (hippocampus: 67% vs 100%; locus coeruleus: 67% vs 77%; and cerebellum: 67% vs 69%) and decreased in the rostral cortex (100% of cats in Prime group vs 92% of cats in Super Senior group) (Figures 2.11A and B).

Intracytoplasmic immunolabelling for the 3-repeat tau isoform (8E6/C11 antibody) was found in cats from all the age groups (Figures 2.11C and D); however, the proportion of cats showing positive immunolabelling tended to decrease with age in some brain regions (rostral cortex: 67% of cats in the Prime group vs 54% in the Super Senior group; occipital cortex: 83% vs 54%; and cerebellum: 17% to 8%) and to increase in the parietal cortex (83% vs 92%) and hippocampus (83% vs 92%).

Similarly, intracytoplasmic immunolabelling for the 4-repeat tau isoform (1E1/A6 antibody) was found in cats from all the age groups and the proportion of cats showing positive immunolabelling tended to increase with age (rostral cortex: none of the cats in the Prime group vs 38% of the cats in the Super Senior group; parietal cortex: 33% vs 62%; occipital cortex: 17% vs 46%; hippocampus: 50% vs 62%; and locus coeruleus: 23% vs 31%) (Figures 2.11E and F).

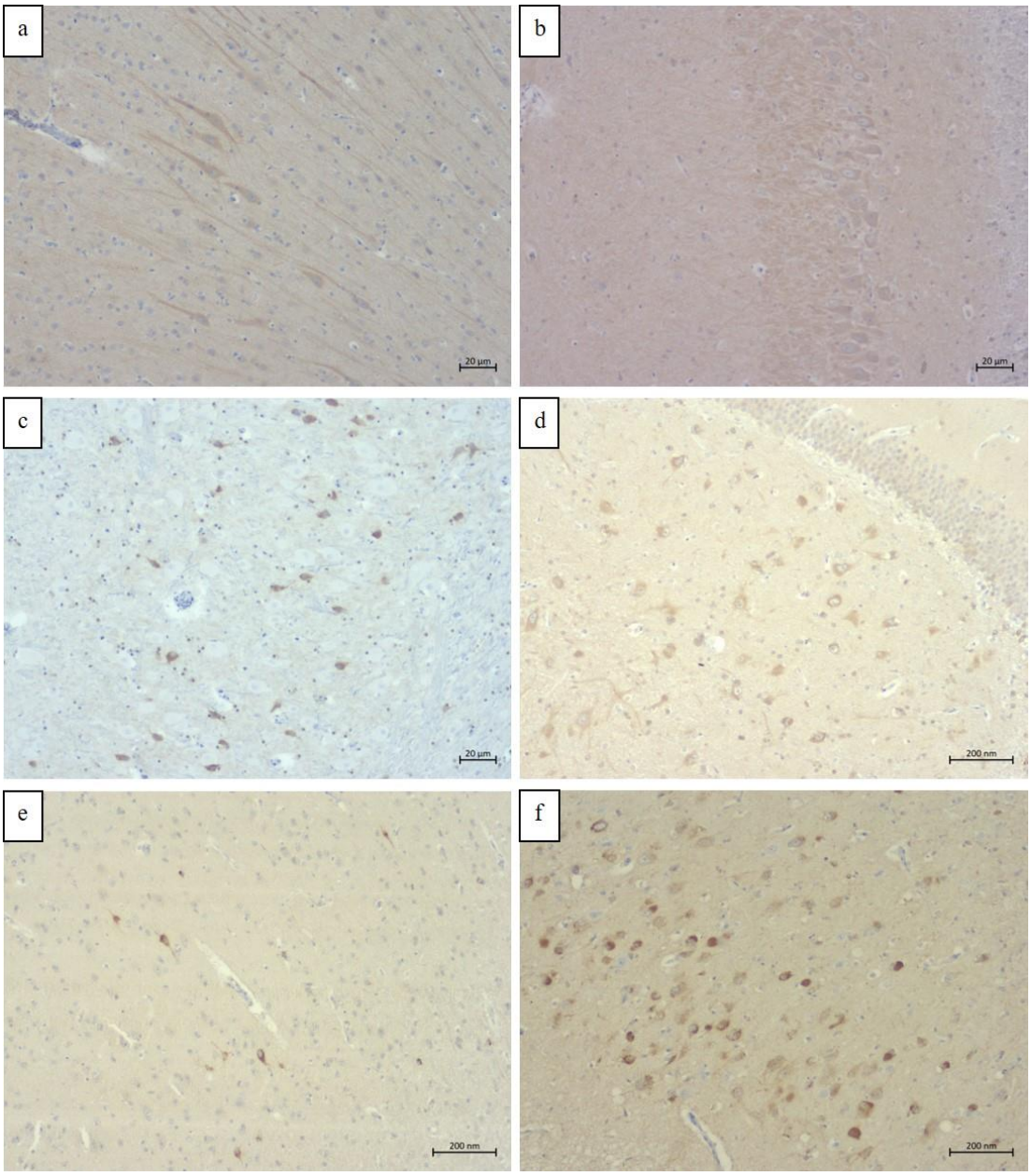

Figure 2.11 Intracytoplasmic labelling of the tau protein and its isoforms in the cat brain. *Intracytoplasmic immunolabelling of a) total tau (Tau46 antibody) in the rostral cortex of a 10-year-old cat; b) total tau (Tau46 antibody) in the hippocampus of a 10-year-old cat; c) 3-repeat tau isoform tau (8E6/C11 antibody) in the parietal cortex of a 11-year-old cat; d) 3-repeat tau isoform tau (8E6/C11 antibody) in the hippocampus of a 7-year-old cat; e) 4-repeat isoform of tau (1E1/A6 antibody) in the rostral cortex of the 19-year-old cat; and f) 4-repeat isoform of tau (1E1/A6 antibody) in the parietal cortex of a 19-year-old cat*

2.3.3.2 Hyperphosphorylated tau

Positive immunolabelling of hyperphosphorylated tau was found within the nucleus and the cytoplasm of the neurons (i.e., AT8, AT100, and A15091A antibodies). Intranuclear immunolabelling was mostly found in younger cats, whereas intracytoplasmic labelling (i.e., pre-tangles) was found in elderly cats.

In this section, results from the intranuclear tau deposits with all three antibodies will be presented first, followed by the results from the intracytoplasmic tau deposits.

Intranuclear immunolabelling with AT8 antibody was most commonly found in younger cats, and the proportion of cats showing positive immunolabelling tended to decrease with age (rostral and occipital cortices: 83% of the cats in the Prime group vs 77% in the Super Senior group; and cerebellum (17% vs 8%). In contrast, the percentage of cats showing intranuclear immunolabelling tended to increase with age in the parietal cortex (67% vs 85%), the hippocampus (50% vs 54%) and the locus coeruleus (33% vs 54%) (Figures 2.12A and B).

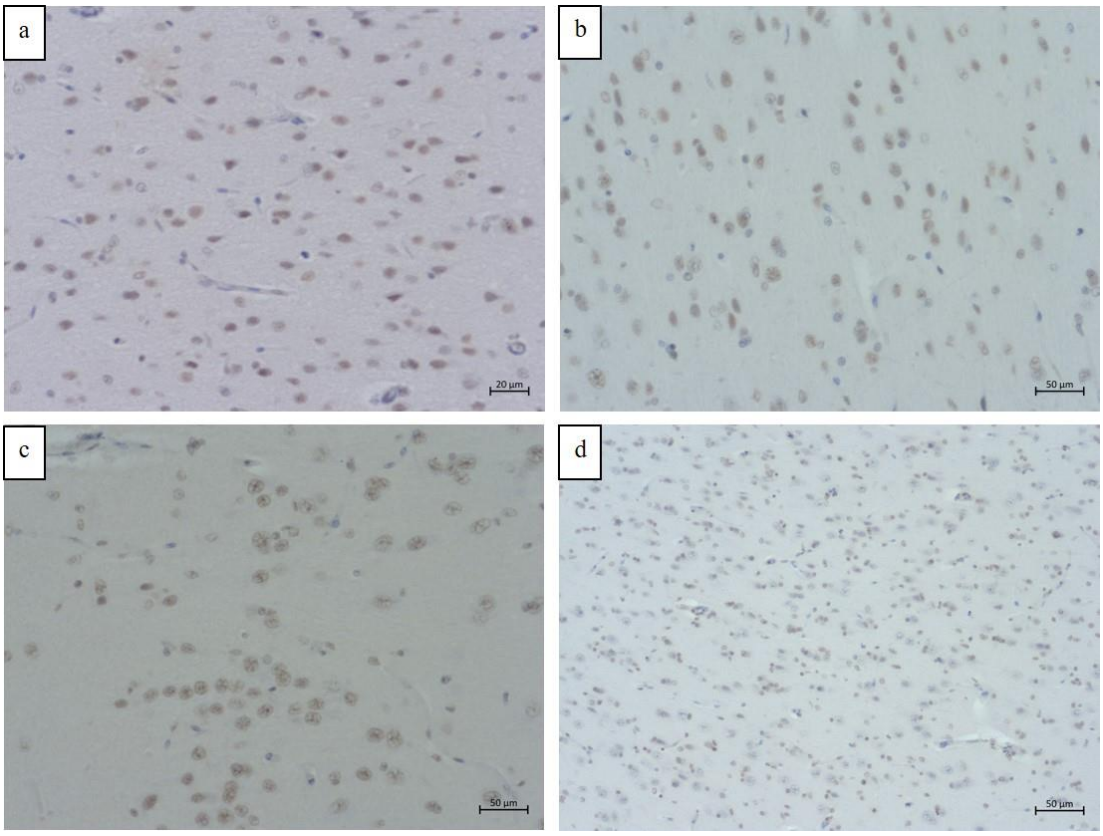

Figure 2.12 Intranuclear immunolabelling of hyperphosphorylated tau. *Intranuclear hyperphosphorylated tau deposits in a) the occipital cortex of a 14-year-old cat (AT8 antibody); and b) the parietal cortex of a 14-year-old cat (AT8 antibody); c) the parietal cortex of a 14-years-old cat (AT100 antibody); and d) the rostral cortex of a 14-years-old cat (A15091 antibody)*

Similarly, intranuclear immunolabelling with AT100 was found in cats of all ages, and in most of the brain regions, with the percentage of cats showing positive immunolabelling decreasing with age in most of them (rostral cortex: 83% of cats in the Prime group vs 69% of cats in the Super Senior group; parietal cortex: 100% vs 92%; occipital cortex: 100% vs 85%; and hippocampus: 83% vs 77%). However, it increased in the locus coeruleus (17% vs 46%), and the cerebellum (33% vs 46%) (Figures 2.12C).

Finally, intranuclear immunolabelling with A15091 antibody was found in cats from all ages, and in most of the brain regions, with the percentage of cats showing positive immunolabelling decreasing with age (rostral cortex: 100% of

the cats in the Prime group vs 92% of cats in the Super Senior group; parietal and occipital cortices: 100% vs 85%; hippocampus, locus coeruleus, and cerebellum 83% vs 77%) (Figure 2.12D).

Intracytoplasmic immunolabelling for hyperphosphorylated tau (i.e., pre-tangles) was found with all three antibodies, and its presence was confirmed by Silver-Gallyas staining (Figure 2.13).

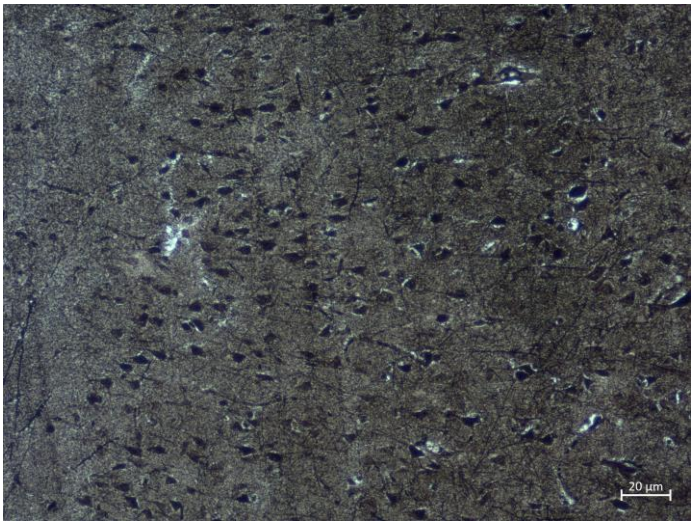

Figure 2.13 Confirmation of the presence of pre-tangles. *Silver-Gallyas staining confirmed the presence of hyperphosphorylated tau pre-tangles (stained in black) in the parietal cortex of a 19-years-old cat*

Elderly cats showed to accumulate pre-tangles with all three antibodies. A total of 14 cats were shown to have pre-tangles with AT8 antibody. Of these, one was in the Prime group; two in the Mature group; one in the Senior group; and ten in the Super Senior group (Figures 2.14A and B). Similarly, 15 cats were shown to have pre-tangles with AT100 antibody. Of these, two were in the Prime group; two in the Mature group; one in the Senior group; and ten in the Super Senior group (Figures 2.14C and D). Of note, almost half of the cats that had pre-tangles with AT8 (n = 6) and AT100 (n = 7) had a confirmed diagnosis of CDS. Moreover, the six cats with CDS that had pre-tangles with the AT8 antibody, had pre-tangles with the AT100 antibody as well, mostly in the cortex

regions. The lowest number of cats with pre-tangles (n = 3) were found with the A15091A antibody. Of these, one was in the Mature group; and two in the Super Senior group. One of these cats from the Super Senior group that had pre-tangles, also had a confirmed diagnosis of CDS; of note, this cat also had pre-tangles with both AT8 and AT100 antibodies (Figure 2.14E).

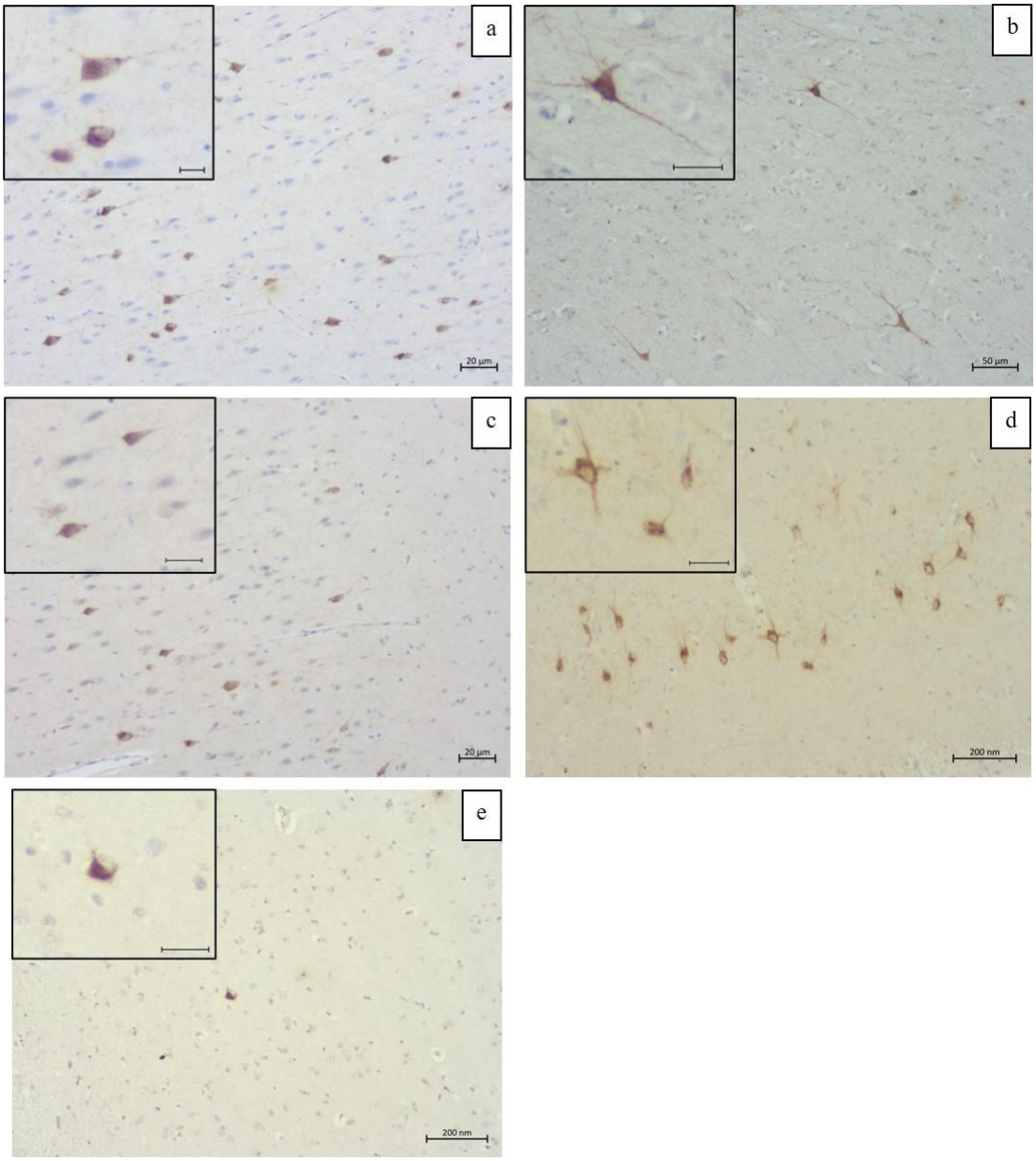

Figure 2.14 Pre-tangles in the cat brain. *Pre-tangles of hyperphosphorylated tau in a) the rostral cortex of a 16-year-old cat (AT8 antibody); b) the rostral cortex of a 10-year-old cat (AT8 antibody); c) the rostral cortex of a 16-year-old cat (AT100 antibody); d) the occipital cortex of a 19-year-old cat (AT100 antibody); and e) the parietal cortex of a 16-year-old cat (A15091A antibody)*

Of note, the presence of pre-tangles with both AT8 and AT100 antibodies tended to increase with age in all of the brain regions (Figure 2.15A and B).

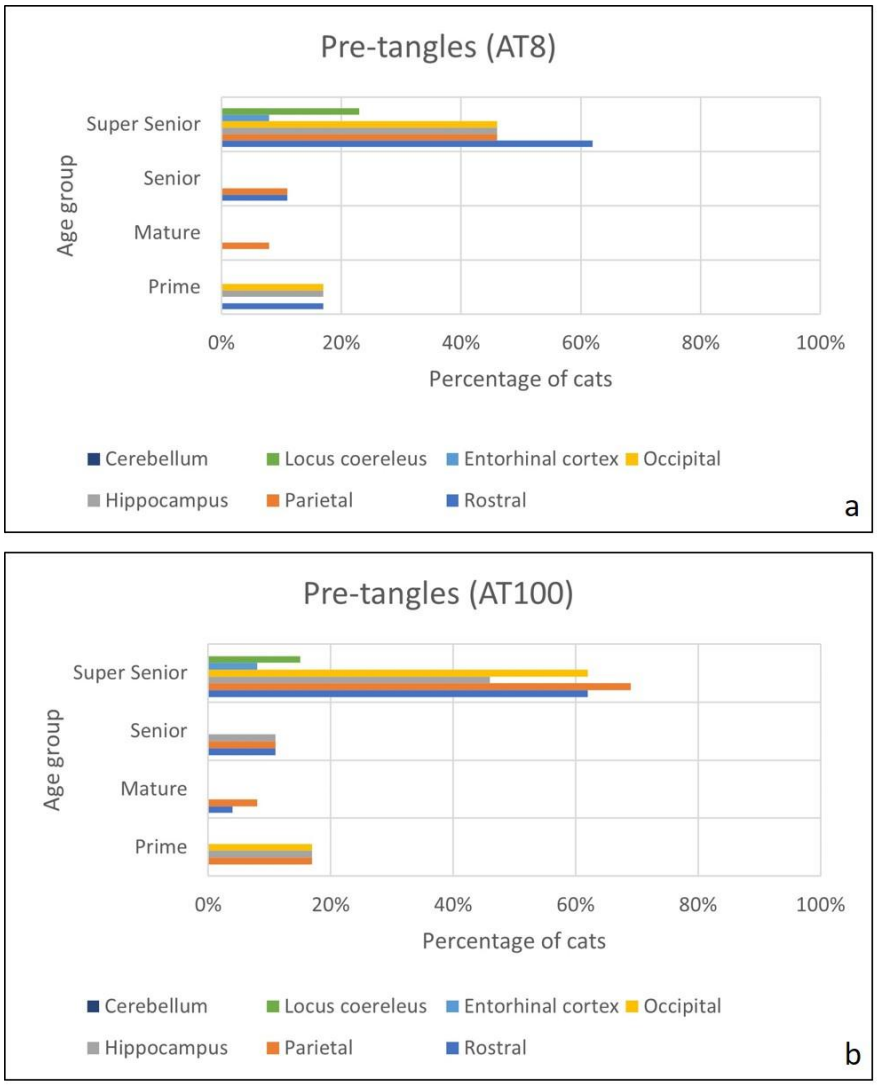

Figure 2.15 Pre-tangles and age. *The presence of pre-tangles tended to increase with age in most of the brain regions with a) AT8 and b) AT100 antibodies*

2.3.3.3 The effect of age in tau pathology

One-way ANOVA were performed to determine whether age has an effect on tau pathology (i.e., intranuclear and intracytoplasmic using AT8 and AT100 antibodies) and to assess whether there were differences between the age groups.

No statistically significant differences were found in the intranuclear immunolabelling between the age groups with neither AT8 nor AT100 antibodies (Table 2.6). The complete lists of means with standard deviations and 95% CI can be found in Appendices 2.3 and 2.4.

Table 2.6 Intranuclear immunolabelling of hyperphosphorylated tau in the different age groups*. One-way ANOVA was performed to determine whether there were differences in the intranuclear immunolabelling of the hyperphosphorylated tau (AT8 and AT100 antibodies) between age groups*

	AT8			AT100		
	F value	**DF**	**p value**	**F value**	**DF**	**p value**
Rostral cortex	0.73	3	0.538	0.49	3	0.689
Parietal cortex	0.97	3	0.409	0.26	3	0.851
Occipital cortex	0.76	3	0.521	1	3	0.396
Hippocampus	1.42	3	0.249	0.05	3	0.985

One-way ANOVA showed statistically significant differences in the positive immunolabelling (i.e., burden) of hyperphosphorylated tau with the AT8 antibody in the hippocampus ($F_3 = 3.08$, $p = 0.036$) (Table 2.7). Tukey tests demonstrated that the differences were between the Prime and Senior groups (Difference of means = -0.284; SE of difference = 0.102; 95% CI [-0.556, -0.012]; t = -2.77; p=0.038) (Figure 2.16). This showed that the Prime group had the greatest burden in the hippocampus (AT8 antibody), when compared to the other age groups. In contrast, there were no statistically significant differences in the positive immunolabelling of hyperphosphorylated tau using the AT100 antibody between the age groups (Table 2.7). The complete list of means with standard deviations and 95% CI can be found in Appendices 2.5 and 2.6.

Table 2.7 Positive immunolabelling of hyperphosphorylated tau in the different age groups. *One-way ANOVA was performed to determine whether there were differences in the positive immunolabelling of the hyperphosphorylated tau using AT8 and AT100 antibodies between age groups*

	AT8			AT100		
	F value	**DF**	**p value**	**F value**	**DF**	**p value**
Rostral cortex						
% Load	0.58	3	0.630	0.23	3	0.876
Burden	1.07	3	0.373	0.37	3	0.775
Parietal cortex						
% Load	0.66	3	0.579	0.04	3	0.991
Burden	1.67	3	0.179	0.66	3	0.584
Occipital cortex						
% Load	0.59	3	0.626	0.06	3	0.980
Burden	1.20	3	0.313	0.66	3	0.581
Hippocampus						
% Load	1.97	3	0.130	0.69	3	0.563
Burden	3.08	3	**0.036**	0.41	3	0.749
Cerebellum						
% Load	0.73	3	0.537	1.20	3	0.320
Burden	n/a	n/a	n/a	n/a	n/a	n/a

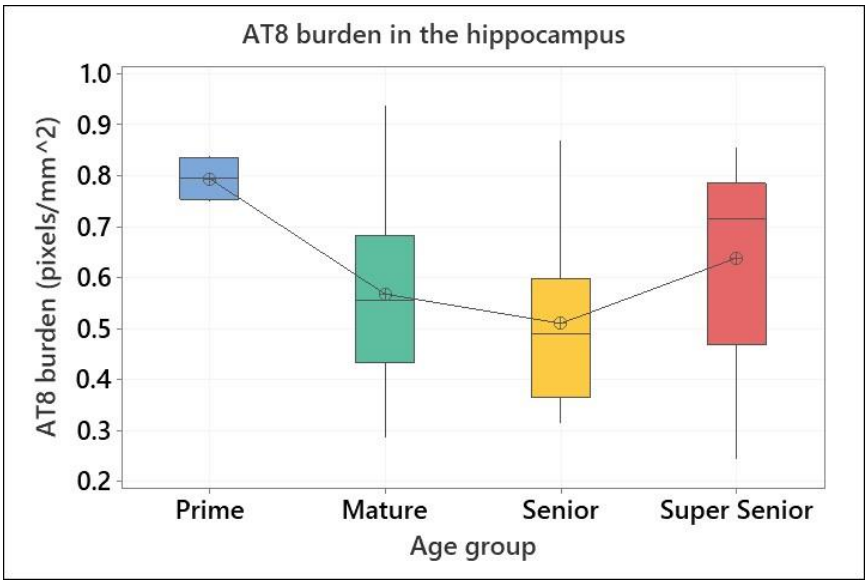

Figure 2.16 Hyperphosphorylated tau burden in the hippocampus in the different age groups. *One-way ANOVA showed that the Prime group had a higher burden of hyperphosphorylated tau (AT8 antibody) in the hippocampus than the Senior group (p = 0.036)*

2.3.3.4 Differences in tau pathology between grey and white matter

Two-samples t-tests were performed to determine whether there were differences in the tau pathology between grey and white matter.

There were statistically significant differences in the intranuclear immunolabelling with AT8 antibody between grey and white matter, with more immunolabelling in the grey matter in all cortical regions (Table 2.8). Similarly, there were statistically significant differences in the intranuclear immunolabelling with AT100 antibody between grey and white matter; however, this antibody showed more immunolabelling in the grey matter in the parietal and occipital cortical regions only (Table 2.8). The complete list of means with standard deviations and standard errors of the means can be found in Appendix 2.7.

Table 2.8 Intranuclear immunolabelling of hyperphosphorylated tau in grey and white matter. *Two-sample t-tests were performed to determine whether there were differences in the intranuclear immunolabelling of hyperphosphorylated tau with both AT8 and AT100 antibodies between grey and white matter*

	AT8			AT100		
	T value	**DF**	**p value**	**T value**	**DF**	**p value**
Rostral cortex	4.73	102	**<0.001**	0.87	98	0.386
Parietal cortex	3.63	104	**<0.001**	5.16	100	**<0.001**
Occipital cortex	4.54	104	**<0.001**	5.22	102	**<0.001**
Hippocampus	-	-	-	-	-	-

Two-sample t-tests showed statistically significant differences in the positive immunolabelling of hyperphosphorylated tau with antibody AT8 in the rostral ($t = 2.12$, $p = 0.036$) and occipital ($t = 2.14$, $p = 0.035$) cortices, with a higher load in the grey matter, when compared to white matter (Table 2.9). Statistically significant differences were also found with the AT100 antibody; however, these were in both the parietal ($t = 2.58$, $p = 0.011$) and occipital ($t = 2.73$, $p = 0.007$) cortex between grey and white matter, being higher in the grey matter (Table 2.9). The complete list of means with standard deviations and standard errors of the means can be found in Appendix 2.8.

Table 2.9 Positive immunolabelling of hyperphosphorylated tau in grey and white matter. *Two-sample t-tests were performed to determine whether there were differences in the positive immunolabelling of hyperphosphorylated tau with both the AT8 and AT100 antibodies between grey and white matter*

	AT8			AT100		
	T value	**DF**	**p value**	**T value**	**DF**	**p value**
Rostral cortex						
% Load	2.12	102	**0.036**	1.36	96	0.176
Burden	0.69	101	0.490	-0.12	96	0.905
Parietal cortex						
% Load	1.74	103	0.084	2.58	102	**0.011**
Burden	-0.05	104	0.960	0.39	101	0.695
Occipital cortex						
% Load	2.14	104	**0.035**	2.73	102	**0.007**
Burden	-0.66	104	0.511	1.34	97	0.183

2.3.3.5 Differences in tau pathology between cats with and without CDS

Two-samples t-tests were performed to determine whether there were differences in the tau pathology between cats with and without CDS.

Two-sample t-tests showed no statistically significant differences in the intranuclear immunolabelling with neither the AT8 nor the AT100 antibodies between cats with and without CDS (Table 2.10). The complete list of means with standard deviations and standard errors of the means can be found in Appendix 2.9.

Table 2.10 Intranuclear immunolabelling of hyperphosphorylated tau in cats with and without cognitive dysfunction syndrome (CDS). *Two-sample t-tests were performed to determine whether there were differences in the intranuclear immunolabelling of hyperphosphorylated tau (AT8 and AT100 antibodies) between cats with and without CDS*

	AT8			AT100		
	T value	**DF**	**p value**	**T value**	**DF**	**p value**
Rostral cortex	-0.86	42	0.394	0.27	42	0.790
Parietal cortex	0.07	42	0.946	0.29	42	0.773
Occipital cortex	-0.40	40	0.690	0.24	40	0.808
Hippocampus	0.65	20	0.526	-0.63	17	0.539

Similarly, no statistically significant differences were found in the positive immunolabelling of hyperphosphorylated tau, with neither the AT8 nor the AT100 antibodies, when comparing the cats with and without CDS (Table 2.11). The complete list of means with standard deviations and standard errors of the means can be found in Appendix 2.10.

Table 2.11 Positive immunolabelling of hyperphosphorylated tau in cats with and without cognitive dysfunction syndrome (CDS). *Two-sample t-tests were performed to determine whether there were differences in the positive immunolabelling of hyperphosphorylated tau with both AT8 and AT100 antibodies between cats with and without CDS*

	AT8			AT100		
	T value	**DF**	**p value**	**T value**	**DF**	**p value**
Rostral cortex						
% Load	-0.53	42	0.598	-0.06	40	0.955
Burden	-0.54	42	0.591	0.44	40	0.659
Parietal cortex						
% Load	-0.26	44	0.793	-0.45	42	0.656
Burden	0.04	44	0.970	-0.01	39	0.990
Occipital cortex						
% Load	-0.61	42	0.543	-0.27	40	0.792
Burden	-0.65	42	0.517	-0.70	39	0.490
Hippocampus						
% Load	0.51	21	0.613	1.58	17	0.132
Burden	-0.21	21	0.833	1.11	17	0.284
Cerebellum						
% Load	-0.57	18	0.578	0.13	18	0.899
Burden	-0.56	18	0.584	-0.07	18	0.944

2.3.3.6 Association between the presence of pre-tangles and CDS

Chi-square tests were performed to determine whether there was an association between cognitive status (i.e., cats with and without CDS) and the presence of pre-tangles.

As previously described, the largest numbers of pre-tangles were found in cats with CDS, which was consistent with the statistically significant association

found between CDS and the presence of pre-tangles with both antibodies (Figure 2.17).

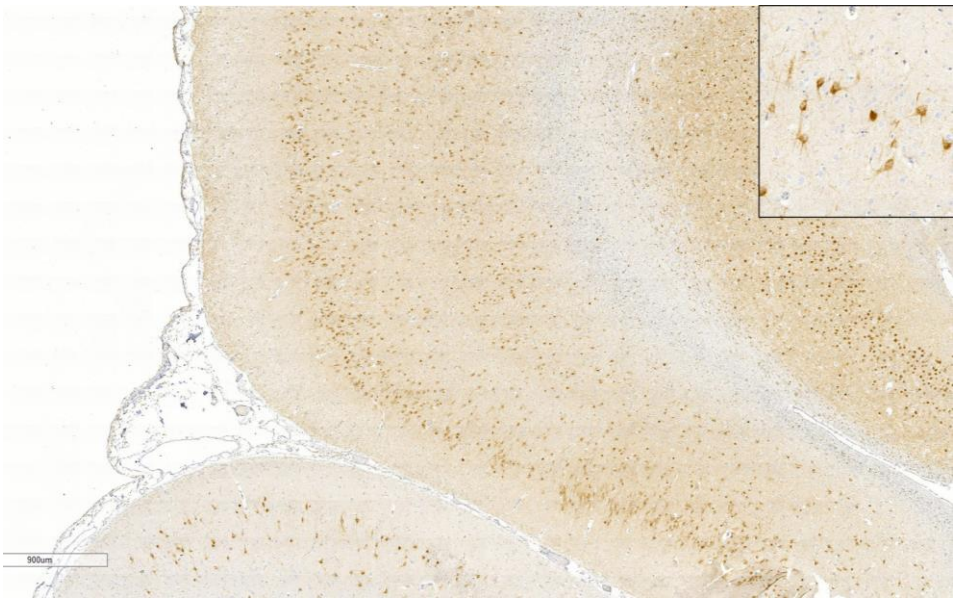

Figure 2.17 Pre-tangles in the brain of a cat with cognitive dysfunction syndrome (CDS). *The largest numbers of pre-tangles were found in the brains of cats with a confirmed diagnosis of CDS. Image from the parietal cortex of a 19-year-old cat with a diagnosis of CDS (stained with AT100 antibody) that also was part of the double-blinded placebo-controlled study (see Chapter 4). Scale bar 900 μm*

Most of the cats with CDS had pre-tangles with both the AT8 and AT100 antibodies in the rostral ($X^2 = 31.534$, $p < 0.001$; and $X^2 = 35.224$, $p < 0.001$, respectively); the parietal ($X^2 = 11.6$, $p = 0.001$; and $X^2 = 19.512$, $p < 0.001$, respectively); the occipital cortex ($X^2 = 22.25$, $p < 0.001$; and $X^2 = 32.245$, $p < 0.001$, respectively); and the hippocampus ($X^2 = 8.709$, $p = 0.003$; and $X^2 = 11.583$, $p = 0.001$, respectively) when compared with cats without CDS.

2.3.4 Validation of intranuclear hyperphosphorylated tau immunolabelling

2.3.4.1 Isotype controls

To validate that intranuclear immunolabelling was not caused by an artifact or non-specific staining, IHC was performed using antibodies with the same isotypes for AT8, AT100, and A15091A antibodies as negative controls (Figures 2.18A and B).

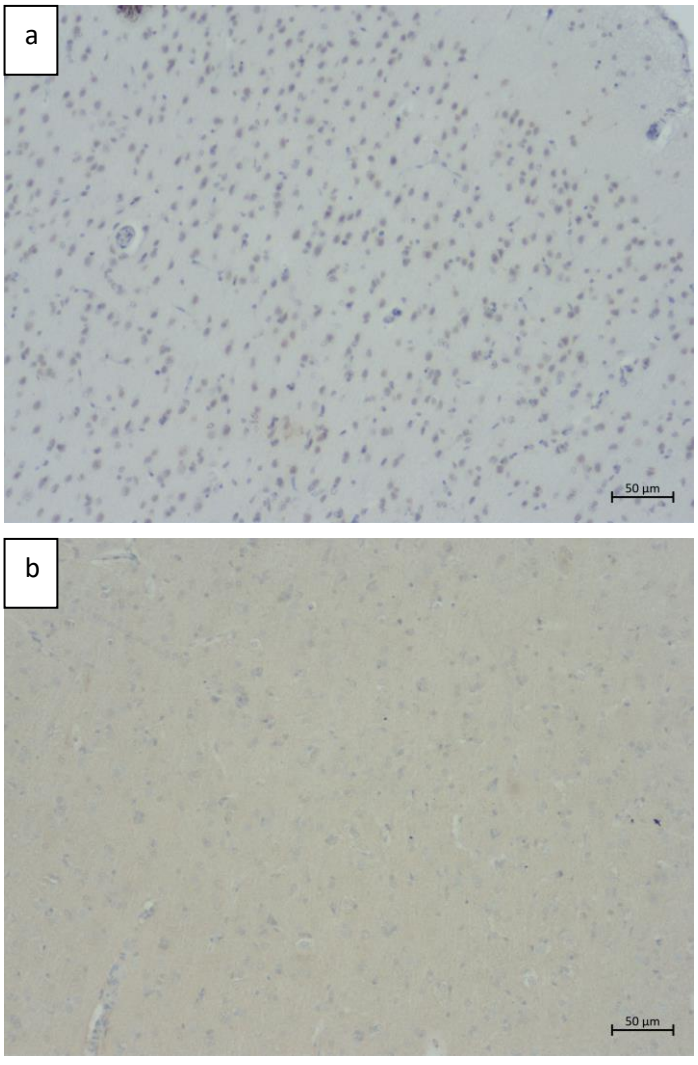

Figure 2.18 Isotype controls for the validation of intranuclear immunolabelling for hyperphosphorylated tau (AT8 antibody) in the rostral cortex of a 14-years-old cat. *A) positive control shows intranuclear immunolabelling using AT8 antibody; and b) the use of the isotype as negative control showed no positive immunolabelling*

2.3.4.2 Dephosphorylation of the tau protein

To validate that the intranuclear labelling obtained was caused by the hyperphosphorylated protein, instead of the non-phosphorylated one, the tau protein was dephosphorylated by using alkaline phosphatase, showing no intranuclear immunolabelling (Figures 2.19A and B).

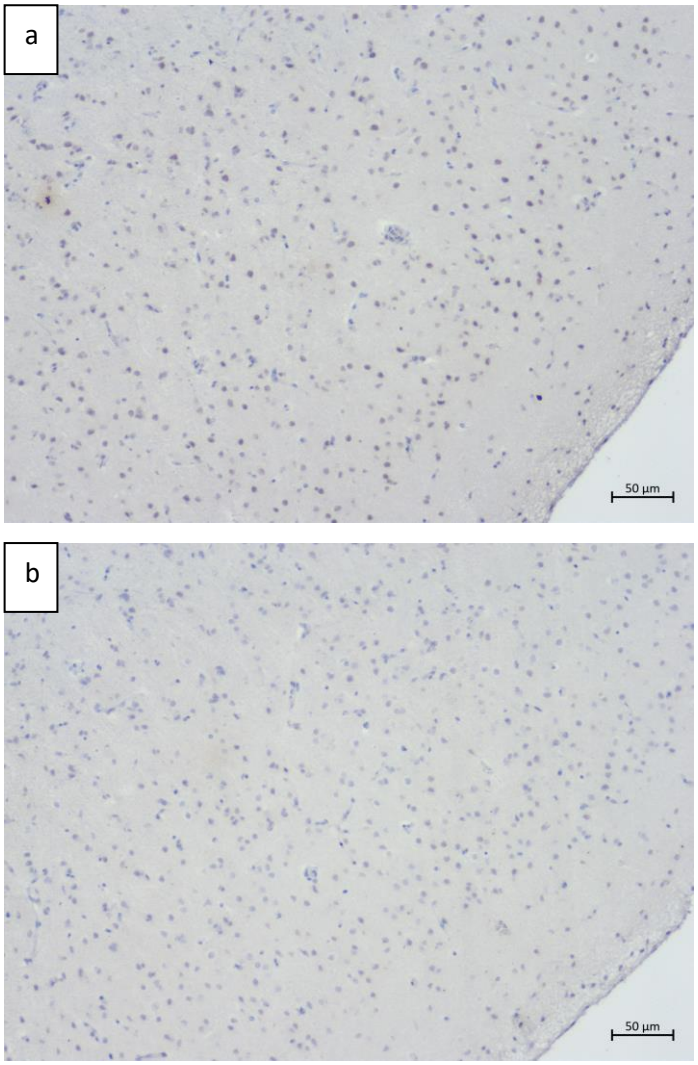

Figure 2.19 Dephosphorylation of the tau protein with alkaline phosphatase for the validation of intranuclear tau immunolabelling (AT8 antibody) in the rostral cortex of an 18-years-old cat*. A) positive control shows intranuclear immunolabelling using AT8 antibody; and b) no positive immunolabelling was found after incubation with alkaline phosphatase*

2.3.4.3 Western blot

Western blots were performed to determine whether the intranuclear tau was in a non-phosphorylated (Tau46 antibody) and/or phosphorylated (AT8 antibody) state. The tau protein has a molecular weight of 79 kDa. A western blot of the different fractions confirmed the presence of intranuclear hyperphosphorylated tau in both the soluble nuclear and the chromatin bound fractions with the AT8 antibody, with a slightly higher expression in the soluble nuclear fraction. Hyperphosphorylated tau was also found in the cytoplasm; however, it was not detected in the cytoskeletal fraction (Figure 2.20). Similarly, a western blot using an antibody against total tau (Tau46) showed the presence of tau protein in both nuclear fractions, with higher levels in the soluble nuclear fraction. As expected, total tau was detected in both the cytoplasm and the cytoskeleton (Figure 2.20).

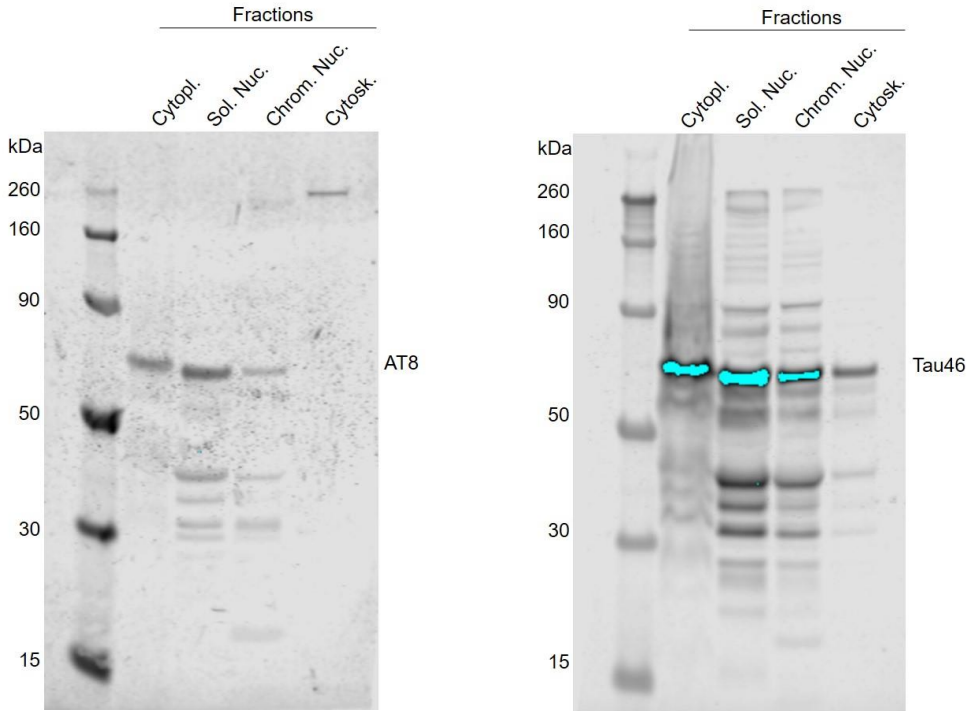

Figure 2.20 Western blot for the validation of intranuclear tau*. Left: the presence of intranuclear hyperphosphorylated tau (AT8 antibody) was confirmed by Western Blot with a slightly higher expression in the soluble nuclear (Sol. Nuc.) fraction, when compared to the chromatin-bound nuclear (Chrom. Nuc.) fraction. Hyperphosphorylated tau was also expressed in the cytoplasm fraction (Cytopl.) but not in she cytoskeleton (Cytosk.). Right: expression of total tau (Tau46 antibody) was observed in all fractions*

2.4 Discussion

The age-related neuropathology seen in the cat brain shares many similarities to that seen in the elderly human brain, including in those with AD. Elderly cats have been shown to develop both Aβ and tau pathologies; with large extracellular Aβ deposits and the presence of pre-tangles. Of note, the largest numbers of pre-tangles were found in the brains of cats with CDS. In contrast, younger cats have mostly intracellular Aβ deposits and no pre-tangles; while young cats did not form pre-tangles, they do have intranuclear tau.

2.4.1 Aβ pathology in the cat brain

Extracellular deposits of Aβ were found in the brains of most of the cats in this study. While there was some variability between individual brains, which also occurs in humans[60], a similar distribution pattern was observed in most (see below).

Cats of all ages had extracellular Aβ deposits; however, the extent of these deposits varied between age groups. Smaller deposits were found in younger cats, whereas the largest deposits were found in cats of the Mature and Super Senior group, particularly in the rostral cortex.

Contrary to what has been reported by others, the youngest cat with extracellular Aβ deposits in this study was four years old; in previous studies Aβ deposits were only found in cats over eight years of age[52, 63, 64, 150]. Extracellular Aβ deposits in cats in the Prime group were only found in the cortical areas (mainly the rostral cortex with 4G8 labelling, and the parietal and occipital cortices with mOC64). It is perhaps not surprising to find Aβ deposits in the brains of young cats. This finding suggests that Aβ deposits start accumulating in these regions in the cat brain which, according to Braak and

Braak, are the same regions in which Aβ deposition starts (i.e., Stage I) in the human brain[60]. Furthermore, in humans, Aβ starts accumulating in individuals as young as 20-years-old, which is decades before they develop any type of cognitive decline and/or dementia[151]. According to International Cat Care and their equivalence table between human and cat years, four cat years correspond to ~32 in humans[148], which correlates to finding Aβ deposits in young individuals.

Extracellular Aβ deposits in the hippocampus were only found in cats in the Super Senior group. Since cats in this age group also had Aβ in all cortical brain regions, it is logical to conclude that, after accumulating in the cortical areas, Aβ pathology in cats progresses to the hippocampus. This same pattern has been described in the human brain during Stages II and III of Aβ progression[60].

While the cortical regions and the hippocampus were the regions most affected by extracellular Aβ deposition in cats, the cerebellum was least affected. In humans, the cerebellum is known to be affected only during the final stage of Aβ progression (i.e., Stage V)[60]. Hence, it is possible that these cats did not live long enough to allow for Aβ deposition to progress to the cerebellum.

Larger extracellular Aβ deposits were found in grey matter, when compared to white matter, and this occurred in all cortical regions. This correlates with the Aβ progression proposed by Braak and Braak, as grey matter is initially affected during Stage I, progressing to the white matter during Stage II of disease[60]. Of note, while Aβ pathology in humans with AD has been mainly reported in grey matter, one study has shown that Aβ deposits also increase in the white matter[152].

Extracellular Aβ deposits in cats have a diffuse pattern, which may explain why they were not detected with Congo red staining. In some cases, however,

these diffuse deposits formed patches (see Figure 2.7 in Results). This distribution pattern, including the formation of patches, has been previously documented in the cat brain[52, 64, 150]. Of note, these deposits are only formed by the Aβ42 peptide[63, 64].

Similarly, diffuse deposits in humans are mainly formed by this same peptide (Aβ42) and are believed to be an early stage of plaques[53, 54]. Interestingly, a 16-year-old cat in this study showed Aβ accumulating into plaque-like structures (see Figure 2.8 in Results). While this might be a sporadic finding, it could also suggest that cats may not only form diffuse Aβ deposits, but they can also develop senile plaques. However, it is likely that most cats do not live long enough to develop these. Nevertheless, further studies are required to confirm this hypothesis.

No statistically significant difference was found in the presence and/or extent of Aβ pathology between cats with and without CDS. This may be due to the low numbers of cats with a confirmed diagnosis of CDS in the present study. It is also plausible that Aβ pathology in cats does not correlate to the behavioural changes related to CDS, as previously suggested[64]. In humans, there is still contradictory evidence regarding the correlation between Aβ pathology and cognitive impairment. It is true that some studies have found a correlation between Aβ deposition and cognitive decline[153]. However, these studies have some caveats; for instance, they used silver stains that do not differentiate between senile plaques and diffuse deposits. In contrast, there is mounting evidence showing that Aβ pathology does not correlate with cognitive decline; moreover, they have shown that there have been individuals with significant Aβ accumulation that did not display any signs of cognitive impairment[55, 79, 154, 155].

In humans, Aβ deposits accumulate in the extracellular spaces, and also inside neurons, within their cytoplasm[156-158]. Initial studies found intracytoplasmic

deposits in the brains of individuals ranging from 38 to 83 years old, with and without AD; while variations were found between the different age groups and across individuals, these deposits did not correlate to AD[156]. However, in these studies, the intracellular accumulation of Aβ was detected by using antibodies against residues 17-24 of Aβ (i.e., 4G8 antibody), which also bind to the amyloid precursor protein (APP). For this reason, recent studies have used antibodies against the C-terminus of Aβ instead. These studies have confirmed that these intracytoplasmic deposits are mainly formed by the γ-cleaved portion of the Aβ42 peptide[157, 158] and can have either a smooth or a granular appearance[158].

The presence of Aβ within the neurons is believed to occur as a result of two processes[56, 157, 159]. It has been suggested that, after the amyloidogenic pathway of APP, some of the Aβ that was released from the neurons into the extracellular spaces is being reabsorbed into the cell by the action of Apolipoprotein E and/or nicotinic and lipoprotein receptors[56, 157, 159]. On the other hand, it has also been suggested that small fragments of Aβ, produced during the amyloidogenic pathway of APP, are not released into the extracellular space and, instead, are retained intracellularly[56, 157, 159].

Intracellular Aβ deposition is believed to be an early event that begins at a young age and progresses with age[157, 158]. For this reason, it has been suggested that intracellular Aβ has a normal function, potentially as an antioxidant[160, 161]. During early years, intracytoplasmic Aβ is expressed at very low concentrations and does not produce significant damage[56]; however, as individuals get older, it continues to accumulate, until it reaches higher concentrations and becomes toxic[162]. High concentrations of intracytoplasmic Aβ have been linked to mitochondrial and cytoskeleton dysfunction, plus synaptic alterations[56, 157, 159]. Furthermore, once a neuron dies, intracytoplasmic Aβ is released into the extracellular space, promoting further Aβ accumulation in this region[159]. Unsurprisingly, correlations between the

concentrations of intra and extracellular Aβ have been reported, as intracellular Aβ tend to decrease when extracellular deposits accumulate[158, 163].

Studies in humans and mice models of AD have shown that intracellular Aβ can abnormally accumulate inside the mitochondria[159, 164, 165]. The accumulation of Aβ in the mitochondria alters glucose metabolism and limits adenosine 5'-triphosphate (ATP) production, promoting further Aβ aggregation[164]. Abnormal Aβ accumulation in the mitochondria alters several of its functions, leading to multiple mitochondrial defects[159, 164, 165]. For instance, in AD, mitochondrial dysfunction plays a vital role in the development of oxidative stress[166]. Accumulation of Aβ in the mitochondria causes hyperpolarization of its membrane potential (i.e., the difference in the electric potential between the interior and the exterior of a cell), increasing the production of reactive oxygen species (ROS), including free radicals, which results in oxidative stress and neuronal death[164]. The exact location of intracellular Aβ in the cat brain was not assessed in the present study; however, it is possible that cats also accumulate Aβ within their mitochondria, increasing the release of free radicals and promoting oxidative stress. Further studies are needed to determine whether Aβ aggregates inside the mitochondria in the cat brain and its involvement in the development of oxidative stress and CDS in this species.

Finally, in the AD brain, intracytoplasmic Aβ has been found in the areas that are affected the earliest, such as the hippocampus and the entorhinal cortex[157]; furthermore, intracytoplasmic Aβ has been found to affect the same cells where NFT are present, although any relationship with the NFT is unclear[156].

In the present study, cats of all ages were shown to have intracytoplasmic Aβ deposition; it had a granular appearance, and the amyloid structure was confirmed by staining with thioflavin-S. These deposits were predominantly

found in the cortical areas. In these regions, intracellular immunolabelling was more pronounced in the Prime group and it tended to decrease with age, especially where large extracellular Aβ deposits were found. However, in other areas, such as the hippocampus, the locus coeruleus, and the cerebellum, it tended to increase with age. It could be argued that this positive intracytoplasmic labelling could be APP, instead of Aβ, especially as the 4G8 antibody binds to the residues 17-24 of Aβ, plus APP; however, intracytoplasmic labelling was also found with the antibody mOC64, which is a conformation-specific antibody that recognises monomers, oligomers, and fibrils of Aβ42. As is the case with humans (as discussed above), these findings suggest that intracytoplasmic Aβ also starts accumulating within cortical areas of the cat brain at a young age, then decreases in these regions as cats get older and extracellular Aβ accumulation increases. However, further longitudinal studies are needed to determine the progression of these deposits both within the cells and in the extracellular spaces, and to determine if there is any correlation between them.

The presence of Aβ oligomers in the cat brain has been previously described[52]. Oligomers in cats consist of hexamers and dodecamers of Aβ[52], which are believed to be the most toxic form of Aβ and are known to be associated with tau pathology in AD[167]. It has been suggested that this form of Aβ may be involved in the development of pre-tangles, as both Aβ oligomers and pre-tangles have been found in the same regions of the aged cat brain[52].

While both the extra and intracellular accumulation of Aβ have been extensively studied in humans, it is still unclear which of them (if any) triggers the process that leads to neurodegeneration and the development of AD[157].

The *amyloid cascade hypothesis* was originally created by Hardy and Higgins (1992) and it proposes that AD is solely triggered by the abnormal production of Aβ[58]. They hypothesised that this abnormal Aβ production is caused by

mutations on the APP gene[58] which, after being cleaved during the amyloidogenic pathway, releases abnormal quantities of Aβ to the extracellular space[56]. Extracellular deposits then trigger further events that may eventually lead to neurodegeneration[56]. While these mechanisms remain unclear, they suggested that this could be due to Aβ being a neurotoxic peptide[58]. Furthermore, they suggested that Aβ neurotoxicity disrupts calcium homeostasis within neurons; hence, promoting hyperphosphorylation of tau and the formation of NFT that will eventually lead to neuronal death[55, 58]. Interestingly, it has been shown that NFT rarely appear in the absence of Aβ pathology[168].

While there is extensive evidence supporting the *amyloid cascade hypothesis*, there is also emerging evidence that challenges it. This new evidence is based in the fact that, while mutations on the APP gene have been proven, these have only been linked to cases of familial AD[55]. However, most of the AD cases are known to be caused by the sporadic form of the disease, in which no mutations on the APP gene have been found[55]. This suggests that there could be different and/or additional mechanisms involved in the development of AD.

2.4.2 Tau pathology in the cat brain

Non-phosphorylated tau protein was detected in the brains of cats of all ages with all three tau antibodies. One of the antibodies, Tau46, binds to the C-terminus of the protein; while the other two (8E6/C11 and 1E1/A6) bind to the three and four repeats isoforms of the protein, respectively. Labelling with these three antibodies showed that tau was highly expressed in all brain regions of cats of all ages, as expected. This correlates to the expression of this protein and its isoforms in the adult human brain[73, 74].

Immunolabelling of hyperphosphorylated tau was detected within the cytoplasm of neurons in some cats. Since these aggregates tended to occupy

the whole cytoplasm and no shape alterations were found, these are thought to be pre-tangles[77], as have been previously described in the cat brain[52, 64, 150].

Pre-tangles were visualised with the three antibodies against hyperphosphorylated tau (i.e., AT8, AT100, and A15091A). The number of pre-tangles appeared to increase with age, although this was not shown to be statistically significant.

Pre-tangles were mostly found in the cortex of cats in the Super Senior group, suggesting that tau pathology starts in the cortical regions of the cat brain. If true, this may represent a difference in the onset of tau pathology between cats and humans, as this pathology starts in the transentorhinal cortex in the human brain[60]. However, since the transentorhinal cortex was not assessed in the present study, it is impossible to say whether tau pathology truly begins in the cortical regions or in the transentorhinal cortex. Of note, a few of the cats in this study were shown to have small numbers of pre-tangles in the entorhinal cortex.

The amount of pre-tangles varied between grey and white matter. Greater numbers of pre-tangles were mostly found in the rostral and occipital cortices in grey matter; far fewer were found in the white matter.

Positive labelling for hyperphosphorylated tau was also found in the hippocampus, where statistically significant differences were found in their burden between the Prime and Senior groups, with more being seen in the Prime group, which was unexpected. However, this finding could be due to inaccuracies in the algorithm created to quantify the protein deposition because the Positive Pixel algorithm only recognises positive pixels. This means that the software efficiently recognises positive staining; however, it fails to recognise the location of this labelling (i.e., cytoplasmic or nuclear). Hence, it is possible that this algorithm recognised both cytoplasmic and nuclear staining when quantifying hyperphosphorylated tau. If this is true, it is

possible that the positive staining seen in the Prime group corresponds to intranuclear staining, rather than pre-tangles. Of note, after visual inspection of the slides, this did appear to be the case, with more intranuclear staining in the cats in the Prime group.

Interestingly, pre-tangles were found in the brains of two cats in the Prime group (i.e., one 4-year-old cat, which was the same cat showing extracellular Aβ deposits; and one 6-year-old cat). While this finding was unexpected, it was not entirely surprising. It is possible that these cats had a non-diagnosed neurological disorder that produced tauopathy, or that their age was miscalculated (particularly if they came from the shelter), and they might have, in fact, been older than recorded. However, it is also plausible that tau pathology starts at a young age in some cats. Braak suggested that the early stages of the tau pathology may occur in younger individuals, as NFT have been found in people under 25 years of age[77]; however, as individuals get older the number of NFT increase, leading to more severe stages of disease[77].

While no statistically significant differences were found in the *load* and *burden* of pre-tangles between cats with and without CDS, associations were found between the *presence* of pre-tangles and CDS; this is nuanced, but important. This is not surprising, as half of the cats with pre-tangles (as shown using all three antibodies) had a confirmed diagnosis of CDS. Chi-square tests showed that cats with CDS had larger numbers of pre-tangles, when compared to cats without CDS. In humans, a strong association has been reported between the presence of NFT and cognitive impairment[169]. Furthermore, the progression stages of tau pathology have been associated with different stages of cognitive impairment and dementia. While cognition is known to remain unaffected during Stages I and II, mild cognitive impairment has been associated with Stages III and IV and, finally, AD has been associated with the most severe stages of pathology (i.e., Stages V and VI)[77].

Of note, three of the cats with CDS included in this study were previously part of the double-blinded placebo-controlled study (see Chapter 4); interestingly, these cats had the largest numbers of pre-tangles within their brains (see Figure 2.17 in Results). To be included in that study the cats had to have significant signs of CDS; hence, as is the case in people, severe clinical impairment is associated with significant tau pathology.

In AD, hyperphosphorylated tau is also known to abnormally accumulate inside the mitochondria, leading to alterations in its morphology (e.g., changes in size and shape) and functions (e.g., alterations in glucose metabolism), plus defects in mitochondrial transport[170]. It is plausible that hyperphosphorylated tau accumulates within the mitochondria of cats; however, this was not assessed in the present study.

The role of tau in the development of AD and cognitive decline remains unclear. On one hand, tau is believed to be the cause and potential trigger of neurodegenerative disease, on the other, more recent evidence proposes that it plays a protective role instead. If tau is believed to be associated with neurodegeneration, any link with APP mutation and Aβ deposition is also debated.

As a potential cause of disease, tau in its hyperphosphorylated state, is believed to be neurotoxic and to trigger neurodegeneration, known as the *tau hypothesis*[70]. Abnormal hyperphosphorylation of tau affects microtubule function and stability, and promotes its disassembly, leading to neuronal death and neurodegeneration[171]. Of note, it has been suggested that APP, with our without mutation, can act as a receptor of abnormal tau, promoting intracellular tau aggregates[172]. However, in some cases, the presence of NFT has been observed in the human brain long before Aβ senile plaques develop[173], suggesting that it may be abnormally hyperphosphorylated tau that triggers the mechanisms that lead to neurodegeneration, rather than APP and Aβ deposition.

A different hypothesis suggests that NFT are not toxic, and instead, tau oligomers (i.e., an intermediate stage between hyperphosphorylated tau monomers and NFT) trigger neurodegeneration[174, 175]. A recent study has shown that mice develop significant cognitive decline and memory impairment, as well as synaptic loss and mitochondrial dysfunction after intracerebral injection of tau oligomers[176]. To date, there is no evidence of the presence of tau oligomers in cats; however, since cats do not produce NFT, it is possible that the pre-tangles seen in the aged cat brain are formed by tau oligomers. In the present study, elderly cats with CDS were shown to have the greatest numbers of pre-tangles, suggesting that these pre-tangles, potentially formed by tau oligomers, are highly toxic in this species. Further studies are needed to confirm which form of tau is present in the pre-tangles and to determine the involvement of tau oligomers in the development of CDS in cats.

An interesting, alternative hypothesis of tau as a cause of disease suggests that tau initially accumulates in a small number of cells, then later propagates to other cells and regions, in a prion-like manner, leading to neurodegeneration[72, 177, 178]. Although the mechanism behind this propagation remains unclear, it is believed that after being released into the extracellular space, either freely or within vesicles, cells nearby uptake the released tau, where it is then seeded. This means that a pathogenic species is created which has the ability to self-amplify. This seed acts as a template for the further recruitment of proteins and formation of aggregates[178]. This is believed to promote further aggregation and spreading to other cells and regions[177]. Of note, while tau seeds are in a non-phosphorylated state, seeded tau aggregates are hyperphosphorylated[177]. Interestingly, the pattern of propagation observed in this hypothesis is identical to the stages of tau pathology proposed by Braak[177].

Contrary to these hypotheses, emerging evidence suggests that tau may not trigger neurodegeneration. Recent studies have found that cells containing NFT can survive for long periods, even decades[70, 77]. For this reason, it has

been proposed that NFT are not, in themselves, neurotoxic and that neuronal death is not produced by the presence of NFT, but by alterations derived by its presence within the cells, such as microtubule instability and impaired axon/dendrite transport[77].

It has also been suggested that NFT provide protection to the cells from oxidative damage, which is one of the earliest events in AD[160, 161]. This hypothesis proposes that tau phosphorylation is upregulated and modified by oxidative stress, which also promotes its aggregation as NFT[179]. Furthermore, it has been found that oxidative damage decreases as the disease progresses and the number of NFT increases[160, 161].

Finally, a reversible tau hyperphosphorylation and formation of NFT has been reported in animals during hibernation, where it has been shown to have a neuroprotective effect[180].

The presence of intranuclear tau has been previously described in both human[181] and mouse brains[182]. While the presence of intranuclear paired helical filaments (PHF) was described in 1988[183], intranuclear non-phosphorylated tau was first reported in the early nineties in human neuroblastoma[184] and other cells, such as fibroblasts and lymphocytes[185]. Interestingly, one of these studies showed that around 16% of the total tau protein is located within the nucleus, particularly in the chromatin[186].

In the present study, in addition to IHC labelling of intranuclear tau, Western blots demonstrated that both phosphorylated and non-phosphorylated states of tau were present in the nuclear fraction of cat brain extracts. In humans, both states of intranuclear tau are also known to exist and are believed to have different functions[181, 186].

Studies in rodents have shown that nuclear non-phosphorylated tau binds and protects DNA after induced oxidative stress or heat-shock[182, 187, 188]. These two

processes promote the translocation and accumulation of non-phosphorylated tau to the nucleus and nucleolus in order to protect and maintain the integrity of DNA[182, 187]. These studies show a significant dephosphorylation of the cytoplasmic tau and an increase in nuclear phosphorylated tau immediately after inducing stress; whereas the opposite was observed after 24 hours, with an increase in phosphorylated cytoplasmic tau and a decrease in nuclear tau[182]. Because of this, it has been suggested that phosphorylation may regulate the translocation of tau between the nucleus and the cytoplasm[182]. Furthermore, abnormal phosphorylation may alter the ability of tau to translocate and/or may affect its ability to bind to DNA and protect it from damage[182, 189-191].

It has been suggested that phosphorylation of tau does not occur in the nucleus, but rather it occurs in the cytoplasm, before it is translocated[186]. However, some other studies have shown that tau phosphorylation at specific residues, such as T181, T212 and S404, is increased within the nucleus[191]. Furthermore, it has been shown that some other epitopes, such as those that recognise Thr212, Ser214 residues (i.e., that are detected by antibody AT100) are located in the nucleus of human neurons[192].

Nuclear tau has also been reported in the AD brain; however, its role in the development of disease remains unclear[188]. Following the amyloid cascade hypothesis, it has been suggested that Aβ deposits promote abnormal phosphorylation of tau, altering its translocation to the nucleus, and impeding its binding and protection to the DNA[188], as described above.

In the present study, intranuclear hyperphosphorylated tau was mostly found in the cortex of cats, particularly in the grey matter. While no statistically significant differences were found between the different age groups, cats in the Prime group tended to have more intranuclear hyperphosphorylated tau, compared to the older age groups. Interestingly, the cats that had pre-tangles showed little or no intranuclear tau immunolabelling. In contrast, intranuclear

non-phosphorylated tau (Tau46 antibody) tended to increase with age in all brain regions. Similarly, studies in humans have shown that nuclear tau increases with age, reaching the highest levels at geriatric stages[193]; however, in AD, intranuclear tau decreases significantly in the hippocampus and cortex, and completely disappears in advanced stages of disease[192].

Even though several hypotheses have been proposed to try to understand the development of AD, the relationship between Aβ and tau pathologies is still under debate. The *amyloid cascade hypothesis* is potentially the most recognised hypothesis, which states that abnormal Aβ accumulation triggers mechanisms that promote tau hyperphosphorylation and aggregation (e.g., mitochondrial dysfunction and release of free radicals), leading to neurodegeneration and eventually to cognitive decline[56, 58]. Similarly, the relationship between Aβ and tau pathologies in the aged cat brain remains unclear. Chambers *et. al.* (2015) suggested that abnormal Aβ accumulation in cats might also trigger hyperphosphorylation of tau and pre-tangle formation[52]; however, further studies are needed to investigate these interactions and the underlying processes that lead to CDS in this species.

The neuroinflammation produced by activation of glial cells (i.e., microglia and astrocytes) is believed to play an important role in the development of AD. Under physiological conditions, microglia are immune cells that provide maintenance of the brain tissue as they respond to injury and the presence of pathogens[194, 195]. When microglial processes detect either injury or a pathogen, microglial cells migrate to the damaged or vulnerable area where they engulf and degrade the debris produced by impaired or dying cells[196, 197]. In AD, abundant glial cells (both microglia and astrocytes) have been found surrounding Aβ plaques[198-200]. It has been suggested that glial cells keep the extracellular spaces clear of Aβ, reducing its deposition and slowing its progression[198, 200-202]. Reactive microglia form a barrier around Aβ deposits, compressing and containing Aβ fibrils inside, and preventing further aggregation into new or existing plaques[203]. However, ageing and/or genetic

factors are known to alter microglial functions, promoting an inadequate degradation and clearance of Aβ, and increasing the risk of developing AD[194, 201]. Furthermore, the presence of protein aggregates in the brain promotes microglial responses that may be damaging. For instance, activation of microglia promotes an inflammatory response and the release of pro-inflammatory mediators (e.g., TNFα and IL-1β) that induce neurotoxicity in reactive astrocytes, leading to neuronal death[204, 205 206, 207]. Astrocytes promote homeostasis of the brain, give shape to grey matter, and mediate neurotransmitters and synaptic activity[199, 208-210]. Similar to microglia, protein deposition in the brain activates astrocytes[199]; reactive astrocytes are also involved in the degradation and clearance of Aβ and promote an inflammatory response[200, 202]. However, abnormal protein deposition may alter calcium signalling in astrocytes affecting neuronal homeostasis that may lead to neuronal death[211]. To date, there is no evidence of the role of neuroinflammation in the aged cat brain. In a separate ongoing study, the author has been assessing neuroinflammatory markers (i.e., microglia, astrocytes, oligodendrocytes, amongst others), their role in ageing and the development of CDS, and their correlation to abnormal Aβ and tau deposition in the cat brain.

Some limitations were identified in the present study. Since the neuropathology was only assessed *post-mortem*, the findings only represent a snapshot of the neuropathology of ageing and CDS in these cats. Further longitudinal studies are needed to determine at what age these changes begin to occur and how the disease progresses over time. Furthermore, since behavioural changes were only fully assessed in three cats (those recruited for the placebo-controlled study; Chapter 4), and cognition was not formally assessed in any cat, no correlation could be made between the cognitive state of these cats and the presence and/or severity of the neuropathology found.

Another limitation arose when trying to trace and retrieve the cats' information, particularly where brains were collected several years before the study started;

no information was recorded for some of the cats (i.e., sex, exact age, or medical record). Having this information would have helped to understand and/or explain the neuropathological changes observed in these cats, especially in the younger ones.

A limitation concerning the sectioning of the brains was also identified. All brains were manually sectioned by the author without using a brain slicer, as had been developed for rats' and mice's brains. This resulted in slight variations in the thickness of the brain slices. In addition, there were natural individual variation in both the size and the shape of the brains. Even though anatomical landmarks were used to minimise variation when slicing the brains, there were differences in the size of some of the structures such as the hippocampus and cerebellum. This impeded analysing these regions in more detail; for example, since the hippocampus was not sectioned in the exact same places for all the cats, assessing differences between the regions of the hippocampus could not be assessed. For future studies, a 3D cat brain slicer could be developed and printed; however, a specialist would be needed to design the template (i.e., veterinary neuroanatomist/neurologist and 3D designer); plus, the high costs of using a 3D printer should be considered.

The inability of the Positive Pixel algorithm to differentiate between nuclear or cytoplasmic immunolabelling represented a limitation. Since the author visually inspected all the slides by light microscopy, this issue was identified promptly. As a result of this limitation, and to examine the nuclear immunolabelling in more detail, the Nuclear algorithm was purchased by Professor Elizabeth Head (The University of California, Irvine). To minimise this problem in the future, different algorithms could be used. A similar algorithm to the Nuclear one, identifies cytoplasmic labelling; however, these algorithms have to be purchased separately and are very expensive.

The relationship between the neuropathological findings described in this chapter and behavioural changes in cats was not assessed. This was due to

the small number of cats with a diagnosis of CDS and the lack of information on some of the cats (i.e., no medical records and/or behavioural assessments). As discussed above, evidence in humans and mice models of AD suggests that the abnormal accumulation of Aβ and tau, particularly of oligomers, triggers different mechanisms (e.g., synaptic loss and mitochondrial dysfunction) that promote neuronal dysfunction and neurodegeneration[167, 174-176]. Furthermore, the abnormal aggregation of Aβ and tau is known to be associated with the appearance of clinical signs in AD[77, 153, 169]. It is possible that similar mechanisms, derived by abnormal deposition of Aβ and tau, may also lead to neurodegeneration and behavioural changes in cats with CDS. Future studies, assessing behavioural changes plus cognition (e.g., visuospatial memory and problem-solving tests), being conducted alongside *in vivo* assessments of protein expression in different body fluids (i.e., levels of Aβ and tau in CSF and/or blood) are needed. These would provide with robust evidence of the initial alterations in Aβ and tau levels in cats and how these change over time until behavioural changes start to appear and CDS is developed, which can be followed by *post-mortem* histopathological examination of the brains.

2.5 Conclusion

Cats have been shown to accumulate diffuse extracellular and intracytoplasmic deposits of Aβ, plus, pre-tangles of hyperphosphorylated tau within their brains. The severity of both pathologies and the number of cats affected were shown to increase with age; three cats with severe CDS had the most pre-tangles. Of note, the presence and progression of these pathologies are similar to those seen in humans with AD, suggesting that the cat has the potential to be a natural model for the study of this condition.

Chapter 3 The use of 1.5T Magnetic Resonance imaging for the assessment of brain atrophy and other age-related brain changes in cats

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"Time spent with cats is never wasted"

-Sigmund Freud

Note:

Parts of this chapter have been submitted for publication to the Journal of Veterinary Radiology and Ultrasound. This paper is currently under review:

Sordo L., Dishong O., Marioni-Henry K., *et al.* Assessment of age-related brain atrophy in live cats and fixed cat brains with and without dementia using 1.5T MRI. *Journal of Veterinary Radiology and Ultrasound.*

Author's contribution to the paper:
The author measured all the regions assessed in the fixed brains and carried out the statistical analyses, as well as writing the paper.

3.1 Introduction

The impact that normal ageing has on the brain, its anatomy, and structures has been widely studied in humans. As individuals get older, vascular changes and shrinkage of the brain may occur that can lead to deterioration of cognition. Recently, imaging techniques have emerged as potential tools for the diagnosis and assessment of brain atrophy, with Magnetic Resonance (MR) imaging and computed tomography (CT) scans being the most commonly used.

In humans over 40 years old, the brain reduces its volume at a rate of 5% per decade[30]; however, not all brain regions shrink at the same rate. The prefrontal cortex is the most affected region, followed by the temporal lobe and the hippocampus[31]; although some contradictory evidence suggests that the hippocampus may be the most affected[33, 34]. In contrast, the occipital cortex is the least affected[31]. Interestingly, sex-related differences may also occur. The frontal and temporal lobes are the most affected in men, compared to the hippocampus and parietal lobes in women[144, 212].

The use of these modern imaging techniques is not limited to the assessment of brain atrophy in normal ageing. Recently, MR imaging has grown as a potential diagnostic tool for the investigation of neurodegenerative disorders, such as Alzheimer's disease (AD). Longitudinal studies have been conducted in cognitively healthy elderly individuals who later developed cognitive decline, including mild cognitive impairment and AD. These studies have shown subtle abnormalities, such as shrinkage, primarily in the medial temporal lobe and parietal regions of the brain[213, 214]. Hippocampal[36-38] and entorhinal[38] atrophy have also been reported, as well as changes in the size of the ventricles[35]. These subtle changes can be detected a decade before these individuals get a diagnosis of AD[91].

The progression of brain atrophy in patients with AD has also been studied (Figure 3.1). In the pre-symptomatic stage of AD, non-specific whole brain atrophy has been reported, along with more specific shrinkage of the medial temporal lobe[39, 92, 215], in early AD, ventrolateral and lateral parietal lobes, along with the posterior cingulate are believed to be most involved[92-95], while in later stages of the disease, non-specific total brain atrophy is again reported[216], along with atrophy of the frontal lobe[217], temporal lobe, and hippocampus[218].

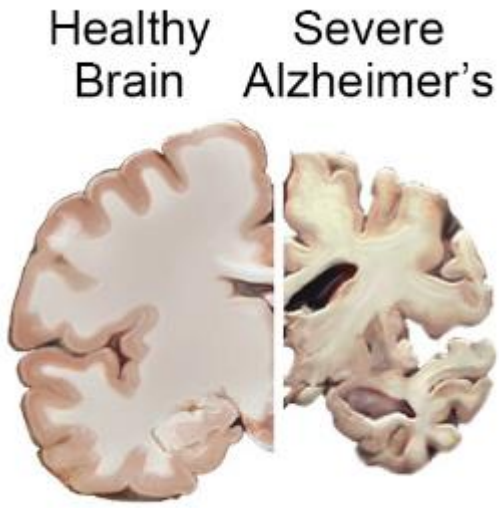

Figure 3.1 Atrophy of the brain in Alzheimer's disease (AD). *Comparative image of a healthy human brain (left) and the brain of a patient with severe AD (right). Image from the National Institute of Aging, USA*

Age-related brain changes have also been studied in other species, such as dogs. Enlargement of the ventricles[219-221], in particular the temporal horn[222], plus reduction of the total brain volume and the hippocampus[220-222] have been reported to occur in aged dogs (Figure 3.2). It has been suggested that the total brain volume remains stable in dogs aged between three months and 11 years of age but begins decreasing at 12 years and older[222]. Atrophy is more evident in the frontal lobe, which is believed to remain the same between six

months and seven years but decreases significantly after eight years old[222, 223]. In addition, dogs have shown a reduction of the interthalamic adhesion thickness[224, 225] associated with ageing. Measuring the thickness of this structure has become a method of diagnosing brain atrophy in dogs as it has been shown to become thinner with age[225], particularly in dogs with cognitive dysfunction syndrome (CDS)[224].

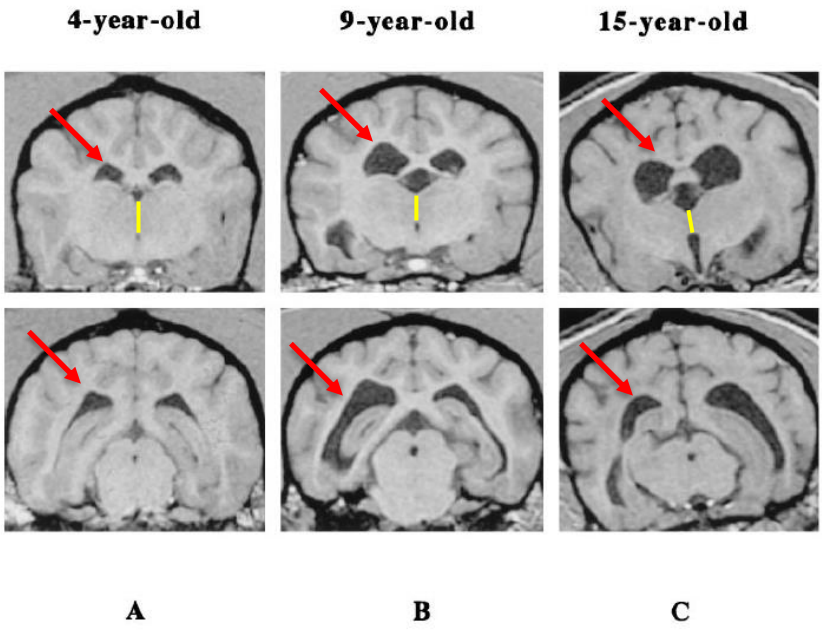

Figure 3.2 Enlargement of the ventricles in the dog brain. *MR images from the brains of A) a four-year-old dog, B) a nine-year-old dog, and C) a 15-year-old dog. Marked increment in the size of the lateral ventricles (red arrows) and cortical atrophy is seen as dogs get older. Measurement of the interthalamic adhesion thickness is represented by yellow lines. Image taken from Su* et. al. *1998*

As with dogs and humans, two studies looking at aged cats demonstrated an increase in the size of the ventricles with age, in particular the right lateral and the third ventricles[40], an increase of the temporal horn[40], a reduction in the total cerebral volume, and a reduced interthalamic adhesion thickness[226].

It is evident that MR imaging and CT scans may potentially be useful tools for the assessment and diagnosis of normal age-related brain changes and

general pathologies in both human and veterinary medicine, including some neurodegenerative diseases, such as AD. However, further studies are needed to fully describe these changes in the cat, and define the changes associated with CDS in this species.

The aim of this study was to ascertain whether there is age-related atrophy of the whole brain and/or specific brain structures, including the lobes, hippocampus, and the interthalamic adhesion, plus enlargement of the ventricles in cats by undertaking measurements of 1.5T MR images. It was hypothesised that elderly cats would have a marked atrophy of the whole brain and some of the different brain structures assessed (i.e., lobes and hippocampus). Furthermore, it was also hypothesised that elderly cats, particularly those with diagnosis of CDS, would have larger lateral ventricles and smaller interthalamic adhesions than younger cats. Finally, it was hypothesised that these age-related neuroanatomical changes will share similarities to those seen in human AD.

3.2 Materials and Methods

3.2.1 Study populations

Two populations were used in this study, one consisting of living feline patients, while the other consisted of brains collected and fixed *post-mortem*.

3.2.1.1 Live cats

Magnetic resonance images of the brains of live cats of various ages were obtained from medical records from the Hospital for Small Animals at the Royal (Dick) School of Veterinary Studies (RDSVS), The University of Edinburgh. A retrospective database search was conducted for all MR images of feline heads obtained from January 2009 to January 2018. Scans of cats that had one or more of the following criteria were not included in the study (Table 3.1):

Table 3.1 Exclusion criteria list. *Scans of cats that were shown to have any of the following were excluded from the study*

Exclusion criteria
• Diagnosis of intracranial mass lesions • Diagnosis of extracranial mass lesions invading the skull • Diagnosis of metabolic and/or inflammatory disease (i.e., meningitis/encephalitis) • Any other obvious brain pathology • Bad quality scans with poor visualisation of the structures

3.2.1.2 Fixed brains

The cats that formed this part of the study came from two different routes. Some of them were pet cats that were patients of the Hospital for Small Animals at the RDSVS, while others were from a shelter in Scotland. Owner and/or shelter consent for the donation and use of the cats' bodies for research was obtained at the time of euthanasia in all cases. No cats were euthanised specifically for this study, rather due to their advanced age, to chronic diseases where further treatment would not improve their quality of life, or if the cats had undesirable behavioural problems (i.e., untreatable aggression) that would impede their rehoming. Brains were collected at routine necropsy at the Pathology Department of the RDSVS. Of note, some brains of cats over 10 years old were collected several years before this study started. No information other than being of over 10 years of age was available for these brains, (e.g., breed, sex, or exact age). However, they were all clinical patients attending the Hospital for Small Animals at the RDSVS that were euthanised because of terminal disease. After collection, brains were fixed by immersion, by placing them inside large plastic containers filled with approximately 200 ml of 10% formalin for no less than one week.

3.2.2 Grouping of cats

Cats of all ages were recruited. Since the exact age of some of the cats was less certain, particularly in those cats coming from the shelter, age groups were created following the American Association of Feline Practitioners (AAFP) and the American Animal Hospital Association (AAHA) life stages guidelines (Table 3.2)[148]. In addition, this allowed the assessment of neuroanatomical changes at the different life stages and to determine whether there were differences between them.

Table 3.2 Grouping of cats. *Cats were grouped into different categories according to their age following the American Association of Feline Practitioners (AAFP) and the American Animal Hospital Association (AAHA) life stages guidelines*

Category	Age	Number of cats	CDS
Kitten	0 to 6 months	n = 0	n = 0
Junior	7 months to 2 years	n = 12	n = 0
Prime	3 to 6 years	n = 13	n = 0
Mature	7 to 10 years	n = 21	n = 0
Senior	11 to 14 years	n = 17	n = 2
Super Senior (Geriatric)	15 years and over	n = 11	n = 2

3.2.3 1.5T MR image acquisition

Since this study proposes the use of MR imaging as a clinical tool for the diagnosis of CDS in cats, it was logical to use equipment that is routinely used by clinicians. Even though there are more powerful MR imaging scanners that provide higher resolution and better image quality (i.e., 3T and 7T), the decision to use a 1.5T in the present study was based on this MRI scanner being the most commonly used in veterinary practice for diagnostic purposes.

Dorsal, sagittal, and transversal T1-weighted (i.e., images produced by using short time to echo [TE; the time elapsed between the delivery of the radiofrequency and obtaining the signal] and repetition time [TR; the amount of time elapsed between successive pulsations that are applied to the same slice]. On T1-weighted images, cerebrospinal fluid (CSF) spaces are dark) and T2-weighted sequences (i.e., images produced by using longer TE and TR times. On T2-weighted images, CSF spaces are bright) for both *in vivo* and *post-mortem* brains were taken using a 1.5 Tesla MRI scanner (Philips Intera, Philips Healthcare™, Reigate, Surrey, UK and Siemens Magnetom Avanto,

Siemens Healthineers™, Erlangen, Germany). For this, scans of the whole head were obtained from anesthetised live cats. Fixed brains were put into plastic containers with plastic lids, filled with formalin; plastic straps were placed around the brains to prevent them from floating and to minimise movement during the scanning procedure (Figure 3.3).

Figure 3.3 Containers used for the acquisition of MRI scans of the fixed brains. *Brains were placed inside plastic containers filled with formalin, and plastic straps were placed around the brains to prevent them from floating and to minimise movement during the scanning procedure*

3.2.4 Image assessment

Images were assessed using Horos™ DICOM viewer v3.3.6 for Mac OS by two different people. The first observer (OD) measured all the *in vivo* scans, and the second observer (author) measured all the *post-mortem* scans. In order to accurately identify each of the structures, anatomical landmarks were determined with the help of an ECVIM Board-certified veterinary neurologist (KMH). Both observers measured the first three scans together. To minimise variability and to ensure consistency, a printed guide with a picture of the annotated brain regions and the description of the anatomical landmarks with the desired structures outlined was created and given to both observers.

Since CSF spaces are brighter on T2-weighted images, the area of the lateral ventricles was measured on this sequence, as well as the interthalamic adhesion thickness. The rest of the measurements were assessed on T1-weighted images.

Nota bene: Both observers remained blinded throughout the image assessment. The age of the cats was not disclosed until all the areas were measured in all the cats, and these were grouped into the different age categories for data analysis.

3.2.4.1 Frontal lobe area

Frontal lobe areas were measured in the T2-weighted sagittal sequence in the slice where the interthalamic adhesion was visible. Using the free-hand drawing tool, the frontal lobe was outlined by drawing a line following the cruciate sulcus until the anterior commissure; at this point the line was drawn in the ventral direction, finishing at the bottom of the cortex, and outlining, in the rostral direction, the lobe until the starting point, without including the olfactory bulb (Figure 3.4A).

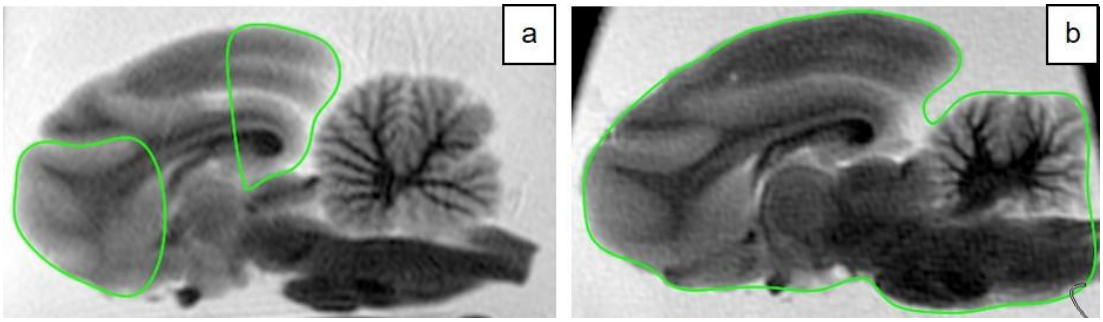

Figure 3.4 Examples of measurements taken on T2 sagittal sequence from a) frontal (left) and occipital (right) lobes areas; and b) total sagittal brain area

3.2.4.2 Occipital lobe area

Occipital lobes were measured in the T2-weighted sagittal sequence in the slice where the interthalamic adhesion is visible. Using the free-hand drawing tool, the occipital lobe was outlined by drawing a straight line starting at the level of the rostral side of the rostral/superior colliculus, continuing in the dorsal direction to the top of the cortex; then continuing in the caudal direction, outlining the lobe until the starting point, excluding the cerebellum and the rostral/superior colliculus (Figure 3.4A).

3.2.4.3 Total sagittal brain area

The total sagittal brain area was measured in the T2-weighted sagittal sequence in the same slice as the frontal and occipital lobes were measured. Using the free-hand drawing tool, the total sagittal brain area was outlined by drawing a line around the brain, including the cerebellum (Figure 3.4B).

3.2.4.4 Temporal lobe area

Temporal lobes were measured in the T2-weighted transversal sequence in the slice where the third ventricle appears for the first time. Using the free-hand drawing tool, the temporal lobes were outlined by drawing a line following the rhinal sulcus until the white matter; continuing in the ventral direction to the bottom of the cortex, outlining the lobe until the starting point (Figure 3.5A). This was measured in both hemispheres.

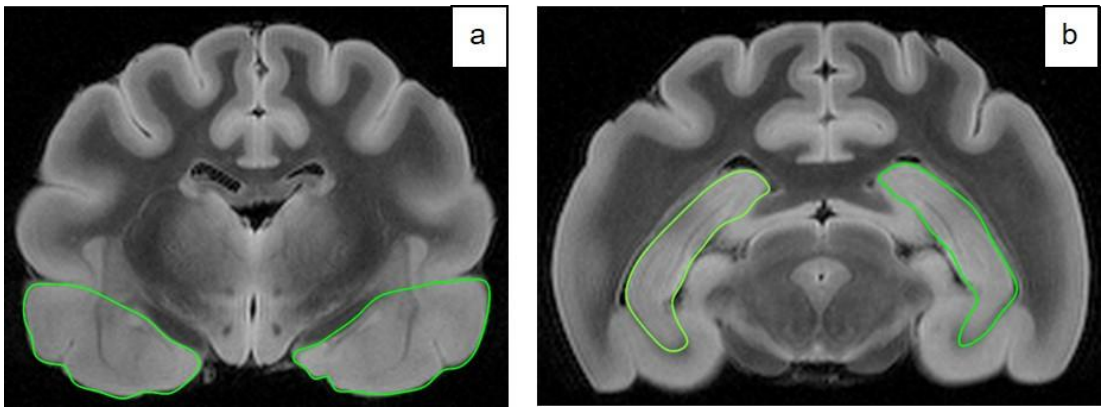

Figure 3.5 Examples of measurements taken on a) T1 transversal sequence of both temporal lobes; and b) T2 transversal sequence of the hippocampal area of both hemispheres

3.2.4.5 Hippocampal area

The hippocampal area was measured in the T2-weighted transversal sequence in the slice where the mesencephalic aqueduct and the rostral/superior colliculus are visible. Using the free-hand drawing tool, the hippocampal area was outlined by drawing a line around the hippocampus in both hemispheres (Figure 3.5B).

3.2.4.6 Left and right hemispheres area

Both hemispheres were measured in the T2-weighted dorsal sequence in the slice where the caudal/inferior colliculus is visible. Using the free-hand drawing tool, the hemispheres area was outlined by drawing a line around the left and the right hemispheres, separately, excluding the caudal/inferior colliculus and the cerebellum (Figure 3.6A).

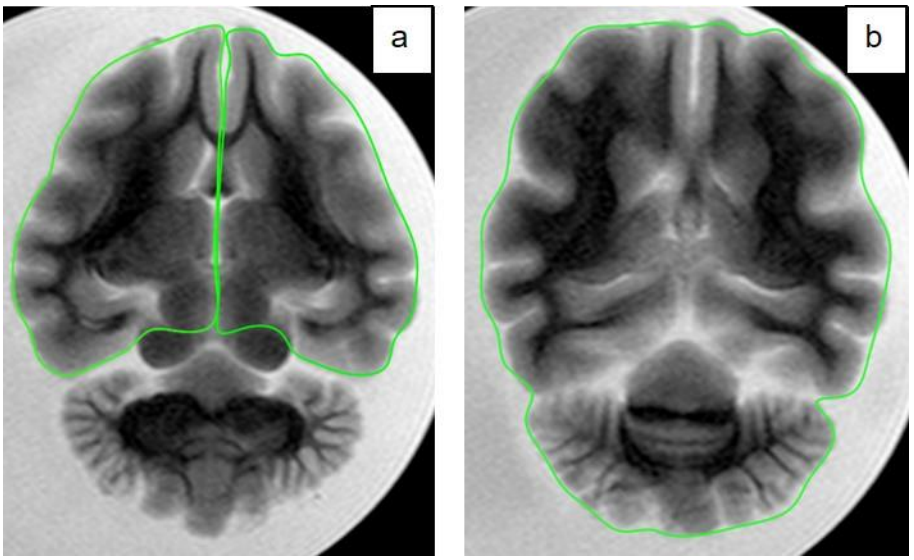

Figure 3.6 Examples of measurements taken on a T2 dorsal sequence of a) both hemispheres area; and b) total dorsal brain area

3.2.4.7 Total dorsal brain area

Total dorsal brain area was measured in the T2-weighted dorsal sequence in the same slice as the hemisphere areas were measured. Using the free-hand drawing tool, the total dorsal brain area was outlined by drawing a line around the whole brain, including the cerebellum (Figure 3.6B).

3.2.4.8 Lateral ventricles area

The area of the lateral ventricles was measured in the T1-weighted transversal sequence in the same slice as the interthalamic adhesion thickness was measured. Using the free-hand drawing tool, both lateral ventricles were outlined (Figure 3.7).

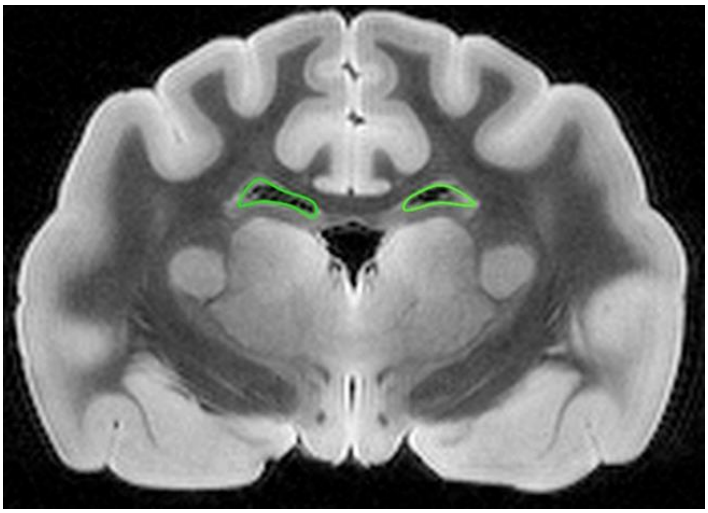

Figure 3.7 Example of measurements taken on a T1 transversal sequence of the lateral ventricles area

3.2.4.9 Interthalamic adhesion thickness

Interthalamic adhesion thickness was measured in the T1-weighted transversal sequence in the slice of its maximum height[224]. Using the straight-line tool, the interthalamic adhesion length was measured by drawing a dorso-ventral straight line on it (Figure 3.8).

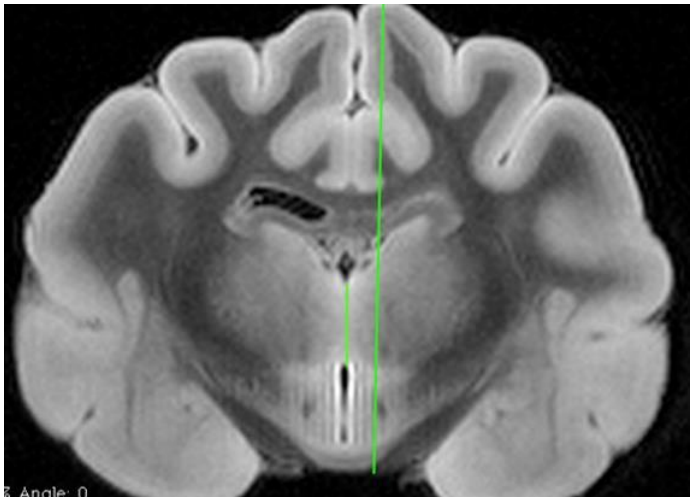

Figure 3.8 Example of measurements taken on a T1 transversal sequence of the interthalamic adhesion length and transversal brain height

The transversal brain height was measured in the T1-weighted transversal sequence in the same slice as the interthalamic adhesion thickness and lateral ventricles were measured[224]. Using the straight-line tool, a line was drawn measuring at midline, from the top to the bottom of the lobes, excluding the pituitary gland (Figure 3.8).

3.2.4.11 Calculation of the total transversal brain area

Since the total transversal brain area was not measured, it had to be calculated using Equation 3.1. This equation was obtained from the standard geometry equation to calculate the area of an ellipse (i.e., A = π ab) created by Archimedes; however, this had to be modified using a three-equation system due to having two unknown variables:

$$A_3 = \pi \left\{ \frac{A_1}{\pi \left[\frac{A_2}{\pi(b_2)} \right]} \right\} \qquad \textit{Equation 3.1}$$

where,

A_3 is the estimated total transversal brain area

A_1 is the total dorsal brain area

A_2 is the total sagittal brain area

b_2 is the transversal brain height

3.2.4.12 Calculation of ratios

For accuracy and to minimise for breed variability (e.g., big breeds of cats having larger brains), corrections were performed by calculating ratios in percentages by using Equation 3.2. This was done by dividing the area of each structure by the total brain area of the same plane (e.g., dividing the frontal lobe ratios by the total sagittal brain area, and multiplying the result by 100 to obtain a percentage). For this, frontal (FROr) and occipital lobes (OCCr) ratios

were obtained using the total sagittal brain area; hemispheres ratio (HEMr) was obtained using the total dorsal brain area; and the temporal lobes (TEMr), hippocampal (HIPr), and lateral ventricles ratios (LVr) were obtained using the estimated total transversal brain area. For bilaterally symmetrical structures (i.e., temporal lobes, hippocampus, hemispheres, and lateral ventricles), both measurements were added to each other and the resulting sum was used to estimate the ratios. Finally, interthalamic adhesion ratio (ITAr) was obtained using the interthalamic adhesion length and transversal brain height.

$$Ratio\ \% = \left(\frac{Area\ of\ structure}{Total\ brain\ area}\right)\ x\ 100 \qquad \textit{Equation 3.2}$$

3.2.5 Data analysis

Data were analysed using Minitab® v19.2020.1 statistical software for Windows.

Data were analysed for both datasets combined (i.e., *in vivo* and *post-mortem* MR images) and separately.

Anderson-Darling tests were performed to assess normality. Non-normal data were transformed using Box-Cox transformation or Johnson transformation, as appropriate. Bartlett tests for equal variances were performed, with 95% confidence level, assuming normal distribution. Non-parametric tests were performed in the variables in which transformation failed.

3.2.5.1 Differences in the size of structures between age groups

To determine whether there were differences in the size of the structures in the different age groups, one-way ANOVA were performed, assuming equal variances (if applicable) with two-sided 95% confidence interval (CI) and a significance level of $\alpha = 0.05$. To determine differences between groups, Tukey tests and Games-Howell *post-hoc* tests were performed for those structures that showed statistically significant differences in the one-way ANOVA. In addition, non-parametric Kruskal-Wallis tests were performed to assess differences in non-normal data.

3.2.5.2 Sex-related differences in the size of structures

Since there were only three intact males and no intact female cats, intact and neutered males were grouped together and were compared against the female cats. To determine whether there were sex-related differences in the size of the structures, two-sample t-tests were performed, with a 95% confidence level and a hypothesized difference of zero, assuming equal variances. Non-parametric Mann-Whitney tests were performed to assess differences in non-normal data.

3.2.5.3 Differences in the size of structures between cats with and without CDS

To determine differences in the size of the structures between cats with and without CDS, cats with CDS were compared to the cats without CDS in the same age-groups; since cats with CDS were in the Senior and Super Senior groups, these cats were compared against the cats without CDS in these two same groups. Two-sample t-tests were performed, as described previously.

3.2.5.4 Correlations between the size of the structures, age, and cognitive status

Spearman rank correlations were performed to determine whether there were correlations between the size of the different structures, the age groups and cognitive status (i.e., cats with and without CDS). For this, cats' ages were used as decimals (e.g., one year six months corresponded to 1.5 years) instead of being grouped into categories.

3.2.5.5 Differences between *in vivo* and *post-mortem* scans

To determine differences between *in vivo* and *post-mortem* scans, two-sample t-tests were performed, as described previously. Given that there were no Junior cats in the *post-mortem* database this group was removed from the *in vivo* dataset; hence, only Prime, Mature, Senior and Super Senior groups were compared.

3.2.5.6 Inter-rater reliability

To assess inter-rater reliability between both observers (i.e., one measuring *in vivo* scans and another measuring *post-mortem* scans) Kendall's coefficients for attribute agreement analysis were performed, with 95% confidence level. For this, the MRI scans of five cats were selected at random and both observers measured all the structures, as described above, independently. The data obtained from the measurements were compared to determine whether there was an agreement between the observers and to confirm that this agreement was not attributed to chance.

3.3 Results

3.3.1 Demographics

3.3.1.1 *In vivo* and *post-mortem* brain scans

MR images from a total of 74 live cats and fixed cat brains were assessed. Most of the cats were non-pedigree cats (77%; n = 57) with 23% (n = 17) being pedigree breeds. Most of the cats were neutered males (55.4%; n = 41), followed by neutered females (33.8%; n = 25), and intact males (4%; n = 3); no intact females were present. However, the sex of some of the cats (6.8%; n = 5) was unknown. Ages ranged between four months and 20 years old. Of these cats, 12 were in the Junior group, 13 in the Prime, 21 in the Mature, 17 in the Senior, and 11 in the Super Senior.

3.3.1.2 *In vivo* scans

The retrospective database search resulted in 90 live cats that had undergone MR imaging of their brain. After inspection of the scans and following the exclusion criteria, MR images from a total of 47 cats were included in this study. Most of the cats (66%; n = 31) were non-pedigree cats (e.g., domestic shorthair [DSH; n = 28]; domestic longhair [DLH; n = 1]; and domestic semi longhair [n = 2]), with 34% (n = 16) being pedigree breeds (e.g., Bengal [n = 1]; Birman [n = 1]; British shorthair [n = 2]; Burmese [n = 2]; Burmilla [n = 1]; Maine Coon [n = 2]; Norwegian Forest [n = 1]; Oriental [n = 1]; Persian [n = 1]; Ragdoll [n = 2]; Siamese [n = 1]; and Siberian [n = 1]). Most of the cats (66%; n = 31) were neutered males, followed by neutered females (27.7%; n = 13), and intact males (6.3%; n = 3); there were no intact female cats. Ages ranged from four months to 17 years. Of these cats, 12 were in the Junior group, 10 in the Prime, nine in the Mature, 13 in the Senior, and three in the Super Senior.

3.3.1.3 Post-mortem scans

A total of 27 cat brains were assessed. The majority of cats (96.3%; n = 26) were non-pedigree cats and only one cat was a pedigree breed (a Bengal cat). Most of the cats (44%; n = 12) were neutered females, followed by neutered males (37%; n = 10); while the sex of the remaining 20% (n = 5) of cats was unknown. Ages ranged from four to 20 years old. Of these cats, none were in the Junior group, three in the Prime, 12 in the Mature, four in the Senior, and eight in the Super Senior. Finally, four of these cats were diagnosed with CDS before euthanasia (see Chapter 4); these cats were in groups Senior (n = 2) and Super Senior (n = 2).

3.3.2 Differences in the size of structures between age groups

One-way ANOVA and Kruskal-Wallis tests were performed to determine differences in the size of the structures in the different cat age groups. Tukey tests and Games-Howell *post-hoc* tests were performed to determine differences between groups.

3.3.2.1 In vivo and *post-mortem* scans

One-way ANOVA showed statistically significant differences in the size of the HEMr ($F_4 = 2.98$, $p = 0.026$) and the OCCr ($F_4 = 5$, $p = 0.003$) between the age groups (Table 3.3). Tukey tests demonstrated that the differences in the HEMr were between the Junior and Super Senior age groups (Difference of means = 3.38; SE of difference = 1.09; 95% CI [0.30, 6.47]; t = 3.09, p = 0.024), with cats in the Junior group having smaller HEMr than cats in the Super Senior group (Figure 3.9).

Table 3.3 Differences in the size of the structures in the different age groups. *One-way ANOVA was performed to determine whether there were differences in the size of the structures between age groups*

Structure	F value	DF	p value
ITAr	1.59	4	0.187
LVr	1.15	4	0.344
TEMr	0.28	4	0.866
Total transversal area	0.82	4	0.516
HEMr	2.98	4	**0.026**
Total dorsal area	0.49	4	0.746
OCCr	5	4	**0.003**
FROr	0.32	4	0.862
Total sagittal area	1.15	4	0.339

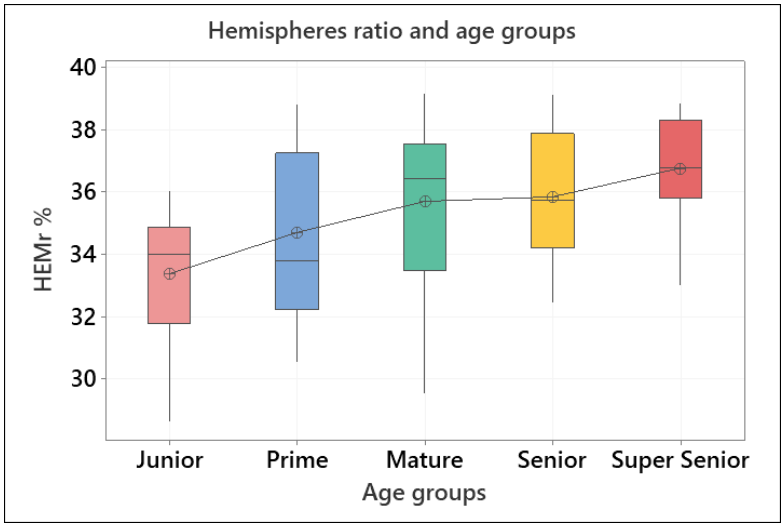

Figure 3.9 Differences in the hemispheres ratios (HEMr) in the different age groups. *The size of the hemispheres tended to increase with age and statistically significances were found between the Junior and the Super Senior groups (p = 0.024)*

Games-Howell tests demonstrated that the statistically significant differences in the OCCr were between groups Junior and Prime age groups (Difference of means = -1.801; SE of difference = 0.558; 95% CI [- 3.491, - 0.112], t = -3.23, p = 0.034); and between the Junior and Super Senior groups (Difference of means = -2.238; SE of difference = 0.653; 95% CI [-4.265, -0.211], t = -3.43,

p = 0.027), with cats in the Junior group having larger OCCr than cats in the Prime and Super Senior groups (Figure 3.10).

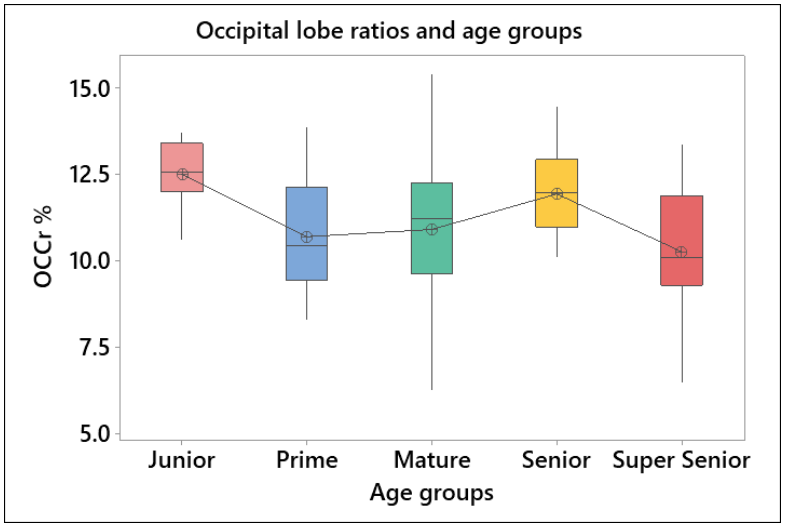

Figure 3.10 Differences in the occipital lobe ratios (OCCr) in the different age groups. *The size of the occipital lobe tended to decrease with age and statistically significant differences were found between groups Junior and Prime (p = 0.034); and between Junior and Super Senior (p = 0.027)*

Furthermore, there were some trends in the means of some structures between the Junior and Super Senior age groups (Table 3.4), including the HEMr, OCCr, and total sagittal brain area (Figure 3.11). The Junior group were shown to have larger OCCr and total sagittal brain area than the Super Senior group; in contrast, the Super Senior group had larger HEMr than the Junior group. The complete list with the means of all structures and age groups with standard deviations and 95% CI can be found in Appendix 3.1.

Table 3.4 Comparison of means between the Junior and the Super Senior groups for hemisphere ratio (HEMr), occipital ratio (OCCr), and Total sagittal brain area. *Only the lowest and the highest means of each structure are shown*

Structure	Lowest mean		Highest mean	
	Age group (n)	***Mean***	***Age group (n)***	***Mean***
HEMr	Junior (11)	33.37	Super Senior (9)	36.75
OCCr	Super Senior (11)	10.26	Junior (11)	12.50
Total sagittal brain area	Super Senior (11)	10.86	Junior (11)	11.87

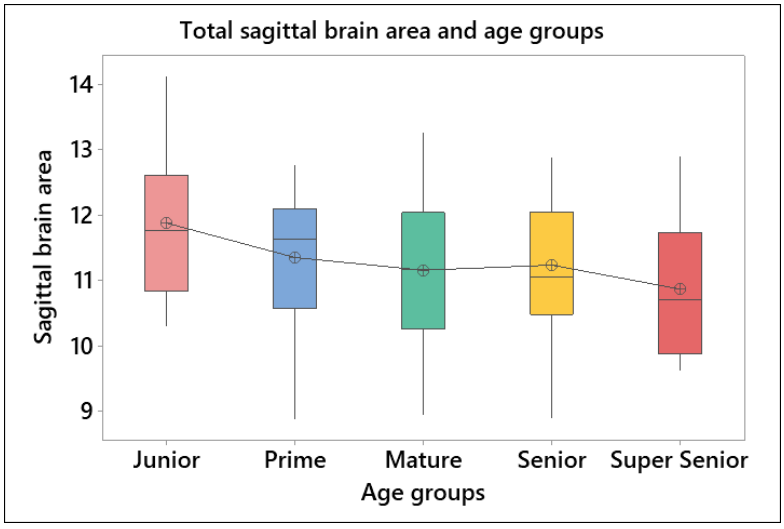

Figure 3.11 Differences in the total sagittal brain areas in the different age groups. *The total sagittal brain area tended to decrease with age*

Kruskal-Wallis tests showed no differences in the size of the hippocampus between age groups ($h_4 = 5.66$, $p = 0.226$).

3.3.2.2 *In vivo* scans

One-way ANOVA showed statistically significant differences in the size of the LVr between the age groups ($F_4 = 5.96$, $p = 0.001$) (Table 3.5). Games-Howell tests demonstrated differences between the Junior and Mature age groups (Difference of means = 0.2277; SE of difference = 0.0770; 95% CI [0.0056, 0.4498]; t = 2.96, p = 0.042); between Prime and Mature groups (Difference of means = 0.3361; SE of difference = 0.0858; 95% CI [0.0887, 0.5835]; t = 3.92, p = 0.004); between Prime and Senior groups (Difference of means = 0.2817; SE of difference = 0.0835; 95% CI [0.0408, 0.5226]; t = 3.37, p = 0.015); and between the Prime and Super Senior groups (Difference of means = 0.357; SE of difference = 0.114; 95% CI [0.027, 0.687]; t = 3.12, p = 0.028), with the Junior and Prime groups having smaller LVr than the rest of the age groups. (Figure 3.12). The complete list with the means of all structures and age groups with standard deviations and 95% CI can be found in Appendix 3.2.

Table 3.5 Differences in the size of the structures in the different age groups in the live cats. *One-way ANOVA was performed to determine whether there were differences in the size of the structures between age groups in the* in vivo *scans*

Structure	F value	DF	p value
ITAr	2.13	4	0.095
LVr	5.23	4	**0.008**
TEMr	0.50	4	0.733
HIPr	1.07	4	0.387
Total transversal area	0.15	4	0.961
HEMr	0.93	4	0.459
Total dorsal area	0.28	4	0.886
OCCr	1.20	4	0.328
FROr	0.81	4	0.529
Total sagittal area	0.31	4	0.868

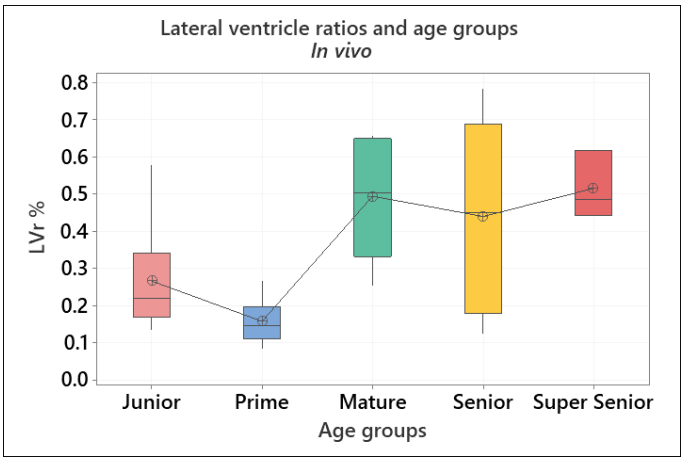

Figure 3.12 Differences in the lateral ventricles ratios (LVr) in the different age groups of live cats. *The LVr tended to increase with age and statistically significant differences were found between groups Junior and Mature (p = 0.042); Prime and Mature (p = 0.004); Prime and Senior (p = 0.015); and between Prime and Super Senior (p = 0.028)*

3.3.2.3 *Post-mortem* scans

One-way ANOVA showed no statistically significant differences in the size of any of the structures between the age groups (Table 3.6). The complete list with the means, standard deviations and 95% CI can be found in Appendix 3.3.

Table 3.6 Differences in the size of the structures in the different age groups in the fixed brains. *One-way ANOVA was performed to determine whether there were differences in the size of the structures between age groups in the* post-mortem *scans*

Structure	F value	DF	p value
ITAr	0.99	3	0.418
LVr	1.44	3	0.262
TEMr	0.50	3	0.689
HIPr	2.09	3	0.136
Total transversal area	0.89	3	0.466
HEMr	0.22	3	0.882
Total dorsal area	0.03	3	0.993
OCCr	1.03	3	0.399
FROr	0.29	3	0.835
Total sagittal area	0.63	3	0.602

While it was not statistically significant, the ITAr (Figure 3.13A) and the LVr (Figure 3.13B) were smaller in the Super Senior group (16.878 ± 2.202; and 4.302 ± 1.428, respectively) when compared to the Prime group (19.025 ± 1.257; and 6.6 ± 2.08, respectively).

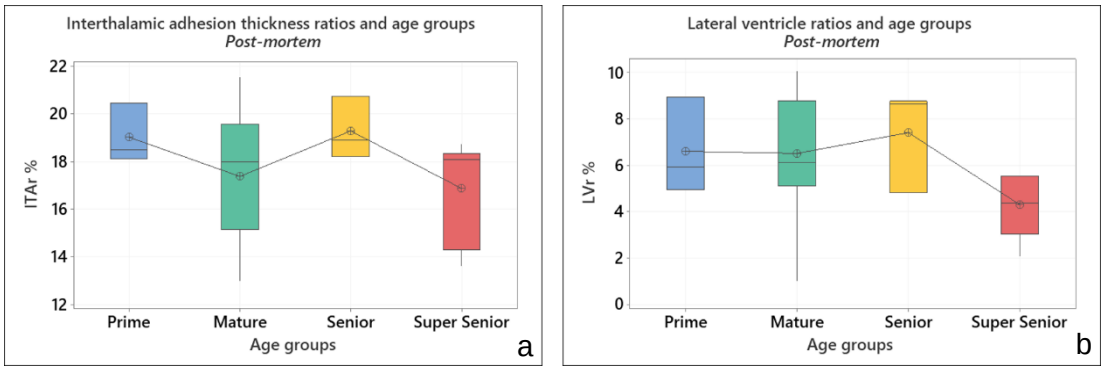

Figure 3.13 Differences in the interthalamic adhesion ratios (ITAr) and lateral ventricles ratios (LVr) in the different age groups of fixed brains. *While no statistically significant differences were found, both a) the ITAr; and b) the LVr tended to decrease more in the Super Senior group*

Both the HIPr (Figure 3.14A) and the OCCr (Figure 3.14B) tended to increase with age and then decrease in the Super Senior group; however, these were not statistically significant.

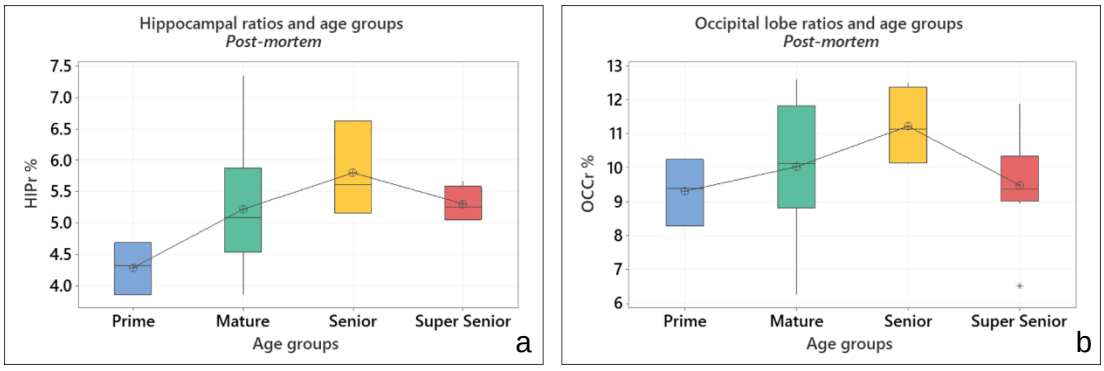

Figure 3.14 Differences in the hippocampal ratios (HIPr) and occipital lobe ratios (OCCr) in the different age groups of fixed brains. *Despite not being statistically significant, both a) the HIPr; and b) the OCCr tended to decrease more in the Super Senior group*

3.3.3 Sex-related differences in the size of structures

Two-sample t-tests and Mann-Whitney tests were performed to determine differences between sexes in the size of the structures. These were performed by grouping all female cats and comparing them to all male cats, regardless of their age.

3.3.3.1 In vivo and *post-mortem* scans

The two-sample t-tests demonstrated statistically significant sex-related differences in the total dorsal brain area ($t_{58} = -2.95$, $p = 0.005$) (Figure 3.15A); and the total sagittal brain area ($t_{63} = -4.16$, $p < 0.001$) (Figure 3.15B) (Table 3.7).

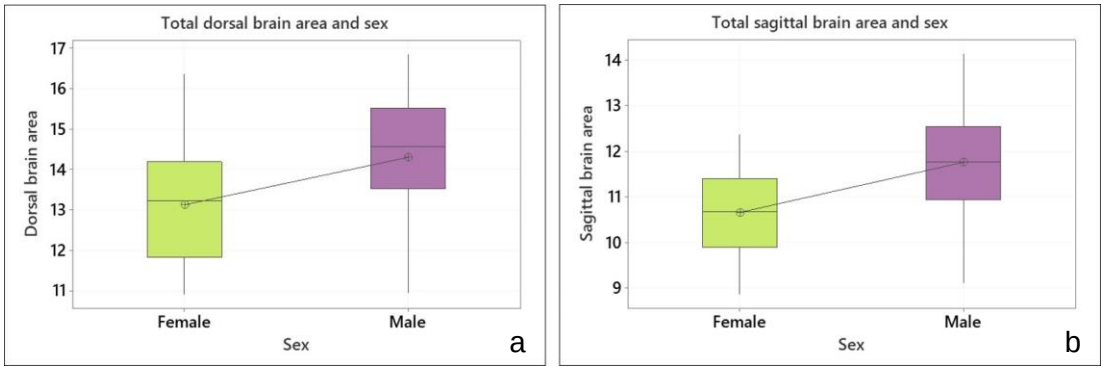

Figure 3.15 Sex-related differences in the total dorsal and sagittal brain areas. *Male cats had larger a) total dorsal ($p = 0.005$); and b) total sagittal brain areas ($p < 0.001$) than female cats*

Table 3.7 Sex-related differences in the size of the structures. *Two-sample t-tests were performed to determine whether there were sex-related differences in the size of structures*

Structure	T value	DF	p value
ITAr	-0.32	63	0.749
LVr	0.94	55	0.350
TEMr	0.11	38	0.911
Total transversal area	-1.05	56	0.297
HEMr	1.95	57	0.056
Total dorsal area	-2.95	58	**0.005**
OCCr	-0.87	51	0.391
FROr	0.22	63	0.826
Total sagittal area	-4.16	63	**<0.001**

Male cats had larger dorsal (14.3 ± 1.45) and sagittal brain areas (11.75 ± 1.03) than female cats (13.13 ± 1.5; and 10.66 ± 1.03, respectively). There were no differences in the ITAr, LVr, TEMr, total transversal area, HEMr, OCCr, and FROr between female and male cats. The complete list with the means of all structures, with standard deviations and SE of the means can be found in Appendix 3.4.

Mann-Whitney tests showed no differences in the size of the hippocampus between female and male cats (W = 1101, p = 0.750).

3.3.3.2 *In vivo* scans

Two-sample t-tests demonstrated that there were statistically significant sex-related differences in the HIPr (t_{38} = 2.04, p = 0.049); total transversal brain area (t_{38} = -2.71, p = 0.010); total dorsal brain area (t_{39} = -4.20, p < 0.001); and the total sagittal brain area (t_{41} = -2.75, p = 0.009) (Table 3.8).

Table 3.8 Sex-related differences in the size of the structures of live cats. *Two-sample t-tests were performed to determine whether there were sex-related differences in the size of structures for* in vivo *scans*

Structure	T value	DF	p value
ITAr	-0.02	44	0.983
LVr	0.12	17	0.904
TEMr	-0.17	37	0.869
HIPr	2.04	38	**0.049**
Total transversal area	-2.71	38	**0.010**
HEMr	0.64	38	0.528
Total dorsal area	-4.20	39	**<0.001**
OCCr	0.02	41	0.988
FROr	0.12	41	0.901
Total sagittal area	-2.75	41	**0.009**

Male cats had larger total dorsal brain areas (14.11 ± 1.36) (Figure 3.16A), total sagittal brain areas (12.071 ± 0.854) (Figure 3.16B), and total transversal brain areas (9.6 ± 0.894) (Figure 3.16C), than female cats (12.25 ± 1.11; 11.287 ± 0.792; and 8.843 ± 0.555, respectively).

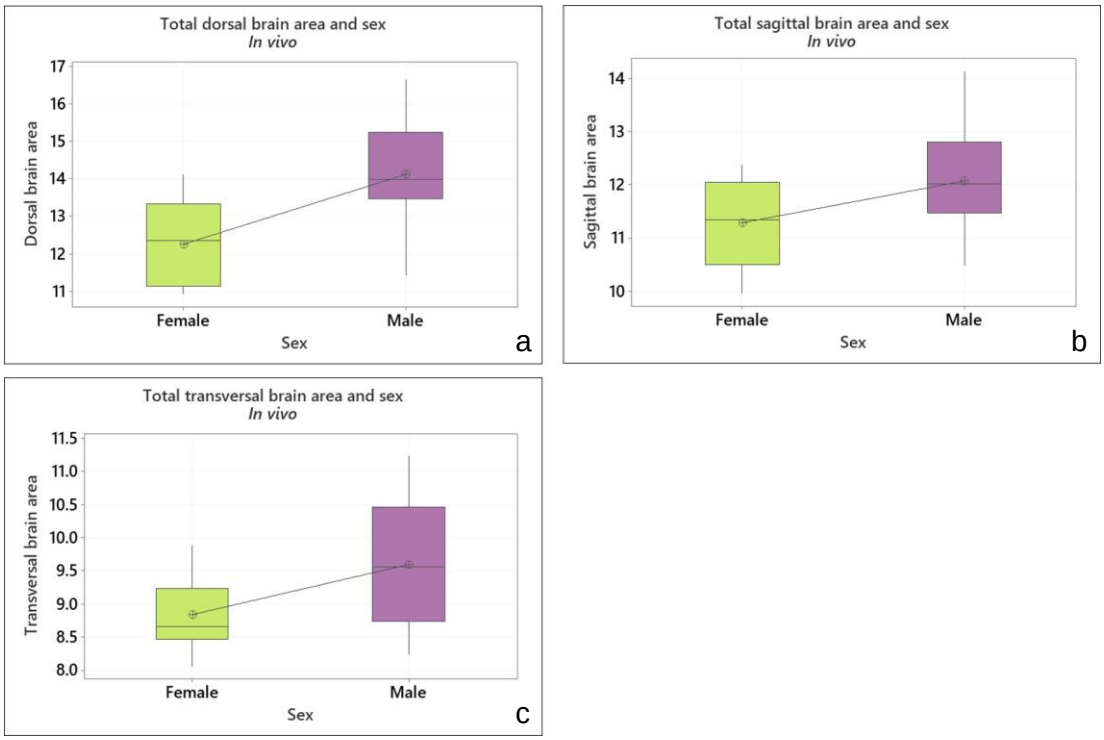

Figure 3.16 Sex-related differences in the total dorsal, sagittal, and transversal brain areas of live cats*. Male cats had larger a) total dorsal (p < 0.001); b) total sagittal (p = 0.009); and c) total transversal brain areas (p = 0.010) than the female cats in the* in vivo *dataset*

In contrast, female cats had larger HIPr (10.48 ± 1.5) (Figure 3.17) than male cats (9.51 ± 1.33). The full list of means with standard deviations and SE of the means can be found in Appendix 3.5.

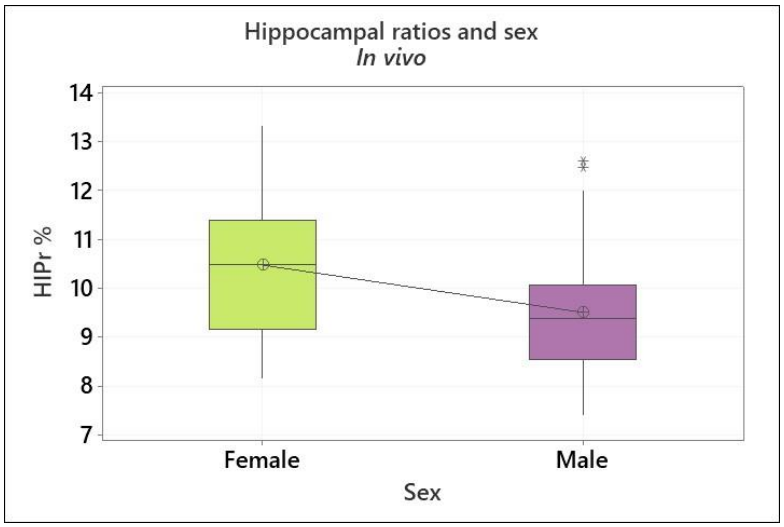

Figure 3.17 Sex-related differences in the size of the hippocampus (HIPr) of live cats. *Female cats had larger HIPr than male cats (p = 0.049) in the* in vivo *dataset*

3.3.3.3 *Post-mortem* scans

The two-sample t-tests demonstrated statistically significant sex-related differences in the HEMr (t_{17} = 2.57, p = 0.020) (Table 3.9) with female cats having larger HEMr (37.96 ± 0.804) than male cats (36.99 ± 0.841) (Figure 3.18).

Table 3.9 Sex-related differences in the size of the structures of fixed brains. *Two-sample t-tests were performed to determine whether there were sex-related differences in the size of structures for* post-mortem *scans*

Structure	T value	DF	p value
ITAr	-0.64	17	0.532
LVr	1.67	16	0.115
TEMr	-0.09	16	0.928
HIPr	0.80	16	0.437
Total transversal area	-0.24	16	0.810
HEMr	2.57	17	**0.020**
Total dorsal area	-0.83	17	0.420
OCCr	0.29	20	0.774
FROr	-0.87	20	0.395
Total sagittal area	-1.98	20	0.061

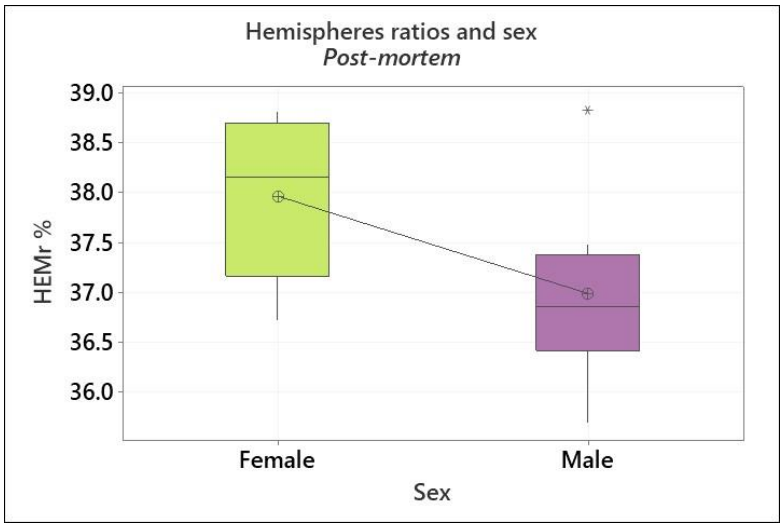

Figure 3.18 Sex-related differences in the size of the hemispheres (HEMr) in the fixed brains. *Female cats had larger HEMr than male cats (p = 0.20) in the* post-mortem *dataset*

While not being statistically significant, male cats had larger total dorsal (14.83 ± 1.66), total sagittal (10.77 ± 0.914), and total transversal brain areas (11.49 ± 1.19), when compared to female cats (14.29 ± 1.14; 10.02 ± 0.841; and 11.37 ± 0.614, respectively) as reported in the live cats. In contrast, female cats had larger HIPr (5.236 ± 0.791) than male cats (4.962 ± 0.667). The full list of means with standard deviations and SE of the means for all the brain structures can be found in Appendix 3.6.

3.3.4 Differences in the size of structures between cats with and without CDS

Two-sample t-tests and Mann-Whitney tests were performed to determine differences in the size of the structures between cats with and without CDS.

3.3.4.1 *In vivo* and *post-mortem* scans

Two-sample t-tests showed differences between cats with and without CDS in the total dorsal brain area (t_{19} = -2.21, p = 0.040), OCCr (t_{24} = 2.47, p = 0.021), and HEMr (t_{18} = -2.12, p = 0.048) (Table 3.10).

Table 3.10 Differences in the size of the structures in cats with and without cognitive dysfunction syndrome (CDS). *Two-sample t-tests were performed to determine whether there were differences in the size of structures between cats with and without CDS*

Structure	T value	DF	p value
ITAr	-0.11	22	0.910
LVr	0.54	18	0.595
TEMr	0.42	18	0.677
HIPr	-0.08	18	0.937
Total transversal area	-1.42	18	0.172
HEMr	-2.12	18	**0.048**
Total dorsal area	-2.21	19	**0.040**
OCCr	2.47	24	**0.021**
FROr	-1.11	24	0.277
Total sagittal area	0.67	24	0.511

Cats without CDS had smaller total dorsal brain areas (13.47 ± 1.56) (Figure 3.19A) and HEMr (35.96 ± 1.91) (Figure 3.19B) than cats with CDS (15.97 ± 0.547; and 38.9 ± 0.344, respectively).

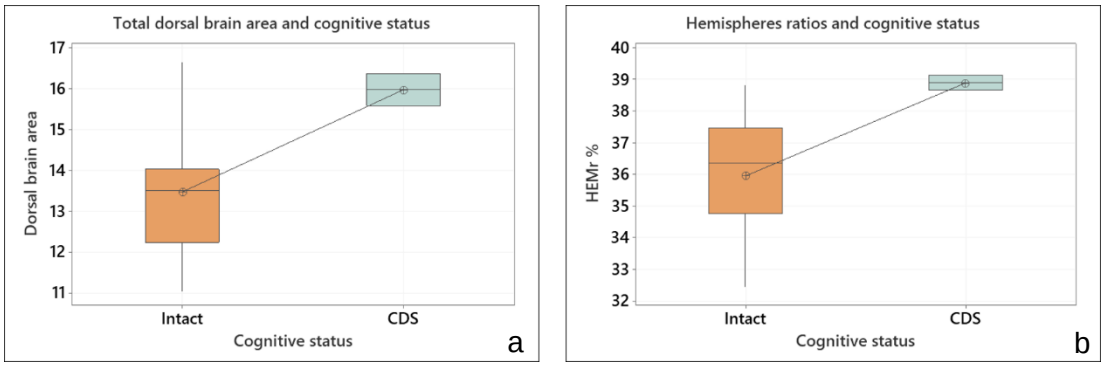

Figure 3.19 Differences in the total dorsal brain area and hemispheres ratios (HEMr) in cats with and without cognitive dysfunction syndrome (CDS). *Cats with CDS had larger a) total dorsal brain areas (p = 0.040); and b) HEMr (p = 0.048) than cats without CDS*

Furthermore, cats with CDS had smaller OCCr (9.43 ± 2.45) than cats without CDS (11.56 ± 1.42) (Figure 3.20). The complete list with the means of all structures and cognitive status with standard deviations and SE of the means can be found in Appendix 3.7.

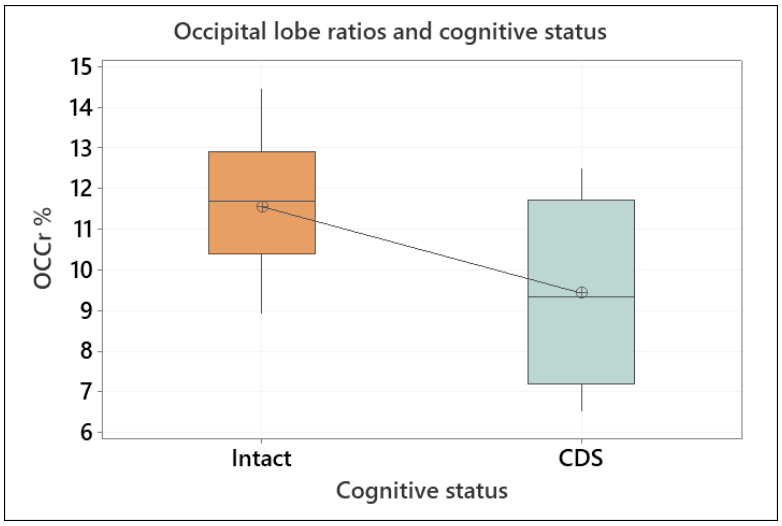

Figure 3.20 Differences in the occipital lobe ratios (OCCr) in cats with and without cognitive dysfunction syndrome (CDS). *Cats with CDS had smaller OCCr (p = 0.021) than cats without CDS*

While not being statistically significant, cats with CDS had smaller total sagittal brain areas (10.73 ± 1.25), LVr (0.283 ± 0.237), and TEMr (6.741 ± 0.543) than cats without CDS (11.14 ± 1.11; 0.372 ± 0.220; and 7.15 ± 1.34, respectively).

3.3.4.2 *In vivo* scans

Since there were no cats with CDS in this database, this was not examined in the live cat data.

3.3.4.3 Post-mortem scans

Since there were not sufficient data, two-sample t-tests were not performed for ITAr, LVr, TEMr, HIPr, and total transversal brain areas. However, one-way ANOVA could be performed to analyse these structures, and showed no differences (Table 3.11).

Table 3.11 Differences in the size of the structures in cats with and without cognitive dysfunction syndrome (CDS) in the fixed brains. *One-way ANOVA was performed to determine whether there were differences in the size of the structures between cats with and without CDS in the* post-mortem *scans*

Structure	F value	DF	p value
ITAr	0.23	1	0.645
LVr	0.07	1	0.803
TEMr	0.13	1	0.729
HIPr	0.21	1	0.664
Total transversal area	0.05	1	0.823

Two-sample t-tests showed differences in the total dorsal brain areas (t_7 = -2.90, p = 0.023) (Table 3.12), which was larger in cats with CDS (15.97 ± 0.547) when compared to cats without CDS (13.96 ± 0.907). Furthermore, despite not being statistically significant, cats with CDS had smaller OCCr (9.43 ± 2.45) and FROr (11.71 ± 1.84) than cats without CDS (10.37 ± 1.08; and 14.36 ± 2.83, respectively). The complete list with the means of all

structures and cognitive status with standard deviations and SE of the means can be found in Appendix 3.8.

Table 3.12 Differences in the size of the structures in cats with and without cognitive dysfunction syndrome (CDS) in the fixed brains. *Two-sample t-tests were performed to determine whether there were differences in the size of structures between cats with and without CDS on the* post-mortem *scans*

Structure	T value	DF	p value
HEMr	-1.95	7	0.092
Total dorsal area	-2.90	7	**0.023**
OCCr	0.95	10	0.363
FROr	1.68	10	0.124
Total sagittal area	-0.88	10	0.400

3.3.5 Correlations between the size of the structures, age, and cognitive status

Spearman rank correlations were performed to determine whether there were associations between the size of the different structures, the age groups and cognitive status (i.e., cats with and without CDS).

3.3.5.1 Correlation between the size of structures and age

3.3.5.1.1 *In vivo* and *post-mortem* scans

There were weak negative linear correlations between age and total sagittal brain area ($r = -0.252$, $p = 0.036$) (Figure 3.21A), OCCr ($r = -0.243$, $p = 0.043$) (Figure 3.21B), and HIPr ($r = -0.248$, $p = 0.05$) (Figure 3.21C). Furthermore, moderate positive linear correlation was found between HEMr and age ($r = 0.452$, $p < 0.001$) (Figure 3.21D). Total sagittal brain area, OCCr, and HIPr tended to decrease with age, while HEMr tended to increase with age. No

correlations were found between age and ITAr, LVr, TEMr, total transversal brain area, total dorsal brain area, and FROr.

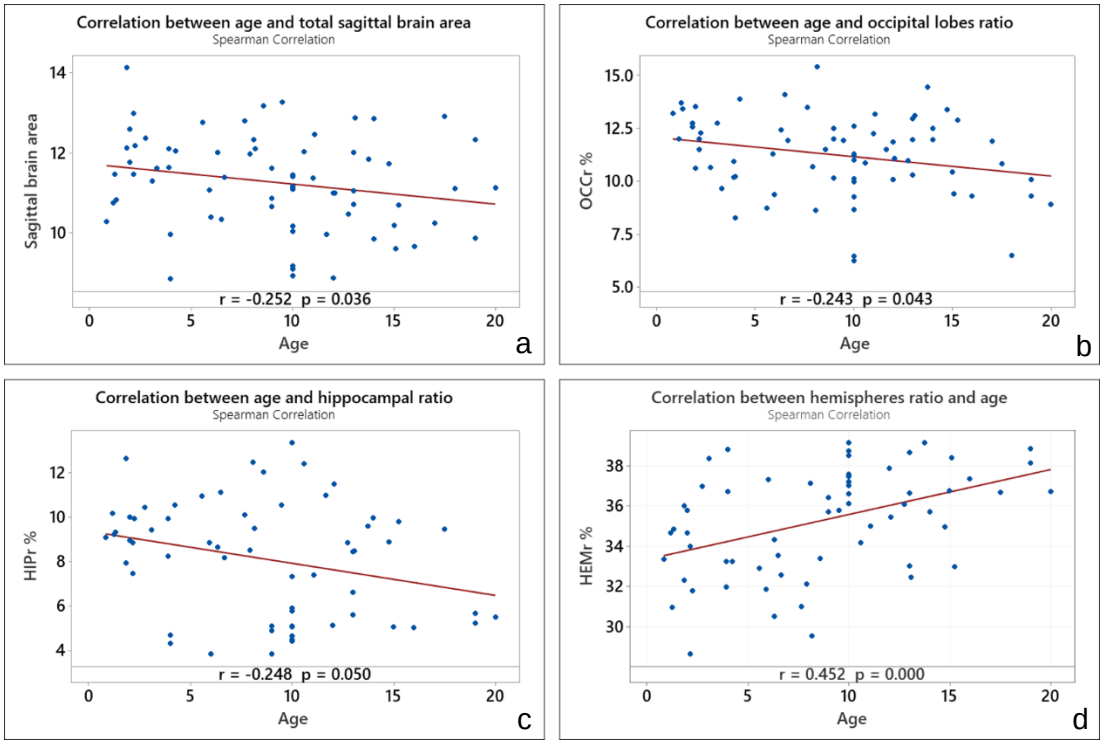

Figure 3.21 Correlations between age and a) the total sagittal brain area (p = 0.036), b) the occipital lobe ratio (OCCr; p = 0.043), c) the hippocampal ratio (HIPr; p = 0.05), and d) the hemispheres ratio (HEMr; p < 0.001)

3.3.5.1.2 In vivo scans

There was a weak positive linear correlation between age and LVr (r = 0.450, p = 0.005) (Figure 3.22); showing that LVr in live cats tended to increase with age. No correlations were found between age and any other brain structure in the scans of live cats only.

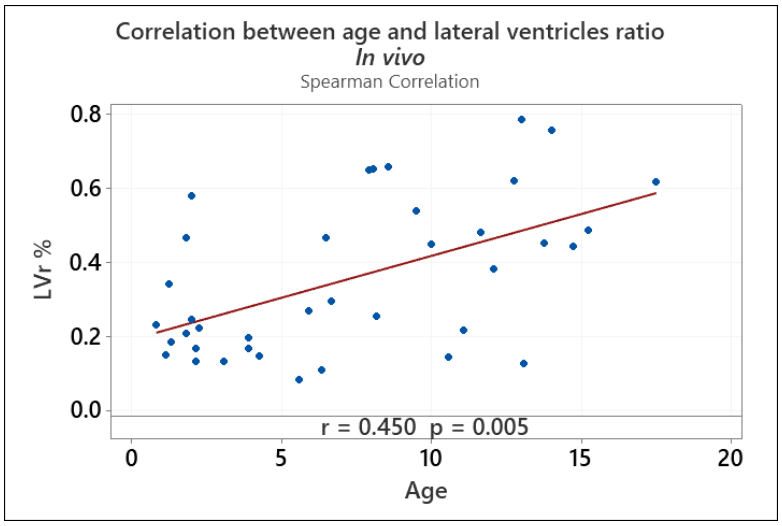

Figure 3.22 Correlation between age and the lateral ventricles ratio (LVr). *There was a positive correlation between age and LVr (p = 0.005) in the* in vivo *scans*

3.3.5.1.3 Post-mortem scans

There was a moderate positive linear correlation between age and HIPr (r = 0.523, p = 0.011) (Figure 3.23); showing that HIPr in the fixed brains tended to increase with age. No correlations were found between age and any other brain structure in the fixed brains only.

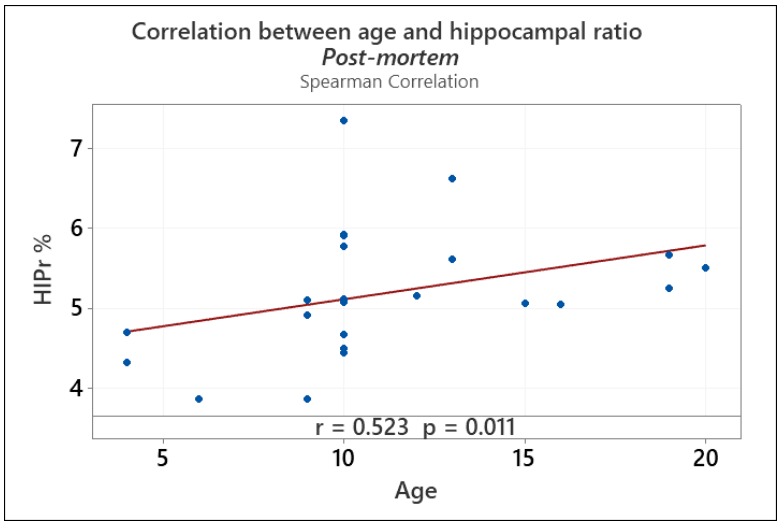

Figure 3.23 Correlation between age and the hippocampal ratio (HIPr). *There was a positive correlation between age and the HIPr (p = 0.011) in* post-mortem *scans*

3.3.5.2 Correlation between the size of structures, age, and sex

3.3.5.2.1 In vivo and *post-mortem* scans

There was a weak negative linear correlation between the total sagittal brain area, age, and sex (r = -0.252, p = 0.036), which was larger in male cats and tended to decrease with age. A weak positive linear correlation was found between sex and HEMr, which was larger in female cats (r = 0.452, p < 0.001) and tended to increase with age. There were no correlations between sex and ITAr, LVr, TEMr, total transversal brain area, OCCr, and FROr.

The total sagittal brain area tended to decrease equally in both male and female cats as they get older (Figure 3.24A). Similarly, HEMr tended to increase equally in both female and male cats as they get older (Figure 3.24B).

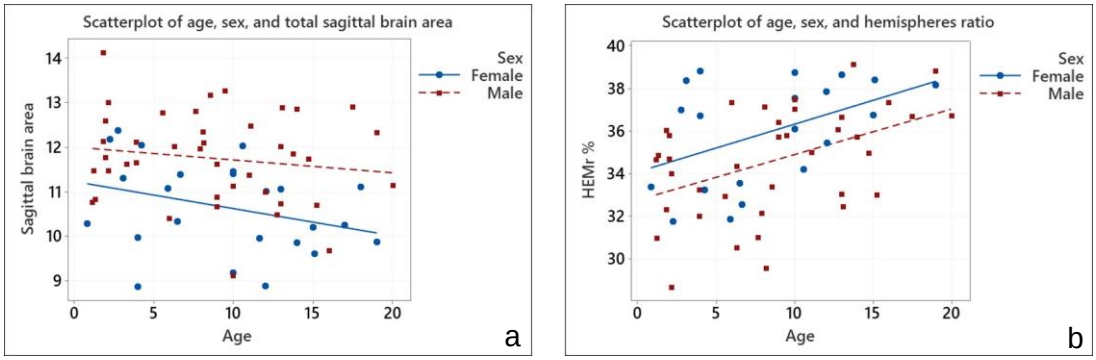

Figure 3.24 Correlations between age, sex, and a) the total sagittal brain area (p = 0.036); and b) the hemispheres ratio (HEMr; p < 0.001)

Despite not being statistically significant, the HIPr tended to decrease with age equally in both female and male cats (Figure 3.25A). Furthermore, ITAr tended to decrease slightly more in male cats (Figure 3.25B); while OCCr tended to decrease more in female cats (Figure 3.25C) as they get older.

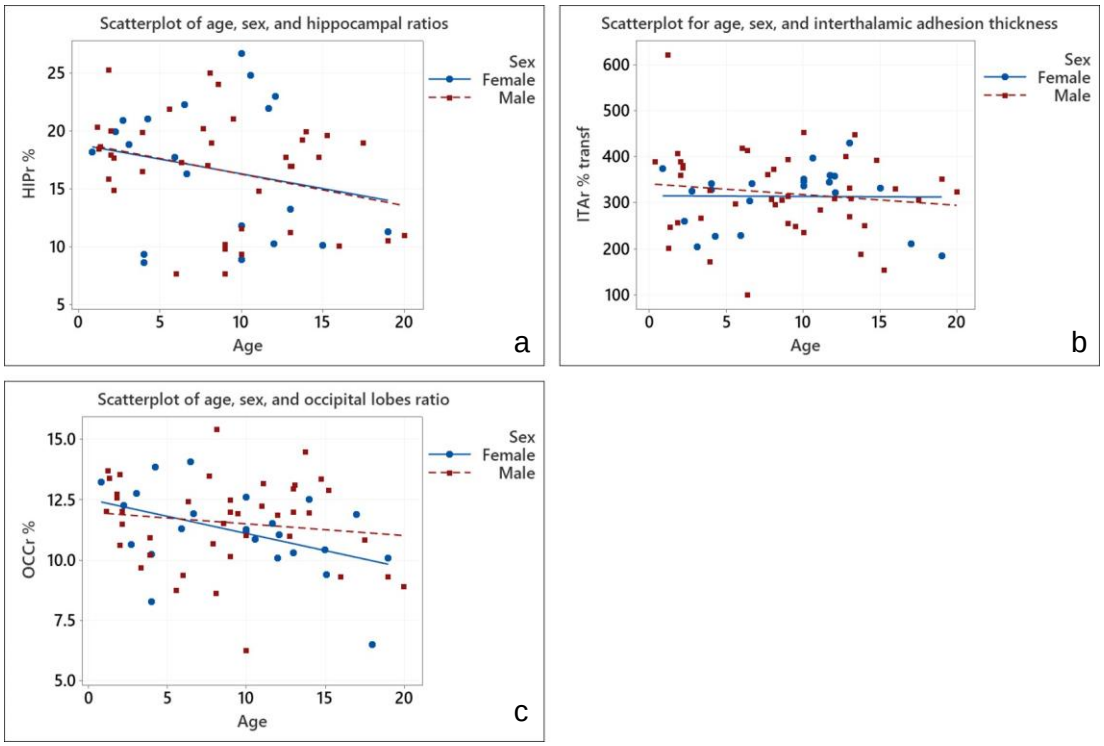

Figure 3.25 Correlations between age, sex, and a) hippocampal ratios (HIPr); b) interthalamic adhesion ratios (ITAr); and c) occipital ratios (OCCr)

3.3.5.2.2 *In vivo* scans

There were no statistically significant correlations between the size of structures, age, and sex in the live cat scans; however, the cortical areas tended to decrease in size more abruptly with age in female than in male cats; particularly the OCCr (Figure 3.26A) and the FROr (Figure 3.26B).

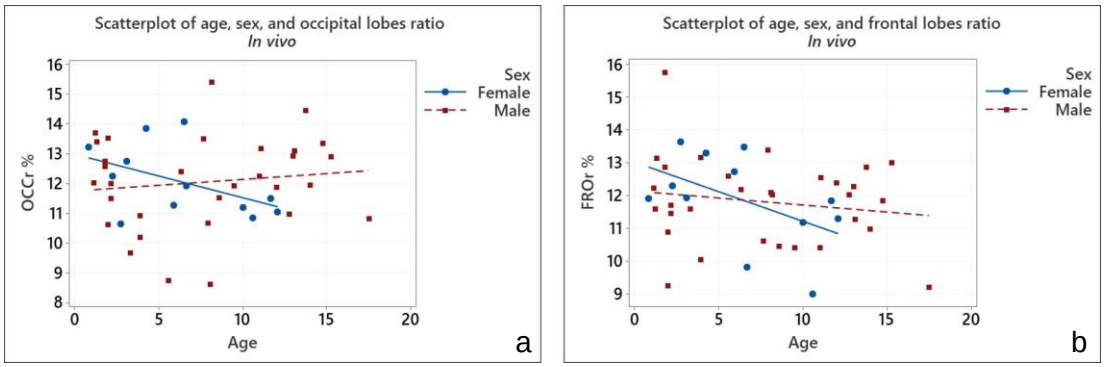

Figure 3.26 Correlations between age, sex, and a) occipital lobe ratios (OCCr); and b) frontal lobe ratios (FROr) for* in vivo *scans

3.3.5.2.3 Post-mortem scans

There were no correlations between the size of structures, age, and sex in the fixed brains.

3.3.5.3 Correlation between the size of structures, age, and cognitive status

3.3.5.3.1 In vivo and *post-mortem* scans

There was a weak positive linear correlation between age and cognitive status ($r = 0.379$, $p = 0.046$), showing that as cats get older, they tended to develop CDS; however, this finding should be interpreted with caution as only four cats (from the *post-mortem* dataset) had a confirmed diagnosis of CDS. Further studies, with larger numbers of cats with CDS are needed to confirm this.

There were no correlations between the size of structures and the cognitive status of the cats. Interestingly and, despite not being statistically significant, the OCCr (Figure 3.27A) and FROr (Figure 3.27B) tended to decrease more with age in the CDS cats, when compared to cats without CDS.

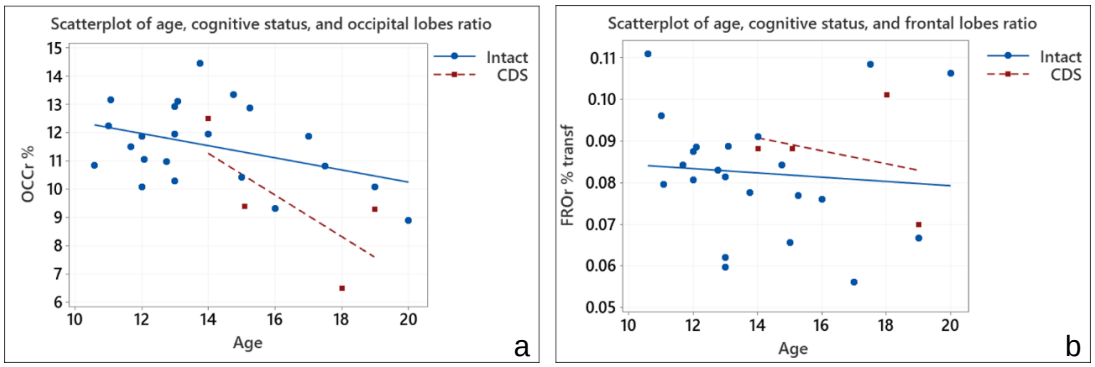

Figure 3.27 Correlations between age, cognitive status, and a) occipital lobe ratios (OCCr); and b) frontal lobe ratios (FROr)

3.3.5.3.2 In vivo scans

Since there were no cats with CDS in this database, correlations between the size of structures, age, and cognitive status were not performed in the *in vivo* scans.

3.3.5.3.3 Post-mortem scans

There were no correlations between the size of structures, age, and cognitive status in the *post-mortem* scans.

3.3.6 Differences between *in vivo* and *post-mortem* scans

Two-sample t-tests showed differences in LVr ($t_{49} = 3.46$, $p = 0.001$), TEMr ($t_{48} = 2.91$, $p = 0.006$), HIPr ($t_{50} = -13.95$, $p < 0.001$), total transversal brain area ($t_{50} = 7.61$, $p < 0.001$), HEMr ($t_{51} = 6.64$, $p < 0.001$), total dorsal brain area ($t_{52} = 2.37$, $p = 0.021$), OCCr ($t_{57} = -4.47$, $p < 0.001$), total sagittal brain area ($t_{57} = -6.55$, $p < 0.001$), and FROr ($t_{57} = 3.26$, $p = 0.002$) (Table 3.13).

Table 3.13 Differences between* in vivo *and* post-mortem *scans. *Two-sample t-tests were performed to determine whether there were differences between* in vivo *and* post-mortem *scans*

Structure	T value	DF	p value
ITAr	0.87	56	0.387
LVr	3.46	49	**0.001**
TEMr	2.91	48	**0.006**
HIPr	-13.95	50	**<0.001**
Total transversal area	7.61	50	**<0.001**
HEMr	6.64	51	**<0.001**
Total dorsal area	2.37	52	**0.021**
OCCr	-4.47	57	**<0.001**
FROr	3.26	57	**0.002**
Total sagittal area	-6.55	57	**<0.001**

For *in vivo* scans, the HIPr, OCCr, and total sagittal brain area were larger, and the FROr, LVr, TEMr, total transversal brain area, HEMr, and total dorsal brain area were smaller, when compared to the *post-mortem* scans (Table 3.14). The complete list with the means of all structures with standard deviations and SE of the means can be found in Appendix 3.9.

Table 3.14 Table of means for* in vivo *and* post-mortem *scans

	In vivo		*Post-mortem*	
Structure	**Mean**	**St. Dev.**	**Mean**	**St. Dev.**
ITAr	17.13	2.41	17.70	2.47
LVr	1.815	0.692	2.426	0.536
TEMr	6.58	2.06	8.00	1.16
HIPr	9.95	1.46	5.193	0.814
Total transversal area	9.337	0.897	11.317	0.975
HEMr	34.15	2.33	37.522	0.948
Total dorsal area	13.54	1.55	14.50	1.39
OCCr	11.89	1.56	9.96	1.76
FROr	11.75	1.25	13.87	3.43
Total sagittal area	11.844	0.841	10.347	0.913

3.3.7 Inter-rater reliability

Kendall's coefficients for attribute agreement analysis with 95% confidence level showed that there was an agreement between observers' measurements that cannot be attributed to chance (W = 0.975, p = 0.048).

3.4 Discussion

This study assessed age-related structural changes in *in vivo* and *post-mortem* MR images from a total of 74 cat brains. Several structures showed statistically significant differences in size between the age groups (i.e., hemispheres, occipital lobe, and lateral ventricles); sexes (i.e., hippocampus, hemispheres, total dorsal brain area, and total sagittal brain area); and cognitive status (i.e., occipital lobe and total dorsal brain area). Furthermore, all brain areas/structures, except the interthalamic adhesion, were statistically different between *in vivo* and *post-mortem* scans. For this reason, the advantages and disadvantages of both *in vivo* and *post-mortem* MR imaging will be discussed first, as well as the effect that chemical fixatives have on brain tissue, which may explain many of these differences.

3.4.1 Differences between *in vivo* and *post-mortem* MR imaging and the effect of fixation on brain tissue

Two-sample t-tests showed statistically significant differences between the *in vivo* and *post-mortem* MR images in all the structures, except in the interthalamic adhesion.

Magnetic resonance imaging is a powerful diagnostic tool used both *in vivo* and *post-mortem* to examine individuals and tissue samples, where it has advantages and disadvantages in both situations.

In vivo MR imaging is widely used in longitudinal studies for which several scans from the same individuals are needed at different time-points. It can also be used in studies that require a number of different assessments from the same live animals, such as MR images plus behavioural changes[227]. In order to perform *in vivo* MR imaging in animals, they need to be anesthetised and

then restrained. While this reduces movement artefacts, it increases the appearance of confounding factors derived from the exposure to stress and drugs[227]. This also has repercussion in the time allowed for the acquisition of the images, which is limited due to animals being anaesthetised, and can result in scans with poor image quality and resolution[227].

In contrast, scans of *post-mortem* tissue use longer acquisition times so they have higher resolution and improved image quality[227, 228] (Figure 3.28); in addition, contrast agents can be perfused into the tissue, enhancing contrast[227]. However, the major disadvantage of using *post-mortem* MR imaging is proving its validity, as there is still uncertainty about the potential morphological changes caused by death and/or the fixation process[227] such as shrinkage of the tissue, alterations of T_1 and T_2 relaxation times, and changes in water diffusion[227].

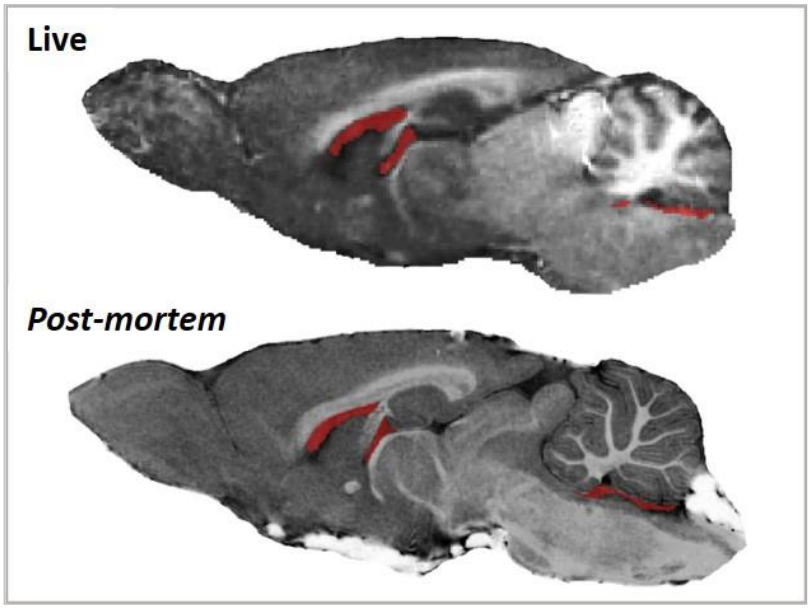

Figure 3.28 Comparison of* in vivo *and* post-mortem *MRI scans. Post-mortem *9.4T MRI scans provide better image quality and resolution as seen in this comparison of* in vivo *and* post-mortem *sagittal sections of a rat's brain. Image taken from Oguz* et al. *2013*

Chemical fixatives, including formalin, preserve tissues after slowly disseminating into it. The decomposition process is prevented by the effect of the cross-linking between formalin and proteins in the tissue[229]. However, since formalin disrupts water motion within the tissue, there may be alterations in the MR features[229].

The effect of death and fixation in different tissues has been studied by using Diffusion Tensor Imaging (DTI), which is a type of MR imaging that measures the diffusion of water within the tissues. For this, DTI evaluates diffusion anisotropy and water diffusivity. Anisotropy assesses how water molecules "move" within the tissue. While under normal conditions water diffuses freely, "obstacles" within the healthy tissue (e.g., cell membranes) or unhealthy tissue (e.g., vascular disease, neoplasia) may impede and/or alter a free diffusion of water leading to variations in anisotropy[230]. While anisotropy assesses how well water "moves" within the tissue, diffusivity assesses the magnitude of this movement[231].

Water content in the brain varies between regions, with most of it corresponding to cerebrospinal fluid (CSF; 99%), followed by grey matter (80%) and white matter (70%)[228]. Interestingly, while anisotropy is almost identical in both *in vivo* and *post-mortem* scans, water diffusivity is known to decrease in fixed *post-mortem* tissues[232]. Of note, water diffusivity decreases more in white matter than in grey matter after fixation[232]; in addition, fixation also reduces the mobility of water between grey and white matter[228]. It has been suggested that this lack of water diffusion is due to limited water motility and the degradation of the tissue structure, particularly in tissue that was fixed by immersion in formalin[228], as opposed to perfused brains[232].

The *post-mortem* interval (PMI) is the time between when an individual dies and the beginning of the fixation process. It is believed that an increased PMI reduces water diffusion and anisotropy, as well as T_1 and T_2 relaxation times, both of which rely on water mobility within the tissue[228]. The T_1 relaxation time

measures longitudinal relaxation (i.e., how quickly the excited protons return to stability and realign with the magnetic field after being hit by the radiofrequency; while T_2 relaxation time measures transverse relaxation (i.e., the rate at which the excited protons dephase from each other). Studies have suggested that this reduction in T_1 and T_2 relaxation times might instead be due to dehydration of the tissue caused by fixation and whether this was performed by immersion or perfusion[233]. Initial reductions in both T_1 and T_2 first occur soon after fixation begins and stabilise after 3-4 weeks[234, 235]. Moreover, it has been proposed that these changes can potentially be reversed by rehydrating the tissue in phosphate-buffered saline (PBS) after fixation has been completed[229].

The effect of fixation has been studied in both immersed and perfused tissues. A study with mice brains showed that the brains swell soon after being immersed in 10% formalin, with a subsequent reduction/shrinkage in both size and weight[236]. This is believed to be due to a sudden increase in water content, and was more evident in the cerebral cortex, while the cerebellum was less affected[236]. After 24 hours of fixation, the mice brains gained on average of 54% of their initial “fresh” weight; however, this swelling quickly reversed within the first nine days, with a further slower reversion over time, reaching their original “fresh” weight after 213 days[236]. Interestingly, while changes in size/weight were consistent between mice brains, their shapes remained unchanged[236]. Finally, these physical changes were more obvious in the brains of younger mice than in older individuals[236], which has also been reported in other mammals, such as macaques[237], dogs[238], and humans[239].

Fixation by perfusion has advantages and disadvantages over fixation by immersion. Perfusion fixation is completed in less time and it does not affect the histological quality of the tissue[240]; however, it is a more expensive technique that requires training and expertise[240]. As reported for fixation by immersion, perfusion promotes swelling of the tissue, increasing its weight[237];

however, unlike with immersion, perfusion can lead to uneven fixation if vascular disease is present[240].

The effect of fixation over time was reported in a study that assessed changes in human brains before and during fixation. For this, brains were extracted and immersed in 20% formalin; several MR scans were then obtained at different time points to assess the progression of the fixative. After the first 18 hours of fixation, a hyperintense band of formalin was detected delimiting the whole surface of the brain in T_1 sequences. This band increased in size over time, from the surface to the centre of the brain, as the formalin progressed through the tissue, reaching a complete diffusion after 14.8 weeks of fixation. In addition, reductions in both T_1 and T_2 relaxation times were observed in grey and white matter. Interestingly, the reduction in T_1 relaxation times and the diffusion of fixative were both simultaneously progressive. Finally, water diffusivity remained unchanged before and during fixation[241].

One of the major physical changes caused by death and fixation, particularly in the brain, is the collapse of CSF spaces. The ventricles increase their size with age, which is used as an indicator of brain atrophy and predictor of AD[35]; however, the opposite has been reported to occur in fixed tissue. In a study that assessed anatomical changes in MR images of *post-mortem* fixed human brains, the size of the ventricles in the fixed brains were significantly smaller than those in living patients. This study found that the ventricles of the fixed brain of a 61-year-old person were similar in size to those of a 25-year-old living person, instead of exhibiting the age-related ventricle dilation normally seen in people in their sixties[228]. Another study in human brains that assessed MR changes pre- and post-fixation by immersion in formalin showed that the ventricles expanded after extraction, with the whole brain expanding up to 5.2% after one day of formalin fixation[242]. These changes are thought to result from pressure changes inside and outside the skull, plus the osmotic pressure of the formalin[242]. However, the total brain volume then shrank up to 8.1%, returning back to normal over time[242].

The effect of fixation on the size and shape of the brain has also been evaluated in a study of rat brains that assessed volumetric differences between *in vivo* and *post-mortem* MR images at three different time points[227] (i.e., one *in vivo* and two *post-mortem* after perfusion fixation). This study showed there were no significant changes in the volumetric measurements of the total brain volume, the ventricles, the neocortex, the hippocampus, the corpus callosum, or the cerebellum[227]. Furthermore, there was a correlation between age, the brain volume, body weight, and brain weight, all being shown to increase with age; of note, these were similar in both *in vivo* and in *post-mortem* scans[227].

Interestingly, similar alterations have also been reported to occur as a result of normal ageing and in AD. In normal ageing, DTI has shown a reduction of white matter anisotropy that is believed to be caused by myelination disturbances, alterations of the microtubule structure and/or changes in axon density[231]. Similarly, in AD, there are reductions in anisotropy and increased water diffusivity due to myelination disturbances; however, the neuroinflammation and glial reaction resulting from cell death may lead to a decrease in both anisotropy and water diffusivity[231].

In conclusion, the available evidence suggests that there may be slight differences between *in vivo* and *post-mortem* MR images. This is supported by the current study, which found that all brain areas/structures (other than the interthalamic adhesion) were statistically different between *in vivo* and *post-mortem* MR scans. Each method has advantages and disadvantages, and both have been shown to be equally useful and valid tools for the assessment of anatomical/structural changes and for the diagnosis of disease; however, they cannot be used interchangeably.

Finally, combining the datasets from live cats and fixed cat brains has its own advantages and disadvantages. For instance, combining datasets increases the sample size of the study, making it more likely to find significant differences between age groups. On the other hand, it can be argued that death and the

fixation process may produce several changes in the *post-mortem* tissue, making the fixed brains dataset unsuitable for combining with the *in vivo* dataset. However, as discussed above, there is a plethora of evidence showing that these changes are minimal and that using fixed tissue is as valid as using scans from live individuals.

3.4.2 Differences in the size of structures between age groups

The size of several structures was assessed and compared between age groups, showing differences in the occipital lobe, the hemispheres, and the lateral ventricles. In addition, correlations with age were demonstrated for the occipital lobe, the hemispheres, the lateral ventricles, the hippocampus, and the total sagittal brain area.

Normal ageing has an impact on brain anatomy and on its different structures. Atrophy of the frontal cortex is the first and most common age-related change reported in dogs[222, 223] and humans[31, 32]. While other brain regions are affected by age in humans, reports have shown that these are more subtle, including atrophy of the temporal and parietal cortices[31, 32]. Interestingly, in the present study, cats were shown to develop atrophy of the occipital cortex, which in humans is the least affected by ageing[31]. This might be the result of combining datasets from *in vivo* and *post-mortem* scans, as atrophy of the occipital lobe was not found in the *in vivo* scans. Alternatively, this might be true age-related atrophy that differs from what has been previously reported in other species. While no statistical difference was found in the size of the occipital lobe between the different age groups in the *post-mortem* scans, there was a clear trend showing an age-related atrophy of this lobe (being more evident in the Super Senior group), which was negatively correlated with age. Furthermore, cats with CDS had smaller occipital lobes than cats without CDS, suggesting that this lobe could be the first brain region affected by ageing and CDS in cats.

The progressive increase in the size of the hemispheres and its positive correlation with age are difficult to explain, as the size of the hemispheres was expected to decrease with age instead. In humans, the hemispheres remain unchanged from ages 20 to 50 years old[29], although an age-related asymmetry has been reported, showing that the left hemisphere tends to be larger than the right one[32]. While hemisphere asymmetry was not assessed in the current study, it has been reported previously in cats[40]; however, unlike in humans, it is the right side of the cat brain that is larger than the left one. Since very young cats were included in this study (the youngest was four months old), it is possible that the brains of these cats had not been fully developed, leading to a significant difference between these and older cats with more developed brains. Studies in dogs support this hypothesis; a study performed in dogs at different stages of development showed there is a linear increase of 10-19% in brain volume from birth to six weeks of age, followed by a gradual growth of up to 20% into adulthood[238]. In humans, full development of the brain occurs around 22 years of age[243], with peaks of growth at 11 and 15 years old[244]. According to the International Cat Care and their equivalence table between human and cat years, 22 human years correspond to 18 to 24 months of age in the cat, which is the Prime group of this study[148]. Another potential explanation suggests that the enlargement of the hemispheres with age might not necessarily be true. Hemispheres were measured on the dorsal plane in which they occupy most of the image, with only the cerebellum being excluded from the measurement (see Figures 3.6A and B in the Material and Methods section). While the size of the cerebellum was not measured for this study, it was included to measure the total dorsal brain area, and thus used for calculating the ratios for the hemispheres. It is therefore possible that there were changes in the size of the cerebellum, including shrinkage due to ageing and/or fixation process, which affected the ratios used for the analysis.

Assessment of i*n vivo* scans showed differences in the size of the lateral ventricles between age groups, which was positively correlated with age. Both Junior and Prime groups had smaller lateral ventricles when compared to the

Mature, Senior and Super Senior groups. It is widely recognised that, as individuals get older, the brain tissue atrophies, and this shrinkage leads to an increase in the size of the CSF spaces, such as the ventricles[32]. In humans, while the brain shrinks at a rate of 2-5% per decade[29, 30], enlargement of the ventricles occurs at a rate of 3-4% per year[245], with this being accelerated by ageing[246]. This increase in the size of the ventricles has been proposed as a predictor for the development of AD[35]. A similar enlargement of the ventricles has also been reported to occur in both cats[40] and dogs[219-221], and is commonly used as a diagnostic criteria for brain atrophy and ageing[225]. A study in dogs showed that the lateral ventricles increase slowly until 10 years of age, followed by a rapid progression thereafter[220]. Interestingly, the opposite was found in the *post-mortem* scans. In these, the Super Senior group had the smallest lateral ventricles of all age groups. This reduction appears to have been caused by the extraction and fixation of the brain; these procedures are known to collapse the ventricles due to the loss of CSF fluid and/or pressure changes[228, 242], as described above.

The interthalamic adhesion is a round structure that connects the left side of the thalamus with the right one. A reduction of the size of this structure has been previously reported to occur in live healthy aged cats[226] and dogs, particularly in those with CDS[224, 225]. The thinning of the interthalamic adhesion is also believed to be correlated with enlargement of the ventricles[224]. While there were no statistically significant changes in the size of this structure in the present study, the interthalamic adhesion thickness tended to reduce with age in the *post-mortem* scans, with the Super Senior group having the smallest interthalamic adhesion of all age groups. As mentioned previously, all of the cats with CDS were part of the *post-mortem* dataset, suggesting that the interthalamic adhesion does reduce in size with age and is potentially linked to CDS in cats.

Atrophy of the hippocampus is another important age-related change reported in humans[31-34] and dogs[219-222]. A study that assessed MR images from people

with and without AD showed that those people with AD had smaller hippocampal volumes (up to 38%) compared with healthy participants[247]. In addition, hippocampal atrophy has been used as an early diagnostic tool, as it has been reported to occur in healthy humans that later develop AD[36-39]. In agreement with reports in dogs and humans, the size of the hippocampus in the *in vivo* cats in the current study was negatively correlated with age, which was expected. Unexpectedly, the opposite was found in the *post-mortem* scans, where a positive correlation was found. In these scans, an initial and progressive increase in size was evident; however, this was followed by a marked decline in the Super Senior group. While the initial increase in hippocampal size could be the result of the tissue expansion caused by death and the fixation process in the younger groups, the drastic decline in the oldest group could also suggest that this atrophy is real, only being evident in the oldest individuals.

Finally, the total sagittal brain area was negatively correlated with age. As discussed previously, age-related shrinkage of the brain has already been reported in humans[30], dogs[219-222] and cats[226]. Interestingly, atrophy of the whole brain is considered an early indicator of AD, as it has been reported to occur in the pre-symptomatic stage of the disease[39, 92, 215].

3.4.3 Sex-related differences in the size of brain structures

It has been proposed that age-related atrophy of the brain is not predominantly produced by neuronal death (i.e., a decline in the number of neurons) but rather by a reduction in their volume instead[144]. This is believed to be linked with sex, affecting different areas of the brain differently in men and women[144]. In the present study, there were sex-related differences in the size of structures, including the three planes of the total brain areas (i.e., dorsal, sagittal, and transverse), the hippocampus and the hemispheres. In addition,

correlations were found between age, sex, and the total dorsal and sagittal brain areas, as well as the hemispheres.

Male cats from the combined datasets had larger total dorsal and sagittal brain areas, compared to female cats; while the male cats from the *in vivo* scans had larger total dorsal, sagittal, and transversal brain areas, when compared to female cats. This could be due to male cats tending to be physically bigger than female cats, hence, they have bigger heads and brains. Since the weight of the cats in this study was not recorded, it was not possible to calculate the correlation between body weight and brain weight. Interestingly, while the dorsal, sagittal, and transversal brain areas were larger in the male cats of the *post-mortem* group, when compared to the females, these were not statistically significant different. This could suggest that removing the brain from the skull and the fixation process produce changes, such as expansion and then shrinkage of the brain, which could mask any potential changes. There were also positive correlations with age and the dorsal brain area which were larger in the male cats and tended to decrease with age. In contrast, female cats from the *in vivo* scans had larger hippocampi than male cats. In humans, sex-related differences have also been identified, showing that the total brain volume and hippocampal size tend to be larger in men than in women[32]. Even though, on average, men have larger hippocampi than women, there is contradictory evidence on the sex-related differences in the size of this structure[248, 249]. These inconsistencies are more evident after adjusting the size of the hippocampus for total brain size[248, 249]; although the different methods used for these adjustments may also contribute to this variation[249]. All the structures assessed in the present study were adjusted for the overall brain size, which might explain why female cats have larger hippocampi than male cats.

Age-related atrophy of the frontal and temporal lobes is most prominent in men[144, 212]; while the hippocampus and parietal lobes are most affected in women[144, 212]. While any reduction in size of the cats' brain structures was not statistically significant between sexes, the assessment of *in vivo* scans showed

that the frontal and occipital lobes tended to decrease more rapidly with age in female cats.

In the *post-mortem* scans, female cats had larger hemispheres than males. This is difficult to explain, especially as male cats from the *in vivo* dataset had larger brain areas. It is possible that this could be due to death and the fixation process. Since the cat brains were not treated in a standard fashion *post-mortem*, some may have been fixed for longer than others prior to sectioning. It is possible that some brains experienced swelling and a further shrinkage, as described above, at different times, potentially having an effect in these results.

3.4.4 Differences in the size of structures between cats with and without CDS

There was a positive correlation between age and cognitive impairment, which adds evidence to support that cats are more likely to develop CDS as they become older. Furthermore, there were statistically significant differences in the size of structures between cats with and without CDS, including the total dorsal brain area, the occipital lobe, and the hemispheres.

As described previously, the main changes associated with ageing and AD in humans are atrophy of the whole brain, which is more evident in the frontal and temporal lobes, plus hippocampi[216-218]. However, while similar changes were expected in the cats of this study, the opposite was found, with cats with CDS having larger dorsal brain areas and hemispheres. As discussed previously, this could be the result of death and fixation, and/or due to differences in the collection and fixation times between the samples. In contrast, cats with CDS had smaller occipital lobes, which the author proposes to be a true change related to ageing and CDS in cats, as discussed previously.

Of note, given the small number of cats with CDS (n = 4) the results of the present study are an under-representation of the changes that can potentially happen in the brains of cats affected by CDS, which is one of the limitations of this study. Further studies, with larger numbers of cats with CDS, are needed.

There are other limitations that have been identified in the present study. Since the collection of brains for the *post-mortem* scans was undertaken at different times, the fixation times changed between samples. Hence, some brains remained immersed in formalin for longer than others. This could potentially had affected the results, as physical changes such as swelling and shrinkage of the tissue could have occurred at different times. Ideally, to minimise this variation, all of the brains should have been collected and fixed using exactly the same procedure; furthermore, brains could have been temporarily placed in PBS before being scanned, in order to reverse the effects of the fixative. To minimise these changes, fixation by perfusion, instead than immersion, could have been performed. However, this is a more expensive technique that requires training and expertise, plus further ethical issues as special consent would have been needed from the cat owners, as would a Home Office license.

It is possible that scanning the *post-mortem* brains while being suspended in formalin could have had an effect in the quality of the image and resolution. To test this theory, agar was assessed as it is commonly used as an MR imaging phantom[250]. For this test, a brain was placed inside a 5% agar block and scanned using the same protocol as described above; however, the image quality and resolution were poor, being insufficient to identify structures or accurately measure them. The use of Fomblin® (Sigma-Aldrich™), a fluorinated lubricant, may have helped to minimise these problems. Since it is a hydrogen-fee fluid, it has been anecdotally recognised as better a suspension media for MR studies; however, it is an expensive fluid.

3.5 Conclusion

Magnetic resonance imaging has the potential to be an accurate tool for the early recognition of age-related changes in the brains of cats. As cats get older, they develop atrophy of the whole brain, particularly of the occipital lobe, with enlargement of the ventricles. It is possible that the live cats whose brain scans were assessed in this study may experience these age-related changes over time and may even develop cognitive decline later in life; however, large longitudinal studies are needed to investigate this possibility, and to determine whether MR imaging could be used as a tool for the early recognition of CDS in cats.

Chapter 4 The effects of Telmisartan (Semintra™, Boehringer Ingelheim) on the clinical signs of CDS in cats

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"The smallest feline is a masterpiece"

-Leonardo da Vinci

Note:

Parts of this chapter will be submitted for publication to the Journal of Veterinary Pharmacology and Therapeutics. The final draft of this paper is currently being revised by the co-authors.

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Author's contribution to the paper:
The author assessed all the questionnaires and carried out the statistical analyses, as well as writing the paper.

4.1 Introduction

Cognitive dysfunction syndrome (CDS) is a complicated and poorly understood condition in cats. Diagnosing CDS is difficult for veterinary practitioners, especially as the diagnosis can only be made by ruling out all other potential causes for the behavioural changes displayed by the affected cats[15, 90]. In addition, these behavioural changes can be misinterpreted as normal ageing; highlighting the importance of accurately differentiating between what is normal, and what is a reason of concern and needs to be explored further. Furthermore, treating CDS in cats may also be challenging. While some interventions have been extrapolated from other species, such as the dog, there is no proven treatment for this condition in cats.

To date, most research has assessed the effect of different drugs on the cognition of non-feline species; recent studies in rodents and humans have shown that drugs that block a specific receptor of the renin-angiotensin system (i.e., the AT_1 receptor) might be beneficial to cognition and memory[131, 251]. It is therefore possible that these drugs could also have positive effects in cats with CDS. If true, this could be a turning point, as a treatment for this condition will significantly improve the quality of life and welfare of affected cats.

4.1.1 Renin-angiotensin system

The renin-angiotensin system (RAS) is a hormone pathway involved in the regulation of many physiological functions, including the modulation of the cardiovascular system, by controlling blood pressure, as well as the body's water balance and thirst[252, 253]. It involves three major hormones (renin, angiotensin II, and aldosterone) which affect the kidneys, lungs, vascular system, and the brain[254].

The RAS is activated by decreased systemic blood pressure. When blood

pressure falls, juxtaglomerular cells in the kidneys activate, cleaving the inactive precursor prorenin into renin[253, 254]. Renin is then released into the blood stream where it cleaves angiotensinogen, a precursor protein produced in the liver and found in circulating plasma, into inactive angiotensin I. Angiotensin I is then converted to angiotensin II (Ang II) by the action of angiotensin converting enzyme (ACE), which is found mainly in the lungs and kidneys. The resulting Ang II binds to angiotensin II type I (AT_1) and type II (AT_2) receptors, and has an effect on various organs, including the kidneys, adrenal cortex, vascular system, and the brain[253, 254] (Table 4.1). In these same organs, the enzyme aminopeptidase A (APA) then converts angiotensin II into angiotensin III, which is later converted into angiotensin IV (Ang IV) by the action of aminopeptidase N (APN)[255] (Figure 4.1).

Table 4.1 Effects of angiotensin II on AT_1 receptors on different organs

Target organ	Effect	Result
Adrenal cortex	Release of aldosterone	Increased sodium reabsorption and potassium excretion from the kidneys
Vascular system	Vasoconstriction of arterioles	Increased blood pressure
Brain	Stimulation of the hypothalamus Stimulation of the posterior pituitary	Stimulates thirst and promotes water intake Release of antidiuretic hormone which increases water reabsorption in the kidneys

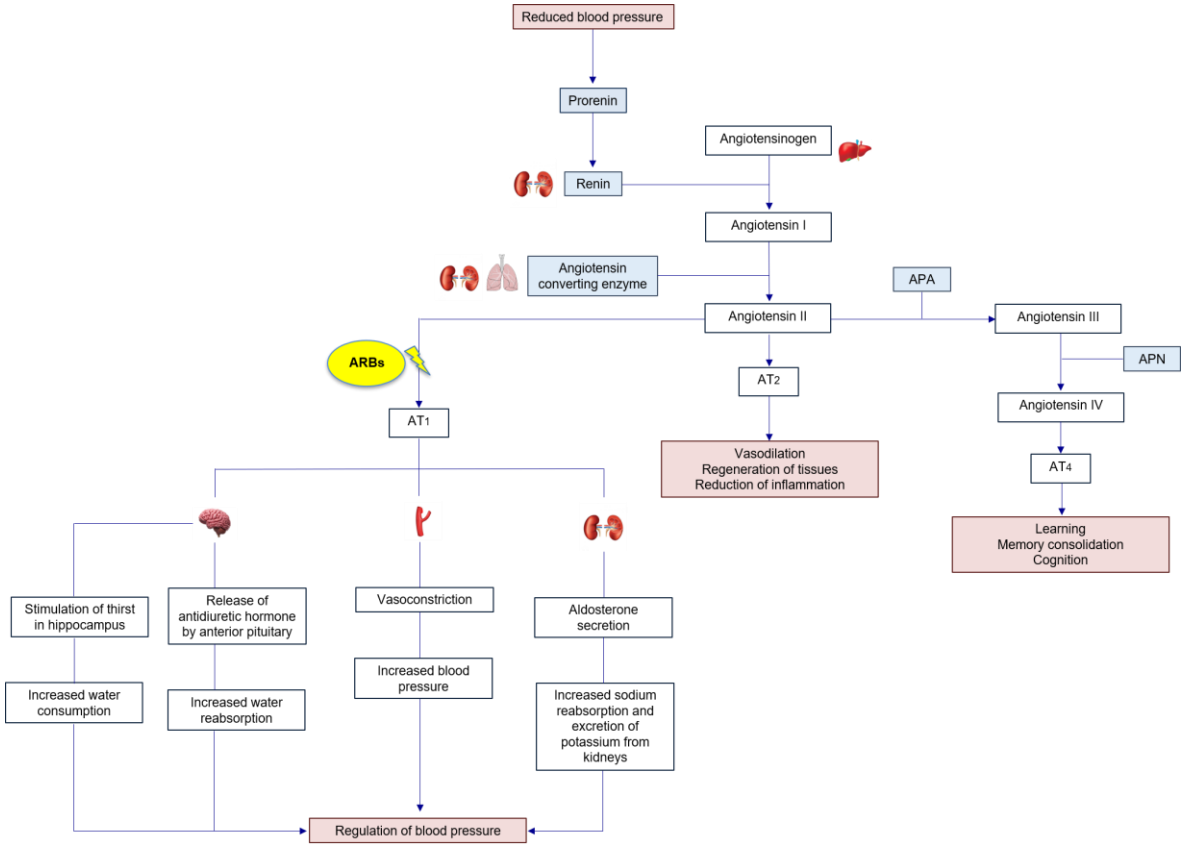


Figure 4.1 Renin-angiotensin system (RAS). *The RAS activates in response to a reduction of blood pressure. Renin converts angiotensinogen into angiotensin I, which is later converted into angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II acts on AT_1 receptors to regulate blood pressure, and on AT_2 receptors to promote vasodilation, regeneration of tissues, and reduction of inflammation. Angiotensin receptor blockers (ARBs) inhibit the effect of angiotensin II on the AT_1 receptors. Angiotensin II can be converted into angiotensin III by aminopeptidase A (APA), and angiotensin III can be converted into angiotensin IV by aminopeptidase N (APN). Angiotensin IV acts on AT_4 receptors, which are involved in learning, memory consolidation, and cognition*

Studies in rats have shown that Ang II plays an important role in maintaining the water and thirst balance[256, 257]. In one of these studies, rats were initially trained to press a bar to be given water. Once they had learned this, the rats were then deprived of water for 24 hours. After the water deprivation period, the rats were placed back in the chamber where they were able to press the bar to obtain water until they were satiated. Once satiated, the rats were then moved to a different chamber and received an intracranial injection of Ang II. Immediately after the injection, rats were moved back into the initial chamber, where they pressed the bar and drank more water. A control experiment was performed seven days later; however, during that experiment, the rats received an intracranial injection of saline instead of Ang II. This study found that, despite being satiated, the motivational levels of the rats after receiving the intracranial injection of Ang II were the same as for when they were water-deprived[256]. This response is believed to be due to Ang II binding to the AT_1 receptors, which are known to control thirst and water intake; hence, the intracranial administration of Ang II resembles the motivation induced by water deprivation[256, 257].

Besides its involvement in regulating blood pressure and water/thirst balance, the RAS is known to be implicated in several other functions and to act in many tissues, including the brain. In this organ, it has been shown to regulate cerebral blood flow, and to be involved in learning and memory consolidation[253]; furthermore, it has been shown to be involved in the etiology of neurodegenerative diseases, such as Alzheimer's disease (AD)[253, 258, 259].

It has been suggested that of the different forms of Angiotensin, it is Ang II, in particular, that affects learning and memory. Some of this evidence comes from the role of Ang II in cognitive disorders and neurodegenerative diseases, such as AD. One of the hypothesized causes of AD is the *cholinergic hypothesis*. This hypothesis suggests that a decreased number of cholinergic fibres in the brain reduces the release of acetylcholine (ACh), leading to further cognitive decline[260]. This correlates with the levels of choline acetyltranferase,

the enzyme that synthethises Ach, which are significantly reduced in people with AD[259]. This may result from the suppressive effect of Ang II on ACh, as high levels of Ang II are known to reduce the release of ACh[259].

However, recent studies have proposed that Ang IV may be involved in learning and memory, instead of Ang II. For instance, one study assessed memory after induced ischemia in rats before and after intracerebral injection of Ang IV[261]. In that study, two different types of memory were evaluated: 1) associative memory was assessed by using the passive avoidance test, in which rats learned to avoid an unpleasant stimulus (i.e., a foot shock); and 2) spatial memory, which was assessed by using the circular water maze. The findings of this study showed that, firstly, induced ischemia produced significant cognitive impairment in the rats; furthermore, injection of Ang IV was shown to reduce this impairment, as animals improved their performance in both tests[261]. This has been suggested to be the result of the agonistic effect of Ang IV on AT_4, which are receptors that are believed to be mostly involved in cognition[255]. Interestingly, unlike Ang II receptors, Ang IV receptors have been identified in several brain areas associated with memory function, such as the hippocampus, facilitating the retrieval and retention of memory[262].

It has been suggested that Ang II and Ang IV might not be the only hormones of the RAS involved in the development of AD. It is believed that ACE also plays a role in this disease; however, this role may not be causative of disease, but rather neuroprotective[263]. *In vitro* studies have shown that ACE can cleave the amyloid-β protein; this cleavage produces less toxic fragments that are less likely to aggregate[263]. Moreover, increased activity levels of ACE have been found in the AD brain; suggesting that ACE may play a protective role in the disease, especially as these increases have been correlated with high amyloid-β burdens and the severity of AD[263].

4.1.2 Angiotensin II receptors

4.1.2.1 Subtype 1: AT_1 receptors

In the brain, these receptors are located in the anterior pituitary gland, the third ventricle, the hypothalamus, the olfactory bulb, and the locus coeruleus, amongst others. These receptors regulate the main functions of the brain RAS, including maintaining cerebral blood flow and the integrity of the blood-brain barrier (BBB), water/thirst balance, blood pressure, and release of hormones, such as vasopressin (also known as antidiuretic hormone) and aldosterone[253, 264, 265].

However, chronic over-activation of AT_1 increases vulnerability to brain ischaemia[130]. Evidence obtained from rat models of human hypertension have shown that over-activation of AT_1 is associated with cerebrovascular stiffness and inflammation[266]. Fortunately, the negative effects produced by this over-activation can be normalised by administrating AT_1 receptor blockers (ARBs) and ACE inhibitors[130, 267].

4.1.2.2 Subtype 2: AT_2 receptors

In the brain, AT_2 receptors are located in the amygdala, caudate putamen, locus coeruleus, and thalamus, amongst other regions. However, there are only low numbers of these receptors within the brain, so little is known about their functions, although they are believed to be involved in the regulation of cerebral blood flow and have been shown to counteract the effect of overactive AT_1 receptors on blood pressure[129, 264, 268].

4.1.2.3 Subtype 3: AT_3 receptors

These receptors have only been identified in cultured mouse neuroblastoma cells[269] and their presence in human or cat tissue has not yet been

described[264].

4.1.2.4 Subtype 4: AT_4 receptors

In the brain, these receptors are located in the cerebral cortex, hippocampus, thalamus, cerebellum, and anterior pituitary gland, amongst others. These receptors are believed to be involved in learning and memory, cognition, and in the regulation of cerebral blood flow[264].

4.1.3 Angiotensin II type 1 receptor blockers

Some drugs are frequently used to manipulate the RAS in order to treat heart disease, hypertension, and diabetes, including ACE inhibitors (e.g., enalapril, benazepril), angiotensin receptor blockers (e.g., losartan, telmisartan), and aldosterone antagonists (e.g., spironolactone)[254].

Angiotensin II type 1 receptor blockers (ARBs) reverse the effects of over-activation of AT_1 by reducing vasoconstriction and inflammation[129, 130]. For this reason, ARBs are commonly used for the treatment of metabolic (e.g., diabetes), and cardiovascular disorders[129, 130].

Similarly, in the brain, ARBs have been shown to reduce inflammation and to slow down the inflammatory cascade by inhibiting the pro-inflammatory effect of microglia[130]. It has been suggested that this anti-inflammatory effect may be neuroprotective, as it prevents vasoconstriction and minimises hypoxia[130]. For these reasons, ARBs have also been proposed as potential treatments to improve cognition in both stroke and AD patients[130].

Sartans (e.g., losartan, candesartan, telmisartan) are ARBs derived from biphenyltetrazole that share similar pharmacological profiles[270]. They are widely used for the treatment of hypertension in people[271] which, if untreated,

is known to increase the risk of vascular dementia and AD[272-274]. Studies in humans have shown that sartans improve cognition and memory in hypertensive patients, as well as reducing the incidence and later development of AD in these patients[275-277].

Moreover, studies in rats have shown that sartans reduce acute brain inflammation after induced stroke[130, 266]. Sartans have also been shown to improve cerebral blood flow and to reduce the risk of ischaemia and brain damage; and to improve the neurological outcome in models of hypertension and stroke[278, 279]. In addition, sartans have been shown to protect cognition and to reduce amyloid-β toxicity after brain inoculation of this protein[251].

In mice, sartans have been shown to improve memory in models of diabetes[280]; and to interrupt the pro-inflammatory response of both microglia and astrocytes in multiple sclerosis models[281].

4.1.4 Telmisartan

The differing pharmacological profiles of the sartans result in differences in their neuroprotective effectiveness[282]. Telmisartan is considered to be the most potent of the sartans[134, 282] as it not only blocks AT_1 receptors, it also activates the peroxisome proliferator activated receptor gamma (PPARγ)[282].

PPARγ is a hormone receptor that plays an important role in the regulation of both carbohydrate and lipid metabolism within the body[283]. When activated, PPARγ improves the sensitivity to insulin, making PPARγ agonists a potential treatment of diabetes[284]. Activation of PPARγ also reduces inflammation[285], provides neuroprotection[134, 137, 139, 286], reduces neurotoxicity induced by glutamate[137] (a neurotransmitter involved in neurological disease) by promoting cell death and release of reactive oxygen species[287], and reduces neuronal damage after focal cerebral ischemia[288, 289]. It also improves memory

and cognition in AD[290].

The beneficial effect of PPARγ agonists in AD is mainly attributed to their anti-inflammatory properties. PPARγ activation suppresses the activation of microglial cells, which occurs as a result of the accumulation of amyloid-β[291], and inhibits their pro-inflammatory effect[292]. Moreover, studies in mice have suggested that PPARγ activation also inhibits the production of amyloid-β protein[293] and promotes its clearance[133].

Activation of PPARγ has also been shown to improve memory and cognition due to its effect on mitochondrial functions. Mitochondria are involved in the energy and glucose metabolism of the cells; this metabolism and the utilisation of glucose are believed to be reduced in the brain of patients with AD[292, 294]. In particular, when this metabolic deficit occurs alongside alterations in the regulation of mitochondrial calcium and increased oxidation, it can lead to hypoxia, brain damage, cognitive impairment, and AD[294]. Studies in mice have shown that activation of PPARγ improves mitochondrial function and utilisation of glucose, leading to an improvement of cognition[295].

As mentioned previously, telmisartan is considered to be the most potent of the sartans as it activates PPARγ[134, 282]. Studies in rodents have shown that treatment with telmisartan restored cognitive function after induced chronic stress[140], induced ischemia[296, 297], and after intra-cerebral injection of amyloid-β[134] that can accumulate abnormally in the brain leading to cognitive decline. Furthermore, in mouse models of AD, intranasal administration of telmisartan reduced amyloid-β deposition and neuronal loss, and improved cognition[298]. Finally, *in vitro* studies have shown that telmisartan reduced inflammatory mediators, such as cytokines and chemokines, that are involved in the aggregation of amyloid-β and promoted its clearance[133]. Telmisartan also reduced the accumulation of phosphorylated tau deposits[299].

The experimental evidence obtained from *in vitro* and rodent models suggest that telmisartan has the potential to be beneficial as a treatment for neurodegenerative diseases and to improve cognition. Currently, there are two ongoing clinical trials assessing the efficacy of telmisartan for the treatment of AD in human patients[142]. One of the studies started in March 2014 and entered Phase 2 at the time the present study was being conducted. That study aims to evaluate the effect of telmisartan administered for one year at a daily dose of 40-80 mg/kg (depending on age and tolerance) on the blood vessels of the brain and whether it slows down brain atrophy in patients with mild to moderate AD[142]. The trial is expected to be completed by March 2022. The second study has been running for eight months and aims to assess the effect of telmisartan (at a daily dose of 20 or 40 mg/kg) on the cognitive abilities and development of AD in African-Americans, an ethnic group known to be at high risk of developing this disease[142]. This study started in April 2015 and was expected to be completed by June 2020.

In cats, telmisartan (Semintra™, Boehringer Ingelheim; at a dose of 1 mg/kg PO q24h) has been shown to be effective in the reduction of proteinuria associated with chronic kidney disease[300]. Furthermore, it has shown to be effective at reducing hypertension in this species, at a dose ranging from 1-3 mg/kg[301-303].

The beneficial effects of telmisartan by reducing neuroinflammation and improving cognition makes this drug a potentially good treatment for CDS in cats.

The aim of this study was to determine the effects of telmisartan (Semintra™, Boehringer Ingelheim) on the clinical signs of CDS in cats at its licensed dose (at the time this study commenced) of 1 mg/kg PO q24h, given for three months. It was hypothesized that the clinical signs of CDS would be significantly reduced in the cats receiving telmisartan (Semintra™, Boehringer Ingelheim).

4.2 Materials and methods

The effects of telmisartan (Semintra™, Boehringer Ingelheim) on the behavioural signs of CDS in cats were assessed by running a double-blinded placebo-controlled study in cats with a confirmed diagnosis of CDS[90]. Changes in the abnormal behaviours were assessed based on the cat owners' responses to a series of questionnaires. The study consisted of a 7-day pre-treatment period, a 90-day treatment period, and a 30-day post-treatment washout period.

Of note, the format of the double-blinded placebo-controlled trial and the questionnaires were designed by Professor Danièlle Gunn-Moore.

4.2.1 Pre-treatment period

4.2.1.1 Recruitment

Cats were recruited through The Hospital for Small Animals of The Royal (Dick) School of Veterinary Studies (RDSVS) and the Bishop's Stortford Veterinary Hospital. To be considered for the study, cats had to be at least 10 years old and have a confirmed diagnosis of CDS (as per the CDS criteria – see below) and to be otherwise healthy. Cats with chronic diseases (i.e., diabetes mellitus, chronic kidney disease, or hyperthyroidism) were considered and recruited only if the disease was stable and had been so for at least three months. The complete inclusion and exclusion criteria for the recruitment of the cats is listed in Table 4.2.

Table 4.2 Inclusion and exclusion criteria for the recruitment of cats

Inclusion criteria
• Age of 10 years or older • Lived with owner for at least six months • Meet the cognitive dysfunction criteria (see below) • Unremarkable physical examination, including retinal examination • Unremarkable routine haematology and serum biochemistry • Normotensive (< 180 mmHg systolic*) • Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) negative on blood • Stable systemic diseases for at least three months (e.g., hyperthyroidism or diabetes mellitus; chronic kidney disease only accepted if IRIS ≤ stage 2 and stable for ≥ 3 months)
Exclusion criteria
• Displaying signs of acute disease • Currently receiving treatment for "old age behaviour" (e.g., selegiline hydrochloride, propentofylline, etc.) • Signs of CDS started before the age of eight years old

* While cats are now considered to have mild hypertension when the systolic blood pressure is > 140 mmHg, the higher cut off of 180 mmHg was accepted when the study started[304].

4.2.1.2 Diagnosis of CDS

At the time of the first consultation, the cat owners responded to the first of a series of questionnaires (Questionnaire 1) (Appendix 4.1) that assessed changes in a total of 27 behaviours (Table 4.3). Some of these behaviours related to CDS (i.e., vocalisation at night, and affection to people, amongst other options), whilst others are not typically associated with CDS (i.e., vomiting, hair loss, etc.); the latter were added so that owners did not know

which were signs of CDS or not, so they acted as distractors; moreover, they also provided evidence of undisclosed signs of ill-health. Cats had to have been displaying behaviours consistent with CDS for at least one month prior to this time point. For this questionnaire, owners were asked to report if these behaviours have decreased significantly (- 2), decreased mildly (- 1), remained the same (0), increased mildly (1), or increased significantly (2), when compared to when the cats were younger (i.e., around five years previously).

Table 4.3 List of the 27 behaviours included in the questionnaires that were assessed by the owners. *The behaviours that are strongly related to cognitive dysfunction syndrome are highlighted in bold*

List of behaviours included in the questionnaires
• Activity levels/time spent playing • ***Affection with people in the house*** • Agitation and restlessness • Aggression towards animals or people • ***Aimless activity (i.e., staring into space, pacing, etc.)*** • Change in appetite • Change in weight • Constipation • Diarrhoea • Drinking • Hair loss or hair thinning • Hearing loss • ***Passing faeces in the house outside litter tray*** • ***Passing urine in the house outside litter tray*** • ***Repetitive or compulsive behavior (e.g., over-grooming, etc.)*** • ***Time spent grooming*** • ***Time spent sleeping at night*** • ***Time spent sleeping during the day*** • ***Tolerance of handling*** • ***Tolerance of being left alone*** • ***Tolerance of other animals in the house*** • Vision loss • ***Vocalisation during the day*** • ***Vocalisation at night*** • Vomiting (including hairballs) • Wanting to spend time outdoors • Willingness to jump up or down (including climbing stairs)

In order to diagnose CDS in the cats, the results from Questionnaire 1 (Q1) were evaluated according to pre-determined cognitive decline criteria (Appendix 4.2). This questionnaire was modified from a similar questionnaire previously used for the diagnosis and severity of CDS in dogs and cats[8], that has been used for similar studies for over a decade; however, it is not yet a fully validated clinical metrology instrument. At present, an improved instrument based on this previous one is being developed. The diagnosis for CDS was made when cats displayed a significant number of CDS-associated behavioural changes for at least one month and medical or stress-related causes of the behaviour could not be found. Given that there is no published clinical metrology instrument for the diagnosis of CDS in cats, a scoring system was developed based on the frequency of the different behaviours and how commonly they were associated with CDS cases in the clinic (DGM, unpublished data). For this, the most frequent behaviours associated with CDS (i.e., vocalisation at night, increased affection to people) were scored as 1*; slightly less frequent behaviours (e.g., vocalisation during day, increased time spent sleeping) were scored as 1; even less frequent behaviours (e.g., activity levels, house-soiling) were scored as 2; and the least frequent behaviours (e.g., willingness to jump, hearing loss) were scored as 3. The number of behavioural changes required for a diagnosis of CDS to be made varied depending on how commonly the behaviours were associated with CDS. For example, cats displaying only one behavioural change that is strongly associated with CDS[90] (scored as 1*) were diagnosed easily. In contrast, cats needed to display a minimum of two of the frequently associated behaviours which were not as strongly associated with CDS (scored as 1), and at least four of the behaviours that are only mildly associated with CDS (scored as 2) (Table 4.4).

Table 4.4 Cognitive dysfunction criteria. *The number of behavioural changes required for a diagnosis of cognitive dysfunction syndrome (CDS) to be made depended on how closely these behaviours are associated with CDS in cats*

Changes in **ONE** of the following
• Affection to people • Tolerance of being left alone • Vocalisation during the night
Changes in **TWO** of the following
• Aimless activities (i.e., staring into space) • Repetitive or compulsive behaviour (i.e., overgrooming, licking objects, etc.) • Time spent grooming • Time spent sleeping during day • Time spent sleeping at night • Vocalisation during the day
Changes in **FOUR** of the following
• Activity levels/time spent playing • Aggression towards animals or people • Agitation/restlessness • Appetite • Tolerance of handling • Wanting to spend time outdoors • Tolerance of other animals in the house • Passing faeces in house outside the litter tray • Passing urine in house outside the litter tray • Vomiting • Weight

4.2.1.3 Medical examination

Only cats displaying significant signs of CDS were recruited to undertake a complete medical examination, including full physical examination, joint palpation, mobility assessment, limited neurological examination (i.e., hands-off examination[305]), assessment of systolic blood pressure using the Doppler method[304], retinal examination (looking for changes suggestive of hypertension, toxoplasmosis, and other pathologies), haematology, serum biochemistry and assessment of serum FeLV and FIV status. Cats with pre-existing diseases, such as diabetes mellitus or hyperthyroidism, could be recruited if their disease had remained stable for at least three months prior to being recruited.

4.2.1.4 Medication and dosage

Recruited cats were randomly separated without replacement into two groups. Group A received the placebo and Group B received telmisartan (Semintra™, Boehringer Ingelheim). Cats from both groups were dosed so they would receive 1 mg/kg of telmisartan or a placebo (Appendix 4.3), orally, once a day, for a total of three months.

The telmisartan and placebo were both provided by Boehringer Ingelheim Animal Health UK Limited. To reduce bias, both compounds were identical, including colour, smell, taste, tub, and packaging; with the only difference that the placebo did not have the active ingredient telmisartan.

Nota bene: Throughout the drug trial and the data analyses, the owners and author remained blinded as to which group received which compound.

4.2.2 Treatment period

Cats from both groups received the randomised trial treatment for a total of three months, and owners were asked to stop the medication exactly 90 days after the treatment began. During the treatment period, owners had to respond to three questionnaires at different time points, at 30 days after the treatment began (i.e., Questionnaire 30 days), and then every 30 days (i.e., Questionnaires 60 and 90 days) (Figure 4.2). These questionnaires were almost identical to Questionnaire 1; the only difference was that the owners were asked to compare their cat's current behaviour to when they began the trial (i.e., Questionnaire 1). The questionnaires were available online at the Vet Professionals website (https://www.vetprofessionals.com/scs-30-120.html; since the study had ended this link is no longer active) or could be posted to the owners.

The follow-up questionnaires also asked owners to report any potential side effects their cat may have been showing, to comment on how easy or difficult it was to give the medication, and how palatable it was for their cat.

4.2.3 Post-treatment period

Once the treatment stopped, cats underwent a second complete medical examination and blood sampling to assess their health. After a washout period of 30-days, owners were asked to respond to a final questionnaire (i.e., Questionnaire 120 days). For this questionnaire, owners had to report changes on their cat's behaviours since the treatment stopped.

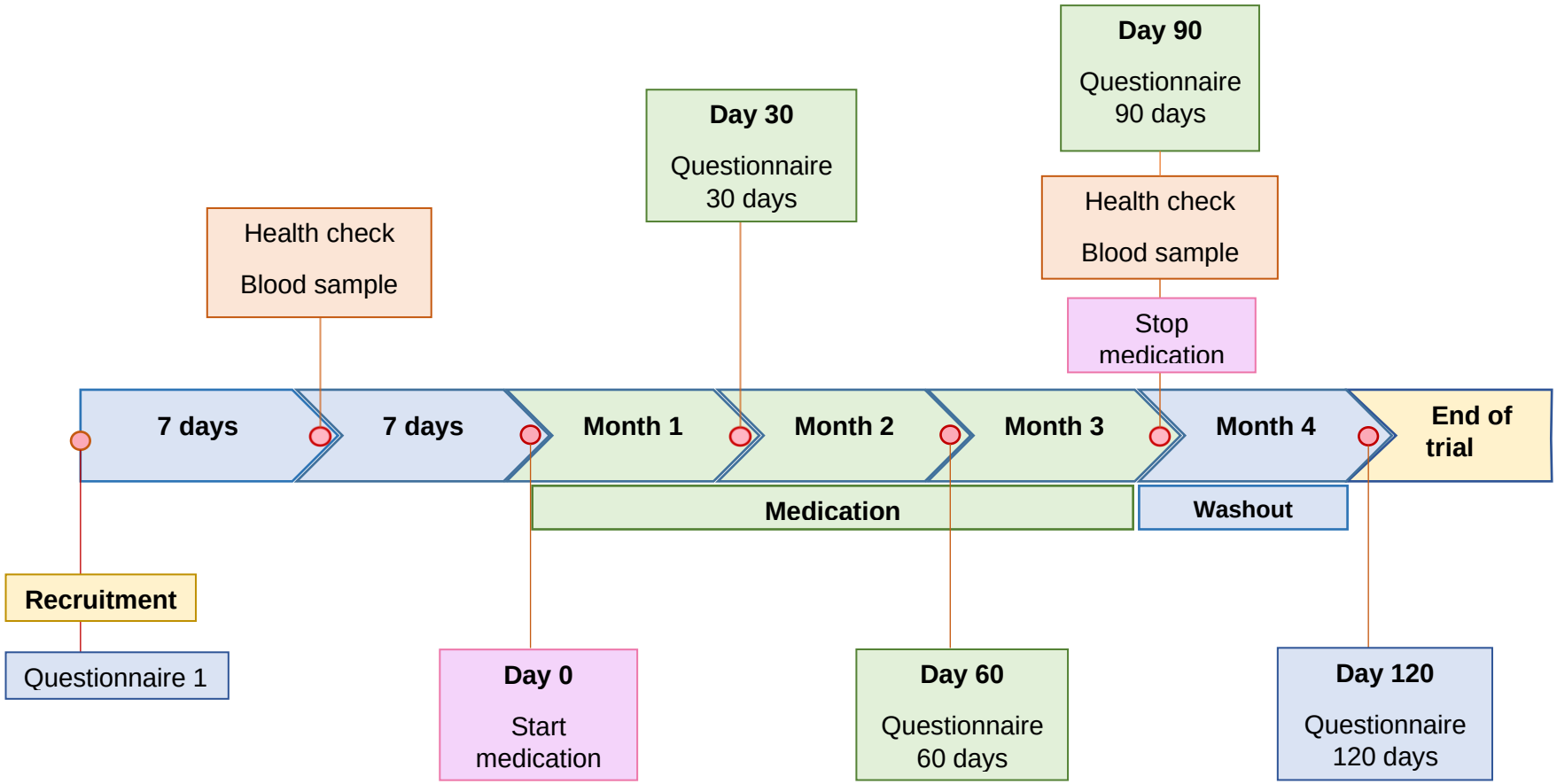


Figure 4.2 Timeline of the double-blinded placebo-controlled study. *Owners were asked to respond to a series of questionnaires. The first one (Questionnaire 30) had to be completed 30 days after starting the administration of the medication/placebo to the cats. Two more questionnaires (Questionnaires 60 and 90) had to be completed every 30 days after that. The administration of both compounds stopped on the 90th day of the trial and after a washout period of 30 days owners had to complete a final questionnaire (Questionnaire 120)*

4.2.4 PPARγ in the cat brain

To assess whether PPARγ was expressed in cat brains, immunohistochemistry was performed on the same brain sections described in Chapter 2, following the same immunohistochemistry protocols, and using a PPARγ monoclonal antibody (1:1000; Clone: K.242.9; ThermoFisher Scientific™; Massachusetts, USA) as the primary antibody.

4.2.5 Ethical approval

Ethical approval was obtained through the Veterinary Ethical Review Committee from the RDSVS, The University of Edinburgh (VERC 98.14).

4.2.6 Data analysis

All data were analysed using SAS v9.4 statistical software for Windows.

4.2.6.1 Correlation between age and behaviours

Ordinal logistic regressions were performed to assess the correlation between age and the different behaviours, and to determine whether the age of the cats had an effect on these behaviours. For this, responses provided by the owners in Questionnaire 1 (Q1) were compared against the age of the cat, regardless of their treatment group.

4.2.6.2 Differences in the behaviours between treatment groups and at different time points

Ordinal categorical data were treated as continuous and fitted into general linear models for repeated measures in which the behaviours were adjusted

for treatment, the different time points, breed, and sex. These models were used to determine whether there were differences in the behaviours between treatment A and B (i.e., all responses at the different time points together for each behaviour for treatment A vs the ones for treatment B; for example, all the responses for changes in activity levels for treatment A vs treatment B); and between treatments at the different time points (i.e., A vs B at Questionnaire 30 days (Q30); A vs B at Questionnaire 60 days (Q60); A vs B at Questionnaire 90 days (Q90); and A vs B at Questionnaire 120 days (Q120); for example, responses from Q30 for changes in activity levels for treatment A vs treatment B.

In addition, ordinal categorical data were treated as continuous and fitted into general linear models for repeated measures to determine whether there were differences in each of the behaviours during and after the treatment. For this, changes in each of the behaviours reported by the owners were compared at the different time points of the treatment period versus the post-treatment period (i.e., Q30 vs Q120; Q60 vs Q120; and Q90 vs Q120) for both treatments, separately. Finally, the means of the three questionnaires completed during the treatment period (i.e., Q30, Q60, and Q90) were compared against Q120, for both treatments, separately.

4.3 Results

4.3.1 Demographics

A total of 60 cats were assessed for this study; however, only 34 of them met the inclusion/exclusion criteria and were recruited. Most of the cats (82.4%; n = 28) were non-pedigree cats (i.e., domestic shorthair [DSH] and domestic longhair [DLH]), with 17.6% (n = 6) being pedigree breeds (i.e., Bengal [n = 2], British Shorthair [n = 1], Devon Rex [n = 1], and Siamese [n = 2]). More than half of the cats (61.8%; n = 21) were females, all neutered except for one; and 38.2% (n = 13) of the cats were neutered males. Ages ranged between 10 and 20 years old, with a mean of 15.3 years old and a median of 16 years old.

4.3.1.1 Group A: Placebo

Most of the cats in Group A (88.2%; n = 15) were non-pedigree cats and 11.8% (n = 2) were pedigree cats. Of the 17 cats in this group, more than half of them (58.8%; n = 10) were females, and 41.2% (n = 7) were males. The ages of cats in Group A ranged between 10 and 18 years old, with a mean of 14.8 years old and a median of 15 years old.

4.3.1.2 Group B: Telmisartan (Semintra™, Boehringer Ingelheim)

More than three quarters of the cats in Group B (76.5%; n = 13) were non-pedigree cats and 23.5% (n = 4) were pedigree cats. Of the 17 cats in this group, 64.7% (n = 11) were females, and 35.3% (n = 6) were males. The ages of cats in Group B ranged between 10 and 20 years old, with a mean of 15.7 years old and a median of 16 years old.

4.3.2 PPARγ in the cat brain

Immunohistochemistry revealed that PPARγ was expressed in all regions of the cat brain, in all age groups (Figure 4.3); however, since the aim of this experiment was only to determine whether or not these receptors are present in the cat brain, differences between the brain regions and the age groups were not assessed.

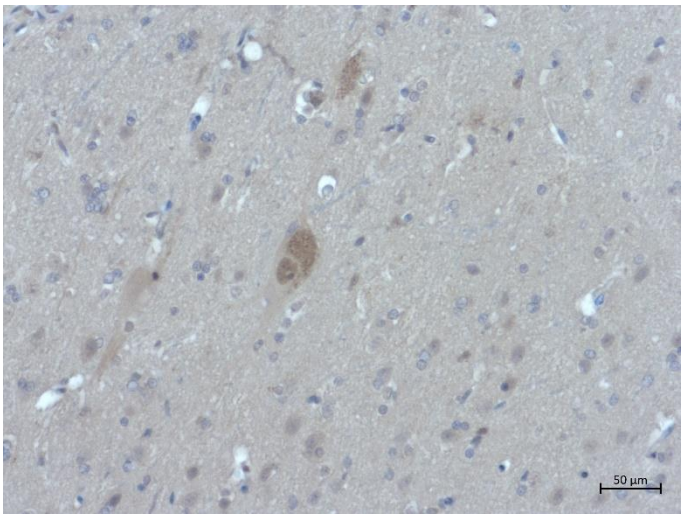


Figure 4.3 PPARγ in the cat brain. *PPARγ were found to be present in all regions of the cat brains, and in all age groups. In this image, nuclear and cytoplasmic immunolabelling of PPARγ is present in the parietal cortex of a 15-years-old cat*

4.3.3 Correlation between age and behaviours

Ordinal logistic regressions were performed to assess any correlation between age and the different behaviours. There was a correlation between age and some behaviours, showing that as cats get older, they are more likely to house-soil (i.e., in this case, to be passing faeces outside the litter box) ($p = 0.05$), to display repetitive and compulsive behaviours ($p = 0.01$), and to lose weight ($p = 0.01$). The complete table of results can be found on Appendix 4.4).

4.3.4 Differences in the behaviours between treatments and at different time points

General linear models for repeated measures were performed to determine whether there were differences in each of the behaviours between the two treatments, and at the different time points. The complete table of results can be found in Appendices 4.5 and 4.6. Furthermore, graphs showing the changes in the different behaviours as reported by the owners and demonstrated by the percentage of cats displaying an increase, decrease or staying the same, for both treatments before, during, and after the treatment can be found in Appendix 4.7.

There were no statistically significant differences between treatment A and B for any of the behaviours assessed. Despite not finding any differences between treatments, there were some statistically significant differences in several of the behaviours in either one or both treatment groups. Interestingly, most of the behaviours that were reported to change were the ones related to CDS (Table 4.5); differences were reported in eight of the 12 behaviours potentially directly related to CDS. In contrast, there were only significant differences in four of the 15 behaviours added as distractors and were not thought to be related to CDS.

Table 4.5 Changes in behaviours as reported by the owners. *Statistically significant differences were mostly found in behaviours believed to be related to cognitive dysfunction syndrome (CDS) in either one or both treatment groups, shown in brackets. In this list, the behaviours related to CDS were listed in order of how frequently they had previously been reported to occur with CDS[20]*

Behaviours related to CDS	Behaviours not related to CDS (Distractors)
• Vocalisation at night A (p = 0.02) and B (p = 0.06) • Vocalisation during the day A (p = 0.05) • Affection with people in the house A (p = 0.03) • Tolerance of handling A (p = 0.04) • Time spent sleeping at night B (p = 0.05) • Time spent sleeping during the day Overall females from A and B (p = 0.007) • Passing urine in the house outside litter tray A (p = 0.05) • Aimless activity (i.e., staring into space, pacing) A (p = 0.02)	• Activity levels/time spent playing A (p = 0.04) and B (p = 0.04) • Agitation and restlessness A (p = 0.01) and B (p = 0.03) • Drinking A (p = 0.04) • Hair loss or hair thinning A (p = 0.03)

The following section presents descriptive statistics showing the percentage of cats displaying changes in each of the behaviours (i.e., increase and

decrease) when the cats were receiving the treatment and after it was finished. Only statistically significant results are presented for any differences in the behaviours for each overall treatment (i.e., if a specific behaviour was different when cats were receiving the treatment vs when it was finished); and when comparing the different time points (i.e., Q30 vs Q60, Q30 vs Q120, and Q60 vs Q120).

4.3.4.1 Activity levels

4.3.4.1.1 Treatment A

During the trial, a small proportion of cats (17.6%; n = 3) were more active, while 5.9% (n =1) of the cats were less active. In contrast, once the treatment finished, 5.9% (n = 1) of the cats were more active than before, and almost a quarter (23.5%; n = 4) were less active at this time point. Overall, cats were more active when receiving treatment A, compared to when it was stopped (p = 0.04) (Figure 4.4A).

4.3.4.1.2 Treatment B

During the treatment, 15.7% (n = 3) of cats were more active than before, and 9.8% (n = 2) were less active. In contrast, after the treatment stopped, none of the cats were more active than before, and 17.7% (n = 3) of cats were less active. When comparing the different time points, cats receiving treatment B were more active in Q30, when compared to Q120 (p = 0.04) (Figure 4.4A).

4.3.4.2 Agitation and restlessness

4.3.4.2.1 Treatment A

During the treatment, 17.6% (n = 3) of cats were less agitated than before, and a similar proportion of cats (13.8%; n = 2) were more agitated than before.

Once the treatment finished, none of the cats were less agitated than before, and 41.2% (n = 7) were more agitated. Cats were less agitated during the treatment, compared to when it was finished (p = 0.01) (Figure 4.4B).

4.3.4.2.2 Treatment B

During the treatment, almost a quarter of the cats (23.5%; n = 4) were less agitated, while 17.7% (n = 3) were more agitated than before. After the treatment finished, none of the cats were less agitated than before, and less than a third of cats (29.4%; n = 5) were more agitated. Cats were less agitated while they were receiving treatment B, when compared to when it was finished (p = 0.03) (Figure 4.4B).

4.3.4.3 Aimless activity

4.3.4.3.1 Treatment A

During the treatment, owners reported that a quarter of the cats (25.5%; n = 4) displayed less aimless activity, and 11.8% (n = 2) displayed more than before. In contrast, after finishing the treatment, 5.9% (n = 1) of the cats displayed less aimless activity, and almost a quarter (23.5%; n = 4) displayed more aimless activity than before. Overall, cats displayed less aimless activity during treatment A, compared to when it was finished (p = 0.02) (Figure 4.4C).

4.3.4.3.2 Treatment B

During the treatment, a small percentage of cats (13.7%; n = 2) displayed less aimless activity than before, and the same percentage of cats (13.7%; n = 2) displayed more aimless activity. However, once the treatment finished, none of the cats displayed less aimless activity than before, and 11.8% (n = 2) displayed more than before (Figure 4.4C).

4.3.4.4 Repetitive/compulsive behaviour

4.3.4.4.1 Treatment A

During the treatment, none of the cats displayed less repetitive or compulsive behaviours than before, and only 7.8% (n = 1) displayed more than before. After the treatment, 11.8% (n = 2) of cats displayed less repetitive or compulsive behaviours than before, and none displayed more than before (Figure 4.4D).

4.3.4.4.2 Treatment B

During the treatment, 7.8% (n = 1) of cats displayed less repetitive or compulsive behaviours than before, and 9.8% (n = 2) display more than before. After the treatment, none of the cats displayed less repetitive or compulsive behaviours than before, and 11.8% (n = 2) displayed more than before (Figure 4.4D).

4.3.4.5 Willingness to go outside

4.3.4.5.1 Treatment A

During the treatment, almost a quarter of the cats (21.6%; n = 4) wanted to spend more time outside, and 7.8% (n = 1) wanted to spend less time outside. In contrast, once the treatment finished, 17.6% (n = 3) of the cats wanted to spend more time outside, and 17.6% (n = 3) of cats wanted to spend less time outside (Figure 4.4E).

4.3.4.5.2 Treatment B

During the treatment, 21.6% (n = 4) of cats wanted to spend more time outside than before, and 9.8% (n = 2) wanted to spend less time outside. In contrast,

once the treatment finished, 23.5% (n = 4) of cats wanted to spend more time outside, and 11.8% (n = 2) wanted to spend less time outside (Figure 4.4E).

4.3.4.6 Willingness to jump

4.3.4.6.1 Treatment A

During the treatment, 7.8% (n = 1) of cats jumped more than before, and the same percentage of cats (7.8%; n = 1) jumped less than before. After the treatment finished, 5.9% (n = 1) of cats jumped more than before, and 5.9% (n = 1) of cats jumped less (Figure 4.4F).

4.3.4.6.2 Treatment B

During the treatment, a small number of cats (11.8%; n = 2) jumped more than before, and 13.7% (n = 2) of cats jumped less than before. Once the treatment finished, none of the cats jumped more than before, and 5.9% (n = 1) of them jumped less than before (Figure 4.4F).

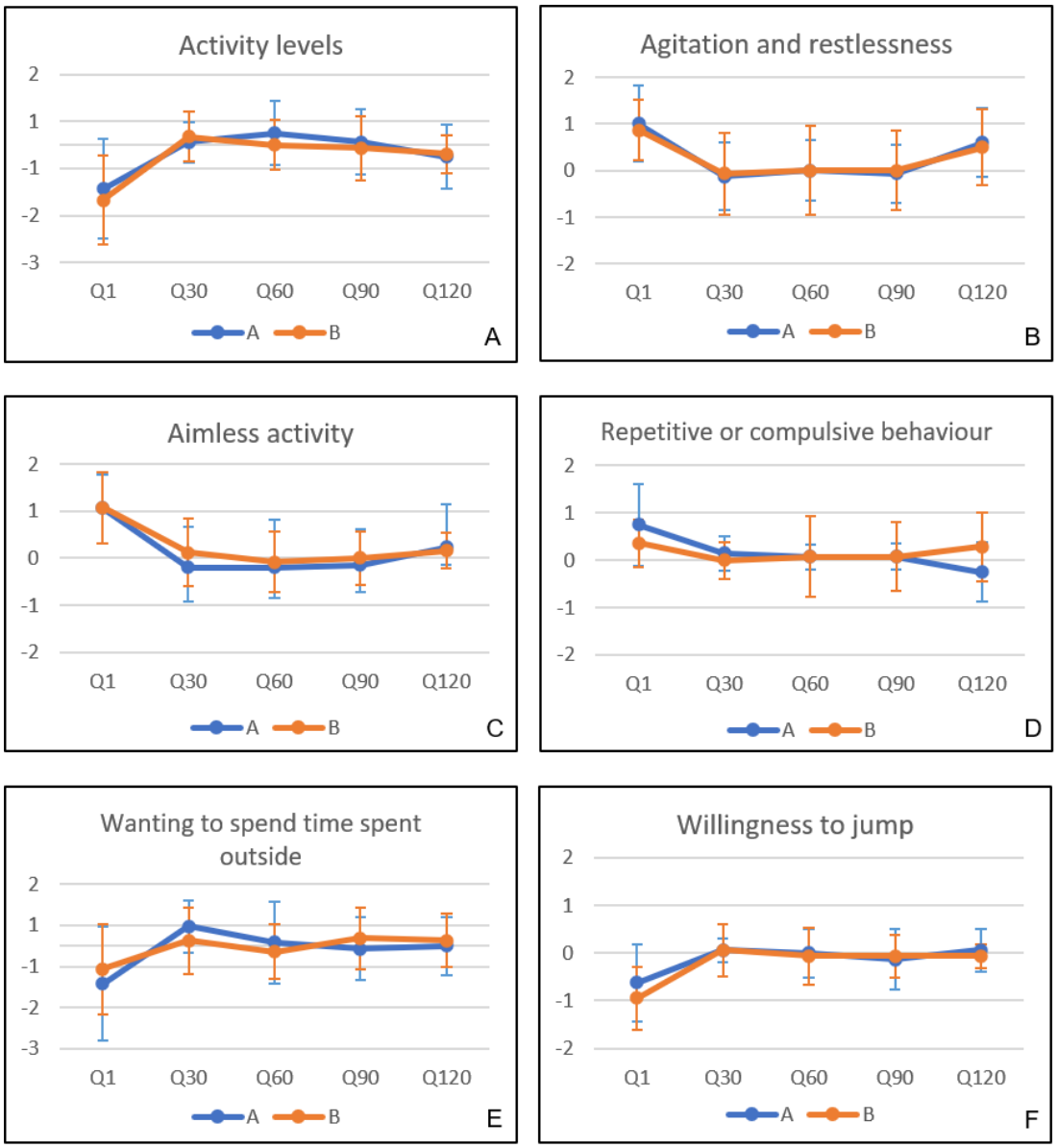


Figure 4.4 Behavioural changes. *Changes through time (mean ± SD), for both treatments, in A) activity levels; B) agitation and restlessness; C) aimless activity; D) repetitive and/or compulsive behaviour; E) wanting to spend time outside; and F) willingness to jump. Negative scores in the y axis represent a decrease (i.e., - 1 = mild, and – 2 = significant), while positive scores represent an increase (i.e., 1 = mild, and 2 = significant) in the behaviours; zero represents no change*

4.3.4.7 Affection with people in the house

4.3.4.7.1 Treatment A

During the treatment, more than a third of the cats (37.2%; n = 6) were more affectionate than before, and 2% (n = 1) of cats were less affectionate than before. After the treatment, 17.6% (n = 3) of cats were more affectionate than before, and 11.8% (n = 2) were less than before. When comparing the different time points, cats on this treatment were more affectionate during Q60, when compared to Q120 (p = 0.03) (Figure 4.5A).

4.3.4.7.2 Treatment B

During the treatment, a third of cats (33.3%; n = 6) were more affectionate than before, and 2% (n = 1) were less than before. In contrast, once they finished the treatment, 5.9% (n = 1) of cats were more affectionate than before, and the same percentage (5.9%; n = 1) were less than before (Figure 4.5A).

4.3.4.8 Aggression towards animals or people

4.3.4.8.1 Treatment A

During the treatment, 9.8% (n = 2) of cats were more aggressive than before, and 2% (n = 1) were less aggressive than before. After the treatment, 11.8% (n = 2) of the cats were more aggressive, and 5.9% (n = 1) were less than before (Figure 4.5B).

4.3.4.8.2 Treatment B

During the treatment, 2% (n = 1) of cats were more aggressive than before, and 5.9% (n = 1) of them were less than before. After receiving the treatment,

none of them were more aggressive than before, and 5.9% (n = 1) were less than before (Figure 4.5B).

4.3.4.9 Tolerance of handling

4.3.4.9.1 Treatment A

During the treatment, almost a quarter of the cats (21.6%; n = 4) tolerated being handled more than before, and 15.7% (n = 3) less than before. After the treatment, none of the cats tolerated being handled more than before, and 17.7% (n = 3) less than before. In addition, cats tolerated being handled more during treatment A, compared to when it was finished (p = 0.04) (Figure 4.5C).

4.3.4.9.2 Treatment B

During the treatment, 15.7% (n = 3) of cats tolerated being handled more than before, and 5.9% (n = 1) less than before. After the treatment, 11.8% (n = 2) of the cats tolerated being handled more than before, and 11.8% (n = 2) less than before (Figure 4.5C).

4.3.4.10 Tolerance of being left alone

4.3.4.10.1 Treatment A

During the treatment, a small proportion of cats (5.9%; n = 1) tolerated being left alone more than before, and 11.8% (n = 2) less than before. After the treatment, none of the cats tolerated being left alone more than before, and 17.7% (n = 3) less than before (Figure 4.5D).

4.3.4.10.2 Treatment B

During the treatment, 7.8% (n = 1) of cats tolerated being left alone more than before, and 7.8% (n = 1) less than before. In contrast, after the treatment, none of these cats tolerated being left alone more than before, and 23.5% (n = 4) less than before (Figure 4.5D).

4.3.4.11 Tolerance of other animals in the household

4.3.4.11.1 Treatment A

During the treatment, a few cats (2%; n = 1) tolerated other animals more than before, and 5.9% (n = 3) less than before. After the treatment, none of the cats tolerated other animals more than before, and 11.8% (n = 2) less than before (Figure 4.5E).

4.3.4.11.2 Treatment B

During the treatment, the same proportion of cats tolerated other animals more (2%; n = 1) and less (2%; n = 1) than before. In contrast, after the treatment, none of the cats tolerated other animals more than before, and 11.8% (n = 2) less than before (Figure 4.5E).

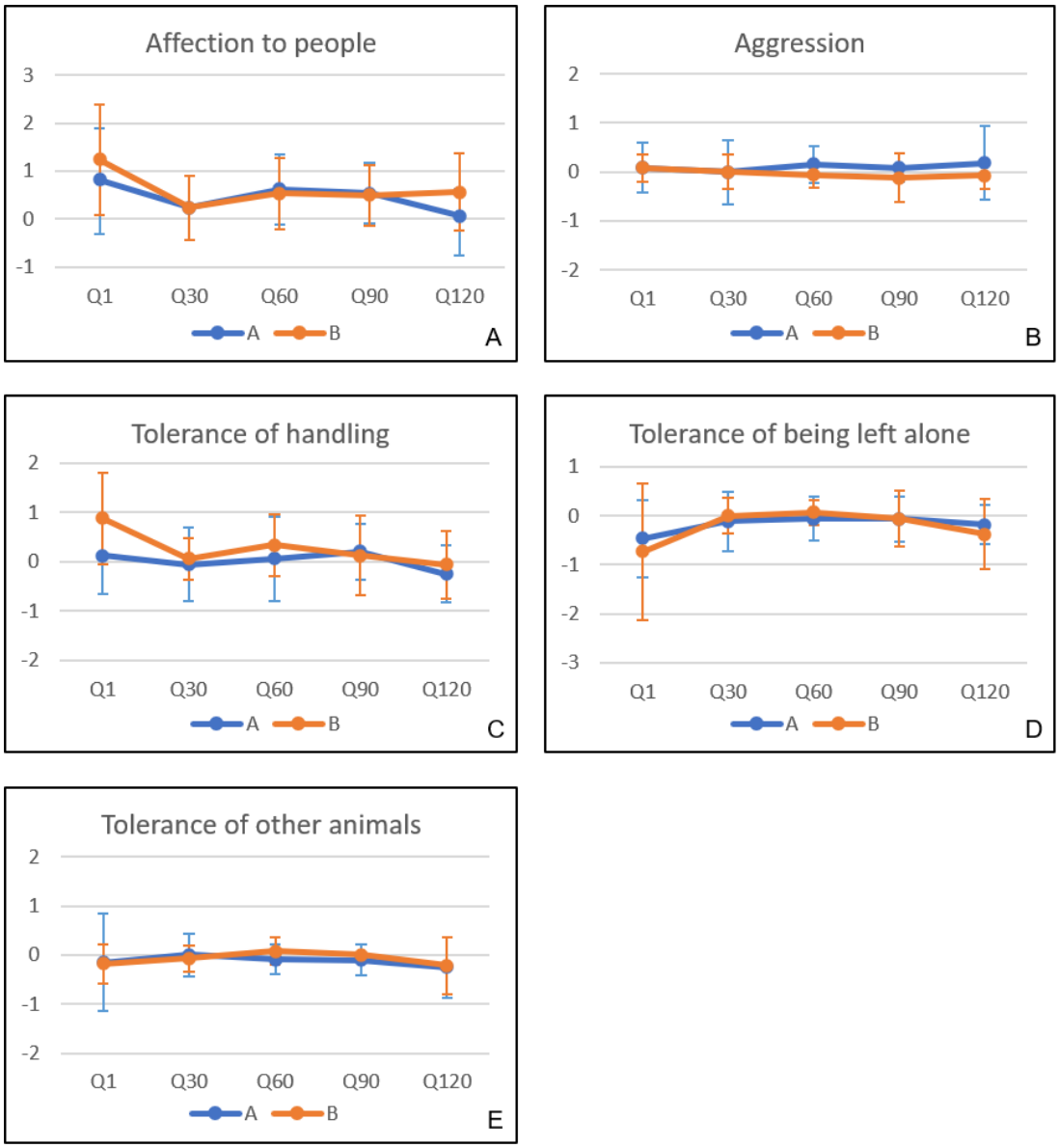


Figure 4.5 Behavioural changes. *Changes through time (mean ± SD), for both treatments, in A) affection with people in the house; B) aggression towards animals or people; C) tolerance of handling; D) tolerance of being left alone; and E) tolerance of other animals in the household. Negative scores in the y axis represent a decrease (i.e., - 1 = mild, and – 2 = significant), while positive scores represent an increase (i.e., 1 = mild, and 2 = significant) in the behaviours; zero represents no change*

4.3.4.12 Time spent grooming

4.3.4.12.1 Treatment A

During the treatment, 15.7% (n = 3) of cats spent more time grooming than before, and 2% (n = 1) of cats spent less time than before. After the treatment, 5.9% (n = 1) of cats spent more time grooming than before, and 23.5% (n = 4) less than before (Figure 4.6A).

4.3.4.12.2 Treatment B

During the treatment, 7.8% (n = 1) of cats spent more time grooming than before, and 3.9% (n = 1) of cats less than before. After the treatment, 5.9% (n = 1) of cats spent more time grooming than before, and the same percentage (5.9%; n = 1) less than before (Figure 4.6A).

4.3.4.13 Time spent sleeping at night

4.3.4.13.1 Treatment A

During the treatment, almost a quarter of the cats (21.6%; n = 4) slept more at night than before, and 7.8% (n = 1) slept less than before. After finishing the treatment, 5.9% (n = 1) of the cats slept more at night than before, and 11.8% (n = 2) slept less than before (Figure 4.6B).

4.3.4.13.2 Treatment B

During the treatment, a quarter of the cats (25.5%; n = 4) slept more at night than before, and 7.8% (n = 1) of cats slept less than before. In contrast, when the treatment finished, 5.9% (n = 1) of cats slept more at night than before, and 29.5% (n = 5) less than before. In addition, cats slept more in general

during treatment B, when compared to when it was finished (p = 0.05) (Figure 4.6B).

4.3.4.14 Time spent sleeping during the day

Overall, female cats from both treatment groups slept more during the day than males at Q60 (p = 0.007).

4.3.4.14.1 Treatment A

During the treatment, almost a third of the cats (29.4%; n = 5) slept more during the day, and 17.6% (n = 3) less than before. After the treatment, 17.6% (n = 3) of cats slept more during the day, and 11.8% (n = 2) less than before (Figure 4.6C).

4.3.4.14.2 Treatment B

During the treatment, 19.6% (n = 3) of cats slept more during the day than before, and 9.8% (n = 2) less than before. After the treatment, 11.8% (n = 2) of cats slept more during the day than before, and 5.9% (n = 1) less than before (Figure 4.6C).

4.3.4.15 Vocalisation during the night

Overall, female cats on both treatments cried less during the night than male cats (p = 0.01). Furthermore, when comparing the different time points, female cats cried less during the night at Q30 (p = 0.03), and at Q60 (p = 0.02), than males.

4.3.4.15.1 Treatment A

During the treatment, almost a quarter of cats (23.5%; n = 4) cried less at night than before, and 9.8% (n = 5) more than before. In contrast, after the treatment, 5.9% (n = 1) of cats cried less at night than before, and 35.3% (n = 6) more than before. In addition, cats cried less during the night while receiving the treatment, when compared to when it was finished (p = 0.02) (Figure 4.6D).

4.3.4.15.2 Treatment B

During the treatment, more than a third of the cats (37.2%, n = 19) cried less at night than before, and 17.7% (n = 9) more than before. After the treatment, none of the cats cried less at night than before, and 35.3% (n = 6) more than before. In addition, cats cried less during the night when receiving the treatment, when compared to when it was finished (p = 0.006) (Figure 4.6D).

4.3.4.16 Vocalisation during the day

4.3.4.16.1 Treatment A

During the treatment, a small number of cats (15.7%; n = 8) cried less during the day than before, and 29.4% (n = 5) more than before. After the treatment, none of the cats cried less during the day than before, and 47.1% (n = 8) more than before. In addition, cats cried less during the day when on treatment A, compared to when the treatment finished (p = 0.05) (Figure 4.6E).

4.3.4.16.2 Treatment B

During the treatment, a quarter of cats (25.5%; n = 4) cried less during the day than before, and 27.5% (n = 5) more than before. In contrast, after the treatment, 5.9% (n = 1) of cats cried less during the day than before, and 35.3% (n = 6) more than before (Figure 4.6E).

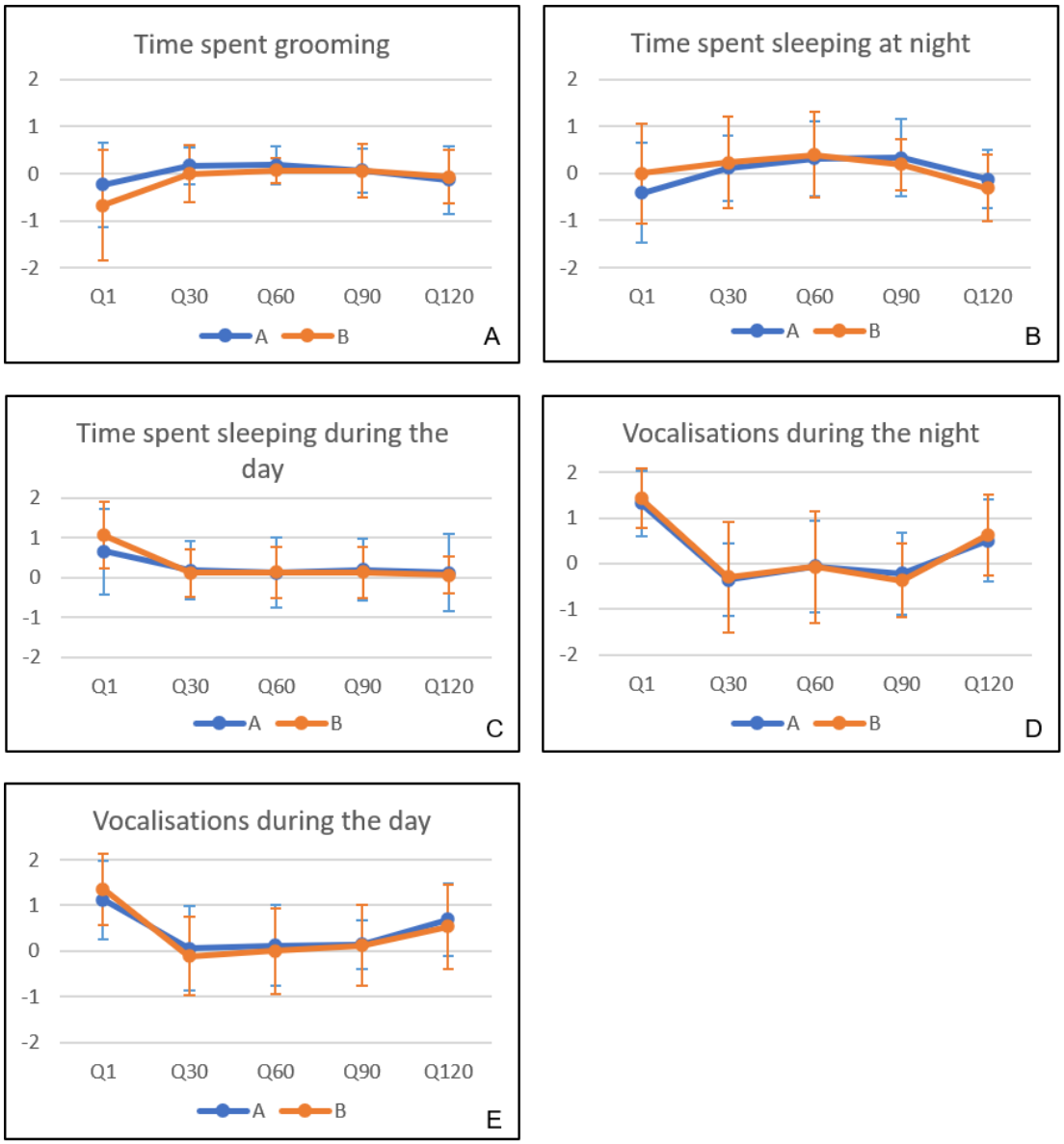


Figure 4.6 Behavioural changes. *Changes through time (mean ± SD), for both treatments, in A) time spent grooming; B) time spent sleeping at night; C) time spent sleeping during the day; D) vocalisations during the night; and E) vocalisations during the day. Negative scores in the y axis represent a decrease (i.e., - 1 = mild, and – 2 = significant), while positive scores represent an increase (i.e., 1 = mild, and 2 = significant) in the behaviours; zero represents no change*

4.3.4.17 Appetite

4.3.4.17.1 Treatment A

During the treatment, almost a third of cats (29.4%; n = 5), ate more than before, and 9.8% (n = 2) less than before. After the treatment, the same percentage (29.4%; n = 5) ate more than before, and 11.8% (n = 2) of cats ate less than before (Figure 4.7A).

4.3.4.17.2 Treatment B

During the treatment, more than a third (35.3%; n = 6) of cats ate more than before, and 19.6% (n = 3) ate less than before. After the treatment, 17.7% (n = 3) ate more than before, and 29.4% (n = 5) ate less than before (Figure 4.7A).

4.3.4.18 Changes in weight

4.3.4.18.1 Treatment A

During the treatment, 15.7% (n = 3) of cats weighed more than before, and 19.6% (n = 3) less than before. After the treatment, 11.8% (n = 2) weighed more than before, and 35.3% (n = 6) less than before (Figure 4.7B).

4.3.4.18.2 Treatment B

During the treatment, 15.7% (n = 3) of cats weighed more than before, and 15.7% (n = 3) less than before. After the treatment, 11.8% (n = 2) weighed more than before, and 29.4% (n = 5) less than before (Figure 4.7B).

4.3.4.19 Drinking

4.3.4.19.1 Treatment A

During the treatment, 29.4% (n = 5) of cats drank more than before, and none of the cats drank less than before. After the treatment, none of the cats drank more than before, and 5.9% (n = 1) drank less than before. Cats drank more water in general during treatment A, compared to when it was finished (p = 0.04) (Figure 4.7C).

4.3.4.19.2 Treatment B

During the treatment, 21.5% (n = 4) of cats drank more than before, and 11.8% (n = 2) less than before. After the treatment, 23.5% (n = 4) of cats drank more than before, and 11.8% (n = 2) less than before (Figure 4.7C).

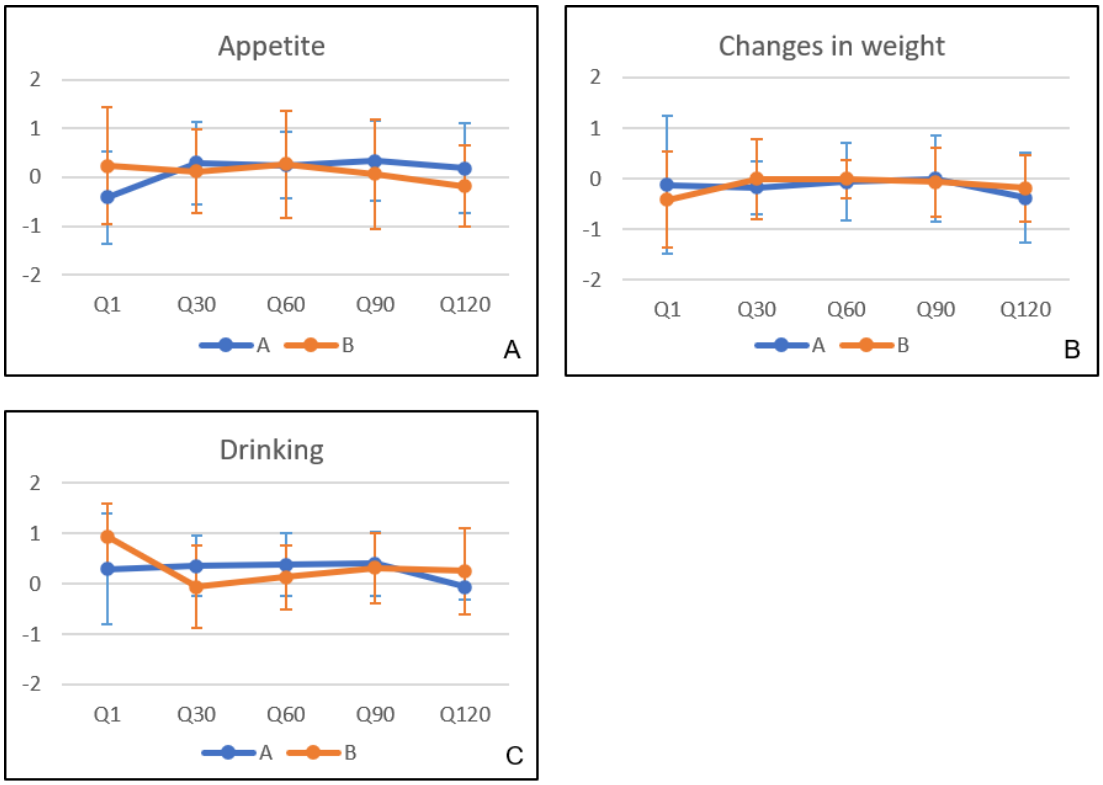


Figure 4.7 Behavioural changes. *Changes through time (mean ± SD), for both treatments, in A) appetite; B) changes in weight; and C) drinking. Negative scores in the y axis represent a decrease (i.e., - 1 = mild, and – 2 = significant), while positive scores represent an increase (i.e., 1= mild, and 2= significant) in the behaviours; zero represents no change*

4.3.4.20 Constipation

4.3.4.20.1 Treatment A

During the treatment, 5.9% (n = 1) of the cats were more constipated than before, and 3.9% (n = 1) were less constipated than before. After the treatment, none of the cats were more constipated than before, and 5.9% (n = 1) less than before (Figure 4.8A).

4.3.4.20.2 Treatment B

During the treatment, 5.9% (n = 1) of cats were more constipated than before, and none of them were less than before. These proportions remained the same after the treatment finished (Figure 4.8A).

4.3.4.21 Diarrhoea

4.3.4.21.1 Treatment A

During the treatment, none of the cats had more diarrhoea than before, and 5.9% (n = 1) had less than before. These percentages remained the same after the treatment finished (Figure 4.8B).

4.3.4.21.2 Treatment B

During the treatment, 7.8% (n = 1) of the cats had more diarrhoea than before, and none of them less than before. These percentages remained the same after the treatment finished (Figure 4.8B).

4.3.4.22 Passing faeces outside litter box

4.3.4.22.1 Treatment A

During the treatment, 7.8% (n = 1) of cats showed less toileting mistakes than before, and 11.8% (n = 2) more than before. After the treatment, 5.9% (n = 1) showed less toileting mistakes than before, and 11.8% (n = 2) of cats made more (Figure 4.8C).

4.3.4.22.2 Treatment B

During the treatment, 2% (n = 1) of cats showed less toileting mistakes than before, and 5.9% (n = 1) made more. After the treatment, none of the cats showed less toileting mistakes than before, and 11.8% (n = 2) made more (Figure 4.8C).

4.3.4.23 Passing urine outside litter box

4.3.4.23.1 Treatment A

During the treatment, 11.8% (n = 2) of the cats showed less toileting mistakes than before, and 7.8% (n = 1) made more. After the treatment, 5.9% (n = 1) of cats showed less toileting mistakes than before, and 17.6% (n = 3) made more. When comparing the different time points, cats had less toileting mistakes (i.e., urination) in Q60 than in Q120 ($p = 0.05$) (Figure 4.8D).

4.3.4.23.2 Treatment B

During the treatment, 9.8% (n = 2) of the cats showed less toileting mistakes than before, and 3.9% (n = 1) made more. After the treatment none of the cats showed less toileting mistakes than before, and 5.9% (n = 1) made more (Figure 4.8D).

4.3.4.24 Vomiting

4.3.4.24.1 Treatment A

During the treatment, 7.8% (n = 1) vomited less than before, and 15.7% (n = 3) more than before. After the treatment, none of the cats vomited less than before, and 11.8% (n = 2) more than before (Figure 4.8E).

4.3.4.24.2 Treatment B

During the treatment, 5.9% (n = 1) of the cats vomited less than before, and 9.8% (n = 2) more than before. After the treatment, none of the cats vomited less than before, and 11.8% (n = 2) more than before (Figure 4.8E).

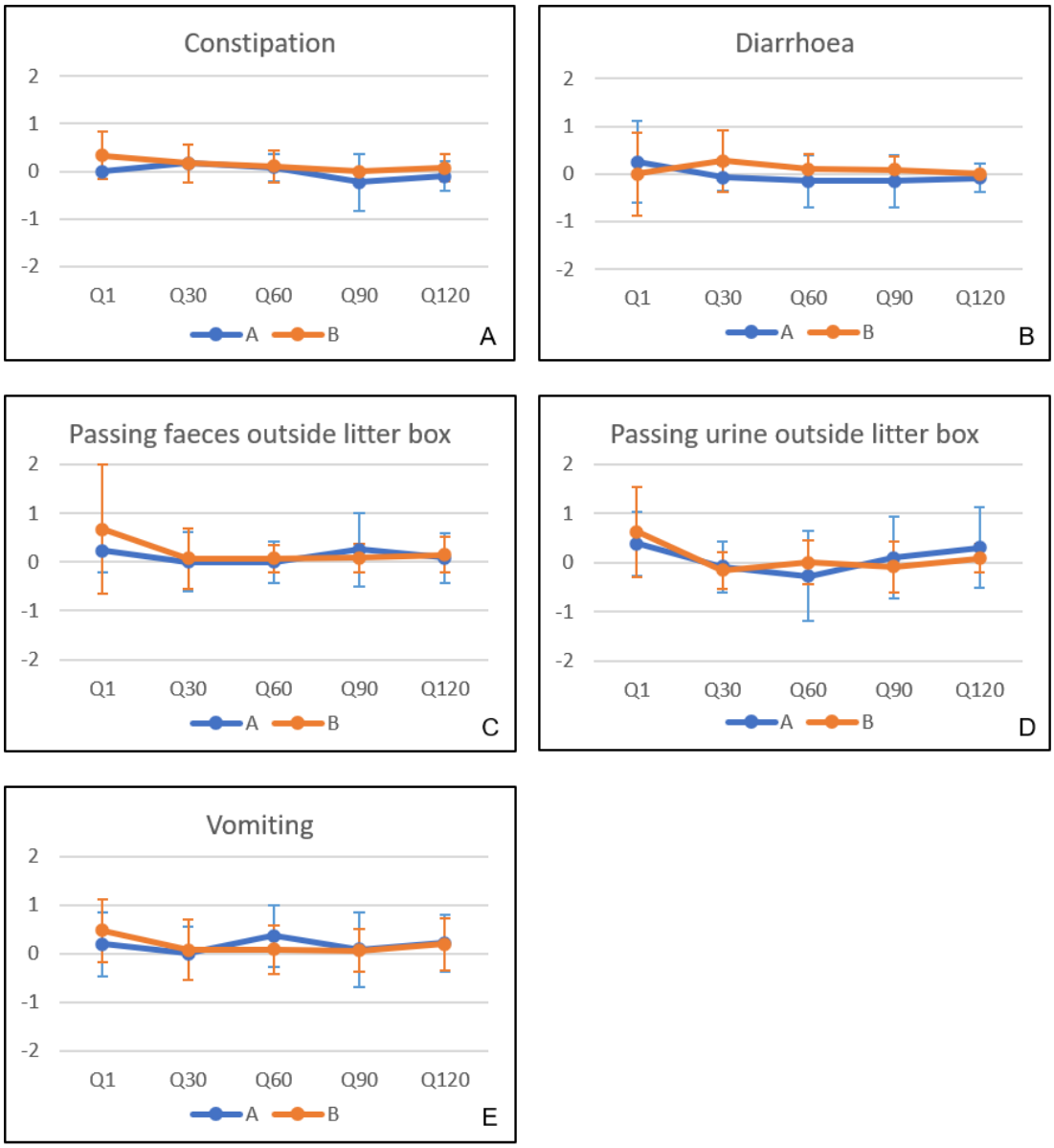


Figure 4.8 Behavioural changes. *Changes through time (mean ± SD), for both treatments, in A) constipation; B) diarrhoea; C) passing faeces outside the litter box; D) passing urine outside the litter box; and E) vomiting. Negative scores in the y axis represent a decrease (i.e., - 1 = mild, and – 2 = significant), while positive scores represent an increase (i.e., 1 = mild, and 2 = significant) in the behaviours; zero represents no change*

4.3.4.25 Hair loss or hair thinning

4.3.4.25.1 Treatment A

During the treatment, 11.8% (n = 2) of cats lost less hair than before, and 3.9% (n = 1) more than before. After the treatment, none of the cats lost less hair than before, and 11.8% (n = 2) more than before. In addition, cats lost less hair during treatment A, than when it was finished (p = 0.03) (Figure 4.9A).

4.3.4.25.2 Treatment B

During the treatment, none of the cats lost less hair than before, and 7.8% (n = 1) more than before. After the treatment, none of the cats lost less hair than before, and 11.8% (n = 2) more than before (Figure 4.9A).

4.3.4.26 Hearing loss

4.3.4.26.1 Treatment A

During the treatment, 1.9% (n = 1) of cats appeared to hear less than before, and 5.9% (n = 1) more than before. After the treatment, none of the cats appeared to hear less than before, and 5.9% (n = 1) more than before (Figure 4.9B).

4.3.4.26.2 Treatment B

During the treatment, none of the cats appeared to hear less than before, and 2% (n = 1) more than before. After the treatment, none of the cats appeared to hear less nor more than before (Figure 4.9B).

4.3.4.27 Vision loss

4.3.4.27.1 Treatment A

No changes in vision were reported at any time in any of the cats (Figure 4.9C).

4.3.4.27.2 Treatment B

Only 2% (n = 1) of cats were reported to see better than before, and none of them less well than before. After the treatment, no changes in vision were reported in any of the cats (Figure 4.9C).

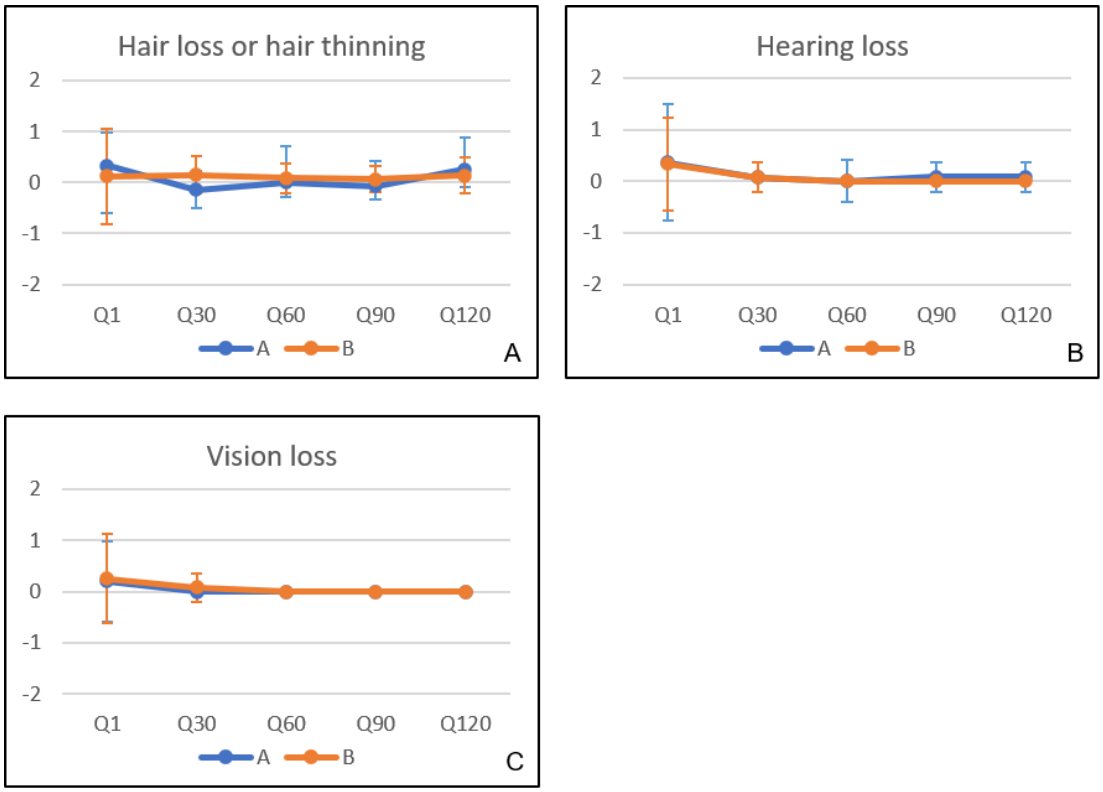


Figure 4.9 Behavioural changes. *Changes through time (mean ± SD) for both treatments, in A) hair loss or hair thinning; B) hearing loss; and C) vision loss. Negative scores in the y axis represent a decrease (i.e., - 1 = mild, and – 2 = significant), while positive scores represent an increase (i.e., 1 = mild, and 2 = significant) in the behaviours; zero represents no change*

4.4 Discussion

This is the first study to show that PPARγ is expressed in the cat brain; however, the administration of telmisartan, a PPARγ agonist, failed to reduce the clinical signs of CDS in affected cats. This study provides additional evidence about the correlation between age and certain physical and behavioural changes in the cat, including weight loss, house-soiling (in this case passing faeces outside the litter box), and repetitive and/or compulsive behaviours.

Immunohistochemistry revealed PPARγ expression in all regions of the cat brain, and in all age groups. This is consistent with rat studies, where PPARs have been found in the cortex, the hippocampus, and the olfactory areas of the brain[306, 307]. Interestingly, the number of these receptors vary with disease in the human brain, being increased in AD[308]. Further studies are needed to determine whether the number of PPARγ increases in the brains of cats with CDS.

That correlations were found between age and certain behaviours, including weight loss is not surprising. Weight loss is observed frequently in elderly cats; in fact, aged cats tend to be underweight[309-311]. In a questionnaire-based study that assessed age-related changes in over 800 cats, owners reported that almost 40% of the cats had lost weight once they were over 11 years of age[18]. This weight loss can be associated with ageing itself (i.e., sarcopenia of ageing[312]), which is associated with normal physiological decline. However, it may be exacerbated by elderly cats having reduced senses of smell and taste[313], and the digestion and absorption of nutrients, as well as intestinal motility and blood flow are considerably reduced with age[314-317]. The presence of disease may also contribute to weight loss, especially those diseases that may lead to hyporexia[313], such as dental disease, inflammatory bowel disease, neoplasia, etc., or diseases that alter metabolism, such as hyperthyroidism and diabetes mellitus. Significant associations have been found between

weight loss and kidney disease, blindness, hyperthyroidism, arthritis, heart disease, and deafness in elderly cats[18]. Significant associations have also been found between a decreased appetite and kidney disease, blindness, lower urinary tract infection, hyperthyroidism, arthritis, deafness, and dental disease[18].

House-soiling (both faeces and urine) has been associated with age in cats. It can be caused by many different interacting factors and illnesses; however, it is considered to be one of the clinical signs of CDS once all other causes have been ruled out[16]. One study looking at behavioural changes in cats over 11 years of age reported that house-soiling occurred in more than half of the cats, regardless of whether they had access outside or to a litterbox[18]. Some of the potential causes for house-soiling include urinary tract disorders (e.g., urinary tract infections, stones or neoplasia); chronic diseases that lead to polyuria and polydipsia (i.e., hyperthyroidism, diabetes mellitus, or chronic kidney disease); gastrointestinal disorders (e.g., lymphoma, or constipation); musculoskeletal disorders (i.e., osteoarthritis) that prevent cats from using cat-flaps or high-sided litterboxes; or true behavioural disorders, such as stress. Furthermore, increased house-soiling in elderly cats has been associated with kidney disease, blindness, lower urinary tract infection, arthritis, and deafness[18].

Repetitive and/or compulsive behaviours were found to increase with age. These types of behaviour can be associated with disorientation, which is another major behavioural sign of CDS in cats[16]. Disorientation (e.g., forgetting if they have eaten, forgetting where the food is, or getting stuck in corners) may lead to confusion and an increase in stress levels, and cats experiencing high levels of stress may display repetitive and/or compulsive behaviours as a coping mechanism[318]. Disorientation may be associated with changes in activity levels (e.g., aimless wandering, restlessness, and repetitive activities), which have also been reported in aged cats[14, 15]. Disorientation is sometimes associated with agitation; this has also been associated with age, as more than

70% of the aged cats included in a questionnaire-based study, appeared to be agitated for no apparent reason[18]. Agitation in elderly cats may be caused by many different and often interacting factors and illnesses, including CDS, and it has previously been shown to be associated with blindness, and arthritis[18].

Despite finding no overall statistically significant differences between the treatment groups, nor clear behavioural improvements during the administration of telmisartan (Semintra™, Boehringer Ingelheim), available evidence suggests that this drug has a strong potential to improve the cognition and clinical signs of cats with CDS. Sartans, including telmisartan, have been shown to improve memory, cerebral blood flow, and cognition in people with AD[290, 319] and in mice models of AD[134, 298]. In addition, studies in humans and mice demonstrated that PPARγ activators, such as telmisartan, reduce the aggregation of amyloid-β[298] and promote its clearance[133], as well as reducing the accumulation of tau deposits[299] and neuronal loss[320, 321].

A number of limitations were identified that could have influenced the findings of the current study, potentially negating any positive effect from the telmisartan.

4.4.1 Dose of medication

In this study, telmisartan was administered at 1 mg/kg PO q24h. However, telmisartan is now licenced at a dosage of up to 3 mg/kg PO q24h for the treatment of hypertension in cats. Telmisartan has a dose response effect in reducing hypertension in cats[303] (i.e., higher doses of telmisartan will reduce high blood pressure more efficiently than lower doses). Similarly, sartans have also been shown to have a dose response efficacy at reducing dementia in people[276]. It is therefore possible that a higher dose of telmisartan may be more effective in reducing signs of CDS in cats.

4.4.2 Sample size

Since this was a pilot study with limited funds, only a limited number of cases were recruited. Recruitment was challenging as many cats with clinical signs of CDS had illnesses that precluded their recruitment. Initial power calculations estimated that 12 to 15 cats in each group should be sufficient to show a statistically significant difference between the groups; however, this was not shown to be the case. This calculation was based on previously published treatment trials on dogs with CDS[103, 105, 322]. Furthermore, retrospective power analyses were performed to determine the sample size needed, for future studies, to show a statistically significant difference between treatment groups. These showed that 17 cats in each group should be sufficient to show statistically significant differences between the groups; since this was the number of cats recruited (per group) in this study, it is possible that other confounding factors such as dose of medication, duration of the trial, and/or placebo effect may have negatively affected the results of the present study.

Another factor that may have reduced the ability to see a significant difference between the groups was the low variability between them, as the values from the responses for all the behaviours were very similar in both groups. In addition, this lack of significance could also be caused by the marked inter-individual variability in the responses within both groups.

4.4.3 Duration of trial

This trial ran for three months. The duration of study was selected based on papers investigating CDS treatments in dogs which showed improvements in as little as two months in trials looking into the efficacy of nutraceuticals[102, 103, 105, 322]. The treatment period was lengthened to three months in the current study because cats show more subtle behavioural changes than dogs and tend to hide their illness. That no positive effect was seen in this study period

may have resulted from cats hiding signs of CDS until more chronic damage had occurred than was present in the dogs; if this is true, a longer treatment period may be needed before improvements are seen after a positive intervention. Alternatively, the duration may have been sufficient, if a higher dose of telmisartan had been selected. Currently, there are two ongoing studies assessing the effect of telmisartan in human patients with AD[142]. These studies will be running for eight months and one year, respectively, which highlights the importance of a long duration of study.

4.4.4 Severity of CDS

Only cats with severe CDS were recruited to ensure that they were clear cases of CDS and reduce the risk of misdiagnosis. It is therefore possible that once CDS is at an advanced stage, the behavioural changes are too severe to be improved, minimising the efficacy of any drug intervention. If true, it is plausible that, if cats with mild, moderate, or early CDS had been recruited, a positive response to treatment may have been detected.

4.4.5 Possibility of misdiagnosis

It is possible that some of the cats did not have CDS, or that it was not the only cause of their behavioural changes. If any of the cats had other causes for their behavioural changes this could have resulted in a heterogeneous group and might explain why the telmisartan appeared not to work. While no cats had lateralising neurological signs or papilledema that might have suggested an intracranial mass lesion or raised intracranial pressure, these conditions could have been present as none of the cats were assessed by MRI. While there were no retinal changes suggestive of Toxoplasmosis, it is not possible to exclude this infection from causing behavioural changes in some of the cats, as Toxoplasmosis serology was not assessed. Likewise, other infectious or

inflammatory conditions (e.g., feline infectious peritonitis, Bartonellosis, etc.) could have been missed if they were causing no ocular changes, or any alterations in haematology or serum biochemistry. The accepted cut off for hypertension was reduced after the study had started, decreasing from < 180 mmHg systolic to < 140 mmHg[304]. It is therefore possible that some of the cats had marginal hypertension. The risk of target organ damage from hypertension is graded as moderate at 160-180 mmHg, so it is possible that some of the cats had hypertensive encephalopathy[304]. However, if that were the case, it could be argued that, since telmisartan is an effective treatment for hypertension in the cat [301-303], this drug should have treated the cats regardless of whether their behavioural changes resulted from hypertension or CDS; that said, higher doses of telmisartan than the one used in the present study (1 mg/kg) may be required to effectively normalise blood pressure.

4.4.6 Placebo effect

Most of the statistically significant behavioural changes were reported in cats from group A (placebo). Interestingly, the majority of these behaviours were associated with CDS, including affection with people within the house, aimless activity, house-soiling (i.e., urination), increased tolerance of handling, and vocalisation during the day. Moreover, two of the behaviours added as distractors were reported to change in the cats of this treatment group: drinking, and hair loss/hair thinning. In contrast, cats from group B (telmisartan) were reported to display changes in only one behaviour related to CDS (i.e., time spent sleeping at night) and none of the distractors. Furthermore, owners of cats from both treatment groups reported changes in some of the same behaviours, regardless of whether they are associated with CDS, including activity levels, time spent sleeping during the day and vocalisation at night.

This placebo effect or bias could have resulted for several reasons.

Hypothesis guessing bias occurs during the process of answering, when respondents of a questionnaire think they know what the hypothesis of the study is and respond accordingly[323]. It is also possible that owners may have been expecting changes in certain behaviours as they had read about CDS in cats.

Another possibility, albeit related, is that too much information may have been given to the owners at the time their cats were recruited. When diagnosing a disease, it is important that veterinarians explain the disease to the owners, its potential causes and clinical signs, plus the available treatments or management. It is particularly important to gain the clients' trust when trying to recruit cases of beloved elderly cats into a long trial. However, by oversharing information during the recruitment process, or if owners explored the topic in detail after their cat has been recruited, this may lead to a respondent's conscious reaction known as *faking good bias* (also known as social desirability), in which the respondents alter their answers in what they believe is the desired direction of the study[323].

Since some of the cases were recruited through the University of Edinburgh email server, with many being doctors and even professors, both of these forms of error are possible as educated people are very likely to read up about their cat's disease, including looking into potential treatment options.

4.4.6.1 Alternative hypothesis

Alternatively, it is possible that the improvements seen in both groups of cats during the treatment period may have been real. Meaning that the cats in *both* groups *did* have reduced signs of CDS. This can be explained by the owners giving their cats more attention, providing them with all of their key resources, plus environmental enrichment, which had a very positive effect in the cats, reducing their signs of CDS. This makes sense, as giving cats with CDS more positive attention can significantly improve their health and well-being[10].

4.4.6.2 Solutions

For future studies, solutions to counter the placebo effects include to avoid oversharing information to minimise respondent bias, plus the addition of measurements that do not rely on owner observations and that provide more objective assessments, such as veterinary or behaviourist reports, GPS trackers to assess activity levels, video and/or audio recorders to assess the amount and frequency of vocalisation, cognitive assessments, etc. Some cognitive tests, originally created for its use in dogs, have been modified and used to assess learning and memory in cats, such as discrimination and reversal learning[15] and the delayed nonmatching to position (DNMP) test that assesses short-term visuospatial working memory[8]. Reversal learning is tested when the cat is first trained to remember under which shaped pot food is hidden (discrimination learning). Once the cat has learned this, the food is then hidden under a different-shaped pot, challenging the cat to reverse or inhibit what they previously learned during the discrimination phase). Similarly, in the DNMP test, cats first learn to displace a pot under which food is hidden (there is only one pot placed in one of three possible locations). The next phase occurs after a delay of five seconds; in this phase, cats are then presented with two pots identical to the one used in the previous phase. One of the pots is placed in the same location as in the previous phase and does not contain any food; the second pot is placed in one of the remaining two positions (nonmatch) and contains the food. While these tests have been successfully used in cats, it is important to consider that testing cognition in cats is far more challenging than in dogs, as they are motivated by different things, with few cats being motivated by food the way many dogs tend to be[8, 14].

Besides the aforementioned solutions, other suggestions to improve future questionnaire-based studies include randomising the order of the responses to prevent *learning bias* in the respondents (i.e., randomly changing the order of the questions in each questionnaire)[323]. This can be particularly effective when owners have to respond to similar questionnaires through time; by doing

this, respondents will not learn the order of the questions being asked. Furthermore, other solutions include increasing the number of outcome measures in order to detect more subtle clinically significant changes (e.g., using a scale from ‘0 to 10’ instead of ‘- 2, - 1, 0, 1, 2’); and using vertical response formats instead of horizontal ones, as the latter are more difficult to read and follow (i.e., to present the answers in a listed format, where the answers are listed from the top to bottom of the sheet, instead of from left to right, as it was used in the present study), and to prevent confusion when responding.

4.5 Conclusion

In conclusion, there is mounting evidence suggesting that telmisartan reduces brain inflammation, provides neuroprotection, restores cognitive function, and reduces the accumulation of both amyloid-β and hyperphosphorylated tau in mammals. However, further studies are needed to determine the true potential of telmisartan as a treatment to improve the behaviour and cognition of cats with CDS.

Chapter 5 Final discussion

“The cat is domestic only as far as suits its own ends…”

-Saki (H. H. Munro)

Cognitive dysfunction syndrome (CDS) is an age-related condition that affects both cats and dogs[8]. In spite of this, research has predominantly been conducted in dogs, with the findings being extrapolated to cats[15]. While a few studies have assessed the clinical presentation of CDS in cats[15, 90], little is known about the neuropathology of the disease in this species. It is important to try to elucidate the mechanisms behind the pathophysiology of this condition, and correlate this with its clinical signs in order to provide early diagnosis and effective treatments in each of the species.

Pet cats are now living longer than ever[1, 324]. This is mainly due to improvements in veterinary medicine, nutrition, and changes in the way we keep our pets (e.g., indoor living)[1, 324]. Increased lifespans result in more elderly cats attending veterinary practices[1, 325-327]; therefore, veterinarians must be capable of recognising the early signs of age-related changes and disease in this species. Moreover, veterinarians should be able to differentiate non-pathological signs and changes caused by normal ageing from age-related diseases.

5.1 Age-related changes in cats

Even though advanced age is known to produce several anatomical and physiological changes, it is argued that ageing, in itself, is not a disease[10]. In cats, some signs of normal ageing can be easily recognised, such as the loss of muscle mass (i.e., sarcopenia), changes in appetite and weight loss, and over-grown nails, amongst others[10]. Some less perceptible changes may also occur, such as alterations in the function of the kidneys, liver, and brain[10, 313, 328]. While some of these changes may not be clinically obvious, others can be detected by veterinarians and even cat owners. In a questionnaire-based study that assessed the prevalence of disease and age-related changes in more than 800 cats, owners were able to recognise age-related changes and signs of disease in their cats[18]. The most common diseases affecting these cats

were arthritis, hyperthyroidism, dental disease, and chronic kidney disease. Of note, the prevalence of these diseases increased significantly in the 20 year period from 1995 to 2015[18], suggesting that both veterinarians and cat owners are becoming more aware of these conditions and have stopped misinterpreting them as normal ageing.

Elderly cats can display a number of age-related behavioural changes. Some of these include reduced grooming, reluctance to eat, and a reduced willingness to play, hunt, and go outside[18, 90, 329]. While some of these behaviours may result from normal ageing, others may be associated with underlying disease, such as increased water intake, reluctance to eat, and weight loss, amongst others[18]. In one study, these behaviours were mostly associated with diseases such as hyperthyroidism, arthritis, blindness, and kidney disease[18].

In addition, some of the behaviours displayed by elderly cats that can be caused by normal ageing, may actually be associated with CDS when they cannot be attributed to underlying medical illness[13, 14]. These include house-soiling, increased affection, and increased vocalisation[18].

5.2 Age-related changes associated with CDS in cats

Cognitive dysfunction is a common age-related syndrome that produces well-recognised behavioural changes and, eventually, leads to cognitive decline and dementia[13, 14]. Importantly, CDS has been shown to be a highly prevalent condition, with around 50% of cats over 15 years of age displaying at least one of these behavioural changes[14, 16]. This underlines the significance of further studies into this condition.

The behavioural changes associated with CDS are summarised under the acronym VISHDAAL[20] and include increased Vocalisation; altered Interactions

with their owners (e.g., increased affection); alterations in Sleep-wake cycles; House-soiling; spatial and/or temporal Disorientation; altered Activity levels; Anxiety; and impaired Learning and memory[20]. Some of these behaviours are more common than others; our most recent study shows that 60% of cats vocalised excessively, with 30% of them doing so mainly at night; 50% of them demanded more attention; and 30% of them house-soiled[18]. It is still unknown whether there is an order of appearance of these behaviours, and it is likely that individual variations occur.

It is important to recognise that some of these behaviours may be caused by other conditions, rather than by CDS. For instance, increased vocalisation could result from pain, especially when caused by arthritis, which is a highly prevalent condition in elderly cats[18, 330, 331]. A recent study focused on trying to understand the cause of increased vocalisation in elderly cats with CDS, based on owners' reports[19]. This study showed that most of the cats primarily vocalised due to disorientation (40%) or attention seeking (40%)[19]. While only 3% of owners reported their cat's vocalised because of pain[19], most of the cats appeared to vocalise for a number of reasons, so pain may have been under-recognised.

It is clear that there is a fine line between what can be considered to be normal and abnormal age-related changes. This highlights the importance of efficiently recognising and differentiating between them. This represents a challenge for veterinary practitioners who need to conduct a careful and exhaustive examination of their elderly patients in order to obtain an adequate diagnosis and so provide early and effective interventions.

5.3 Neuropathology of ageing and CDS in cats and its similarities to humans

Besides the clinical signs and behavioural abnormalities, cats with CDS also develop neuropathology, including the accumulation of amyloid-β (Aβ) and hyperphosphorylated tau deposits[52, 63-67].

According to the UniProt Consortium database, the Aβ protein has a similar amino acid sequence in humans, cats and wild-type mice; the Aβ sequences in cats and mice are 96.8% similar to human Aβ[332]. In cats and humans, the Aβ sequence is only one amino acid different, versus three amino acids different between mice and humans[52].

The amino acid sequence for the tau protein is more similar between cats and humans (i.e., 84.4% similarity), than between wild-type mice and humans (i.e., 74.2% similarity)[332]. In addition, unlike mice, that produce only three isoforms of tau (i.e., 4-repeats), cats produce the same six tau isoforms as humans (i.e., 3 and 4-repeats)[52]. The complete amino acid sequences for both Aβ and tau proteins, in humans, cats, and wild type mice can be found in Appendix 5.1.

The present study assessed the presence and distribution of Aβ and hyperphosphorylated tau in the cat brain. For this, immunohistochemistry of seven different brain regions was performed in cats of various ages, with and without CDS. The cats were shown to accumulate both intracytoplasmic and extracellular deposits of Aβ, as well as nuclear and cytoplasmic hyperphosphorylated tau, which formed pre-tangles.

5.3.1 Aβ pathology in cats and its similarities to Alzheimer's disease (AD)

Cats of all ages were shown to accumulate intracellular and extracellular Aβ deposits; however, individual variations were found, as well as differences between the age groups.

While intracytoplasmic Aβ was found within the neurons of cats of all ages, younger cats were shown to have more of these aggregates than older cats. Most of the young cats were shown to have these intracytoplasmic Aβ deposits in the cortical brain regions, whereas only a few young cats were shown to have them in other brain regions, such as the hippocampus, locus coeruleus, and cerebellum. In contrast, the proportion of cats with intracytoplasmic Aβ in the hippocampus, locus coeruleus, and cerebellum, increased with age. In humans, intracytoplasmic Aβ is known to start accumulating at young ages and to progress with age[157, 158]. Furthermore, as intracytoplasmic Aβ pathology progresses and neurons die, Aβ is then released into the extracellular spaces, leading to the formation of extracellular Aβ deposits and plaques, promoting further aggregation[159]. The same pattern was observed in the cats in this study, as intracytoplasmic Aβ deposits tended to decrease with age; in addition, those elderly cats with the largest extracellular deposits had very few to no intracytoplasmic Aβ.

The early stage of intracytoplasmic Aβ accumulation within neurons has been shown to be a harmless event[56]; in fact, it has been suggested that intracellular Aβ may confer neuroprotection as it has antioxidant properties[160, 161]. However, as Aβ pathology progresses and intracytoplasmic Aβ increases, it becomes toxic altering synapsis and promoting cytoskeleton and mitochondrial dysfunction[56, 157, 159]. In AD, intracytoplasmic Aβ has been reported to accumulate in the areas that are first affected by disease, such as hippocampus and entorhinal cortex[56, 159]. A similar pattern was found in the

present study; as cats became older, intracytoplasmic Aβ deposits appeared in the hippocampus, cerebellum, and locus coeruleus.

As is the case in humans, extracellular Aβ deposits in cats are formed from the Aβ42 peptide[63, 64]. In contrast, Aβ40 deposits, which are commonly found in the blood vessels of elderly humans (i.e., cerebral amyloid angiopathy)[54, 56], have been only rarely described in elderly cats[65].

Extracellular diffuse deposits of Aβ were also found in cats of all ages. Younger cats had smaller extracellular Aβ deposits, whereas older cats had larger Aβ deposits. Of note, the youngest cat shown to have extracellular Aβ deposition was a 4-years-old, which contradicts previous reports showing that Aβ deposits started to accumulate at the age of eight years in the cat brain[52, 63, 64, 150]. This finding suggests that extracellular Aβ in cats can start aggregating earlier than has been previously observed. The location of these deposits was found to vary between the age groups. Younger cats accumulated few extracellular Aβ deposits, and they were mainly in the cortex, whereas older cats had larger deposits, which were mainly in the cortex, and also in the hippocampus. This suggests that Aβ pathology in cats begins in the cortical brain regions and later progresses to the hippocampus, as it happens in humans with AD[60]. Deposits of Aβ in humans start aggregating in cortical areas of individuals as young as 20 years old, decades before developing signs of AD[151]; as the disease progresses, these deposits tend to spread to the hippocampus[60].

In accordance with previous studies[52, 64, 150], extracellular Aβ in cats was shown to accumulate in a diffuse pattern, occasionally forming patches. This diffuse pattern of deposition is also known to occur in humans, and is thought to be an early stage of plaque formation.[53, 54] Interestingly, a 16-year-old cat in the current study accumulated Aβ in a more typical plaque-like pattern, suggesting

that cats can potentially develop senile plaques; however, they might not normally live long enough for this to occur.

While behavioural changes were not extensively assessed for this part of the present study, previous studies have shown that cats with Aβ pathology can display abnormal behaviours that are associated with CDS, such as confusion, disorientation, and increased vocalisation[63, 67]; however, neither the severity or the prevalence of these behaviours have been correlated with the extent of Aβ deposition[64]. The same lack of correlation has also been reported in humans with AD, where the severity of Aβ pathology has not been correlated with the clinical signs displayed by affected people[54, 60, 64, 147].

5.3.2 Tau pathology in cats and its similarities to AD

The current study found that the brains of cats of all ages strongly expressed the same tau isoforms that are found in humans (i.e., 3 and 4-repeat isoforms), as has been previously reported by others[52].

In the present study, some elderly cats were shown to accumulate intracytoplasmic deposits of hyperphosphorylated tau. Since these deposits occupied most of the cytoplasm and there were no changes in the shape of the affected neurons, these are believed to be pre-tangles, which are considered to be an early stage of neurofibrillary tangles (NFT)[77], as previously reported by others[52, 64, 150].

In the current study, the largest number of pre-tangles were mostly found in the cortex of elderly cats, whereas lower numbers were found in other regions, such as the entorhinal cortex and the hippocampus, suggesting that tau pathology starts in the cortical regions and later progresses to the hippocampus and entorhinal cortex. If true, this differs from the initial stage of disease in humans, which starts in the transentorhinal cortex[60], a region that

was not assessed in the present study. Therefore, it is impossible to say whether tau pathology truly begins in the cortical regions or in the transentorhinal cortex. Further studies are needed to determine the regions in which tau pathology begins in the cat brain and its progression with age.

Even though tau pathology was predominantly found in the brains of elderly cats, two young cats, aged four and 6-years-old, were found to have occasional pre-tangles, which occurred within the cortical brain regions. The presence of pre-tangles at this young age could be the result of a non-diagnosed neurological disorder that produces tauopathy, and/or an age miscalculation, with the cats being older than originally recorded. Alternatively, it could potentially suggest that in some cases, and similarly to Aβ, tau pathology in cats might begin at a young age. In humans, early stages of NFT have been reported in people as young as 25 years old, and this increases with age, leading to more severe stages of disease[77].

For the first time, significant associations were found between the presence of pre-tangles and CDS. The present study demonstrated that cats with CDS had larger numbers of pre-tangles than cats without CDS. In humans, strong correlations have been reported between NFT and cognitive decline[169]. However, due to the small number of CDS cases, further studies are needed to corroborate these findings, and to determine whether there is a correlation between the presence of pre-tangles, and the prevalence and severity of behavioural changes.

In addition, this was also the first time that intranuclear tau was found in both phosphorylated and non-phosphorylated states, within the cat brain. This was validated by performing a number of experiments, such as using isotype controls for each of the antibodies; dephosphorylating the tau protein using alkaline phosphatase; and performing Western blots on different cell structures after fractionation (e.g., nuclear fraction, cytoplasmic fraction, etc.).

Intranuclear tau is known to be present in the human brain and is believed to have a neuroprotective role[181, 182, 187, 188]. Tau can translocate to the nucleus to bind and protect DNA[182, 187]. Since this process is believed to be regulated by phosphorylation[182, 187], abnormal phosphorylation of tau may alter its ability to translocate to the nucleus and to bind to DNA[182, 189-191].

The highest number of intranuclear deposits was found mainly in the cortex of younger cats, and the proportion of cats showing intranuclear deposits tended to decrease with age. In contrast, elderly cats with pre-tangles were shown to have few to no nuclear labelling. This suggests that intranuclear tau is predominantly found in younger cats and, as animals' age, it decreases as the pathology progresses and pre-tangles form. The picture appears to be more complex in humans, where intranuclear tau increases with age, reaching its most prevalent in geriatric people[193]; however, in people with AD, intranuclear tau decreases with age, disappearing completely in the advanced stages of disease[192].

5.4 Diagnosis of CDS in cats

The diagnosis of CDS can only be made by exclusion; ruling out all other potential causes for the behavioural changes displayed by the affected cats[13, 14]. This is a challenging task for veterinary practitioners, who need to have a high index of suspicion to investigate the cause of the cats' behavioural abnormalities and then have the ability to perform thorough and extensive examinations. New diagnostic tools are therefore needed to facilitate efficient and early diagnosis.

Normal ageing alters the anatomy of the brain and its various structures. As individuals get older, age-related changes start to appear, such as shrinkage of the brain. As these changes progress, they may later lead to cognitive decline. Imaging techniques, such as computed tomography (CT) and

especially magnetic resonance imaging (MRI), have shown great potential as diagnostic tools for the assessment of age-related brain changes in humans.

The present study assessed brain atrophy and other age-related brain changes in cats of various ages, with and without CDS, by using a 1.5T MRI.

5.4.1 Age-related brain changes in cats and their similarities to humans

As mentioned previously, imaging techniques have been widely used for the diagnosis of age-related brain changes in humans. They are particularly useful with AD, where some of the changes can be seen decades before people develop clinical signs[91], and hence, can be considered as early diagnostic criteria for AD[36-39, 92, 215]. These changes include shrinkage of the brain[213, 214], hippocampal atrophy[36-38], and enlargement of the ventricles[35].

In the present study, brain changes were mostly detected in elderly cats, as expected. In accordance with previous reports, elderly cats in this study were shown to develop total brain atrophy[226] and enlargement of the lateral ventricles[40], plus age-related changes in other brain structures, such as the occipital lobe and the hippocampus. Elderly cats were also shown to have larger lateral ventricles than younger cats, which correlates with age. In agreement with this, in humans, enlargement of the ventricles occurs with increasing age as the brain shrinks and the size of the cerebrospinal fluid (CSF) spaces increases[32]. It has been proposed that enlargement of the ventricles, in addition to the reducing size of the interthalamic adhesion (a structure that has shown to become smaller with age[224, 226]), could be diagnostic criteria for brain atrophy and ageing in pets[225]. However, in the current study, the enlargement of the ventricles was not correlated to the size of the interthalamic adhesion.

Age-related changes were also found in regions of the brain not reported previously. For instance, atrophy of the occipital lobe was found in cats with CDS, suggesting that this structure could be affected earliest in this condition. However, in humans, the occipital lobe is the least affected region[31]. This may represent a difference in the onset and progression of disease between species, or be an error, related to the small number of cats with CDS.

Sex-related differences are also of interest. Male cats were found to have larger brains than females, which can be explained because male cats are physically bigger than female cats. Similarly, male humans have larger total brain volumes and hippocampi than females[32]. In the cats, both the frontal and occipital lobes tended to decrease more abruptly with age in female cats. In humans, differences in the rate of atrophy have also been reported. Atrophy of frontal and temporal lobes is more prominent in men[144, 212], whereas atrophy of the hippocampi and parietal lobes is more prominent in women[144, 212].

Even though cats and humans have been shown to develop similar age-related brain changes, further studies are needed to assess similarities in the progression of these changes and the regions that are mostly affected in both species. Since there were only a small number of cats with CDS in this study, it is possible that these findings were not a true representation of CDS-related brain changes. Furthermore, some of the brains were assessed *post-mortem*; hence, the fixation process may have altered their structure, especially as there was no set fixation period. Further longitudinal studies are needed to determine the onset and progression of the age-related brain changes that could lead to CDS in cats. Moreover, simultaneous assessments of behavioural and/or cognitive changes are needed to help elucidate the relationship between these and the anatomical age-related changes.

5.5 Treatment for CDS in cats

To date, there is no treatment available for CDS in cats[13, 14]; however, interventions including environmental enrichment, dietary supplementation, specific diets, and potentially, non-licenced drugs, can be offered to try to reduce the clinical signs of CDS in cats.

Studies in humans and rodents have shown that drugs that block certain renin-angiotensin receptors (i.e., AT_1) have a positive effect on cognition and memory[131, 251]. Telmisartan is a potent AT_1 receptor blocker, plus an activator of the peroxisome proliferator activated receptors gamma (PPARγ)[134, 282]. It has been shown to reduce neuroinflammation[133], provide neuroprotection, and to improve cognition in humans[290, 319] and rodents[140, 297]. Telmisartan has also been shown to reduce the accumulation of Aβ and phosphorylated tau deposits, and to promote their clearance in *in vitro* and mice models of AD[133, 298, 299]. It is therefore plausible that telmisartan could also have positive effects in cats with CDS.

The present study assessed the effects of telmisartan (Semintra™, Boehringer Ingelheim) on the clinical signs of CDS in cats by conducting a double-blinded placebo-controlled study. To be recruited, cats had to have displayed signs of CDS for at least one month prior to this study, but to be otherwise healthy. Behavioural changes were assessed using owners' reports conducted every 30 days for a total of four months. During the first three months the cats received the treatment (i.e., telmisartan or placebo), followed by a wash-out period of a further month.

This study demonstrated that some of the behavioural changes associated with CDS are also strongly associated with age, for example, weight loss, house-soiling (i.e., passing faeces outside the litter box), and repetitive/compulsive behaviours.

Despite finding no statistically significant differences between the treatment groups, other available evidence suggests that telmisartan has a strong potential to improve cognition and to reduce the clinical signs of CDS in cats. Further studies are needed to prove this; however, these will need to use higher doses of telmisartan and/or for longer treatment periods, a larger sample size, and to avoid or minimise placebo effects by not oversharing information with the owners. Including more objective measurements, such as behaviourists and/or veterinarian assessments would be highly advisable.

5.6 The cat as a model for AD

Animal models have been widely used to try to understand the mechanisms behind AD and to develop potential therapeutic treatments. However, extrapolating findings from animal models to humans has been challenging due to natural differences between species[333].

Alzheimer's disease is only one of the many different types of dementia that exist in people[333]; however, it is the most common type, causing around two thirds of all dementia cases[333]. Most cases of AD result from the sporadic form of the disease, with far fewer cases of the familial form[333]. While the mechanism behind the development of both types of AD remain unknown[55, 333], the neuropathology produced is very similar[333]; however, they differ by the age of onset, the progression of disease, and their cognitive symptoms[55, 333].

The most used animal model for the study of AD is the mouse model; however, mice do not naturally develop either AD or its pathology[56, 333]. For this reason, transgenic mice with genetic mutations were developed to recreate the neuropathology of the disease[333]. Of note, these mouse models mimic the genetics of familial AD, instead of the more common sporadic form of the disease[55, 333].

Mutations on the amyloid precursor protein (APP) gene that lead to abnormal Aβ production are known to exist in the familial form of AD[58]. For this reason, and to reproduce it, transgenic mice that overproduce APP were initially created. These mice develop severe Aβ pathology, which is very similar to that seen in the human brain[56, 333]. However, this strain of mice does not develop NFT[333]. Hence, another double transgenic mouse model had to be developed that overexpressed APP plus developed NFT[333]. These models have been shown to be useful in elucidating both Aβ and tau pathologies, plus the interaction between them[333].

Genetically modified mice lacking specific genes associated with AD have also been created[333]. Even though these mice do not produce neuropathology, they have been used to try to elucidate the mechanisms behind the pathology, the progression of disease and to identify targets for potential drug treatments[333].

For decades, transgenic mouse models have been widely used to help elucidate some of the mechanisms behind AD and to identify potential treatments; however, there is still no cure for AD[333]. Some of the drugs used in pre-clinical trials that have been successful in mouse models, have been unsuccessful in clinical trials with people with AD[56]. For instance, in mouse models some drugs have been shown to reduce the production, aggregation, and clearance of Aβ, plus reverse cognitive damage[56]; however, these positive changes were not seen in humans with AD[56]. Moreover, tau mutations in some transgenic mice are mostly associated with frontotemporal dementia, rather than with AD[55]. For all the aforementioned, it has been questioned whether mice models are a good representation of human AD and/or whether there could be more accurate animal models[55].

Recent evidence has demonstrated that there are other animals that develop the neuropathology seen in AD. For example, horses and dogs accumulate Aβ deposits and develop senile plaques[334, 335]. Dogs have been proposed to be a good model for AD, especially as they display similar cognitive impairment,

they develop senile plaques and cerebral amyloid angiopathy, plus they share the same environment as humans[336]; however, they do not produce NFT[3]. In contrast, there are several other animals that naturally and spontaneously develop both Aβ and tau pathologies, and could potentially be accurate animal models, such as cheetahs[337], leopards[338], dolphins[339], donkeys (data submitted) and cats[64, 150], amongst others.

The evidence provided by the present study suggests that the domestic cat has a strong potential to be a natural model for AD, especially as cats display several age-related behavioural changes, plus the neuropathology of ageing and CDS, which shares many similarities to that seen in humans. In addition, since the owners of cats with CDS are highly motivated to find a treatment that reduces their cat's distress, many are keen to have their cat involved in potential treatment trials.

5.7 Conclusion

The present study provides evidence of the most common age-related brain changes in cats with and without CDS. These changes include brain atrophy, atrophy of the occipital lobe and the hippocampus, plus enlargement of the ventricles. In addition, it proposes the use of MRI as a potential *in vivo* tool for the diagnosis of age-related brain changes and the early recognition of CDS in cats.

This study has also shown that cats accumulate Aβ as intracytoplasmic deposits within neurons and diffuse extracellular deposits, and accumulate hyperphosphorylated tau within neurons as intranuclear deposits and intracytoplasmic pre-tangles. Hence, there are several similarities between the neuropathologies of ageing and CDS with AD.

While there is still no treatment for CDS, this study has proposed the use of telmisartan as a potential treatment, as this drug has been shown to have several beneficial effects on cognition in other species.

There is mounting evidence to suggest that mice are probably not the best model for the study of AD. In contrast, there are many other animals that naturally and spontaneously produce the neuropathology of the disease and may be more accurate models for the study of this condition. However, it is important to consider the sustainability and welfare implications involved in using these animals as potential models. Here the role of elderly cats with CDS could be significant, as the owners of these cats are typically very keen for them to be enrolled in any study that may potentially reduce their clinical signs of dementia.

Finally, all the evidence presented in the current study helps to further our understanding of CDS in cats. It will help owners and veterinary practitioners to distinguish between normal and abnormal age-related changes; it will help veterinarians to reach the diagnosis by facilitating them with tools that can be used in practice; furthermore, it may help to provide early and efficient interventions that will ultimately improve the quality of life and the welfare of the affected cats.

Bibliography

1. O'Neill DG, Church DB, McGreevy PD, et al. Longevity and mortality of cats attending primary care veterinary practices in England. *Journal of Feline Medicine and Surgery* 2015; 17: 125-133.

2. Vogt AH, Rodan I, Brown M, et al. AAFP-AAHA feline life stage guidelines. *Journal of the American Animal Hospital Association* 2010; 46: 70-85. DOI: 10.5326/0460070.

3. Vite CH and Head E. Aging in the Canine and Feline Brain. *Veterinary Clinics of North America: Small Animal Practice* 2014; 44: 1113-1129. DOI: 10.1016/j.cvsm.2014.07.008.

4. Harrison J and Buchwald J. Eyeblink conditioning deficits in the old cat. *Neurobiology of Aging* 1983; 4: 45-51.

5. Levine MS, Lloyd RL, Fisher RS, et al. Sensory, motor and cognitive alterations in aged cats. *Neurobiology of Aging* 1987; 8: 253-263.

6. Harrison J and Buchwald J. Auditory brainstem responses in the aged cat. *Neurobiology of Aging* 1982; 3: 163-171.

7. Landsberg GM. Behavior problems of older cats. In: *Schaumburg I (ed): Proceedings of the 135th Anual Meeting of the American Veterinary Medical Association* San Diego, CA, 1998 1998, pp.317-320.

8. Landsberg GM, Nichol J and Araujo JA. Cognitive Dysfunction Syndrome. A Disease of Canine and Feline Brain Aging. *Veterinary Clinics: Small Animal Practice* 2012; 42: 749-768.

9. Sparkes A and Manley DS. From small acorns... the new Cat Friendly Clinic/Cat Friendly Practice programmes. *Journal of Feline Medicine and Surgery* 2012; 14: 180-181. DOI: 10.1177/1098612X12439264.

10. Miele A, Sordo L and Gunn-Moore DA. Feline Aging: Promoting Physiologic and Emotional Well-Being. *Veterinary Clinics of North America - Small Animal Practice* 2020; 50: 719-748.

11. van Haaften KA, Eichstadt Forsythe LR, Stelow EA, et al. Effects of a single preappointment dose of gabapentin on signs of stress in cats during transportation and veterinary examination. *Journal of the American Veterinary Medical Association* 2017; 251: 1175-1181. DOI: 10.2460/javma.251.10.1175.

12. Stevens BJ, Frantz EM, Orlando JM, et al. Efficacy of a single dose of trazodone hydrochloride given to cats prior to veterinary visits to reduce signs of transport- and examination-related anxiety. *Journal of the*

American Veterinary Medical Association 2016; 249: 202-207. DOI: 10.2460/javma.249.2.202.

13. Landsberg GL and Araujo JA. Behavior problems in geriatric pets. *Veterinary Clinics of North America-Small Animal Practice* 2005; 35: 675-698.

14. Gunn-Moore D, Moffat K, Christie LA, et al. Cognitive dysfunction and the neurobiology of ageing in cats. *Journal of Small Animal Practice* 2007; 48: 546-553. DOI: 10.1111/j.1748-5827.2007.00386.x.

15. Landsberg GM, Denenberg S and Araujo JA. Cognitive dysfunction in cats: a syndrome we used to dismiss as 'old age'. *Journal of Feline Medicine and Surgery* 2010; 12: 837-848.

16. Moffat K and Landsberg G. An investigation of the prevalence of clinical signs of cognitive dysfunction syndrome (CDS) in cats. *Journal of the American Animal Hospital Association* 2003; 39: 512 (abstract).

17. Landsberg GM, Hunthausen W and Ackerman L. The effects of aging on the behavior of senior pets. *Handbook of Behavior problems of the dog and cat*. 2nd ed ed. Oxford: Saunders, 2003, pp.269-304.

18. Sordo L, Breheny C, Halls V, et al. Prevalence of disease and age-related behavioural changes in cats: past and present. *Veterinary Sciences* 2020; 7: 1-19.

19. Cerna P, Gardiner H, Sordo L, et al. Potential causes of increased vocalisation in elderly cats with cognitive dysfunction syndrome as assessed by their owners. *Animals* 2020; 10: 1092. DOI: https://doi.org/10.3390/ani10061092.

20. Sordo L and Gunn-Moore D. Cognitive dysfunction in cats: update on neuropathological and behavioural changes plus clinical management. *Veterinary Record* 2021: 1-12.

21. Milgram NW, Landsberg GM, De Rivera C, et al. Age and cognitive dysfunction in the domestic cat. In: *Proceedings of the ACVB/AVSAB Symposium* St. Louis, 2011 2011, pp.28-29.

22. Cotman CW and Head E. The canine (dog) model of human aging and disease: dietary, environmental and immunotherapy approaches. *Journal of Alzheimer's Disease* 2008; 15: 685-707.

23. Head E, Liu J, Hagen TM, et al. Oxidative damage increases with age in a canine model of human brain aging. *Journal of Neurochemistry* 2002; 82: 375-381.

24. Scheff SW, Price DA, Schmitt FA, et al. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiology of Aging* 2006; 27: 1372-1384.

25. West MJ, Coleman PD, Flood DG, et al. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *The Lancet* 1994; 344: 769-772.

26. Juraska JM and Lowry NC. Neuroanatomical changes associated with cognitive aging. *Current topics in behavioral neurosciences* 2012; 10: 137-162.

27. Zhang C, Hua T, Zhu Z, et al. Age related changes of structures in cerebellar cortex of cat. *Journal of Biosciences* 2006; 31: 55-60.

28. Dobson H and Denenberg S. Ageing and imaging based neuropathology in the cat. In: *Programs and abstracts of the 17th Congress of ESVCE and 1st Congress of ECAWBM* Avignon, 2011 2011.

29. Miller A, Alston R and Corsellis J. Variation with age in the volumes of grey and white matter in the cerebral hemispheres of man: measurements with an image analyser. *Neuropathology and applied neurobiology* 1980; 6: 119-132.

30. Svennerholm L, Bostrom K and B J. Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes. *Acta Neuropathologica* 1997; 94: 345-352.

31. Raz N. The ageing brain: structural changes and their implications for cognitive ageing. In: Dixon R, Backman L and Nilsson L (eds). Oxford: Oxford University Press, 2004, pp.115-134.

32. Raz N, Gunning FM, Head D, et al. Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. *Cerebral cortex (New York, NY: 1991)* 1997; 7: 268-282.

33. Anderton B. Ageing of the brain. *Mechanisms of Ageing and Development* 2002; 123: 811-817.

34. Barnes C. Long-term potentiation and the ageing. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 2003; 358: 765-772.

35. Jack CR, Jr., Shiung MM, Weigand SD, et al. Brain atrophy rates predict subsequent clinical conversion in normal elderly and amnestic MCI. *Neurology* 2005; 65: 1227-1231.

36. Kaye JA, Swihart T, Howieson D, et al. Volume loss of the hippocampus and temporal lobe in healthy elderly persons destined to develop dementia. *Neurology* 1997; 48.

37. den Heijer T, Geerlings MI, Hoebeek FE, et al. Use of hippocampal and amygdalar volumes on magnetic resonance imaging to predict dementia in cognitively intact elderly people. *Archives of General Psychiatry* 2006; 63: 57-62.

38. Martin SB, Smith CD, Collins HR, et al. Evidence that volume of anterior medial temporal lobe is reduced in seniors destined for mild cognitive impairment. *Neurobiology of Aging* 2011; 31: 1099-1106.

39. Fox NC, Warrington EK, Freeborough PA, et al. Presymptomatic hippocampal atrophy in Alzheimer's disease: a longitudinal MRI study. *Brain* 1996; 119: 2001-2007.

40. Babicsak VR, Klein AV, Tsunemi MH, et al. Age-related changes of the cerebral ventricles of healthy domestic cats. *Pesq Vet Bras* 2018; 38: 1935-1941.

41. Levine MS, Adinolfi AM, Fisher RS, et al. Ultrastructural alterations in caudate nucleus in aged cats. *Brain Research* 1988; 440: 267-279.

42. Levine MS, Lloyd RL, Hull CD, et al. Neurophysiological and morphological alterations in caudate neurons in aged cats. *Brain Research* 1987; 401: 213-230.

43. Levine MS, Adinolfi AM, Fisher RS, et al. Quantitative morphology of medium-sized caudate spiny neurons in aged cats. *Neurobiology of Aging* 1986; 7: 277-286.

44. Levine MS, Hull CD, Villablanca JR, et al. Effects of caudate nuclear or frontal cortical ablation in neonatal kittens or adult cats on the spontaneous firing of forebrain neurons. *Brain Research* 1982; 256: 129-138.

45. Levine MS, Hull CD, Buchwald NA, et al. Effects of caudate nuclei or frontal cortical ablations in kittens: motor activity and visual discrimination performance in neonatal and juvenile kittens. *Experimental Neurology* 1978; 62: 555-569.

46. Villablanca JR, Olmstead CE, Levine MS, et al. Effects of caudate nuclei or frontal cortical ablations in kittens: neurology and gross behavior. *Experimental Neurology* 1978; 61: 615-634.

47. Muir JL. Acetylcholine, aging, and Alzheimer's disease. *Pharmacology, Biochemistry, and Behavior* 1997; 56: 687-696.

48. Bartus RT, Dean RL, Beer B, et al. The cholinergic hypothesis of geriatric memory dysfunction. *Science* 1982; 217: 408-417.

49. Coyle JT, Price DL and DeLong MR. Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* 1983; 219: 1184-1190.

50. Zhang JH, Sampogna S, Morales FR, et al. Age-related changes in cholinergic neurons in the laterodorsal and the pedunculo-pontine tegmental nuclei of cats: a combined light and elecetron microscope study. *Brain Research* 2005; 1052: 47-55.

51. Chase MH. Sleep patterns in old cats. In: Chase MH (ed) *Sleep Disorders: Basic and Clinical Research*. New York: Spectrum Publications, Inc., 1983, pp.445-448.

52. Chambers JK, Tokuda T, Uchida K, et al. The domestic cat as a natural animal model of Alzheimer's disease. *Acta neuropathologica communications* 2015; 3: 78-78.

53. Hardy J and Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends in pharmacological sciences* 1991; 12: 383-388.

54. Perl DP. Neuropathology of Alzheimer's disease. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine: A Journal of Translational and Personalized Medicine* 2010; 77: 32-42.

55. Ricciarelli R and Fedele E. The amyloid cascade hypothesis in Alzheimer's disease: it's time to change our mind. *Current neuropharmacology* 2017; 15: 926-935.

56. Volloch V, Olsen B and Rits S. Alzheimer’s Disease is Driven by Intraneuronally Retained Beta-Amyloid Produced in the AD-Specific, βAPP-Independent Pathway: Current Perspective and Experimental Models for Tomorrow. *Annals of integrative molecular medicine* 2020; 2: 90.

57. Hu Y, Kienlen-Campard P, Tang T-C, et al. β-Sheet structure within the extracellular domain of C99 regulates amyloidogenic processing. *Scientific reports* 2017; 7: 1-11.

58. Hardy JA and Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 1992; 256: 184-186.

59. Murray CE, Gami-Patel P, Gkanatsiou E, et al. The presubiculum is preserved from neurodegenerative changes in Alzheimer’s disease. *Acta neuropathologica communications* 2018; 6: 62.

60. Braak H and Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta neuropathologica* 1991; 82: 239-259.

61. Thal DR, Rüb U, Orantes M, et al. Phases of Aβ-deposition in the human brain and its relevance for the development of AD. *Neurology* 2002; 58: 1791-1800.

62. Jouanne M, Rault S and Voisin-Chiret A-S. Tau protein aggregation in Alzheimer's disease: an attractive target for the development of novel therapeutic agents. *European journal of medicinal chemistry* 2017; 139: 153-167.

63. Cummings BJ, Satou T, Head E, et al. Diffuse plaques contain C-terminal A beta 42 and not A beta 40: evidence from cats and dogs. *Neurobiology of Aging* 1996; 17: 653-659.

64. Head E, Moffat KS, Das P, et al. Beta-amyloid deposition and tau phosphorylation in clinically characterized aged cats. *Neurobiology Aging* 2005; 26: 749-763.

65. Nakamura S, Nakayama H, Kiatipattanasakul W, et al. Senile plaques in very aged cats. *Acta Neuropathologica* 1996; 91: 437-439.

66. Brellou G, Vlemmas I, Lekkas S, et al. Immunohistochemical investigation of amyloid beta-protein (Abeta) in the brain of aged cats. *Histology and Histopathology* 2005; 20: 725-731.

67. Gunn-Moore D, McVee J, Bradshaw JM, et al. Ageing changes in cat brains demonstrated by beta-amyloid and AT8-immunoreactive phosphorylated tau deposits. *Journal of Feline Medicine and Surgery* 2006; 8: 234-242.

68. Wisniewski T, Lalowski M, Bobik M, et al. Amyloid Beta 1-42 deposits do not lead to Alzheimer's neuritic plaques in aged dogs. *Biochemical Journal* 1996; 313: 575-580.

69. Hyman BT, Marzloff K and Arriagada PV. The lack of accumulation of senile plaques or amyloid burden in Alzheimer's disease suggests a dynamic balance between amyloid deposition and resolution. *Journal of Neuropathology and Experimental Neurology* 1993; 52: 594-600.

70. Lee H-g, Perry G, Moreira PI, et al. Tau phosphorylation in Alzheimer's disease: pathogen or protector? *Trends in molecular medicine* 2005; 11: 164-169.

71. O'Connor CM, Adams JU and Fairman J. Essentials of cell biology. *Cambridge, MA: NPG Education* 2010; 1: 54.

72. Kametani F and Hasegawa M. Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Frontiers in neuroscience* 2018; 12: 25.

73. Goedert M, Spillantini M, Jakes R, et al. Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron* 1989; 3: 519-526.

74. Šimić G, Babić Leko M, Wray S, et al. Tau protein hyperphosphorylation and aggregation in Alzheimer's disease and other tauopathies, and possible neuroprotective strategies. *Biomolecules* 2016; 6: 6.

75. Guo T, Noble W and Hanger DP. Roles of tau protein in health and disease. *Acta neuropathologica* 2017; 133: 665-704.

76. Kurihara M, Mano T, Saito Y, et al. Colocalization of BRCA1 with Tau Aggregates in Human Tauopathies. *Brain Sciences* 2020; 10: 7.

77. Braak H and Del Tredici K. Neurofibrillary tangles. *Encyclopedia of movement disorders, vol 2: HP* 2010: 265-269.

78. Metaxas A and Kempf SJ. Neurofibrillary tangles in Alzheimer's disease: elucidation of the molecular mechanism by immunohistochemistry and tau protein phospho-proteomics. *Neural regeneration research* 2016; 11: 1579.

79. Braak H and Braak E. Evolution of the neuropathology of Alzheimer's disease. *Acta Neurologica Scandinavica* 1996; 94: 3-12.

80. Kuroki K, Uchida K, Kiatipattanasakul W, et al. Immunohistochemical detection of tau proteins in various non-human animal brains. *Neuropathology* 1997; 17: 174-180.

81. Riederer BM, Mourton-Gilles C, Frey P, et al. Differential phosphorylation of tau proteins during kitten brain development and Alzheimer's disease. *Journal of Neurocytology* 2001; 30: 145-158.

82. Selkoe DJ, Bell DS, Podlisny MB, et al. Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. *Science* 1987; 235: 873-877. DOI: 10.1126/science.3544219.

83. Selkoe DJ. Alzheimer's disase: genotypes, phenotype, and treatments. *Science* 1997; 275: 630-631.

84. Attems J. Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms. *Acta Neuropathologica* 2005; 110: 345-359.

85. Attems J, Jellinger KA and Lintner F. Alzheimer's disease pathology influences severity and topographical distribution of cerebral amyloid angiopathy. *Acta Neuropathologica* 2005; 110: 222-231.

86. Serrano-Pozo A, Frosch MP, Masliah E, et al. Neuropathological alterations in Alzheimer disease. *Cold Spring Harbor Perspectives In Biology* 2011; 3.

87. Slingerland LI, Hazewinkel HAW, Meij BP, et al. Cross-sectional study of the prevalence and clinical features of osteoarthritis in 100 cats. *The Veterinary Journal* 2011; 187: 304-309.

88. Lascelles BDX, Dong Y, Marcellin-Little DJ, et al. Relationship of orthopedic examination, goniometric measurements, and radiographic signs of degenerative joint disease in cats. *BMC Veterinary Research* 2012; 8: 10.

89. Enomoto M, Lascelles BDX and Gruen ME. Development of a checklist for the detection of degenerative joint disease-associated pain in cats. *Journal of Feline Medicine and Surgery* 2020. DOI: https://doi.org/10.1177/1098612X20907424.

90. Gunn-Moore DA. Cognitive Dysfunction in Cats: Clinical Assessment and Management. *Topics in Companion Animal Medicine* 2011; 26: 17-24. DOI: 10.1053/j.tcam.2011.01.005.

91. Dickerson BC, Stoub TR, Shah RC, et al. AD signature MRI biomarker predicts AD dementia in cognitively normal adults. *Neurology* 2011; 76: 1395-1402.

92. Fox NC, Crum WR, Scahill RI, et al. Imaging of onset and progression of Alzheimer's disease with voxel-compression mapping of serial magnetic resonance images. *Lancet* 2001; 358: 201-205.

93. Smith CD, Chebrolu H, Wekstein DR, et al. Brain structural alterations before mild cognitive impairment. *Neurology* 2007; 68: 1268-1273.

94. Thompson PM, Mega MS, Woods RP, et al. Cortical change in Alzheimer's disease detected with a disease- specific population-based brain atlas. *Cerebral Cortex* 2001; 11: 1-16.

95. Good CD, Scahill RI, Fox NC, et al. Automatic differentiation of anatomical patterns in the human brain: validation with studies of degenerative dementias. *NeuroImage* 2002; 17: 29-46.

96. Head E. Combining an antioxidant-fortified diet with behavioral enrichment leads to cognitive improvement and reduced brain pathology in aging canines: strategies for healthy aging. *Annals of the New York Academy of Sciences* 2007; 1114: 398-406.

97. Gunn-Moore D. Cognitive Dysfunction in the Cat. In: Little SE and August JR (eds) *August´s Consultation in Feline Internal Medicine Volume 7*. St. Louis: Elsevier, 2016, pp.977-985.

98. Houpt KA and Beaver B. Behavioral problems of geriatric dogs and cats. *The Veterinary clinics of North America Small animal practice* 1981; 11: 643-652. DOI: 10.1016/S0195-5616(81)50076-3.

99. Landsberg GM, DePorter T and Araujo JA. Management of anxiety, sleeplessness and cognitive dysfunction in the senior pet. *Veterinary Clinics of North America-Small Animal Practice* 2011; 41: 565-590.

100. Griffith CA, Steigerwald ES and Buffington CA. Effects of a synthetic facial pheromone on behavior of cats. *Journal of the American Veterinary Medical Association* 2000; 217: 1154-1156.

101. Ellis SL, Rodan I, Carney HC, et al. AAFP and ISFM feline environmental needs guidelines. *Journal of Feline Medicine and Surgery* 2013; 15: 219-230.

102. Bottiglieri T. S-Adenosyl-L-methionine (SAMe): from the bench to the bedside - molecular basis of a pleiotrophic molecule. *American Journal of Clinical Nutrition* 2002; 76: 1151S(1157)-1151S(1157).

103. Araujo JA, Faubert ML, Brooks M, et al. NOVIFIT (NoviSAMe) Tablets improve executive function in aged dogs and cats: implications for treatment of cognitive dysfunction syndrome. *Intern J Appl Res Vet Med* 2012; 10: 90-98.

104. Araujo JA, Landsberg GM, Milgram NW, et al. Improvement of short-term memory performance in aged beagles by a nutraceutical supplement containing phosphatidylserine, Ginkgo biloba, vitamin E, and pyridoxine. *Can Vet J* 2008; 49.

105. Heath SE, Barabas S and Craze PG. Nutritional supplementation in cases of canine cognitive dysfunction-A clinical trial. *Applied Animal Behaviour Science* 2007; 105: 284-296. DOI: 10.1016/j.applanim.2006.11.008.

106. Birks J and Evans JG. Ginkgo biloba for cognitive impairment and dementia. *Cochrane Database of systematic reviews* 2009.

107. Salehi B, Mishra AP, Nigam M, et al. Resveratrol: A double-edged sword in health benefits. *Biomedicines* 2018; 6: 91.

108. Hill AS, Werner JA, Rogers QR, et al. Lipoic acid is 10 times more toxic in cats than reported in humans, dogs or rats. *Journal of Animal Physiology and Animal Nutrition* 2004; 88: 150-156.

109. Meyer HP and Bečvářová I. Effects of a Urinary Food Supplemented with Milk Protein Hydrolysate and L-tryptophan on Feline Idiopathic Cystitis - Results of a Case Series in 10 cats. *International Journal of Applied Research in Veterinary Medicine* 2016; 14: 59-65.

110. Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the three-city cohort study. *Neurology* 2007; 69: 1921-1930.

111. Butterfield DA, Castegna A, Pocernich CB, et al. Nutritional approaches to combat oxidative stress in Alzheimer's disease. *The Journal of Biochemistry* 2003; 14: 444-461.

112. Trevizan L, de Mello Kessler A, Bigley KE, et al. Effects of dietary medium-chain triglycerides on plasma lipids and lipoprotein distribution and food aversion in cats. *American Journal of Veterinary Research* 2010; 71: 435-440.

113. Cupp CJ, Jean-Phillipe C, Kerr WW, et al. Effect of nutritional interventions on longevity of senior cats. *International Journal of Applied Research in Veterinary Medicine* 2006; 4: 34-50.

114. Cupp CJ, Kerr WW, Jean-Philippe C, et al. The role of nutritional interventions in the longevity and maintenance of long-term health in aging cats. *International Journal of Applied Research in Veterinary Medicine* 2008; 6: 69-81.

115. Pan Y, Araujo JA, Burrows J, et al. Cognitive enhancement in middle-aged and old cats with dietary supplementation with a nutrient blend containing fish oil, B vitamins, antioxidants and arginine. *British Journal of Nutrition* 2013; 110: 40-49.

116. Houpt K, Levine E, Landsberg G, et al. Antioxidant fortified food improves owner perceived behaviour in the aging cat. In: *Proceedings of the ESFM Conference* Prague, Czech Republic, 2007 2007.

117. Landsberg G, Milgram B, Mougeot I, et al. Therapeutic effects of an alpha-casozepine and L-tryptophan supplemented diet on fear and anxiety in the cat. *Journal of Feline Medicine and Surgery* 2017; 19: 594-602.

118. Naarden B and Corbee RJ. The effect of a therapeutic urinary stress diet on the short-term recurrence of feline idiopathic cystitis. *Veterinary Medicine and Science* 2019. DOI: 10.1002/vms3.197.

119. Westropp JL, Kass PH and Buffington CAT. Evaluation of the effects of stress in cats with idiopathic cystitis. *American Journal of Veterinary Research* 2006; 67: 731-736. DOI: 10.2460/ajvr.67.4.731.

120. Ruehl WW, Bruyette DS, DePaoli A, et al. Canine cognitive dysfunction as a model for human age-related cognitive decline, dementia, and Alzheimer's disease: clinical presentation, cognitive testing, pathology and response to l-deprenyl therapy. *Progress in brain research* 1995; 106: 217-225.

121. Campbell S, Trettien A and Kozan B. A noncomparative open-label study evaluating the effect of selegiline hydrochloride in a clinical setting. *Veterinary Therapeutics: Research in Applied Veterinary Medicine* 2001; 2: 24-39.

122. Parkinson FE, Rudophi KA and Fredholm BB. Propentofylline: a nucleoside transport inhibitor with neuroprotective effects in cerebral ischemia. *Gen Pharmacol* 1994; 25: 1053-1058.

123. Milgram NW, Ivy GO, Head E, et al. The effect of l-deprenyl on behavior, cognitive function, and biogenic amines in the dog. *Neurochemical Research* 1993; 18: 1211-1219.

124. Landsberg GM. Therapeutic options for cognitive decline in senior pets. *Journal of American Animal Hospital Association* 2006; 42: 407-413.

125. Studzinski CM, Araujo JA and Milgram NW. The canine model of human cognitive aging and dementia: pharmacological validity of the model for assessment of human cognitive-enhancing drugs. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2005; 29: 489-498.

126. Center SA, Elston TH, Rowland PH, et al. Fulminant hepatic failure associated with oral administration of diazepam in 11 cats. *Journal of American Veterinary Medical Association* 1996; 209: 618-625.

127. Araujo JA, Greig NH, Ingram DK, et al. Cholinesterase inhibitors improve both memory and complex learning in aged Beagle dogs. *Journal of Alzheimer's Disease* 2011; 26: 143-155.

128. Doody RS. Current treatments for Alzheimer's disease: cholinesterase inhibitor. *Journal of Clinical Psychiatry* 2003; 64: S7-S7.

129. Saavedra JM, Sánchez-Lemus E and Benicky J. Blockade of brain angiotensin II AT1 receptors ameliorates stress, anxiety, brain inflammation and ischemia: Therapeutic implications. *Psychoneuroendocrinology* 2011; 36: 1-18. DOI: 10.1016/j.psyneuen.2010.10.001.

130. Saavedra JM. Angiotensin II AT1 Receptor blockers ameliorate inflammatory stress: A beneficial effect for the treatment of brain disorders. *Cellular and Molecular Neurobiology* 2012; 32: 667-681.

131. Fogari R, Mugellini A, Zoppi A, et al. Influence of losartan and atenolol on cognitive function in very elderly hypertensive patients. *Journal of Human Hypertension* 2003; 17: 781-785.

132. Papademetriou V. Hypertension and cognitive function. Blood pressure regulation and cognitive function: a review of the literature. *Geriatrics* 2005; 60: 20-22.

133. Camacho IE, Serneels L, Spittaels K, et al. Peroxisome-proliferator-activated receptor gamma induces a clearance mechanism for the amyloid-beta peptide. *The Journal Of Neuroscience: the official journal of the Society for Neuroscience* 2004; 24: 10908-10917.

134. Tsukuda K, Mogi M, Iwanami J, et al. Cognitive deficit in amyloid-beta-injected mice was improved by pretreatment with a low dose of telmisartan partly because of peroxisome proliferator-activated receptor-gamma activation. *Hypertension* 2009; 54: 782-787.

135. Pang T, Wang J, Benicky J, et al. Telmisartan directly ameliorates the neuronal inflammatory response to IL-1b partly through the JNK/c-Jun and NADPH oxidase pathways. *Journal of Neuroinflammation* 2012; 9: 1-19.

136. Kasahara Y, Taguchi A, Uno H, et al. Telmisartan suppresses cerebral injury in a murine model of transient focal ischemia. *Brain Research* 2010; 1340: 70-80.

137. Wang J, Pang T, Hafko R, et al. Telmisartan ameriolates glutamate-induced neurotoxicity: roles of AT(1) receptor blockade and PPARγ activation. *Neuropharmacology* 2014; 79: 249-261.

138. Eslami H, Sharifi AM, Rahimi H, et al. Protective effect of telmisartan against oxidative damage induced by high glucose in neuronal PC12 cell. *Neuroscience Letters* 2014; 558: 31-36.

139. Pang T, Sun LX, Wang T, et al. Telmisartan protects central neurons against nutrient deprivation-induced apoptosis in vitro through activation of PPARγ and the Akt/GSK-3β pathway. *Acta Pharmacologica Sinica* 2014; 35: 727-737.

140. Wincewicz D and Braszko JJ. Telmisartan attenuates cognitive impairment caused by chronic stress in rats. *Pharmacol Rep* 2014; 66: 436-441.

141. Mogi M, Li JM, Tsukuda K, et al. Telmisartan prevented cognitive decline partly due to PPAR-gamma activation. *Biochemical and Biophysical Research Communications* 2008; 375: 446-449.

142. de Barros A. Telmisartan, https://alzheimersnewstoday.com/telmisartan/ (2019, accessed October, 25th 2019).

143. Glabe CG. Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. *Neurobiology of Aging* 2006; 27: 570-575.

144. Murphy D, DeCarli C, McIntosh A, et al. Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of ageing. *Archives of General Psychiatry* 1996; 53: 585-594.

145. Head E, McCleary R, Hahn FF, et al. Region-specific age at onset of beta-amyloid in dogs. *Neurobiology of Aging* 2000; 21: 89-96.

146. Colle MA, Hauw JJ, Crespeau F, et al. Vascular and parenchymal beta-amyloid deposition in the aging dog: correlation with behavior. *Neurobiology of Aging* 2000; 21: 695-704.

147. Braak H and Braak E. Morphological criteria for the recognition of Alzheimer's disease and the distribution pattern of cortical changes related to this disorder. 1994.

148. Vogt AH, Rodan I, Brown M, et al. AAFP-AAHA: Feline Life Stage Guidelines. *Journal of Feline Medicine and Surgery* 2017; 12: 43-54. DOI: https://doi.org/10.1016/j.jfms.2009.12.006.

149. Kraszpulski M, Soininen H, Riekkinen Sr P, et al. Pitfalls in the quantitative estimation of β-amyloid immunoreactivity in human brain tissue. *Histochemistry and cell biology* 1998; 110: 439-445.

150. Gunn-Moore D, McVee J, Bradshaw JM, et al. Beta amyloid and hyper-phosphorylated tau deposition in cat brains. *Journal of Feline Medicine and Surgery* 2006; 8: 234-242.

151. Gonneaud J, Arenaza-Urquijo EM, Mézenge F, et al. Increased florbetapir binding in the temporal neocortex from age 20 to 60 years. *Neurology* 2017; 89: 2438-2446.

152. Collins-Praino LE, Francis YI, Griffith EY, et al. Soluble amyloid beta levels are elevated in the white matter of Alzheimer's patients, independent of cortical plaque severity. *Acta neuropathologica communications* 2014; 2: 1-10.

153. Blessed G, Tomlinson BE and Roth M. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *The British journal of psychiatry* 1968; 114: 797-811.

154. Villemagne VL, Pike KE, Chételat G, et al. Longitudinal assessment of Aβ and cognition in aging and Alzheimer disease. *Annals of neurology* 2011; 69: 181-192.

155. Aizenstein HJ, Nebes RD, Saxton JA, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. *Archives of neurology* 2008; 65: 1509-1517.

156. Grundke-Iqbal I, Iqbal K, George L, et al. Amyloid protein and neurofibrillary tangles coexist in the same neuron in Alzheimer disease. *Proceedings of the National Academy of Sciences* 1989; 86: 2853-2857.

157. Gouras GK, Tsai J, Naslund J, et al. Intraneuronal Aβ42 accumulation in human brain. *The American journal of pathology* 2000; 156: 15-20.

158. Baker-Nigh A, Vahedi S, Davis EG, et al. Neuronal amyloid-β accumulation within cholinergic basal forebrain in ageing and Alzheimer's disease. *Brain* 2015; 138: 1722-1737.

159. LaFerla FM, Green KN and Oddo S. Intracellular amyloid-β in Alzheimer's disease. *Nature Reviews Neuroscience* 2007; 8: 499-509.

160. Nunomura A, Tamaoki T, Tanaka K, et al. Intraneuronal amyloid β accumulation and oxidative damage to nucleic acids in Alzheimer disease. *Neurobiology of disease* 2010; 37: 731-737.

161. Smith MA, Casadesus G, Joseph JA, et al. Amyloid-β and τ serve antioxidant functions in the aging and Alzheimer brain. *Free Radical Biology and Medicine* 2002; 33: 1194-1199.

162. Puzzo D and Arancio O. Amyloid-β peptide: Dr. Jekyll or Mr. Hyde? *Journal of Alzheimer's Disease* 2013; 33: S111-S120.

163. Mori C, Spooner ET, Wisniewski KE, et al. Intraneuronal Aβ42 accumulation in Down syndrome brain. *Amyloid* 2002; 9: 88-102.

164. Butterfield DA, Reed T, Newman SF, et al. Roles of amyloid β-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radical Biology and Medicine* 2007; 43: 658-677.

165. Manczak M, Anekonda TS, Henson E, et al. Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Human molecular genetics* 2006; 15: 1437-1449.

166. Beal MF. Mitochondria take center stage in aging and neurodegeneration. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society* 2005; 58: 495-505.

167. Bernstein SL, Dupuis NF, Lazo ND, et al. Amyloid-β protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease. *Nature chemistry* 2009; 1: 326-331.

168. Nelson PT, Alafuzoff I, Bigio EH, et al. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *Journal of Neuropathology & Experimental Neurology* 2012; 71: 362-381.

169. Dickson DW, Crystal HA, Bevona C, et al. Correlations of synaptic and pathological markers with cognition of the elderly. *Neurobiology of Aging* 1995; 16: 285-304.

170. Pérez MJ, Jara C and Quintanilla RA. Contribution of tau pathology to mitochondrial impairment in neurodegeneration. *Frontiers in neuroscience* 2018; 12: 441.

171. Alonso AdC, Grundke-Iqbal I and Iqbal K. Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nature medicine* 1996; 2: 783-787.

172. Takahashi M, Miyata H, Kametani F, et al. Extracellular association of APP and tau fibrils induces intracellular aggregate formation of tau. *Acta neuropathologica* 2015; 129: 895-907.

173. Braak H and Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta neuropathologica* 2011; 121: 171-181.

174. Maeda S, Sahara N, Saito Y, et al. Increased levels of granular tau oligomers: an early sign of brain aging and Alzheimer's disease. *Neuroscience research* 2006; 54: 197-201.

175. Santacruz K, Lewis J, Spires T, et al. Tau suppression in a neurodegenerative mouse model improves memory function. *Science* 2005; 309: 476-481.

176. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, et al. Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Molecular neurodegeneration* 2011; 6: 1-14.

177. Goedert M and Spillantini MG. Propagation of Tau aggregates. *Molecular brain* 2017; 10: 18.

178. Clavaguera F, Bolmont T, Crowther RA, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nature cell biology* 2009; 11: 909-913.

179. Perez M, Cuadros R, Smith M, et al. Phosphorylated, but not native, tau protein assembles following reaction with the lipid peroxidation product, 4-hydroxy-2-nonenal. *Febs Letters* 2000; 486: 270-274.

180. Arendt T, Stieler J, Strijkstra AM, et al. Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *Journal of Neuroscience* 2003; 23: 6972-6981.

181. Rady RM, Zinkowski RP and Binder LI. Presence of tau in isolated nuclei from human brain. *Neurobiology of aging* 1995; 16: 479-486.

182. Sultan A, Nesslany F, Violet M, et al. Nuclear Tau, a Key Player in Neuronal DNA Protection. *Journal of Biological Chemistry* 2011; 286: 4566-4575.

183. Metuzals J, Robitaille Y, Houghton S, et al. Paired helical filaments and the cytoplasmic-nuclear interface in Alzheimer's disease. *Journal of neurocytology* 1988; 17: 827-833.

184. Loomis P, Howard T, Castleberry R, et al. Identification of nuclear tau isoforms in human neuroblastoma cells. *Proceedings of the National Academy of Sciences* 1990; 87: 8422-8426.

185. Thurston VC, Zinkowski RP and Binder LI. Tau as a nucleolar protein in human nonneural cells in vitro and in vivo. *Chromosoma* 1996; 105: 20-30.

186. Greenwood JA and Johnson GV. Localization and in situ phosphorylation state of nuclear tau. *Experimental cell research* 1995; 220: 332-337.

187. Papasozomenos SC and Su Y. Altered phosphorylation of tau protein in heat-shocked rats and patients with Alzheimer disease. *Proceedings of the National Academy of Sciences* 1991; 88: 4543-4547.

188. Maina MB, Al-Hilaly YK and Serpell LC. Nuclear tau and its potential role in alzheimer's disease. *Biomolecules* 2016; 6: 2-20. DOI: 10.3390/biom6010009.

189. Lefebvre T, Ferreira S, Dupont-Wallois L, et al. Evidence of a balance between phosphorylation and O-GlcNAc glycosylation of Tau proteins—a role in nuclear localization. *Biochimica et Biophysica Acta (BBA)-General Subjects* 2003; 1619: 167-176.

190. Qi H, Cantrelle F-X, Benhelli-Mokrani H, et al. Nuclear magnetic resonance spectroscopy characterization of interaction of Tau with DNA and its regulation by phosphorylation. *Biochemistry* 2015; 54: 1525-1533.

191. Ulrich G, Salvadè A, Boersema P, et al. Phosphorylation of nuclear Tau is modulated by distinct cellular pathways. *Scientific reports* 2018; 8: 1-14.

192. Hernández-Ortega K, Garcia-Esparcia P, Gil L, et al. Altered machinery of protein synthesis in Alzheimer's: from the nucleolus to the ribosome. *brain pathology* 2016; 26: 593-605.

193. Gil L, Federico C, Pinedo F, et al. Aging dependent effect of nuclear tau. *Brain Research* 2017; 1677: 129-137. DOI: 10.1016/j.brainres.2017.09.030.

194. Cameron B and Landreth GE. Inflammation, microglia, and Alzheimer's disease. *Neurobiology of disease* 2010; 37: 503-509.

195. Colonna M and Butovsky O. Microglia function in the central nervous system during health and neurodegeneration. *Annual review of immunology* 2017; 35: 441-468.

196. Cantoni C, Bollman B, Licastro D, et al. TREM2 regulates microglial cell activation in response to demyelination in vivo. *Acta neuropathologica* 2015; 129: 429-447.

197. Davalos D, Grutzendler J, Yang G, et al. ATP mediates rapid microglial response to local brain injury in vivo. *Nature neuroscience* 2005; 8: 752-758.

198. Hamelin L, Lagarde J, Dorothée G, et al. Early and protective microglial activation in Alzheimer's disease: A prospective study using 18F-DPA-714 PET imaging. *Brain* 2016; 139: 1252-1264. DOI: 10.1093/brain/aww017.

199. Verkhratsky A, Olabarria M, Noristani HN, et al. Astrocytes in Alzheimer's disease. *Neurotherapeutics* 2010; 7: 399-412.

200. Guénette SY. Astrocytes: a cellular player in Aβ clearance and degradation. *Trends in molecular medicine* 2003; 9: 279-280.

201. Hansen DV, Hanson JE and Sheng M. Microglia in Alzheimer's disease. *Journal of Cell Biology* 2018; 217: 459-472.

202. Mohamed A and Posse de Chaves E. Aβ internalization by neurons and glia. *International journal of Alzheimer's disease* 2011; 2011.

203. Condello C, Yuan P, Schain A, et al. Microglia constitute a barrier that prevents neurotoxic protofibrillar Aβ42 hotspots around plaques. *Nature Communications* 2015; 6: 6176-6176. DOI: 10.1038/ncomms7176.

204. Bornemann KD, Wiederhold K-H, Pauli C, et al. Aβ-induced inflammatory processes in microglia cells of APP23 transgenic mice. *The American journal of pathology* 2001; 158: 63-73.

205. Heneka MT, Golenbock DT and Latz E. Innate immunity in Alzheimer's disease. *Nature immunology* 2015; 16: 229-236.

206. Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 2017; 541: 481-487.

207. Dá Mesquita S, Ferreira AC, Sousa JC, et al. Insights on the pathophysiology of Alzheimer's disease: The crosstalk between amyloid pathology, neuroinflammation and the peripheral immune system. *Neuroscience & Biobehavioral Reviews* 2016; 68: 547-562.

208. Iglesias J, Morales L and Barreto GE. Metabolic and inflammatory adaptation of reactive astrocytes: role of PPARs. *Molecular neurobiology* 2017; 54: 2518-2538.

209. González-Reyes RE, Nava-Mesa MO, Vargas-Sánchez K, et al. Involvement of astrocytes in Alzheimer's disease from a

neuroinflammatory and oxidative stress perspective. *Frontiers in Molecular Neuroscience* 2017; 10: 427.

210. Nedergaard M, Ransom B and Goldman SA. New roles for astrocytes: redefining the functional architecture of the brain. *Trends in neurosciences* 2003; 26: 523-530.

211. Lee L, Kosuri P and Arancio O. Picomolar amyloid-β peptides enhance spontaneous astrocyte calcium transients. *Journal of Alzheimer's Disease* 2014; 38: 49-62.

212. Compton J, Van Amelsoort T and Murphy D. HRT and its effect on normal ageing of the brain and dementia. *British Journal of Clinical Pharmacology* 2001; 52.

213. Morris JC, Roe CM, Grant EA, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. *Archives of Neurology* 2009; 66: 1469-1475.

214. Csernansky JG, Wang L, Swank J, et al. Preclinical detection of Alzheimer's disease: hippocampal shape and volume predict dementia onset in the elderly. *NeuroImage* 2005; 25: 783-792.

215. Schott JM, Fox NC, Frost C, et al. Assessing the onset of structural change in familial Alzheimer's disease. *Annals of Neurology* 2003; 53: 181-188.

216. Chan D, Fox NC, Jenkins R, et al. Rates of global and regional cerebral atrophy in AD and frontotemporal dementia. *Neurology* 2001; 57: 1756-1763.

217. Resnick SM, Pham DL, Kraut MA, et al. Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. *Journal of Neuroscience* 2003; 23: 3295-3301.

218. Chan D, Fox NC, Scahill RI, et al. Patterns of temporal lobe atrophy in semantic dementia and Alzheimer's disease. *Annals of Neurology* 2001; 49: 433-442.

219. Pugliese M, Carrasco JL, Gomez-Anson B, et al. Magnetic resonance imaging of cerebral involutional changes in dogs as markers of aging: an innovative tool adapted from a human visual rating scale. *The Veterinary Journal* 2010; 186: 166-171.

220. Su MY, Head E, Brooks WM, et al. Magnetic resonance imaging of anatomic and vascular characteristics in a canine model of human aging. *Neurobiology of Aging* 1998; 19: 479-485.

221. Vite CH, Insko EK, Schotland HM, et al. Quantification of cerebral ventricular volume in English bulldogs. *Veterinary Radiology and Ultrasound* 1997; 38: 437-443.

222. Tapp PD, Siwak CT, Gao FQ, et al. Frontal lobe volume, function, and beta-amyloid pathology in a canine model of aging. *J Neurosci* 2004; 24: 8205-8213. 2004/09/24. DOI: 10.1523/JNEUROSCI.1339-04.2004.

223. Fox MW. Integrative development of brain and behavior in the dog. Chicago: University of Chicago Press, 1971.

224. Noh D, Choi S, Choi H, et al. Evaluation of interthalamic adhesion size as an indicator of brain atrophy in dogs with and without cognitive dysfunction. *Veterinary Radiology and Ultrasound* 2017; 58: 581-587.

225. Hasegawa D, Yayoshi N, Fujita Y, et al. Measurement of interthalamic adhesion thickness as a criteria for brain atrophy in dogs with and without cognitive dysfunction (dementia). *Veterinary Radiology and Ultrasound* 2005; 46: 452-457.

226. Babicsak VR, Klein AV, Tsunemi MH, et al. Brain parenchymal changes during normal aging in domestic cats. *Pesq Vet Bras* 2018; 38: 1196-1202.

227. Oguz I, Yaxley R, Budin F, et al. Comparison of magnetic resonance imaging in live vs. post mortem rat brains. *PloS one* 2013; 8: e71027.

228. Pfefferbaum A, Sullivan EV, Adalsteinsson E, et al. Postmortem MR imaging of formalin-fixed human brain. *Neuroimage* 2004; 21: 1585-1595.

229. van Duijn S, Nabuurs RJ, van Rooden S, et al. MRI artifacts in human brain tissue after prolonged formalin storage. *Magnetic Resonance in Medicine* 2011; 65: 1750-1758.

230. Le Bihan D and Iima M. Diffusion magnetic resonance imaging: what water tells us about biological tissues. *PLoS biology* 2015; 13: e1002203.

231. Sullivan EV and Pfefferbaum A. Diffusion tensor imaging in normal aging and neuropsychiatric disorders. *European journal of radiology* 2003; 45: 244-255.

232. Sun SW, Neil JJ and Song SK. Relative indices of water diffusion anisotropy are equivalent in live and formalin-fixed mouse brains. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* 2003; 50: 743-748.

233. Shepherd T, Flint J, Thelwall P, et al. Postmortem interval alters the water relaxation and diffusion properties of nervous tissue: implications for high resolution MRI of human autopsy samples. In: *Proc Intl Soc Magn Reson Med* 2006, p.139.

234. Tovi M and Ericsson A. Measurements of T1 and T2 over time in formalin-fixed human whole-brain specimens. *Acta Radiologica* 1992; 33: 400-404.

235. Boyko O, Alston S, Fuller GN, et al. Utility of postmortem magnetic resonance imaging in clinical neuropathology. *Archives of Pathology and Laboratory Medicine* 1994; 118: 219-225.

236. Weisbecker V. Distortion in formalin-fixed brains: using geometric morphometrics to quantify the worst-case scenario in mice. *Brain Structure and Function* 2012; 217: 677-685.

237. Frontera JG. The effects of prolonged fixation on the measurements of the brain of macaques. *The Anatomical Record* 1959; 133: 501-511.

238. Fox M. Uptake of formalin by brain tissue from dogs killed at various stages of development. *Nature* 1965; 205: 1221-1221.

239. Quester R and Schröder R. The shrinkage of the human brain stem during formalin fixation and embedding in paraffin. *Journal of neuroscience methods* 1997; 75: 81-89.

240. McFadden WC, Walsh H, Richter F, et al. Perfusion fixation in brain banking: a systematic review. *Acta neuropathologica communications* 2019; 7: 146.

241. Yong-Hing CJ, Obenaus A, Stryker R, et al. Magnetic resonance imaging and mathematical modeling of progressive formalin fixation of the human brain. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* 2005; 54: 324-332.

242. Schulz G, Crooijmans HJ, Germann M, et al. Three-dimensional strain fields in human brain resulting from formalin fixation. *Journal of neuroscience methods* 2011; 202: 17-27.

243. Hudspeth WJ and Pribram KH. Stages of brain and cognitive maturation. 1990.

244. Epstein HT. Stages in human brain development. *Developmental brain research* 1986; 30: 114-119.

245. Sullivan E, Pfefferbaum A, Adalsteinsson E, et al. Differential rates of regional brain change in callosal and ventricular size: a 4-year longitudinal MRI study of elderly men. *Cerebral Cortex* 2002; 12: 438-445.

246. Salat DH, Kaye JA and Janowsky JS. Prefrontal gray and white matter volumes in healthy aging and Alzheimer disease. *Archives of neurology* 1999; 56: 338-344.

247. Laakso MP, Soininen H, Partanen K, et al. Volumes of hippocampus, amygdala and frontal lobes in the MRI-based diagnosis of early Alzheimer's disease: correlation with memory functions. *Journal of Neural Transmission-Parkinson's Disease and Dementia Section* 1995; 9: 73-86.

248. Ritchie SJ, Cox SR, Shen X, et al. Sex differences in the adult human brain: evidence from 5216 UK biobank participants. *Cerebral Cortex* 2018; 28: 2959-2975.

249. van Eijk L, Hansell NK, Strike LT, et al. Region-specific sex differences in the hippocampus. *Neuroimage* 2020; 215: 116781.

250. Hellerbach A, Schuster V, Jansen A, et al. MRI phantoms–are there alternatives to agar? *PloS one* 2013; 8: e70343.

251. Doens D and Fernández PL. Microglia receptors and their implications in the response to amyloid β for Alzheimer's disease pathogenesis. *Journal of Neuroinflammation* 2014; 11: 1-14. DOI: 10.1186/1742-2094-11-48.

252. Gyurko R, Wielbo D and Ian Phillips M. Antisense inhibition of AT1 receptor mRNA and angiotensinogen mRNA in the brain of spontaneously hypertensive rats reduces hypertension of neurogenic origin. *Regulatory Peptides* 1993; 49: 167-174. DOI: 10.1016/0167-0115(93)90438-E.

253. Wright JW and Harding JW. The brain renin-angiotensin system: A diversity of functions and implications for CNS diseases. *Pflugers Archiv European Journal of Physiology* 2013; 465: 133-151. DOI: 10.1007/s00424-012-1102-2.

254. Fountain JH and Lappin SL. Physiology, Renin Angiotensin System. *StatPearls* 2018.

255. Braszko JJ, Walesiuk A and Wielgat P. Cognitive Effects Attributed to Angiotensin II may Result from its Conversion to Angiotensin IV. *Journal of the Renin-Angiotensin-Aldosterone System* 2006; 7: 168-174.

256. Graeff FG, Gentil CG, Peres VL, et al. Lever-pressing behavior caused by intraseptal angiotensin II in water satiated rats. *Pharmacology Biochemistry and Behavior* 1973; 1: 357-359.

257. Rolls BJ, Jones BP and Fallows DJ. A comparison of the motivational properties of thirst. *Physiology of Behaviour* 1972; 9.

258. AbdAlla S, Lother H, el Missiry A, et al. Angiotensin II AT2 receptor oligomers mediate G-protein dysfunction in an animal model of Alzheimer disease. *Journal of Biological Chemistry* 2009; 284: 6554-6565.

259. Kehoe PG, Miners S and Love S. Angiotensins in Alzheimer’s disease-friend or foe? *Trends in Neuroscice* 2009; 32: 619-628.

260. Barnes JM, Barnes NM, Costall B, et al. Angiotensin-Converting Enzyme Inhibition, Angiotensin, and Cognition. *Journal of Cardiovascular Pharmacology* 1992; 19: S63-S71.

261. Wright JW, Clemens JA, Panetta JA, et al. Effects of LY231617 and angiotensin IV on ischemia-induced deficits in circular water maze and passive avoidance performance in rats. *Brain Research* 1996; 717: 1-11.

262. Wright J, Miller-Wing A, Shaffer M, et al. Angiotensin II (3–8)(ANG IV) hippocampal binding: potential role in the facilitation of memory. *Brain research bulletin* 1993; 32: 497-502.

263. Miners JS, Ashby E, Van Helmond Z, et al. Angiotensin-converting enzyme (ACE) levels and activity in Alzheimer's disease, and relationship of perivascular ACE-1 to cerebral amyloid angiopathy. *Neuropathology and Applied Neurobiology* 2008; 34: 181-193.

264. Wright JW and Harding JW. Important roles for angiotensin III and IV in the brain renin-angiotensin system. *Brain Research Reviews* 1997; 25: 96-124.

265. Saavedra JM. Evidence to Consider Angiotensin II Receptor Blockers for the Treatment of Early Alzheimer's Disease. *Cellular and Molecular Neurobiology* 2016; 36: 259-279. DOI: 10.1007/s10571-015-0327-y.

266. Nishimura Y, Ito T and Saavedra JM. Angiotensin II AT 1 blockade normalizes cerebrovascular autoregulation and reduces cerebral ischemia in spontaneously hypertensive rats. *Stroke* 2000; 31: 2478-2486. DOI: 10.1161/01.STR.31.10.2478.

267. Saavedra Juan M. Angiotensin II AT 1 receptor blockers as treatments for inflammatory brain disorders. *Clinical Science* 2012; 123: 567-590. DOI: 10.1042/cs20120078.

268. Singh KD and Karnik SS. Angiotensin Receptors: Structure, Function, Signaling and Clinical Applications. *Journal of Cell Signaling* 2016; 1: 111.

269. Chaki S and Inagami T. Identification and characterization of a new binding site for angiotensin II in mouse neuroblastoma neuro-2A cells. *Biochemical and Biophysical Research Communications* 1992; 182: 388-394.

270. Neldam S. Choosing an angiotensin-receptor blocker: Blood pressure lowering, cardiovascular protection or both? *Future Cardiology* 2010; 6: 129-135. DOI: 10.2217/fca.09.61.

271. Ogihara T, Kikuchi K, Matsuoka H, et al. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). *Hypertension Research* 2009; 32: 3-107.

272. Launer LJ, Masaki K, Petrovitch H, et al. The association between midlife blood pressure levels and late-life cognitive function. The Honolulu-Asia Aging Study. *JAMA* 1995; 274: 1846-1851.

273. Skoog I, Nilsson L, Persson G, et al. A 15 year longitudinal study of blood pressure and dementia. *Lancet* 1996; 347: 1141-1145.

274. Kilander L, Nyman H, Boberg M, et al. Hypertension is related to cognitive impairment: a 20-year follow-up of 999 men. *Hypertension* 1998; 31: 780-786.

275. Fogari R, Mugellini A, Zoppi A, et al. Effects of valsartan compared with enalapril on blood pressure and cognitive function in elderly patients with essential hypertension. *European Journal of Clinical Pharmacology* 2004; 59: 863-868. DOI: 10.1007/s00228-003-0717-9.

276. Li NC, Lee A, Whitmer RA, et al. Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: Prospective cohort analysis. *BMJ (Online)* 2010; 340: 141-141. DOI: 10.1136/bmj.b5465.

277. Davies NM, Kehoe PG, Ben-Shlomo Y, et al. Associations of anti-hypertensive treatments with Alzheimer's disease, vascular dementia, and other dementias. *Journal of Alzheimer's Disease* 2011; 26: 699-708. DOI: 10.3233/JAD-2011-110347.

278. Ito T, Yamakawa H, Bregonzio C, et al. Protection against ischemia and improvement of cerebral blood flow in genetically hypertensive rats by chronic pretreatment with an angiotensin II AT1 antagonist. *Stroke* 2002; 33: 2297-2303. DOI: 10.1161/01.STR.0000027274.03779.F3.

279. Krikov M, Thone-Reineke C, Müller S, et al. Candesartan but not Ramipril pretreatment improves outcome after stroke and stimulates neurotrophin BNDF/TrkB system in rats. *Journal of Hypertension* 2008; 26: 544-552.

280. Tota S, Kamat PK, Awasthi H, et al. Candesartan improves memory decline in mice: Involvement of AT1 receptors in memory deficit induced by intracerebral streptozotocin. *Behavioural Brain Research* 2009; 199: 235-240. DOI: 10.1016/j.bbr.2008.11.044.

281. Lanz TV, Ding Z, Ho PP, et al. Angiotensin II sustains brain inflammation in mice via TGF-β. *Journal of Clinical Investigation* 2010; 120: 2782-2794. DOI: 10.1172/JCI41709.

282. Benson SC, Pershadsingh HA, Ho CI, et al. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension* 2004; 43: 993-1002.

283. Rotman N and Wahli W. PPAR Modulation of Kinase-Linked Receptor Signaling in Physiology and Disease. *Physiology* 2010; 25: 176-185. DOI: 10.1152/physiol.00018.2010.

284. Fiévet C, Fruchart JC and Staels B. PPARalpha and PPARgamma dual agonists for the treatment of type 2 diabetes and the metabolic syndrome. *Current Opinion in Pharmacology* 2006; 6: 604-614.

285. Duan SZ, Usher MG and Mortensen RM. PPARs: The vasculature, inflammation and hypertension. *Current Opinion in Nephrology and Hypertension* 2009; 18: 128-133. DOI: 10.1097/MNH.0b013e328325803b.

286. Chen YC, Wu JS, Tsai HD, et al. Peroxisome proliferator-activated receptor gamma (PPAR-γ) and neurodegenerative disorders. *Molecular Neurobiology* 2012; 46: 114-124.

287. Atlante A, Calissano P, Bobba A, et al. Glutamate neurotoxicity, oxidative stress and mitochondria. *FEBS Letters* 2001; 497: 1-5. DOI: https://doi.org/10.1016/S0014-5793(01)02437-1.

288. Zhao Y, Patzer A, Herdegen T, et al. Activation of cerebral peroxisome proliferator-activated receptors gamma promotes neuroprotection by attenuation of neuronal cyclooxygenase-2 overexpression after focal cerebral ischemia in rats. *The FASEB Journal* 2006; 20: 1162-1175. DOI: 10.1096/fj.05-5007com.

289. Zuhayra M, Zhao Y, von Forstner C, et al. Activation of cerebral peroxisome proliferator-activated receptors γ (PPARγ) reduces neuronal damage in the substantia nigra after transient focal cerebral ischaemia in the rat. *Neuropathology and Applied Neurobiology* 2011; 37: 738-752. DOI: 10.1111/j.1365-2990.2011.01169.x.

290. Sodhi RK, Singh N and Jaggi AS. Neuroprotective mechanisms of peroxisome proliferator-activated receptor agonists in Alzheimer's disease. *Naunyn-Schmiedeberg's Archives of Pharmacology* 2011; 384: 115-124. DOI: 10.1007/s00210-011-0654-6.

291. Jiang Q, Heneka M and Landreth GE. The role of peroxisome proliferator-activated receptor-gamma (PPARgamma) in Alzheimer's disease: therapeutic implications. *CNS Drugs* 2008; 22: 1-14.

292. Landreth G, Jiang Q, Mandrekar S, et al. PPARgamma agonists as therapeutics for the treatment of Alzheimer's disease. *Neurotherapeutics* 2008; 5: 481-489. 2008/07/16. DOI: 10.1016/j.nurt.2008.05.003.

293. Heneka MT, Sastre M, Dumitrescu-Ozimek L, et al. Acute treatment with the PPARgamma agonist pioglitazone and ibuprofen reduces glial inflammation and Abeta1-42 levels in APPV717I transgenic mice. *Brain* 2005; 128: 1442-1453. 2005/04/09. DOI: 10.1093/brain/awh452.

294. Blass JP. The mitochondrial spiral: an adequate cause of dementia in the Alzheimer's syndrome. *Annals of the New York Academy of Sciences* 2000; 924: 170-183.

295. Strum JC, Shehee R, Virley D, et al. Rosiglitazone induces mitochondrial biogenesis in mouse brain. *J Alzheimers Dis* 2007; 11: 45-51. 2007/03/16. DOI: 10.3233/jad-2007-11108.

296. Haraguchi T, Iwasaki K, Takasaki K, et al. Telmisartan, a partial agonist of peroxisome proliferator-activated receptor γ, improves impairment of spatial memory and hippocampal apoptosis in rats treated with repeated cerebral ischemia. *Brain Research* 2010; 1353: 125-132. DOI: 10.1016/j.brainres.2010.07.017.

297. Washida K, Ihara M, Nishio K, et al. Nonhypotensive Dose of Telmisartan Attenuates Cognitive Impairment Partially Due to Peroxisome Proliferator-Activated Receptor-γ Activation in Mice With Chronic Cerebral Hypoperfusion. *Stroke* 2010; 41: 1798-1806.

298. Torika N, Asraf K, Cohen H, et al. Intranasal telmisartan ameliorates brain pathology in five familial Alzheimer's disease mice. *Brain, Behavior, and Immunity* 2017; 64: 80-90. DOI: 10.1016/j.bbi.2017.04.001.

299. D'Abramo C, Ricciarelli R, Pronzato MA, et al. Troglitazone, a peroxisome proliferator-activated receptor-γ agonist, decreases tau phosphorylation in CHOtau4R cells. *Journal of Neurochemistry* 2006; 98: 1068-1077.

300. Sent U, Gössl R, Elliott J, et al. Comparison of Efficacy of Long-term Oral Treatment with Telmisartan and Benazepril in Cats with Chronic Kidney Disease. *Journal of Veterinary Internal Medicine* 2015; 29: 1479-1487. DOI: 10.1111/jvim.13639.

301. Glaus TM, Elliott J, Herberich E, et al. Efficacy of long-term oral telmisartan treatment in cats with hypertension: Results of a prospective European clinical trial. *Journal of Veterinary Internal Medicine* 2019; 33: 413-422. DOI: 10.1111/jvim.15394.

302. Coleman AE, Brown SA, Stark M, et al. Evaluation of orally administered telmisartan for the reduction of indirect systolic arterial blood pressure in awake, clinically normal cats. *Journal of Feline Medicine and Surgery* 2019; 21: 109-114. DOI: 10.1177/1098612X18761439.

303. Coleman AE, Brown SA, Traas AM, et al. Safety and efficacy of orally administered telmisartan for the treatment of systemic hypertension in cats: Results of a double-blind, placebo-controlled, randomized clinical trial. *Journal of Veterinary Internal Medicine* 2019; 33: 478-488. DOI: 10.1111/jvim.15429.

304. Acierno MJ, Brown S, Coleman AE, et al. ACVIM consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *Journal of Veterinary Internal Medicine* 2018; 32: 1803-1822. DOI: 10.1111/jvim.15331.

305. Garosi L. Neurological examination of the cat. How to get started. 2009.

306. Braissant O, Foufelle F, Scotto C, et al. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology* 1996; 137: 354-366.

307. Moreno S, Farioli-vecchioli S and Cer' u MP. Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS. *Neuroscience* 2004; 123: 1131-1145.

308. Kummer MP and Heneka MT. PPARs in Alzheimer's Disease. *PPAR Res* 2008; 2008: 403896. 2008/07/23. DOI: 10.1155/2008/403896.

309. Scarlett JM, Donoghue S, Saidla J, et al. Overweight cats: prevalence and risk factors. *International Journal of Obesity* 1994; 18: S22-S28.

310. Harper EJ. Changing Perspectives on Aging and Energy Requirements: Aging and Digestive Function in Humans, Dogs and Cats. *The Journal of Nutrition* 1998; 128: 2632S-2635S. DOI: 10.1093/jn/128.12.2632s.

311. Perez-Camargo G, Patil AR and Cupp CJ. Body composition changes in aging cats. *Compend Contin Educ Pract Vet* 2004; 26: 71-71.

312. Cruz J, Martin FC, Schneider SM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing* 2010; 39: 412-423. DOI: 10.1093/ageing/afq034.

313. Bellows J, Center S, Daristotle L, et al. Evaluating aging in cats: How to determine what is healthy and what is disease. *Journal of Feline Medicine and Surgery* 2016; 18: 551-570. DOI: 10.1177/1098612X16649525.

314. Anantharaman-Barr HG, Gicquello P and Rabot R. The effect of age on the digestibility of macronutrients and energy in cats. *Proceedings of the British Small Animal Veterinary Association* 1991: 164-164.

315. Taylor EJ, Adams C and Neville R. Some nutritional aspects of ageing in dogs and cats. *Proceedings of the Nutrition Society* 1995; 54: 645-656. DOI: 10.1079/pns19950064.

316. Peachey SE, Dawson JM and Harper EJ. Gastrointestinal transit times in young and old cats. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 2000; 126: 85-90. DOI: 10.1016/S1095-6433(00)00189-6.

317. Peachey SE and Harper EJ. Aging Does Not Influence Feeding Behavior in Cats. *The Journal of Nutrition* 2002; 132: 1735S-1739S. DOI: 10.1093/jn/132.6.1735s.

318. Landsberg G, Hunthausen W and Ackerman L. Stereotypic and compulsive disorders. In: Landsberg G, Hunthausen W and Ackerman L (eds) *Handbook of behavior problems of the dog and cat*. 2nd ed. ed.: Elsevier Saunders, 2003, pp.195-225.

319. Kume K, Hanyu H, Sakurai H, et al. Effects of telmisartan on cognition and regional cerebral blood flow in hypertensive patients with Alzheimer's disease. *Geriatric Gerontology International* 2012; 1: 207-214.

320. Combs CK, Johnson DE, Karlo C, et al. Inflammatory Mechanisms in Alzheimer's Disease: Inhibition of β-Amyloid-Stimulated Proinflammatory Responses and Neurotoxicity by PPARγ Agonists. *Journal of Neuroscience* 2000; 20: 558-567.

321. Sastre M, Dewachter I, Rossner S, et al. Nonsteroidal anti-inflammatory drugs repress β-secretase gene promoter activity by the activation of PPARγ. *Proceedings of the National Academy of Sciences of the United States of America* 2006; 103: 443-448.

322. Rème CA, Dramard V, Kern L, et al. Effect of S-adenosylmethionine tablets on the reduction of age-related mental decline in dogs: a double-blinded, placebo-controlled trial. *Veterinary Therapeutics: Research in Applied Veterinary Medicine* 2007; 9: 69-82.

323. Choi BCK and Pak AWP. A Catalog of Biases in Questionnaires. *Preventing Chronic Disease* 2005; 2: A13.

324. Laflamme DP, Abood SK, Fascetti AJ, et al. Pet feeding practices of dog and cat owners in the United States and Australia. *Journal of American Veterinary Medical Association* 2008; 232: 687-694.

325. Gunn-Moore D. Increased vocalisation in elderly cats. *European Journal of Companion Animal Practice* 2015; 25: 20-29.

326. Egenvall A, Bonnett BN, Häggström J, et al. Morbidity of insured Swedish cats during 1999-2006 by age, breed, sex, and diagnosis. *Journal of Feline Medicine and Surgery* 2010; 12: 948-959. DOI: 10.1016/j.jfms.2010.08.008.

327. Laflamme D and Gunn-Moore D. Nutrition of Aging Cats. *Veterinary Clinics of North America Small Animal Practice* 2014; 44: 761-774. DOI: 10.1016/j.cvsm.2014.03.001.

328. Bellows J, Center S, Daristotle L, et al. Aging in cats: Common physical and functional changes. *Journal of Feline Medicine and Surgery* 2016; 18: 533-550. DOI: 10.1177/1098612X16649523.

329. Landsberg GM, Hunthausen WL and Ackerman LJ. Behavior Problems of the Dog and Cat. 2013: 454-454.

330. Bennett D, Zainal Ariffin SMb and Johnston P. Osteoarthritis in the cat: 1. How common is it and how easy to recognise? *Journal of feline medicine and surgery* 2012; 14: 65-75.

331. Hardie EM, Roe SC and Martin FR. Radiographic evidence of degenerative joint disease in geriatric cats: 100 cases (1994–1997). *Journal of the American Veterinary Medical Association* 2002; 220: 628-632.

332. Consortium TU. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Research* 2018; 47: D506-D515. DOI: 10.1093/nar/gky1049.

333. LaFerla FM and Green KN. Animal models of Alzheimer disease. *Cold Spring Harbor perspectives in medicine* 2012; 2: a006320.

334. Capucchio MT, Márquez M, Pregel P, et al. Parenchymal and Vascular Lesions in Ageing Equine Brains: Histological and Immunohistochemical Studies. *Journal of Comparative Pathology* 2010; 142: 61-73. DOI: 10.1016/j.jcpa.2009.07.007.

335. Jahns H, Callanan JJ, MaElroy MC, et al. Age-related and non age-related changes in 100 surveyed horse brains. *Veterinary Pathology* 2006; 43: 740-750.

336. Youssef SA, Capucchio MT, Rofina JE, et al. Pathology of the aging brain in domestic and laboratory animals, and animal models of human neurodegenerative diseases. *Veterinary Pathology* 2016; 53: 327-348.

337. Serizawa S, Chambers JK and Une Y. Beta amyloid deposition and neurofibrillary tangles spontaneously occur in the brains of captive cheetahs (Acinonyx jubatus). *Veterinary Pathology* 2012; 49: 304-312.

338. Chambers JK, Uchida K, Harada T, et al. Neurofibrillary tangles and the deposition of a beta amyloid peptide with a novel N-terminal epitope in the brains of wild Tsushima leopard cats. *PLoS ONE* 2012; 7: e46452-e46452.

339. Gunn-Moore D, Kaidanovich-Beilin O, Gallego Iradi MC, et al. Alzheimer's disease in humans and other animals: A consequence of postreproductive life span and longevity rather than aging. *Alzheimer's & Dementia* 2018; 14: 195-204.

Appendices

“Cats are like music; it’s foolish to try to explain their worth to those who don’t appreciate them”

-Anonymous

1.1. Review article.

Sordo L. and Gunn-Moore D. (2021). Cognitive dysfunction in cats: update on neuropathological and behavioural changes plus clinical management. *Veterinary Record*, 1-12.

2.1. Positive amyloid-β (Aβ) immunolabelling in the different age groups.
Means of the one-way ANOVA and Kruskal-Wallis for positive Aβ labelling (mOC64 antibody) in all the age groups with standard deviations and 95% confidence interval (CI).

	Group (n)	Mean	St. Dev.	95% CI
Rostral				
% Load	Prime (5)	4.194	2.831	(2.957, 5.431)
	Mature (25)	5.28	1.903	(4.726, 5.833)
	Senior (9)	4.809	1.913	(3.887, 5.732)
	Super Senior (14)	5.183	1.775	(4.444, 5.922)
Burden	Prime (5)	9.65	6.06	(4.58, 17.71)
	Mature (25)	19.89	8.76	(17.62, 22.15)
	Senior (9)	17.18	8.2	(13.41, 20.96)
	Super Senior (14)	17.58	7.25	(14.55, 20.61)
Parietal				
% Load	Prime (5)	4.291	3.067	(2.892, 5.690)
	Mature (25)	5.158	2.137	(4.533, 5.784)
	Senior (9)	4.665	2.21	(3.622, 5.707)
	Super Senior (14)	5.093	2.071	(4.257, 5.929)
Burden	Prime (5)	4.268	1.91	(2.884, 5.652)
	Mature (25)	6.23	2.475	(5.611, 6.849)
	Senior (9)	5.76	2.266	(4.719, 6.792)
	Super Senior (14)	6.056	1.682	(5.229, 6.884)
Occipital				
% Load	Prime (5)	5.353	1.681	(4.188, 6.518)
	Mature (25)	5.473	1.894	(4.952, 5.994)
	Senior (9)	4.665	2.052	(3.796, 5.533)
	Super Senior (12)	4.995	1.679	(4.243, 5.747)
Burden	Prime (5)	15.19	6.46	(10.07, 20.31)
	Mature (25)	19	8.6	(16.71, 21.29)
	Senior (9)	16.2	9.41	(12.38, 20.01)
	Super Senior (12)	17.23	6.66	(13.92, 20.53)
Hippocampus				
% Load	Prime (4)	28.81	16.65	(6.98, 50.64)
	Mature (24)	35.99	20.54	(27.08, 44.90)
	Senior (7)	37.43	23.92	(20.93, 53.93)
	Super Senior (11)	38.11	23.85	(24.95, 51.28)
Burden	Prime (4)	13.75	3.13	(5.41, 22.09)
	Mature (24)	18.13	8.63	(14.72, 21.53)
	Senior (7)	18.63	11.17	(12.33, 24.94)
	Super Senior (11)	20.02	6.17	(14.99, 25.05)

	Group (n)	Median	Median Rank	Z-value
Cerebellum				
% Load	Prime (4)	24.3881	26.5	0.3
	Mature (23)	26.4364	25.2	0.34
	Senior (8)	17.4322	20.4	-0.91
	Super Senior (13)	27.2351	25.2	0.2

2.2. Positive amyloid-β (Aβ) immunolabelling in grey matter (GM) and white matter (WM), and in cats with and without cognitive dysfunction syndrome (CDS). Means of the two-sample t-tests for the positive Aβ labelling (mOC64 antibody) in all the age groups with standard deviations and standard errors of the means for GM and WM; and for cats with and without CDS.

GM vs WM

	GM				WM			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
ROSTRAL								
% Load	53	6	1.87	0.26	53	4.15	1.61	0.22
Burden	53	21.75	8.41	1.2	53	13.96	6.58	0.9
PARIETAL								
% Load	53	5.76	2.21	0.3	53	4.19	1.94	0.27
Burden	53	6.73	2.13	0.29	53	5.11	2.07	0.28
OCCIPITAL								
% Load	51	5.99	1.77	0.25	51	4.42	1.61	0.23
Burden	51	21.1	7.96	1.1	51	14.33	6.9	0.97

CDS vs non-CDS

	CDS				Intact			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
ROSTRAL								
% Load	9	5.08	1.47	0.35	14	5.01	2.04	0.38
Burden	9	18.23	5.15	1.2	14	16.91	8.8	1.7
PARIETAL								
% Load	9	4.62	1.74	0.41	14	5.12	2.33	0.44
Burden	9	15.26	5.42	1.3	14	16.29	8.22	1.6
OCCIPITAL								
% Load	7	4.77	1.41	0.38	14	4.9	2.03	0.38
Burden	7	18.43	5.45	1.5	14	15.96	8.81	1.7
HIPPOCAMPUS								
% Load	5	22.22	3.25	1.5	13	43.9	24.8	6.9
Burden	5	295	141	63	13	499	367	102
CEREBELLUM								
% Load	8	23.2	21	7.4	13	23.4	19.3	5.3
Burden	8	5.66	1.54	0.55	13	5.3	1.56	0.43

2.3. Intranuclear immunolabelling of hyperphosphorylated tau (AT8 antibody) in the different age groups. Means of the one-way ANOVA for the intranuclear immunolabelling of hyperphosphorylated tau (AT8 antibody) in all age groups with standard deviations and 95% confidence interval (CI).

	Group (n)	Mean	St. Dev.	95% CI
Rostral				
	Prime (6)	21.42	16.71	(11.58, 31.27)
	Mature (24)	15.92	17.7	(11.00, 20.84)
	Senior (9)	19.99	15.73	(11.95, 28.03)
	Super Senior (13)	18.15	17.4	(11.46, 24.84)
Parietal				
	Prime (6)	1.951	0.472	(1.659, 2.244)
	Mature (25)	1.7879	0.5702	(1.6447, 1.9310)
	Senior (9)	2.006	0.473	(1.768, 2.245)
	Super Senior (13)	1.8336	0.4435	(1.6351, 2.0321)
Occipital				
	Prime (6)	2.494	0.892	(1.826, 3.161)
	Mature (26)	2.156	1.299	(1.835, 2.476)
	Senior (9)	2.592	0.953	(2.047, 3.137)
	Super Senior (12)	2.277	1.11	(1.805, 2.749)
Hippocampus				
	Prime (5)	1.753	0.388	(1.226, 2.280)
	Mature (24)	2.006	0.589	(1.765, 2.246)
	Senior (9)	2.374	0.519	(1.981, 2.766)
	Super Senior (13)	2.117	0.669	(1.791, 2.444)

2.4. Intranuclear immunolabelling of hyperphosphorylated tau (AT100 antibody) in the different age groups. Means of the one-way ANOVA and Kruskal-Wallis for the intranuclear immunolabelling of hyperphosphorylated tau (AT100 antibody) in all age groups with standard deviations and 95% confidence interval (CI).

	Group (n)	Mean	St. Dev.	95% CI
Rostral				
	Prime (6)	4.867	2.011	(3.732, 6.001)
	Mature (22)	4.475	1.999	(3.883, 5.068)
	Senior (9)	4.297	2.068	(3.371, 5.224)
	Super Senior (13)	4.916	1.869	(4.146, 5.687)
Parietal				
	Prime (6)	2.251	0.507	(1.949, 2.554)
	Mature (22)	2.1062	0.5483	(1.9519, 2.2605)
	Senior (9)	2.146	0.535	(1.900, 2.393)
	Super Senior (13)	2.1694	0.4917	(1.9641, 2.3747)
Occipital				
	Prime (6)	32.88	26.1	(21.05, 44.71)
	Mature (25)	23.29	18.44	(17.50, 29.09)
	Senior (9)	24.14	22.72	(14.49, 33.80)
	Super Senior (12)	27.31	20.55	(18.95, 35.67)
Hippocampus				
	Prime (6)	47.14	13.74	(27.73, 66.54)
	Mature (22)	44.47	22.02	(34.34, 54.61)
	Senior (7)	46.7	28.9	(28.7, 64.6)
	Super Senior (12)	47.36	26.54	(33.64, 61.09)

2.5. Positive immunolabelling of hyperphosphorylated tau (AT8 antibody) in the different age groups. Means of the one-way ANOVA and Kruskal-Wallis for the positive immunolabelling for hyperphosphorylated tau (AT8 antibody) in all age groups with standard deviations and 95% confidence interval (CI).

	Group (n)	Mean	St. Dev.	95% CI
Rostral				
% Load	Prime (5)	-0.088	0.506	(-0.711, 0.535)
	Mature (25)	-0.048	1.077	(-0.327, 0.230)
	Senior (9)	0.29	1.009	(-0.175, 0.754)
	Super Senior (13)	-0.03	0.94	(-0.416, 0.356)
Burden	Prime (5)	-0.1256	0.3051	(-0.3438, 0.0927)
	Mature (25)	0.047	0.967	(-0.227, 0.322)
	Senior (9)	0.292	1.054	(-0.232, 0.816)
	Super Senior (13)	-0.153	0.99	(-0.553, 0.247)
Parietal				
% Load	Prime (5)	-0.208	0.431	(-0.516, 0.100)
	Mature (25)	0.009	0.996	(-0.274, 0.292)
	Senior (9)	0.006	0.874	(-0.428, 0.441)
	Super Senior (14)	-0.261	1.118	(-0.695, 0.173)
Burden	Prime (5)	-0.26	0.464	(-0.839, 0.320)
	Mature (25)	0.185	0.863	(-0.075, 0.444)
	Senior (9)	0.137	1.021	(-0.295, 0.569)
	Super Senior (14)	-0.24	1.071	(-0.586, 0.107)
Occipital				
% Load	Prime (5)	2.458	2.478	(-3.344, 8.259)
	Mature (26)	7.26	9.81	(4.71, 9.80)
	Senior (9)	9.21	10.61	(4.88, 13.53)
	Super Senior (13)	5.62	8.59	(2.02, 9.22)
Burden	Prime (5)	0.7674	0.0789	(0.6735, 0.8612)
	Mature (26)	0.6873	0.1517	(0.6462, 0.7285)
	Senior (9)	0.6566	0.1695	(0.5866, 0.7265)
	Super Senior (13)	0.6951	0.1505	(0.6368, 0.7533)
Hippocampus				
% Load	Prime (4)	1.203	1.062	(-10.865, 13.271)
	Mature (25)	7.78	9.02	(2.95, 12.60)
	Senior (9)	9.12	6	(1.07, 17.16)
	Super Senior (14)	9.13	18.96	(2.68, 15.58)
Burden	Prime (4)	0.7939	0.0439	(0.6227, 0.9651)
	Mature (25)	0.5677	0.1644	(0.4992, 0.6362)
	Senior (9)	0.5101	0.1682	(0.3960, 0.6243)
	Super Senior (14)	0.6382	0.1983	(0.5467, 0.7297)
Cerebellum				
% Load	Prime (4)	2.58	2.72	(-6.44, 11.59)
	Mature (23)	8.67	10.58	(4.91, 12.43)
	Senior (7)	7.19	8.06	(0.37, 14.00)
	Super Senior (13)	3.73	6.84	(-1.27, 8.73)

	Group (n)	Median	Median Rank	Z-value
Cerebellum				
Burden	Prime (4)	13.5352	21.8	-0.34
	Mature (23)	37.3083	26.2	1.09
	Senior (7)	69.8205	24.9	0.18
	Super Senior (13)	5.6716	20.3	-1.14

2.6. Positive immunolabelling of hyperphosphorylated tau (AT100 antibody) in the different age groups. Means of the one-way ANOVA and Kruskal-Wallis for the positive immunolabelling of hyperphosphorylated tau (AT100 antibody) in all age groups with standard deviations and 95% confidence interval (CI).

	Group (n)	Mean	St. Dev.	95% CI
Rostral				
% Load	Prime (6)	1.476	0.758	(0.771, 2.182)
	Mature (22)	1.331	1.241	(0.963, 1.699)
	Senior (8)	1.103	1.427	(0.492, 1.714)
	Super Senior (13)	1.291	1.252	(0.812, 1.770)
Burden	Prime (6)	0.6184	0.0834	(0.5466, 0.6903)
	Mature (22)	0.6053	0.1229	(0.5678, 0.6428)
	Senior (8)	0.6291	0.154	(0.5669, 0.6914)
	Super Senior (13)	0.636	0.1259	(0.5872, 0.6848)
Parietal				
% Load	Prime (6)	0.953	1.006	(0.195, 1.711)
	Mature (24)	1.089	1.314	(0.710, 1.468)
	Senior (9)	1.086	1.57	(0.467, 1.705)
	Super Senior (13)	1.052	1.281	(0.537, 1.567)
Burden	Prime (6)	2.614	1.144	(1.887, 3.341)
	Mature (24)	3.195	1.673	(2.709, 3.681)
	Senior (9)	3.062	2.452	(1.843, 4.282)
	Super Senior (13)	2.968	1.516	(2.356, 3.581)
Occipital				
% Load	Prime (6)	1.2257	0.1737	(1.0850, 1.3664)
	Mature (25)	1.2222	0.245	(1.1532, 1.2911)
	Senior (9)	1.2025	0.301	(1.0876, 1.3174)
	Super Senior (12)	1.2353	0.2306	(1.1358, 1.3348)
Burden	Prime (6)	2.948	0.913	(2.368, 3.528)
	Mature (25)	3.414	1.593	(2.961, 3.867)
	Senior (9)	3.361	2.148	(2.293, 4.430)
	Super Senior (12)	3.362	1.298	(2.814, 3.910)
Hippocampus				
% Load	Prime (5)	1.193	0.255	(0.781, 1.606)
	Mature (24)	1.4478	0.3911	(1.2595, 1.6362)
	Senior (7)	1.307	0.532	(0.958, 1.656)
	Super Senior (12)	1.497	0.583	(1.230, 1.763)
Burden	Prime (5)	1.408	0.283	(1.022, 1.793)
	Mature (24)	1.63	0.3776	(1.4540, 1.8060)
	Senior (7)	1.539	0.546	(1.213, 1.864)
	Super Senior (12)	1.599	0.492	(1.350, 1.848)
Cerebellum				
% Load	Prime (5)	1.51	0.322	(1.050, 1.970)
	Mature (22)	1.67	0.539	(1.450, 1.889)
	Senior (8)	1.379	0.414	(1.015, 1.743)
	Super Senior (12)	1.365	0.562	(1.068, 1.662)

	Group (n)	Median	Median Rank	Z-value
Cerebellum				
Burden	Prime (5)	35.541	18.4	-0.97
	Mature (22)	153.8	28.1	1.92
	Senior (8)	60.695	22.5	-0.34
	Super Senior (12)	43.738	19.8	-1.22

2.7. Intranuclear immunolabelling of hyperphosphorylated tau in grey matter (GM) and white matter (WM). Means of the two-sample t-tests for the intranuclear immunolabelling of hyperphosphorylated tau with both AT8 and AT100 antibodies with standard deviations and SE of the means, between GM and WM.

AT8

	GM				WM			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
Rostral	52	25.5	19.7	2.7	52	10.13	8.83	1.2
Parietal	53	2.025	0.485	0.067	53	1.685	0.481	0.066
Occipital	53	2.77	1.07	0.15	53	1.83	1.07	0.15
Hippocampus	-	-	-	-	-	-	-	-

AT100

	GM				WM			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
Rostral	50	4.78	2.04	0.29	50	4.43	1.89	0.27
Parietal	51	2.385	0.501	0.07	51	1.908	0.428	0.06
Occipital	52	35.6	22	3	52	15.4	12.8	1.8
Hippocampus	-	-	-	-	-	-	-	-

2.8. Positive immunolabelling of hyperphosphorylated tau in grey matter (GM) and white matter (WM). Means of the two-sample t-tests for the positive immunolabelling of hyperphosphorylated tau deposits with both AT8 and AT100 antibodies with standard deviations and SE of the means, between GM and WM.

AT8

	GM				WM			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
ROSTRAL								
% Load	52	8	10.1	1.4	52	6.11	9.05	1.3
Burden	52	0.088	0.908	0.13	52	-0.041	0.99	0.14
PARIETAL								
% Load	53	7.3	10.4	1.4	53	5.88	8.7	1.2
Burden	53	91	188	26	53	118	242	33
OCCIPITAL								
% Load	53	7.8	9.27	1.3	53	5.67	9.28	1.3
Burden	53	134	232	32	53	191	494	68

AT100

	GM				WM			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
ROSTRAL								
% Load	49	1.467	0.961	0.14	49	1.13	1.42	0.2
Burden	49	0.617	0.109	0.016	49	0.62	0.139	0.02
PARIETAL								
% Load	52	1.39	1.09	0.15	52	0.74	1.43	0.2
Burden	52	3.12	1.68	0.23	52	2.98	1.79	0.25
OCCIPITAL								
% Load	52	1.285	0.207	0.029	52	1.159	0.26	0.036
Burden	52	3.54	1.39	0.19	52	3.13	1.72	0.24

2.9. Intranuclear immunolabelling of hyperphosphorylated tau in cats with and without cognitive dysfunction syndrome (CDS). Means of the two-sample t-tests for the intranuclear immunolabelling of hyperphosphorylated tau with both AT8 and AT100 antibodies with standard deviations and standard errors (SE) of the means, between cats with and without CDS.

AT8

	CDS				Intact			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
Rostral	9	16.3	15.5	3.6	13	20.7	17.4	3.4
Parietal	9	17.300	18.1	4.3	13	17.4	12.5	2.5
Occipital	8	16.4	17	4.3	13	18.8	17	3.3
Hippocampus	9	32.7	28.9	9.6	13	26.5	16.6	4.6

AT100

	CDS				Intact			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
Rostral	9	25.1	18.8	4.4	13	25.7	20.1	4
Parietal	9	23.4	18.9	4.4	13	23.1	18.8	3.7
Occipital	8	25.7	21.8	5.4	13	26.1	21.4	4.2
Hippocampus	7	42	31.3	12	12	50.1	24.5	7.1

2.10. Positive immunolabelling of hyperphosphorylated tau in cats with and without cognitive dysfunction syndrome (CDS). Means of the two-sample t-tests for the positive immunolabelling of hyperphosphorylated tau with both AT8 and AT100 antibodies with standard deviations and standard errors (SE) of the means, between cats with and without CDS.

AT8

	CDS				Intact			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
ROSTRAL								
% Load	8	0.92	1.61	0.4	14	1.18	1.58	0.3
Burden	8	-0.08	1.05	0.26	14	0.1	1.04	0.2
PARIETAL								
% Load	9	0.69	1.53	0.36	14	0.81	1.5	0.28
Burden	9	0.048	0.843	0.2	14	0.038	0.876	0.17
OCCIPITAL								
% Load	8	0.81	1.4	0.35	14	1.1	1.61	0.3
Burden	8	91	202	50	14	303	621	117
HIPPOCAMPUS								
% Load	9	1.5	1.6	0.53	14	1.18	1.32	0.35
Burden	9	0.551	0.214	0.071	14	0.57	0.2	0.053
CEREBELLUM								
% Load	8	-0.27	1.25	0.44	12	-0.003	0.866	0.25
Burden	8	-0.15	1.24	0.44	12	0.121	0.9	0.26

AT100

	CDS				Intact			
	N	Mean	StDev	SE Mean	N	Mean	StDev	SE Mean
ROSTRAL								
% Load	8	1.2	1.06	0.27	13	1.23	1.46	0.29
Burden	8	0.471	0.111	0.028	13	0.448	0.19	0.037
PARIETAL								
% Load	9	0.95	1.16	0.27	13	1.14	1.54	0.3
Burden	9	3	1.22	0.29	13	3.01	2.32	0.46
OCCIPITAL								
% Load	8	1.04	1.19	0.3	13	1.16	1.48	0.29
Burden	8	3.16	1.08	0.27	13	3.49	1.98	0.39
HIPPOCAMPUS								
% Load	7	1.838	0.671	0.25	12	1.353	0.629	0.18
Burden	7	1.964	0.609	0.23	12	1.626	0.661	0.19
CEREBELLUM								
% Load	7	1.1	1.57	0.59	13	1.01	1.64	0.45
Burden	7	3.73	1.51	0.57	13	3.79	2.05	0.57

3.1. Differences in the size of the structures in the different age groups. Means of the one-way ANOVA and Kruskal-Wallis for all structures and age groups with standard deviations and 95% confidence interval (CI) for live cats and fixed brains.

Structure	Group (n)	Mean	St. Dev.	95% CI
ITAr	Junior (12)	355.3	109.1	(305.9, 404.6)
	Prime (13)	280.9	92.6	(233.5, 328.4)
	Mature (21)	313.9	77.7	(276.5, 351.2)
	Senior (15)	333.6	69.8	(289.4, 377.7)
	Super Senior (9)	287.2	82.4	(230.1, 344.2)
LVr	Junior (11)	1.4766	0.3144	(1.1923, 1.7609)
	Prime (11)	1.714	0.524	(1.430, 1.999)
	Mature (19)	1.508	0.532	(1.297, 1.718)
	Senior (12)	1.402	0.508	(1.130, 1.675)
	Super Senior (8)	1.277	0.312	(0.943, 1.610)
TEMr	Junior (11)	13.57	6.58	(9.15, 17.99)
	Prime (11)	14.99	5.09	(11.57, 18.41)
	Mature (19)	14.265	3.976	(12.349, 16.182)
	Senior (12)	13.776	2.156	(12.406, 15.146)
	Super Senior (8)	14.89	3.09	(12.31, 17.48)
Total transversal area	Junior (11)	-0.318	0.552	(-0.868, 0.232)
	Prime (11)	-0.119	0.996	(-0.669, 0.431)
	Mature (21)	0.247	1.033	(-0.151, 0.645)
	Senior (12)	-0.111	0.873	(-0.637, 0.416)
	Super Senior (8)	0.071	0.884	(-0.574, 0.716)
HEMr	Junior (11)	33.371	2.247	(31.861, 34.880)
	Prime (12)	34.694	2.808	(32.910, 36.478)
	Mature (21)	35.692	2.64	(34.490, 36.893)
	Senior (11)	35.836	2.159	(34.386, 37.287)
	Super Senior (9)	36.756	1.819	(35.350, 38.161)
Total dorsal area	Junior (11)	13.659	1.577	(12.715, 14.604)
	Prime (12)	14.033	1.081	(13.129, 14.937)
	Mature (21)	14.181	1.666	(13.498, 14.865)
	Senior (12)	13.477	1.678	(12.573, 14.381)
	Super Senior (9)	14.017	1.693	(12.973, 15.061)
OCCr	Junior (11)	12.503	0.945	(11.868, 13.138)
	Prime (12)	10.702	1.662	(9.645, 11.758)
	Mature (21)	10.911	2.263	(9.881, 11.941)
	Senior (15)	11.934	1.19	(11.275, 12.593)
	Super Senior (11)	10.265	1.948	(8.956, 11.574)
FROr	Junior (11)	0.08387	0.01092	(0.07467, 0.09307)
	Prime (12)	0.0779	0.01147	(0.06909, 0.08671)
	Mature (21)	0.08239	0.01914	(0.07574, 0.08905)
	Senior (15)	0.08396	0.01237	(0.07608, 0.09184)
	Super Senior (11)	0.08178	0.01751	(0.07258, 0.09099)
Total sagittal area	Junior (11)	11.874	1.103	(11.177, 12.571)
	Prime (12)	11.348	1.118	(10.680, 12.015)
	Mature (21)	11.154	1.24	(10.649, 11.658)
	Senior (15)	11.232	1.149	(10.635, 11.829)
	Super Senior (11)	10.866	1.095	(10.169, 11.563)

Structure	Group (n)	Median	Mean Rank	Z-Value
HIPr	Junior (11)	18.4517	40.6	1.72
	Prime (11)	17.7237	32.1	0.02
	Mature (21)	11.8367	28	-1.21
	Senior (12)	17.3137	36.3	0.91
	Super Senior (8)	11.1698	23.9	-1.34

3.2. Differences in the size of the structures in the different age groups of live cats. Means of the one-way ANOVA for all structures and age groups with standard deviations and 95% confidence interval (CI) for live cats.

Structure	Group (n)	Mean	St. Dev.	95% CI
ITAr	Junior (12)	18.654	2.817	(17.220, 20.088)
	Prime (10)	15.767	2.926	(14.196, 17.338)
	Mature (9)	17.843	1.078	(16.187, 19.499)
	Senior (12)	17.885	2.043	(16.451, 19.319)
	Super Senior (3)	16.56	3.77	(13.69, 19.43)
LVr	Junior (11)	0.2666	0.1406	(0.1649, 0.3682)
	Prime (7)	0.1581	0.0606	(0.0307, 0.2855)
	Mature (8)	0.4942	0.1595	(0.3751, 0.6134)
	Senior (9)	0.4398	0.2479	(0.3275, 0.5522)
	Super Senior (3)	0.5154	0.0906	(0.3208, 0.7100)
TEMr	Junior (11)	6.784	3.292	(5.259, 8.310)
	Prime (8)	7.32	3	(5.53, 9.11)
	Mature (8)	5.609	1.787	(3.820, 7.398)
	Senior (9)	6.753	1.191	(5.067, 8.440)
	Super Senior (3)	6.637	1.685	(3.716, 9.559)
HIPr	Junior (11)	9.414	1.348	(8.539, 10.289)
	Prime (8)	9.625	0.978	(8.599, 10.651)
	Mature (9)	10.635	1.767	(9.667, 11.602)
	Senior (9)	9.732	1.63	(8.764, 10.699)
	Super Senior (3)	9.377	0.464	(7.702, 11.053)
Total transversal area	Junior (11)	0.01138	0.00185	(0.010057, 0.012703)
	Prime (8)	0.01148	0.00216	(0.009935, 0.013037)
	Mature (9)	0.01184	0.00223	(0.010384, 0.013309)
	Senior (9)	0.01175	0.00218	(0.010297, 0.013222)
	Super Senior (3)	0.01232	0.00304	(0.00979, 0.01486)
HEMr	Junior (11)	33.371	2.247	(31.955, 34.787)
	Prime (9)	33.721	2.508	(32.155, 35.287)
	Mature (9)	33.469	2.498	(31.903, 35.034)
	Senior (8)	35.128	2.065	(33.468, 36.789)
	Super Senior (3)	34.87	1.84	(32.16, 37.58)
Total dorsal area	Junior (11)	13.659	1.577	(12.685, 14.634)
	Prime (9)	13.901	1.233	(12.824, 14.978)
	Mature (9)	13.639	1.504	(12.562, 14.716)
	Senior (9)	13.17	1.734	(12.093, 14.247)
	Super Senior (3)	13.24	2.47	(11.37, 15.10)
OCCr	Junior (11)	12.503	0.945	(11.629, 13.377)
	Prime (9)	11.167	1.608	(10.202, 12.133)
	Mature (9)	12.095	1.997	(11.130, 13.061)
	Senior (11)	12.195	1.127	(11.321, 13.068)
	Super Senior (3)	12.36	1.349	(10.687, 14.032)
FROr	Junior (11)	12.108	1.597	(11.284, 12.931)
	Prime (9)	12.36	1.093	(11.450, 13.271)
	Mature (9)	11.505	1.326	(10.594, 12.416)
	Senior (11)	11.545	1.12	(10.721, 12.369)
	Super Senior (3)	11.37	1.94	(9.79, 12.94)
Total sagittal area	Junior (11)	11.874	1.103	(11.305, 12.443)
	Prime (9)	11.882	0.528	(11.254, 12.511)
	Mature (9)	12.091	0.941	(11.462, 12.719)
	Senior (11)	11.63	0.954	(11.062, 12.199)
	Super Senior (3)	11.776	1.104	(10.687, 12.866)

3.3. Differences in the size of the structures in the different age groups of fixed brains. Means of the one-way ANOVA for all structures and age groups with standard deviations and 95% confidence interval (CI) for fixed brains.

Structure	Group (n)	Mean	St. Dev.	95% CI
ITAr	Prime (3)	19.025	1.257	(16.050, 21.999)
	Mature (12)	17.381	2.879	(15.894, 18.868)
	Senior (3)	19.286	1.312	(16.312, 22.260)
	Super Senior (6)	16.878	2.202	(14.775, 18.981)
LVr	Prime (3)	6.6	2.08	(3.76, 9.44)
	Mature (12)	6.509	2.666	(5.088, 7.930)
	Senior (3)	7.4	2.25	(4.56, 10.25)
	Super Senior (5)	4.302	1.428	(2.101, 6.504)
TEMr	Prime (3)	7.967	0.633	(6.504, 9.429)
	Mature (11)	8.241	1.292	(7.477, 9.004)
	Senior (3)	7.292	0.623	(5.829, 8.754)
	Super Senior (5)	7.933	1.403	(6.800, 9.065)
HIPr	Prime (3)	4.292	0.416	(3.374, 5.210)
	Mature (12)	5.22	0.914	(4.761, 5.679)
	Senior (3)	5.799	0.753	(4.881, 6.717)
	Super Senior (5)	5.307	0.273	(4.596, 6.018)
Total transversal area	Prime (3)	-0.02	0.539	(-1.310, 1.269)
	Mature (12)	0.126	1.329	(-0.518, 0.771)
	Senior (3)	0.336	0.506	(-0.924, 1.655)
	Super Senior (5)	-0.703	0.522	(-1.701, 0.296)
HEMr	Prime (3)	37.614	1.07	(36.410, 38.818)
	Mature (12)	37.359	1.03	(36.757, 37.961)
	Senior (3)	37.724	1.014	(36.520, 38.928)
	Super Senior (6)	37.699	0.892	(36.848, 38.551)
Total dorsal area	Prime (3)	14.428	0.1671	(12.634, 16.222)
	Mature (12)	14.588	1.726	(13.691, 15.484)
	Senior (3)	14.398	1.325	(12.604, 16.192)
	Super Senior (6)	14.407	1.266	(13.138, 15.675)
OCCr	Prime (3)	9.304	0.978	(7.207, 11.401)
	Mature (12)	10.022	2.098	(8.974, 11.071)
	Senior (4)	11.216	1.2	(9.400, 13.032)
	Super Senior (8)	9.48	1.523	(8.196, 10.764)
FROr	Prime (3)	15.5	3.84	(11.23, 19.78)
	Mature (12)	13.85	4.06	(11.71, 15.99)
	Senior (4)	13.91	2.92	(10.21, 17.61)
	Super Senior (8)	13.26	2.88	(10.65, 15.88)
Total sagittal area	Prime (3)	9.744	0.788	(8.630, 10.858)
	Mature (12)	10.451	0.945	(9.894, 11.008)
	Senior (4)	10.135	0.969	(9.170, 11.100)
	Super Senior (8)	10.524	0.936	(9.842, 11.207)

3.4. Sex-related differences in the size of the structures. Means of the two-sample t-tests and medians of Mann-Whitney tests for all structures and sex with standard deviations and standard error (SE) of the means for live cats and fixed brains.

Structure	Lowest mean				Highest mean			
	Group (n)	Mean	St. Dev.	SE Mean	Group (n)	Mean	St. Dev.	SE Mean
ITAr	Female (22)	314.1	65.8	14	Male (43)	321.3	92.5	14
LVr	Male (37)	1.412	0.484	0.08	Female (20)	1.536	0.46	0.1
TEMr	Male (37)	14.08	4.41	0.73	Female (20)	14.22	4.46	1
HIPr	Male (38)	16.62	4.69	0.76	Female (20)	16.76	5.82	1.3
Total transversal area	Female (20)	-0.23	1.01	0.23	Male (38)	0.039	0.869	0.14
HEMr	Male (39)	34.61	2.52	0.4	Female (20)	35.96	2.48	0.56
Total dorsal area	Female (21)	13.13	1.5	0.33	Male (39)	14.3	1.45	0.23
OCCr	Female (24)	11.18	1.7	0.35	Male (41)	11.57	1.83	0.29
FROr	Male (41)	12.53	2.42	0.38	Female (24)	12.67	2.57	0.52
Total sagittal area	Female (24)	10.66	1.03	0.21	Male (41)	11.75	1.03	0.16

Structure	Group (n)	Median	Difference	CI difference	Achieved confidence
HIPr	Female (20)	17.9492	-0.46445	(-3.05195, 2.87652)	95.13%
	Male (38)	17.6897			

3.5. Sex-related differences in the size of the structures of live cats. Means of the two-sample t-tests for all structures and sex with standard deviations and standard error (SE) of the means for live cats.

Structure	Lowest mean				Highest mean			
	Group (n)	Mean	St. Dev.	SE Mean	Group (n)	Mean	St. Dev.	SE Mean
ITAr	Female (13)	17.52	1.78	0.49	Male (33)	17.54	2.86	0.5
LVr	Male (27)	-1.167	0.68	0.13	Female (12)	-1.134	0.827	0.24
TEMr	Female (12)	6.54	2.68	0.77	Male (27)	6.68	2.35	0.45
HIPr	Male (28)	9.51	1.33	0.25	Female (12)	10.48	1.5	0.43
Total transversal area	Female (12)	8.843	0.555	0.16	Male (28)	9.6	0.894	0.17
HEMr	Male (29)	33.79	2.38	0.44	Female (11)	34.42	2.15	0.65
Total dorsal area	Female (12)	12.25	1.11	0.32	Male (29)	14.11	1.36	0.25
OCCr	Female (12)	12.05	1.18	0.34	Male (31)	12.05	1.55	0.28
FROr	Male (31)	11.82	1.33	0.24	Female (12)	11.88	1.41	0.41
Total sagittal area	Female (12)	11.287	0.792	0.23	Male (31)	12.071	0.854	0.15

3.6. Sex-related differences in the size of the structures of fixed brains. Means of the two-sample t-tests for all structures and sex with standard deviations and standard error (SE) of the means for fixed brains.

Structure	Lowest mean				Highest mean			
	Group (n)	Mean	St. Dev.	SE Mean	Group (n)	Mean	St. Dev.	SE Mean
ITAr	Female (9)	17.78	2.24	0.75	Male (10)	18.37	1.84	0.58
LVr	Male (10)	5.06	2.53	0.8	Female (8)	6.97	2.26	0.8
TEMr	Female (8)	7.975	0.881	0.31	Male (10)	8.03	1.42	0.45
HIPr	Male (10)	4.962	0.667	0.21	Female (8)	5.236	0.791	0.28
Total transversal area	Female (8)	11.37	0.614	0.22	Male (10)	11.49	1.19	0.37
HEMr	Male (10)	36.99	0.841	0.27	Female (9)	37.96	0.804	0.27
Total dorsal area	Female (9)	14.29	1.14	0.38	Male (10)	14.83	1.66	0.52
OCCr	Male (10)	10.08	1.87	0.59	Female (12)	10.3	1.73	0.5
FROr	Female (12)	13.47	3.23	0.93	Male (10)	14.74	3.61	1.1
Total sagittal area	Female (12)	10.02	0.841	0.24	Male (10)	10.77	0.914	0.29

3.7. Differences in the size of the structures in cats with and without cognitive dysfunction syndrome (CDS). Means of the two-sample t-tests for all structures and cognitive status with standard deviations and standard error (SE) of the means for live cats and fixed brains.

Structure	Lowest mean				Highest mean			
	Group (n)	Mean	St. Dev.	SE Mean	Group (n)	Mean	St. Dev.	SE Mean
ITAr	Intact (20)	17.62	2.19	0.49	CDS (4)	17.76	2.97	1.5
LVr	CDS (2)	0.283	0.237	0.17	Intact (18)	0.372	0.22	0.052
TEMr	CDS (2)	6.741	0.543	0.38	Intact (18)	7.15	1.34	0.32
HIPr	Intact (18)	7.97	2.45	0.58	CDS (2)	8.11	2.1	1.5
Total transversal area	Intact (18)	9.93	1.27	0.3	CDS (2)	11.26	0.811	0.57
HEMr	Intact (18)	35.96	1.91	0.45	CDS (2)	38.9	0.334	0.24
Total dorsal area	Intact (19)	13.47	1.56	0.36	CDS (2)	15.97	0.547	0.39
OCCr	CDS (4)	9.43	2.45	1.2	Intact (22)	11.56	1.42	0.3
FROr	Intact (23)	12.24	2.29	0.48	CDS (3)	13.79	2.02	1.2
Total sagittal area	CDS (4)	10.73	1.25	0.63	Intact (22)	11.14	1.11	0.24

3.8. Differences in the size of the structures in cats with and without cognitive dysfunction syndrome (CDS) in the fixed brains. Means of the one-way ANOVA for all structures and cognitive status with standard deviations and 95% confidence interval (CI); and the means of the two-sample t-tests for all structures and cognitive status with standard deviations and standard error (SE) of the means for fixed brains.

One-way ANOVA

Structure	Lowest mean				Highest mean			
	Group (n)	Mean	St. Dev.	95% CI	Group (n)	Mean	St. Dev.	95% CI
ITAr	Intact (8)	17.55	2.33	(15.600, 19.497)	CDS (1)	18.74	*	(13.23, 24.25)
LVr	Intact (7)	0.216	0.1254	(0.0998, 0.3317)	CDS (1)	0.2507	*	(-0.0561, 0.5575)
TEMr	Intact (7)	7.632	1.24	(6.486, 8.779)	CDS (1)	8.113	*	(5.079, 11.146)
HIPr	Intact (7)	5.525	0.551	(5.015, 6.035)	CDS (1)	5.256	*	(3.907, 6.604)
Total transversal area	Intact (7)	11.19	0.697	(10.541, 11.829)	CDS (1)	11.36	*	(9.65, 13.06)

Two-sample t-test

Structure	Lowest mean				Highest mean			
	Group (n)	Mean	St. Dev.	SE Mean	Group (n)	Mean	St. Dev.	SE Mean
HEMr	Intact (7)	37.45	0.798	0.3	CDS (2)	38.618	0.297	0.21
Total dorsal area	Intact (7)	13.96	0.907	0.34	CDS (2)	15.97	0.547	0.39
OCCr	CDS (4)	9.43	2.45	1.2	Intact (8)	10.37	1.08	0.38
FROr	CDS (4)	11.71	1.814	0.92	Intact (8)	14.36	2.83	1
Total sagittal area	Intact (8)	10.23	0.754	0.27	CDS (4)	10.73	1.25	0.63

3.9. Differences in the size of the structures in live cats and fixed brains. Means of the two-sample t-tests for all structures with standard deviations and standard error (SE) of the means for the comparison of live cats and fixed brains scans.

Structure	Lowest mean				Highest mean			
	Group (n)	Mean	St. Dev.	SE Mean	Group (n)	Mean	St. Dev.	SE Mean
ITAr	Live (34)	17.13	2.41	0.41	Fixed (24)	17.7	2.47	0.5
LVr	Live (28)	1.815	0.692	0.13	Fixed (23)	2.426	0.536	0.11
TEMr	Live (28)	6.58	2.06	0.39	Fixed (22)	8	1.161	0.25
HIPr	Fixed (23)	5.193	0.814	0.17	Live (29)	9.95	1.46	0.27
Total transversal area	Live (29)	9.337	0.897	0.17	Fixed (23)	11.317	0.975	0.2
HEMr	Live (29)	34.15	2.33	0.43	Fixed (24)	37.522	0.948	0.19
Total dorsal area	Live (30)	13.54	1.55	0.28	Fixed (24)	14.5	1.39	0.28
OCCr	Fixed (27)	9.96	1.76	0.34	Live (32)	11.89	1.56	0.28
FROr	Fixed (27)	13.87	3.43	0.66	Live (32)	11.75	1.25	0.22
Total sagittal area	Fixed (27)	10.347	0.913	0.18	Live (32)	11.844	0.841	0.15

4.1 Questionnaire 1

Surname ……..……

Address ……

……..……..….Post Code ……..…..……

Telephone ……

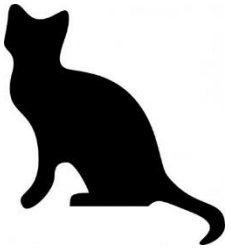
Email ……..……

Cat's Name ……..

Breed ……..…..

Age ……..…years …….months

How long has this cat been living with you? ..…years ….months



Gender: Male ☐ Male neutered ☐

Female ☐ Female neutered ☐

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1) Does your cat have outdoor access? Yes ☐ No

2) Does your cat have a litter tray indoors? Yes ☐ No ☐

3) Has your cat been diagnosed with any new medical conditions? Yes ☐ No ☐

If yes, please state what: ……

4) Is your cat currently on any medication? Yes ☐ No ☐

If yes, please state what: ……

(Include all food supplements, herbal remedies, over-the-counter medications, and any medications prescribed by your vet)

5) Would you be willing to answer several questionnaires, every 3 months, for no more than a year, as part of a study into senility (cognitive decline) in elderly cats? Yes ☐ No ☐

Please answer the questions below by placing a tick in the appropriate column. ***For each question, think about how your cat used to be (perhaps a few years ago) and compare this with how he/she is now.*** If you have noticed a change please indicate how significant you think this change has been.

	Significant decrease	**Mild decrease**	**No change**	**Mild increase**	**Significant increase**
Change in weight					
Appetite					
Drinking					
Time spent grooming					
Time spent sleeping at night					
Time spent sleeping during the day					
Vocalisation at night					
Vocalisation during the day					
Affection with people in the house					
Tolerance of handling					
Aggression towards animals or people					
Wanting to spend time outdoors					
Activity levels/time spent playing					
Tolerance of being left alone					

(Please read and complete the next page)

	Significant decrease	Mild decrease	No change	Mild increase	Significant increase
Tolerance of other animals in the house					
Willingness to jump up or down (including climbing stairs)					
Aimless activity (e.g. pacing, staring into space)					
Repetitive or compulsive behaviour (e.g. grooming, licking inanimate objects)					
Agitation and restlessness					
Passing faeces in the house outside litter tray					
Passing urine in the house outside litter tray					
Vomiting (including fur balls)					
Diarrhoea					
Constipation					
Vision loss					
Hearing loss					
Hair loss or hair thinning					

How long ago did you first notice any changes listed above?...……...……...

Are there any other problems you think it may be useful for us to know?……..…

Thank you for completing this survey. Please return all pages in the stamped self-addressed envelope as soon as possible.

4.2. Cognitive decline inclusion criteria

	Weighting	Case Number
Change in weight	2	
Appetite	2	
Drinking	3	
Time spent grooming	1	
Time spent sleeping at night	1	
Time spent sleeping during the day	1	
Vocalisation at night	1*	
Vocalisation during the day	1	
Affection with people in the house	1*	
Tolerance of handling	2	
Aggression towards animals or people	2	
Wanting to spend time outdoors	2	
Activity levels/time spent playing	2	
Tolerance of being left alone	1*	
Tolerance of other animals in the house	2	
Willingness to jump up or down (including climbing stairs)	3	
Aimless activity (eg pacing, staring into space)	1	
Repetitive or compulsive behaviour (eg grooming, licking inanimate objects)	1	
Agitation and restlessness	2	
Passing faeces in house outside litter tray	2	
Passing urine in house outside litter tray	2	
Vomiting (including fur-balls)	2	
Diarrhoea	3	
Constipation	3	
Vision loss	3	
Hearing loss	3	
Hair loss or hair thinning	3	
TOTAL of each number FOR THIS CAT:		See below:

1* = 1

1 = 3
2 = 5
3 = 3

To be included in the trial a cat must score a minimum of:

- one x1* or
- two x 1 or
- four x 2.

4.3. Medication information

Feline Cognitive Dysfunction Study

Medication Information

Dose: 1 mg/kg Telmisartan

Bottle concentration: placebo or 4 mg/ml Telmisartan → 0.25 ml/kg PO SID

Bottle volume: 30 mL in each

Bottles of medication needed per cat:

Weight	Number of Bottles
2.6 kg or less	2
2.7 kg – 4.0 kg	3
4.1 kg – 5.3 kg	4
5.4 kg – 6.6 kg	5
6.7 kg or more	6

4.4. Correlation between age and behaviours. Ordinal logistic regression for the correlation between age and the different behaviours; statistically significant behaviours are shown in bold

Ordinal logistic regression		
Behaviour	**Coef.**	***p* value**
Activity levels	0.043	0.71
Affection with people in the house	-0.014	0.91
Agitation and restlessness	0.151	0.25
Aggression	0.061	0.75
Aimless activity	0.057	0.65
Appetite	0.111	0.33
Diarrhoea	0.021	0.91
Drinking	-0.131	0.26
Grooming	0.194	0.13
Hair loss	0.048	0.72
Hearing loss	0.060	0.70
Passing faeces outside litter box	-0.421	**0.05**
Passing urine outside litter box	-0.262	0.22
Repetitive/compulsive behaviour	0.590	**0.01**
Sleep during the day	0.068	0.55
Sleep during the night	-0.045	0.70
Time spent outside	0.064	0.61
Tolerance of being handled	0.063	0.59
Tolerance of being left alone	-0.156	0.20
Tolerance of other animals	-0.151	0.37
Vision loss	0.204	0.25
Vocalisations during the night	-0.011	0.94
Vocalisations during the day	-0.115	0.33
Vomiting	-0.137	0.29
Weight	0.323	**0.01**
Willingness to jump	0.192	0.12

4.5. Differences in the behaviours for treatment A. Table with the least squares means, standard error and p value of the general linear model for repeated measures, for all the behaviours at the different time points for treatment A

Treatment A			
Behaviour	**Least Squares Means**	**Standard Error**	***p* value**
Activity levels			
30 days	0.066	0.121	0.587
60 days	0.289	0.152	0.066
90 days	0.117	0.170	0.496
120 days	-0.276	0.142	0.062
Affection			
30 days	0.221	0.157	0.168
60 days	0.641	0.182	**0.001**
90 days	0.513	0.162	**0.003**
120 days	0.035	0.181	0.849
Agitation/restlessness			
30 days	-0.096	0.181	0.597
60 days	0.045	0.213	0.834
90 days	-0.071	0.196	0.718
120 days	0.631	0.202	**0.004**
Aimless activity			
30 days	-0.150	0.193	0.446
60 days	-0.282	0.227	0.225
90 days	-0.131	0.176	0.462
120 days	0.324	0.186	0.093
Appetite			
30 days	0.310	0.214	0.159
60 days	0.132	0.228	0.566
90 days	0.309	0.245	0.218
120 days	0.137	0.222	0.543
Drinking			
30 days	0.363	0.183	0.056
60 days	0.392	0.157	**0.018**
90 days	0.395	0.174	**0.030**
120 days	-0.044	0.162	0.787
Grooming			
30 days	0.166	0.907	0.076
60 days	0.195	0.087	**0.032**
90 days	0.048	0.138	0.729
120 days	-0.087	0.164	0.599
Hair loss			
30 days	-0.149	0.088	0.102
60 days	-0.078	0.153	0.615
90 days	-0.065	0.105	0.539
120 days	0.265	0.143	0.075
Passing faeces outside litter box			
30 days	0.001	0.130	0.997
60 days	0.101	0.103	0.335
90 days	0.236	0.158	0.147
120 days	0.231	0.136	0.100
Passing urine outside litter box			
30 days	-0.081	0.124	0.518
60 days	-0.214	0.191	0.273
90 days	0.158	0.192	0.418
120 days	0.315	0.176	0.085
Repetitive/compulsive behaviour			
30 days	0.141	0.086	0.113
60 days	0.101	0.157	0.523
90 days	0.055	0.142	0.701
120 days	-0.245	0.193	0.215

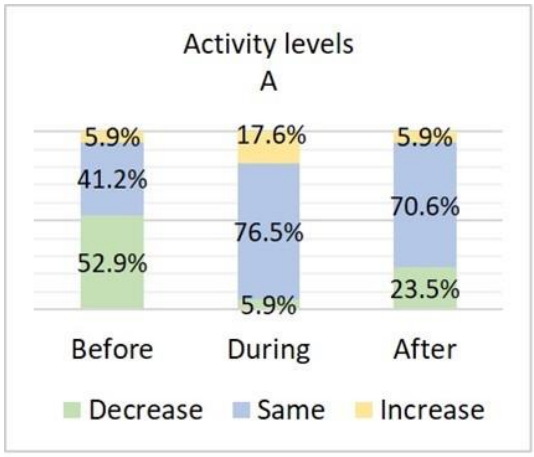
Treatment A			
Behaviour	**Least Squares Means**	**Standard Error**	***p* value**
Sleeping during the day			
30 days	0.166	0.168	0.329
60 days	0.032	0.172	0.852
90 days	0.148	0.174	0.404
120 days	0.159	0.193	0.417
Sleeping during the night			
30 days	0.110	0.187	0.562
60 days	0.288	0.208	0.175
90 days	0.265	0.172	0.133
120 days	-0.135	0.169	0.432
Time spent outside			
30 days	0.475	0.192	**0.020**
60 days	0.038	0.220	0.864
90 days	-0.187	0.208	0.378
120 days	-0.011	0.186	0.954
Tolerance of being handled			
30 days	-0.034	0.150	0.821
60 days	0.012	0.186	0.949
90 days	0.266	0.184	0.157
120 days	-0.264	0.161	0.113
Tolerance of being left alone			
30 days	-0.131	0.123	0.294
60 days	-0.054	0.091	0.559
90 days	-0.088	0.129	0.502
120 days	-0.177	0.148	0.241
Tolerance of other animals			
30 days	0.002	0.103	0.983
60 days	-0.095	0.081	0.255
90 days	-0.103	0.058	0.086
120 days	-0.228	0.173	0.200
Vocalisations during night			
30 days	-0.287	0.217	0.196
60 days	0.053	0.254	0.837
90 days	-0.202	0.221	0.370
120 days	0.564	0.219	**0.015**
Vocalisations during day			
30 days	0.096	0.218	0.663
60 days	0.178	0.216	0.415
90 days	0.170	0.199	0.399
120 days	0.685	0.221	**0.004**
Vomiting			
30 days	-0.016	0.121	0.897
60 days	0.335	0.151	**0.035**
90 days	0.115	0.165	0.492
120 days	0.216	0.152	0.166
Weight			
30 days	-0.158	0.140	0.270
60 days	-0.067	0.154	0.666
90 days	-0.150	0.212	0.485
120 days	-0.382	0.198	0.063
Willingness to jump			
30 days	0.058	0.097	0.551
60 days	0.006	0.139	0.968
90 days	-0.113	0.140	0.425
120 days	0.060	0.093	0.520

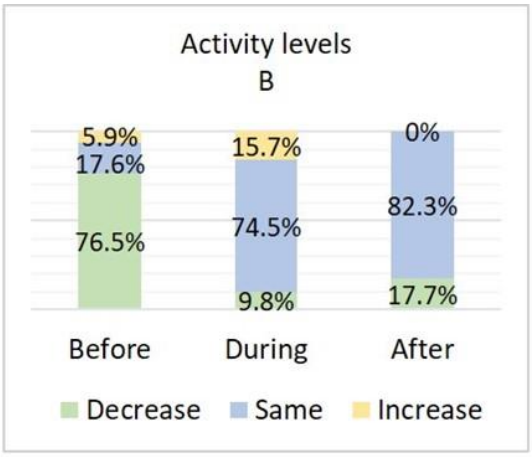
4.6. Differences in the behaviours for treatment B. Table with the least squares means, standard error and p value of the general linear model for repeated measures, for all the behaviours at the different time points for treatment B

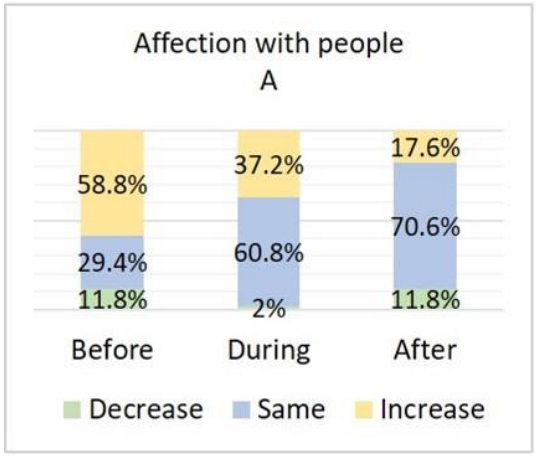
Treatment B			
Behaviour	**Least Squares Means**	**Standard Error**	***p* value**
Activity levels			
30 days	-0.276	0.142	0.122
60 days	0.020	0.160	0.903
90 days	-0.007	0.176	0.967
120 days	-0.228	0.145	0.127
Affection			
30 days	0.283	0.166	0.099
60 days	0.487	0.192	**0.017**
90 days	0.492	0.166	**0.006**
120 days	0.530	0.185	**0.008**
Agitation/restlessness			
30 days	-0.146	0.192	0.451
60 days	0.059	0.219	0.791
90 days	0.045	0.196	0.821
120 days	0.549	0.202	**0.011**
Aimless activity			
30 days	-0.029	0.198	0.887
60 days	-0.079	0.241	0.747
90 days	-0.062	0.184	0.738
120 days	0.204	0.187	0.284
Appetite			
30 days	0.158	0.227	0.493
60 days	0.203	0.240	0.405
90 days	0.035	0.254	0.890
120 days	-0.247	0.227	0.285
Drinking			
30 days	-0.042	0.194	0.831
60 days	0.131	0.165	0.433
90 days	0.299	0.176	0.099
120 days	0.279	0.165	0.102
Grooming			
30 days	0.104	0.096	0.289
60 days	0.075	0.091	0.412
90 days	0.066	0.137	0.633
120 days	-0.008	0.168	0.961
Hair loss			
30 days	0.063	0.091	0.498
60 days	0.055	0.158	0.729
90 days	0.070	0.103	0.501
120 days	0.134	0.132	0.321
Passing faeces outside litter box			
30 days	-0.078	0.131	0.556
60 days	0.085	0.102	0.411
90 days	0.040	0.159	0.803
120 days	0.186	0.130	0.164
Passing urine outside litter box			
30 days	-0.187	0.128	0.155
60 days	0.004	0.197	0.983
90 days	0.005	0.196	0.988
120 days	0.139	0.164	0.404
Repetitive/compulsive behaviour			
30 days	-0.094	0.090	0.303
60 days	0.120	0.164	0.469
90 days	0.110	0.147	0.460
120 days	0.275	0.185	0.149

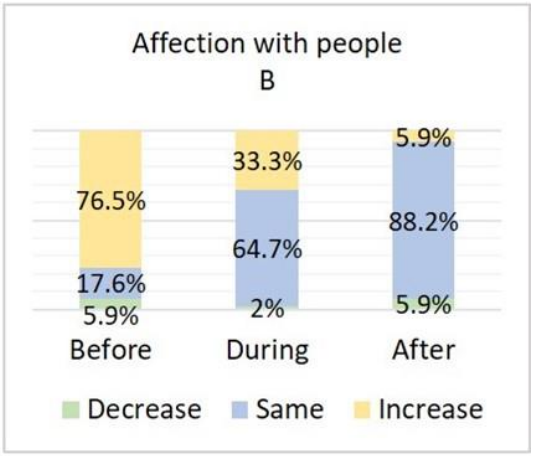
Treatment B			
Behaviour	**Least Squares Means**	**Standard Error**	***p* value**
Sleeping during the day			
30 days	0.104	0.178	0.564
60 days	-0.036	0.181	0.842
90 days	0.064	0.182	0.729
120 days	0.126	0.207	0.548
Sleeping during the night			
30 days	0.358	0.198	0.080
60 days	0.386	0.219	0.089
90 days	0.137	0.176	0.444
120 days	-0.330	0.174	0.068
Time spent outside			
30 days	0.152	0.198	0.449
60 days	-0.165	0.215	0.448
90 days	0.156	0.204	0.450
120 days	0.109	0.180	0.548
Tolerance of being handled			
30 days	0.115	0.159	0.477
60 days	0.280	0.195	0.162
90 days	0.148	0.184	0.428
120 days	-0.070	0.166	0.678
Tolerance of being left alone			
30 days	-0.029	0.130	0.824
60 days	0.067	0.096	0.490
90 days	-0.097	0.132	0.468
120 days	-0.350	0.152	**0.028**
Tolerance of other animals			
30 days	-0.069	0.100	0.498
60 days	0.070	0.076	0.364
90 days	-0.016	0.051	0.753
120 days	-0.169	0.161	0.305
Vocalisations during night			
30 days	-0.297	0.230	0.206
60 days	0.098	0.267	0.715
90 days	-0.350	0.219	0.121
120 days	0.725	0.224	**0.003**
Vocalisations during day			
30 days	-0.018	0.227	0.937
60 days	0.097	0.227	0.671
90 days	0.187	0.195	0.346
120 days	0.547	0.237	**0.028**
Vomiting			
30 days	-0.074	0.123	0.549
60 days	0.103	0.154	0.509
90 days	0.103	0.157	0.515
120 days	0.235	0.146	0.119
Weight			
30 days	0.165	0.149	0.276
60 days	-0.009	0.162	0.955
90 days	-0.089	0.213	0.679
120 days	-0.207	0.204	0.318
Willingness to jump			
30 days	0.124	0.103	0.235
60 days	-0.059	0.146	0.687
90 days	-0.036	0.141	0.799
120 days	-0.064	0.095	0.502

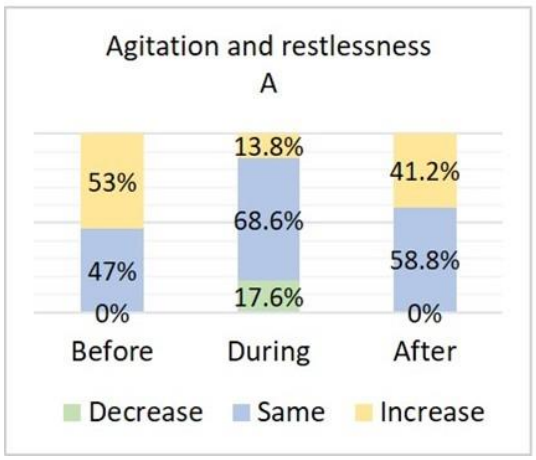
4.7. Changes in the behaviours through time. Changes in the different behaviours (in alphabetical order) as reported by the owners and demonstrated by the percentage of cats displaying an increase, decrease, or the same for both treatments, before, during, and after the trial

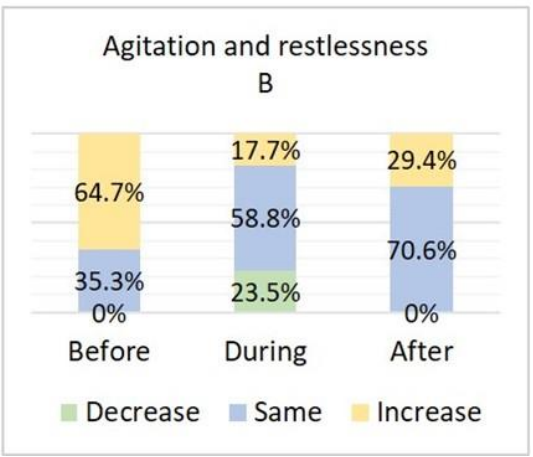


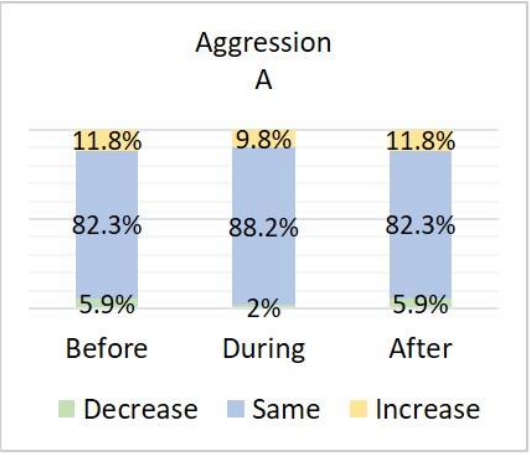


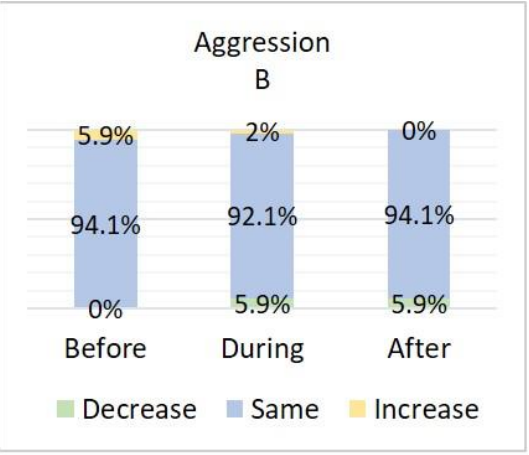


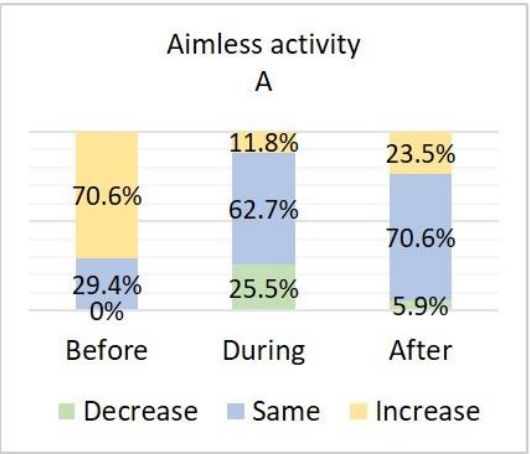


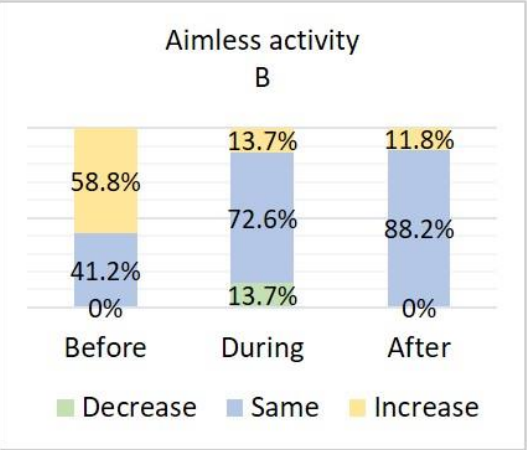


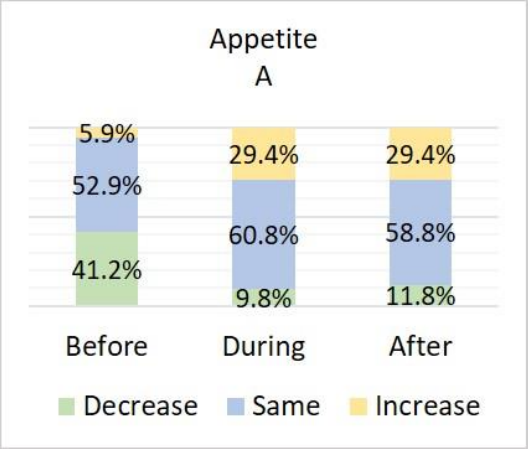


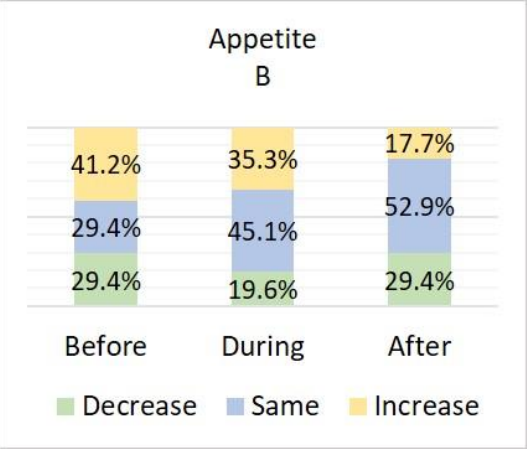

Aggression
A
11.8%
82.3%
5.9%
9.8%
88.2%
2%
11.8%
82.3%
5.9%
Before
During
After
Decrease
Same
Increase

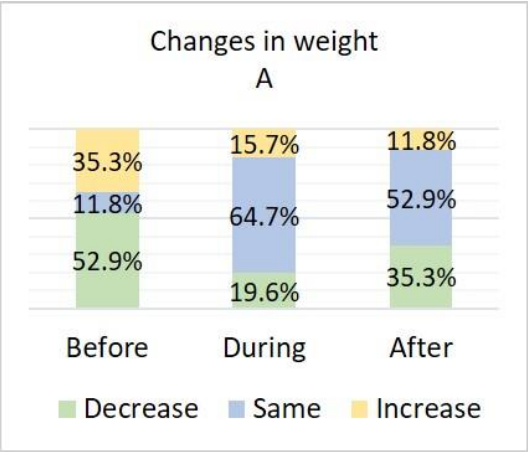

Aggression
B
5.9%
94.1%
0%
2%
92.1%
5.9%
0%
94.1%
5.9%
Before
During
After
Decrease
Same
Increase

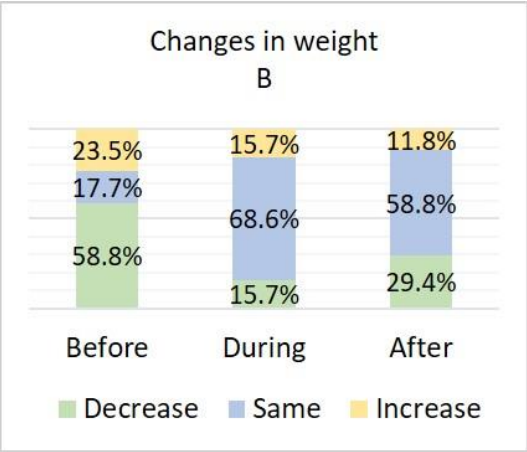

Aimless activity
A
70.6%
29.4%
0%
11.8%
62.7%
25.5%
23.5%
70.6%
5.9%
Before
During
After
Decrease
Same
Increase

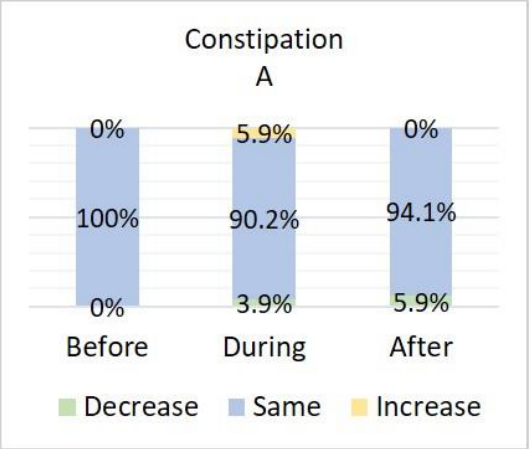

Aimless activity
B
58.8%
41.2%
0%
13.7%
72.6%
13.7%
11.8%
88.2%
0%
Before
During
After
Decrease
Same
Increase

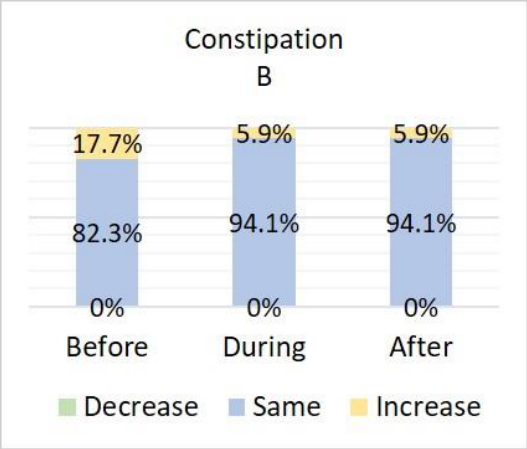

Appetite
A
5.9%
52.9%
41.2%
29.4%
60.8%
9.8%
29.4%
58.8%
11.8%
Before
During
After
Decrease
Same
Increase

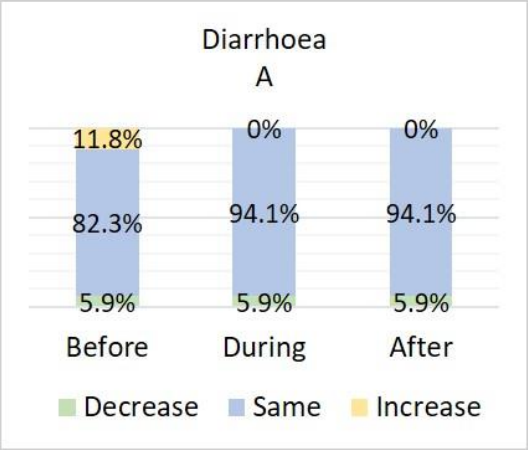

Appetite
B
41.2%
29.4%
29.4%
35.3%
45.1%
19.6%
17.7%
52.9%
29.4%
Before
During
After
Decrease
Same
Increase

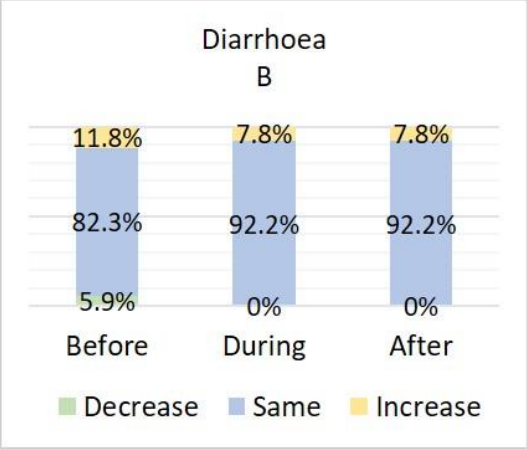

Changes in weight
A
35.3%
11.8%
52.9%
15.7%
64.7%
19.6%
11.8%
52.9%
35.3%
Before
During
After
Decrease
Same
Increase

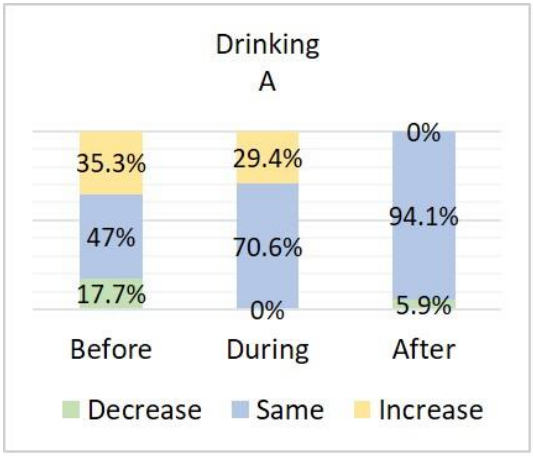

Changes in weight
B
23.5%
17.7%
58.8%
15.7%
68.6%
15.7%
11.8%
58.8%
29.4%
Before
During
After
Decrease
Same
Increase

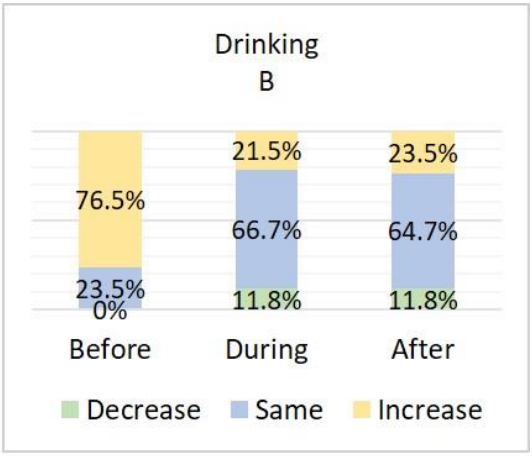

Constipation
A
0%
100%
0%
5.9%
90.2%
3.9%
0%
94.1%
5.9%
Before
During
After
Decrease
Same
Increase

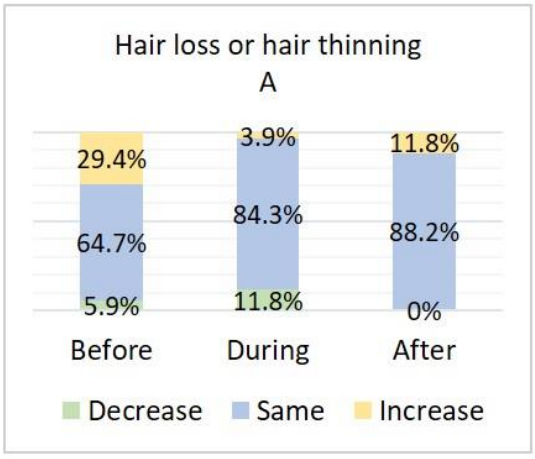

Constipation
B
17.7%
82.3%
0%
5.9%
94.1%
0%
5.9%
94.1%
0%
Before
During
After
Decrease
Same
Increase

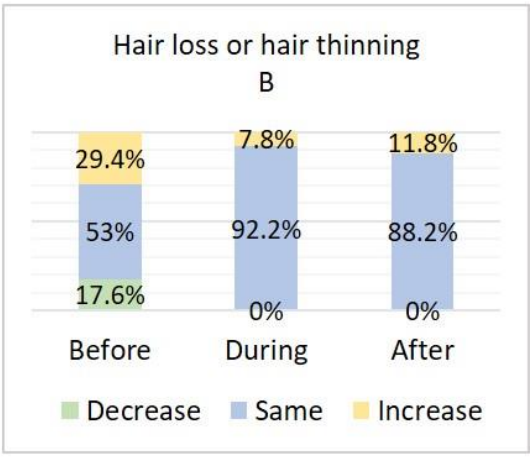

Diarrhoea
A
11.8%
82.3%
5.9%
0%
94.1%
5.9%
0%
94.1%
5.9%
Before
During
After
Decrease
Same
Increase

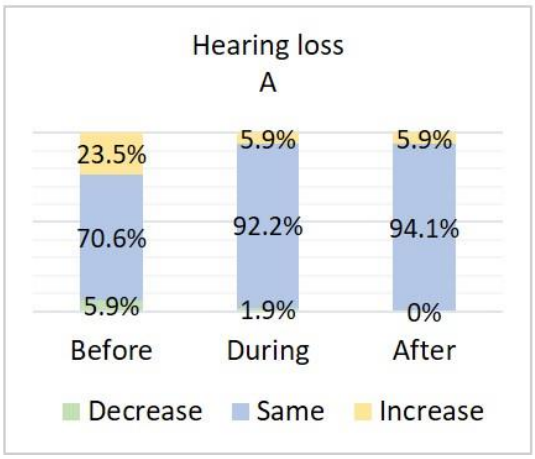

Diarrhoea
B
11.8%
82.3%
5.9%
7.8%
92.2%
0%
7.8%
92.2%
0%
Before
During
After
Decrease
Same
Increase

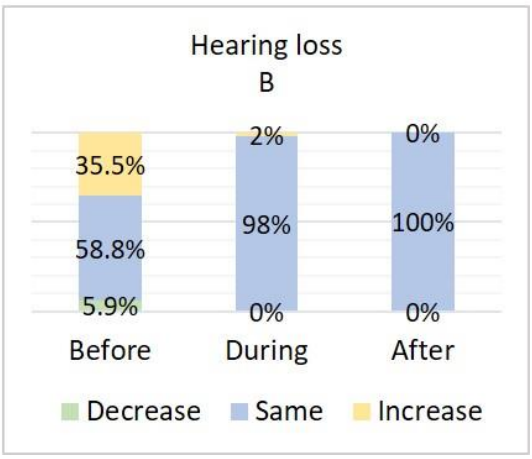

Drinking
A
35.3%
47%
17.7%
29.4%
70.6%
0%
0%
94.1%
5.9%
Before
During
After
Decrease
Same
Increase

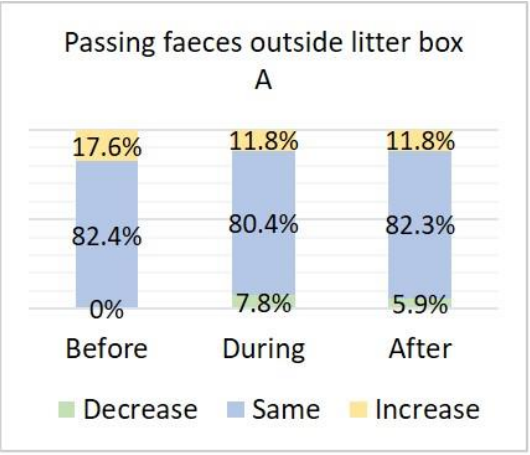

Drinking
B
76.5%
23.5%
0%
21.5%
66.7%
11.8%
23.5%
64.7%
11.8%
Before
During
After
Decrease
Same
Increase

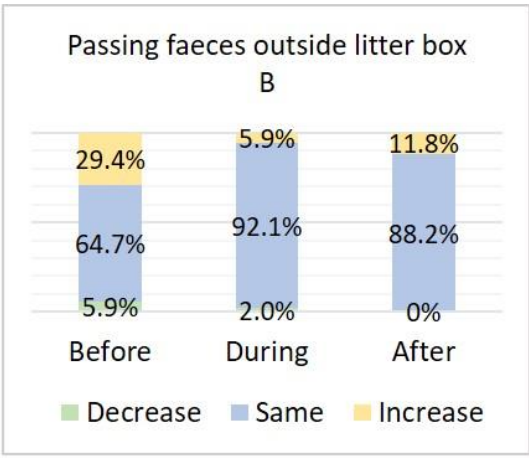

Hair loss or hair thinning
A
29.4%
64.7%
5.9%
3.9%
84.3%
11.8%
11.8%
88.2%
0%
Before
During
After
Decrease
Same
Increase

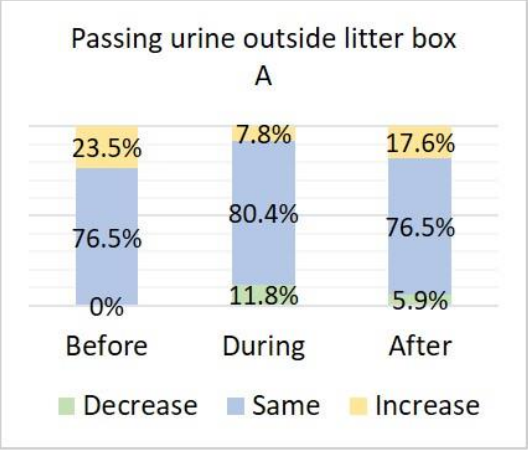

Hair loss or hair thinning
B
29.4%
53%
17.6%
7.8%
92.2%
0%
11.8%
88.2%
0%
Before
During
After
Decrease
Same
Increase

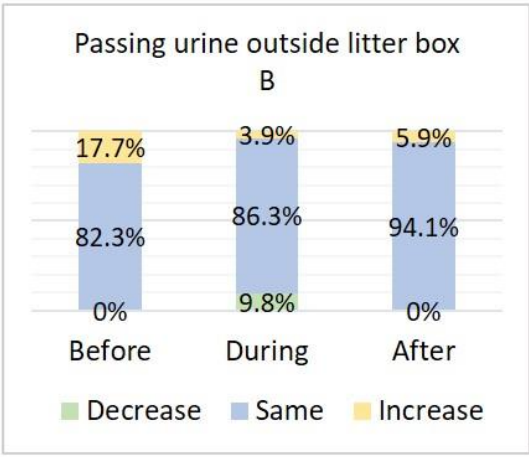

Hearing loss
A
23.5%
70.6%
5.9%
5.9%
92.2%
1.9%
5.9%
94.1%
0%
Before
During
After
Decrease
Same
Increase

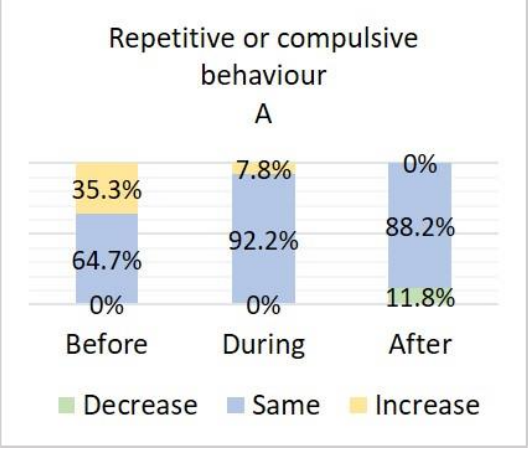

Hearing loss
B
35.5%
58.8%
5.9%
2%
98%
0%
0%
100%
0%
Before
During
After
Decrease
Same
Increase

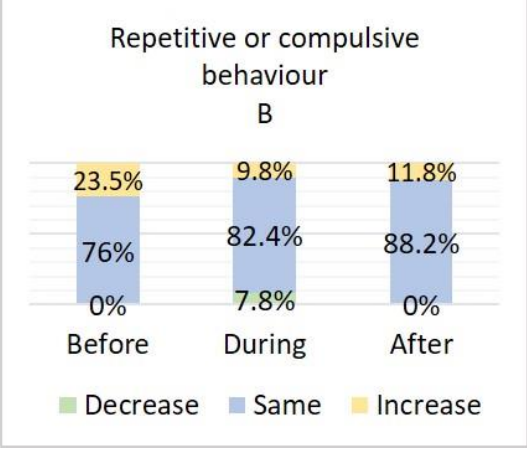

Passing faeces outside litter box
A
17.6%
82.4%
0%
11.8%
80.4%
7.8%
11.8%
82.3%
5.9%
Before
During
After
Decrease
Same
Increase

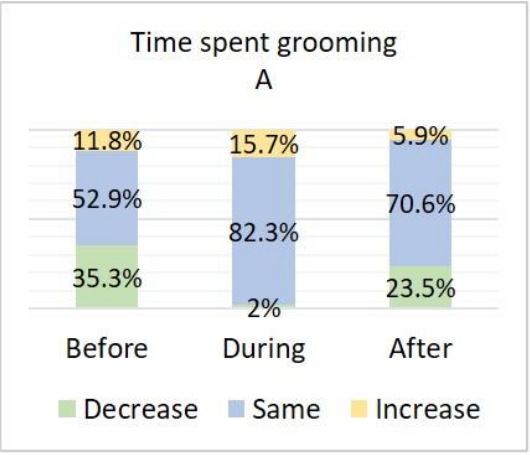

Passing faeces outside litter box
B
29.4%
64.7%
5.9%
5.9%
92.1%
2.0%
11.8%
88.2%
0%
Before
During
After
Decrease
Same
Increase

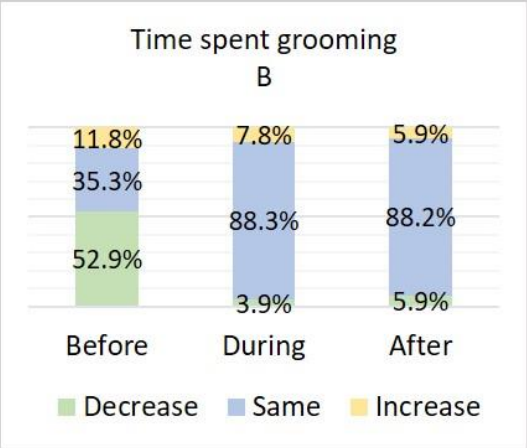

Passing urine outside litter box
A
23.5%
76.5%
0%
7.8%
80.4%
11.8%
17.6%
76.5%
5.9%
Before
During
After
Decrease
Same
Increase

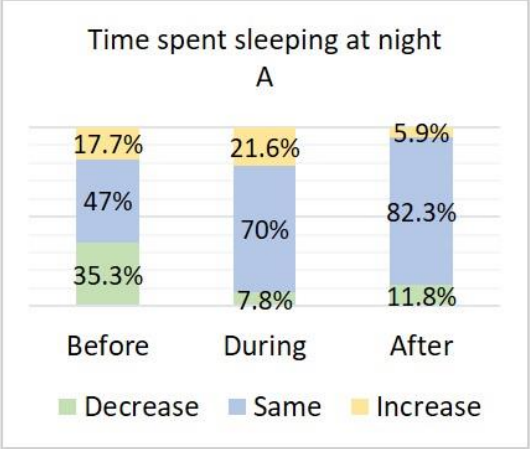

Passing urine outside litter box
B
17.7%
82.3%
0%
3.9%
86.3%
9.8%
5.9%
94.1%
0%
Before
During
After
Decrease
Same
Increase

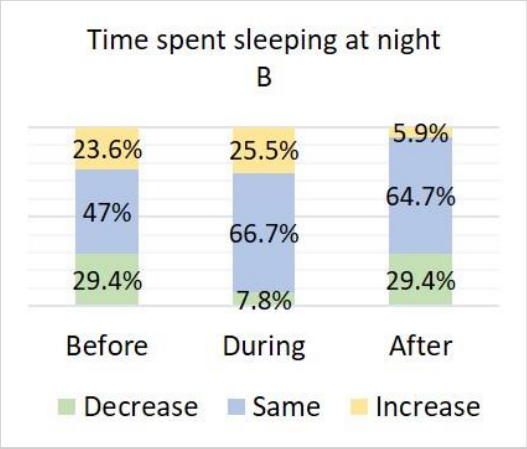

Repetitive or compulsive behaviour
A
35.3%
64.7%
0%
7.8%
92.2%
0%
0%
88.2%
11.8%
Before
During
After
Decrease
Same
Increase

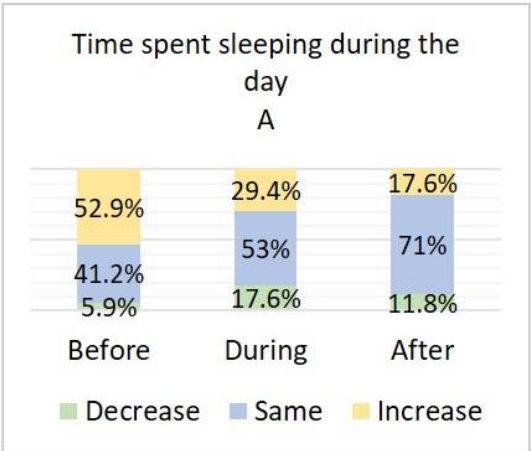

Repetitive or compulsive behaviour
B
23.5%
76%
0%
9.8%
82.4%
7.8%
11.8%
88.2%
0%
Before
During
After
Decrease
Same
Increase

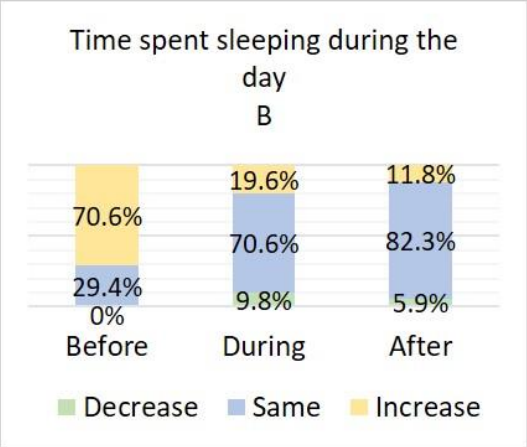

Time spent grooming
A
11.8%
52.9%
35.3%
15.7%
82.3%
2%
5.9%
70.6%
23.5%
Before
During
After
Decrease
Same
Increase

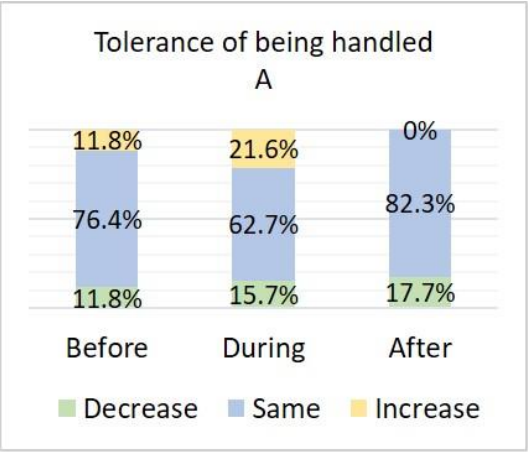

Time spent grooming
B
11.8%
35.3%
52.9%
7.8%
88.3%
3.9%
5.9%
88.2%
5.9%
Before
During
After
Decrease
Same
Increase

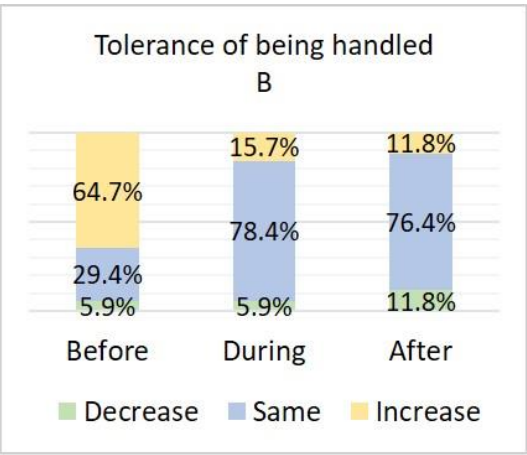

Time spent sleeping at night
A
17.7%
47%
35.3%
21.6%
70%
7.8%
5.9%
82.3%
11.8%
Before
During
After
Decrease
Same
Increase

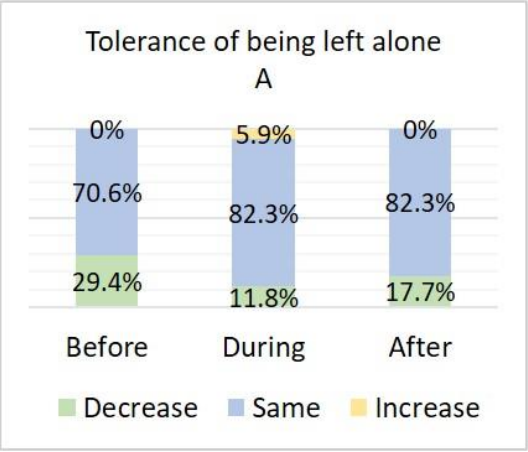

Time spent sleeping at night
B
23.6%
47%
29.4%
25.5%
66.7%
7.8%
5.9%
64.7%
29.4%
Before
During
After
Decrease
Same
Increase

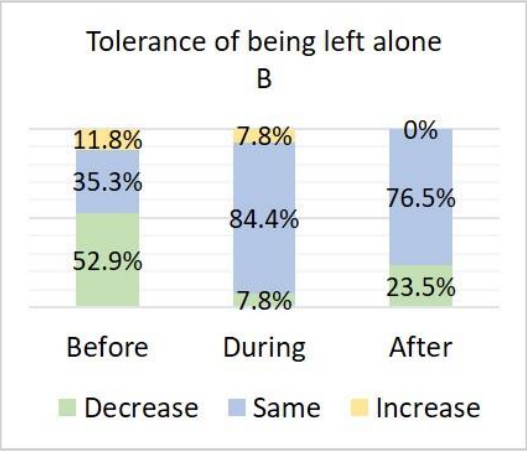

Time spent sleeping during the day
A
52.9%
41.2%
5.9%
29.4%
53%
17.6%
17.6%
71%
11.8%
Before
During
After
Decrease
Same
Increase

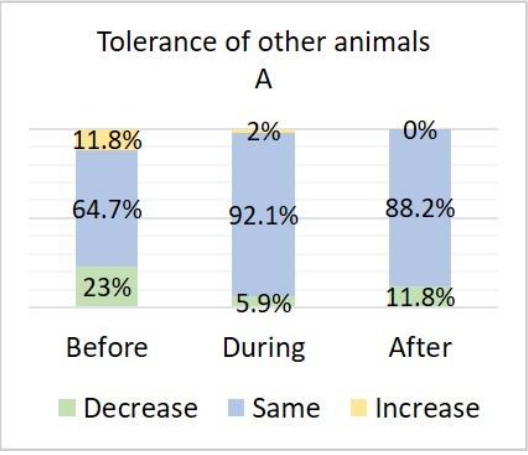

Time spent sleeping during the day
B
70.6%
29.4%
0%
19.6%
70.6%
9.8%
11.8%
82.3%
5.9%
Before
During
After
Decrease
Same
Increase

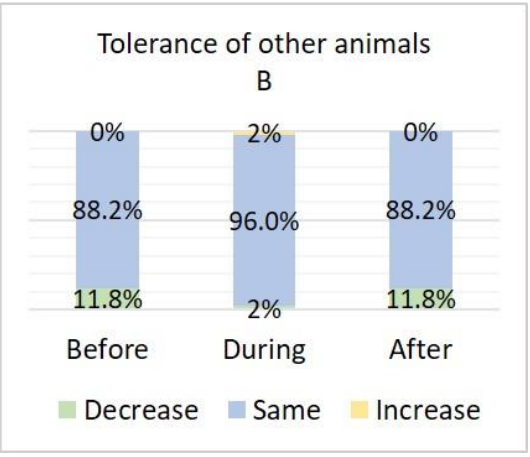

Tolerance of being handled
A
11.8%
76.4%
11.8%
21.6%
62.7%
15.7%
0%
82.3%
17.7%
Before
During
After
Decrease
Same
Increase

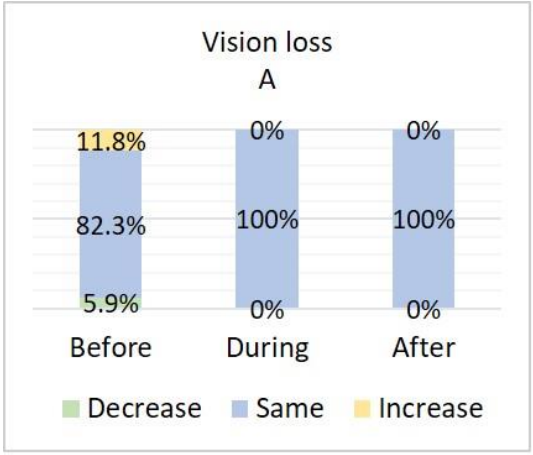

Tolerance of being handled
B
64.7%
29.4%
5.9%
15.7%
78.4%
5.9%
11.8%
76.4%
11.8%
Before
During
After
Decrease
Same
Increase

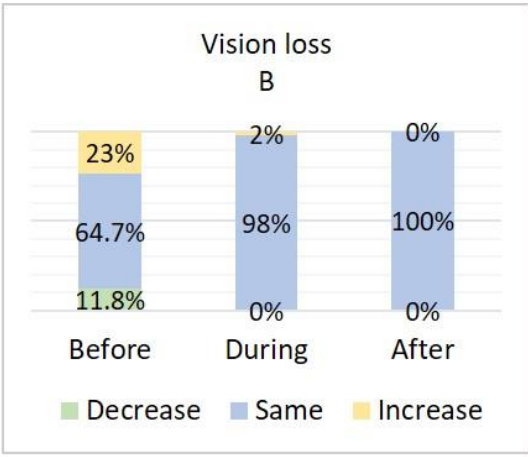

Tolerance of being left alone
A
0%
70.6%
29.4%
5.9%
82.3%
11.8%
0%
82.3%
17.7%
Before
During
After
Decrease
Same
Increase

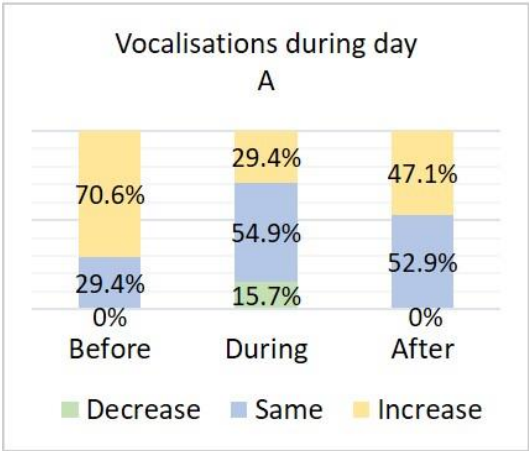

Tolerance of being left alone
B
11.8%
35.3%
52.9%
7.8%
84.4%
7.8%
0%
76.5%
23.5%
Before
During
After
Decrease
Same
Increase

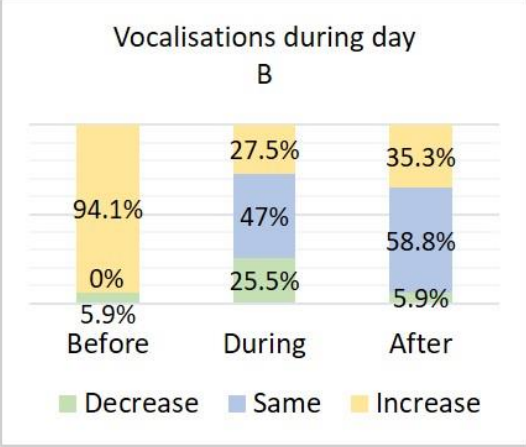

Tolerance of other animals
A
11.8%
64.7%
23%
2%
92.1%
5.9%
0%
88.2%
11.8%
Before
During
After
Decrease
Same
Increase

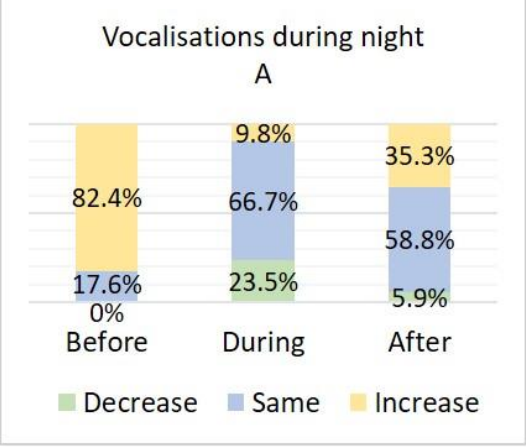

Tolerance of other animals
B
0%
88.2%
11.8%
2%
96.0%
2%
0%
88.2%
11.8%
Before
During
After
Decrease
Same
Increase

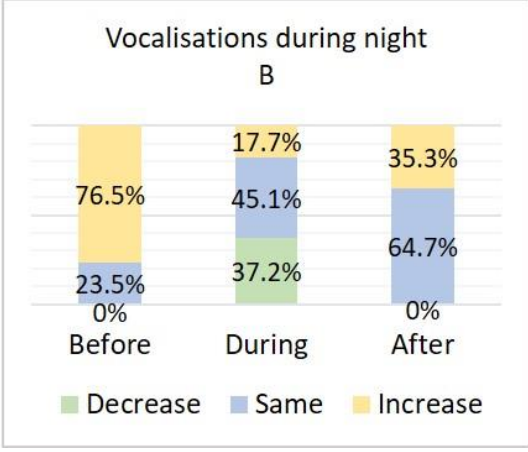

Vision loss
A
11.8%
82.3%
5.9%
0%
100%
0%
0%
100%
0%
Before
During
After
Decrease
Same
Increase

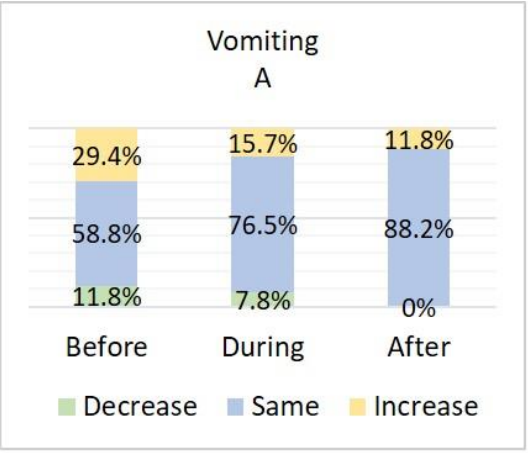

Vision loss
B
23%
64.7%
11.8%
2%
98%
0%
0%
100%
0%
Before
During
After
Decrease
Same
Increase

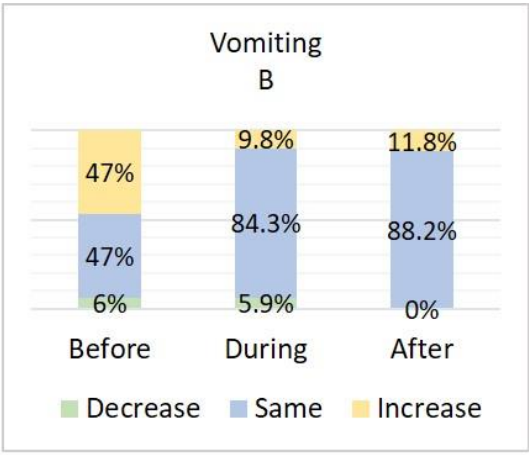

Vocalisations during day
A
70.6%
29.4%
0%
29.4%
54.9%
15.7%
47.1%
52.9%
0%
Before
During
After
Decrease
Same
Increase

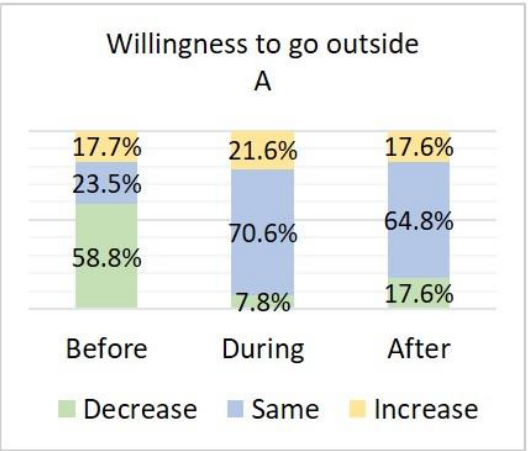

Vocalisations during day
B
94.1%
0%
5.9%
27.5%
47%
25.5%
35.3%
58.8%
5.9%
Before
During
After
Decrease
Same
Increase

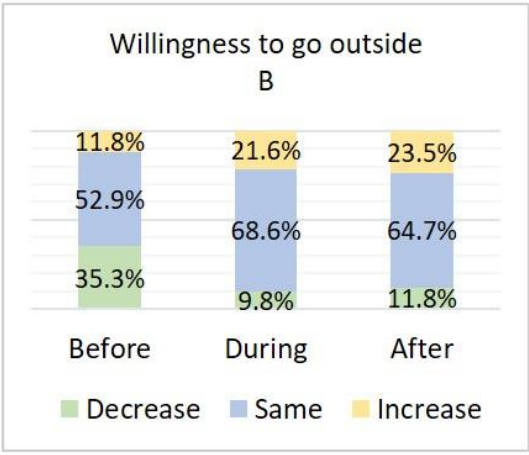

Vocalisations during night
A
82.4%
17.6%
0%
9.8%
66.7%
23.5%
35.3%
58.8%
5.9%
Before
During
After
Decrease
Same
Increase

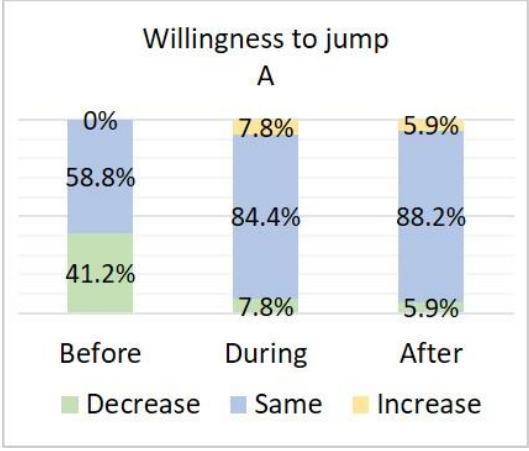

Vocalisations during night
B
76.5%
23.5%
0%
17.7%
45.1%
37.2%
35.3%
64.7%
0%
Before
During
After
Decrease
Same
Increase

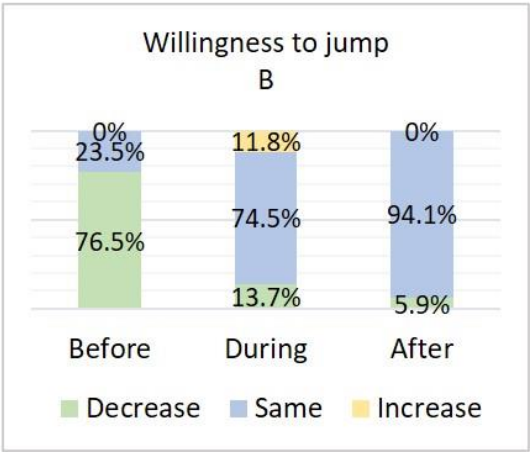

Vomiting
A
29.4%
58.8%
11.8%
15.7%
76.5%
7.8%
11.8%
88.2%
0%
Before
During
After
Decrease
Same
Increase


Vomiting
B
47%
47%
6%
9.8%
84.3%
5.9%
11.8%
88.2%
0%
Before
During
After
Decrease
Same
Increase


Willingness to go outside
A
17.7%
23.5%
58.8%
21.6%
70.6%
7.8%
17.6%
64.8%
17.6%
Before
During
After
Decrease
Same
Increase


Willingness to go outside
B
11.8%
52.9%
35.3%
21.6%
68.6%
9.8%
23.5%
64.7%
11.8%
Before
During
After
Decrease
Same
Increase


Willingness to jump
A
0%
58.8%
41.2%
7.8%
84.4%
7.8%
5.9%
88.2%
5.9%
Before
During
After
Decrease
Same
Increase


Willingness to jump
B
0%
23.5%
76.5%
11.8%
74.5%
13.7%
0%
94.1%
5.9%
Before
During
After
Decrease
Same
Increase

5.1. Comparison of the amino acid sequences for amyloid-β between humans and cats.

```
P05067 A4_HUMAN                1    MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWDSDPSGTK   60
                                    MLP LAL+LLAAWTARALEVPTDGNAGLLAEPQ+AMFCG+LNMHMNVQNGKW+SDPSGTK
A0A0A0MPX8 A0A0A0MPX8_FELCA  166    MLPALALVLLAAWTARALEVPTDGNAGLLAEPQVAMFCGKLNMHMNVQNGKWESDPSGTK  225

P05067 A4_HUMAN               61    TCIDTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHPHFVIPYRCLVG  120
                                    TCI TKE ILQYCQEVYPELQITNVVEANQPVTIQNWCKRG KQCKTH   VIPYRCLVG
A0A0A0MPX8 A0A0A0MPX8_FELCA  226    TCIGTKEDILQYCQEVYPELQITNVVEANQPVTIQNWCKRGHKQCKTHARIVIPYRCLVG  285

P05067 A4_HUMAN              121    EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR  180
                                    EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKST+LHDYGMLLPCGIDKFR
A0A0A0MPX8 A0A0A0MPX8_FELCA  286    EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTSLHDYGMLLPCGIDKFR  345

P05067 A4_HUMAN              181    GVEFVCCPLAEESDNVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVAEVEEE  240
                                    GVEFVCCPLAEESDN+DSADAEEDDSDVWWGGAD DYADGSEDKVVEVAEEEE A+VE+E
A0A0A0MPX8 A0A0A0MPX8_FELCA  346    GVEFVCCPLAEESDNIDSADAEEDDSDVWWGGADADYADGSEDKVVEVAEEEEGADVEDE  405

P05067 A4_HUMAN              241    EADDDEDDEDGDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPC  300
                                    EA+DDEDDEDGDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPC
A0A0A0MPX8 A0A0A0MPX8_FELCA  406    EAEDDEDDEDGDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPC  465

P05067 A4_HUMAN              301    RAMISRWYFDVTEGKCAPFFYGGCGGNRNNFDTEEYCMAVCGSAMSQSLLKTTQEPLARD  360
                                    RAMISRWYFDVTEGKCAPFFYGGCGGNRNNFDTEEYCMAVCGS MSQSLLKTTQEPL +D
A0A0A0MPX8 A0A0A0MPX8_FELCA  466    RAMISRWYFDVTEGKCAPFFYGGCGGNRNNFDTEEYCMAVCGSVMSQSLLKTTQEPLPQD  525

P05067 A4_HUMAN              361    PVKLPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEEAERQA  420
                                     VKLPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEEAERQA
A0A0A0MPX8 A0A0A0MPX8_FELCA  526    AVKLPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEEAERQA  585

P05067 A4_HUMAN              421    KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL  480
                                    KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL
A0A0A0MPX8 A0A0A0MPX8_FELCA  586    KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL  645

P05067 A4_HUMAN              481    QAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER  540
                                    QAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER
A0A0A0MPX8 A0A0A0MPX8_FELCA  646    QAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER  705

P05067 A4_HUMAN              541    MNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTET  600
                                    MNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTET
A0A0A0MPX8 A0A0A0MPX8_FELCA  706    MNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTET  765

P05067 A4_HUMAN              601    KTTVELLPVNGEFSLDDLQPWHSFGADSVPANTENEVEPVDARPAADRGLTTRPGSGLTN  660
                                    KTTVELLPVNGEFSLDDLQPWH FG DSVPANTENEVEPVDARPAADRGLTTRPGSGLTN
A0A0A0MPX8 A0A0A0MPX8_FELCA  766    KTTVELLPVNGEFSLDDLQPWHPFGVDSVPANTENEVEPVDARPAADRGLTTRPGSGLTN  825

P05067 A4_HUMAN              661    IKTEEISEVKMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL  720
                                    IKTEEISEVKMDAEFRH+SGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL
A0A0A0MPX8 A0A0A0MPX8_FELCA  826    IKTEEISEVKMDAEFRHESGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL  885

P05067 A4_HUMAN              721    VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN            770
                                    VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN
A0A0A0MPX8 A0A0A0MPX8_FELCA  886    VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN            935
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Comparison of the amino acid sequences for amyloid-β between humans and mice.

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P05067 A4_HUMAN          1    MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWDSDPSGTK   60
                              MLP LALLLLAAWT RALEVPTDGNAGLLAEPQIAMFCG+LNMHMNVQNGKW+SDPSGTK
Q53ZT3 Q53ZT3_MOUSE      1    MLPSLALLLLAAWTVRALEVPTDGNAGLLAEPQIAMFCGKLNMHMNVQNGKWESDPSGTK   60

P05067 A4_HUMAN         61    TCIDTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHPHFVIPYRCLVG  120
                              TCI TKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTH H VIPYRCLVG
Q53ZT3 Q53ZT3_MOUSE      61    TCIGTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHTHIVIPYRCLVG  120

P05067 A4_HUMAN         121    EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR  180
                              EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR
Q53ZT3 Q53ZT3_MOUSE      121    EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR  180

P05067 A4_HUMAN         181    GVEFVCCPLAEESDNVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVAEVEEE  240
                              GVEFVCCPLAEESD+VDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVA+VEEE
Q53ZT3 Q53ZT3_MOUSE      181    GVEFVCCPLAEESDSVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVADVEEE  240

P05067 A4_HUMAN         241    EADDDEDDEDGDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPC  300
                              EADDDED EDGDEVEEEAEEPYEEATERTTS ATTTTTTTESVEEVVREVCSEQAETGPC
Q53ZT3 Q53ZT3_MOUSE      241    EADDDEDVEDGDEVEEEAEEPYEEATERTTSTATTTTTTTESVEEVVREVCSEQAETGPC  300

P05067 A4_HUMAN         301    RAMISRWYFDVTEGKCAPFFYGGCGGNRNNFDTEEYCMAVCGSAMSQSLLKTTQEPLARD  360
                              RAMISRWYFDVTEGKC PFFYGGCGGNRNNFDTEEYCMAVCGS  +QSLLKTT EPL +D
Q53ZT3 Q53ZT3_MOUSE      301    RAMISRWYFDVTEGKCVPFFYGGCGGNRNNFDTEEYCMAVCGSVSTQSLLKTTSEPLPQD  360

P05067 A4_HUMAN         361    PVKLPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEEAERQA  420
                              P KLPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEEAERQA
Q53ZT3 Q53ZT3_MOUSE      361    PDKLPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEEAERQA  420

P05067 A4_HUMAN         421    KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL  480
                              KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL
Q53ZT3 Q53ZT3_MOUSE      421    KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL  480

P05067 A4_HUMAN         481    QAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER  540
                              QAVPPRP HVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER
Q53ZT3 Q53ZT3_MOUSE      481    QAVPPRPHHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER  540

P05067 A4_HUMAN         541    MNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTET  600
                              MNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTET
Q53ZT3 Q53ZT3_MOUSE      541    MNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTET  600

P05067 A4_HUMAN         601    KTTVELLPVNGEFSLDDLQPWHSFGADSVPANTENEVEPVDARPAADRGLTTRPGSGLTN  660
                              KTTVELLPVNGEFSLDDLQPWH FG DSVPANTENEVEPVDARPAADRGLTTRPGSGLTN
Q53ZT3 Q53ZT3_MOUSE      601    KTTVELLPVNGEFSLDDLQPWHPFGVDSVPANTENEVEPVDARPAADRGLTTRPGSGLTN  660

P05067 A4_HUMAN         661    IKTEEISEVKMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL  720
                              IKTEEISEVKMDAEF HDSG+EV HQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL
Q53ZT3 Q53ZT3_MOUSE      661    IKTEEISEVKMDAEFGHDSGFEVRHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL  720

P05067 A4_HUMAN         721    VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN            770
                              VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN
Q53ZT3 Q53ZT3_MOUSE      721    VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN            770
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Comparison of the amino acid sequences for tau between humans and cats.

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P10636 TAU_HUMAN          1    MAEPRQEFEVMEDHAGTYGLGDRKD---QGGYTMHQDQEGDTDAGLKESPLQTPTEDGSE   57
                               MAEPRQ+F VM+DHAGTYG G+RKD   QG YT+ QD EGD D GLKESPLQTP +DGSE
A0A337SA80 A0A337SA80_FELCA 1    MAEPRQDFTVMDDHAGTYGTGERKDLPSQGSYTLMQDHEGDVDQGLKESPLQTPADDGSE   60

P10636 TAU_HUMAN          58   EPGSETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDE  117
                               EPGSETSDAKSTPTAED TAPLVDEGAPG+QAAAQPHTEIPEGTTAEEAGIGDTP+LED+
A0A337SA80 A0A337SA80_FELCA 61   EPGSETSDAKSTPTAEDATAPLVDEGAPGEQAAAQPHTEIPEGTTAEEAGIGDTPNLEDQ  120

P10636 TAU_HUMAN          118  AAGHVTQEPESGKVVQEGFLREPGPPGLSHQLMSGMPGAPLLPEGPREATRQPSGTGPED  177
                               AAGHVTQEP S KVVQE  L EPG PGL  Q     PGAP+LP GPREAT QPSGT PED
A0A337SA80 A0A337SA80_FELCA 121  AAGHVTQEPGSVKVVQEILLGEPGTPGLPRQ---HAPGAPILPHGPREATHQPSGTEPED  177

P10636 TAU_HUMAN          178  TEGGRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKA  237
                               TEG R   ELL++QL+GDL QEGPPLKG  GKER G K+ VDEDRDVDESSPQDSP S
A0A337SA80 A0A337SA80_FELCA 178  TEGSRRDTELLEYQLVGDLRQEGPPLKGDHGKERRGGKD-VDEDRDVDESSPQDSPRSHV  236

P10636 TAU_HUMAN          238  SPAQDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSVG-RAKGQD  296
                               SP + G PPQT +R AT + GF AEGAI LPVDFLS+VSTEI ASEP  P  G  A+GQD
A0A337SA80 A0A337SA80_FELCA 237  SPVRGGPPPQTVSRGATGLSGFTAEGAIRLPVDFLSQVSTEIEASEPPRPGAGPTAEGQD  296

P10636 TAU_HUMAN          297  APLEFTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSE  356
                                P  FTFHVEI  NVQKEQA SE  L  AA PG PGE  E +GPS GEDTK++DLPEPSE
A0A337SA80 A0A337SA80_FELCA 297  TPPGFTFHVEIKANVQKEQARSEVDLEGAALPGPPGEEQEPQGPSEGEDTKKSDLPEPSE  356

P10636 TAU_HUMAN          357  KQPAAAPRGKPVSRVPQLKARMVSKSKDGTGSDDKKAKTSTRSSAKTLKNRPCLSPKHPT  416
                               KQPAA   GKP+SRVPQLKARMVSK +DGTG+DDKKAKTST SSAKTLKNRPCLSPK PT
A0A337SA80 A0A337SA80_FELCA 357  KQPAAVLPGKPISRVPQLKARMVSKGRDGTGADDKKAKTSTPSSAKTLKNRPCLSPKRPT  416

P10636 TAU_HUMAN          417  PGSSDPLIQPSSPAVCPEPPSSPKYVSSVTSRTGSSGAKEMKLKGADGK--TKIATPRGA  474
                               PGSSDPLI+PSSPAVCPE  SSPK+VSSVT RTGSSGAKEMK+KGADGK  TKIATPRGA
A0A337SA80 A0A337SA80_FELCA 417  PGSSDPLIKPSSPAVCPESSSSPKHVSSVTPRTGSSGAKEMKVKGADGKAGTKIATPRGA  476

P10636 TAU_HUMAN          475  APPGQKGQANATRIPAKTPPAPKTPPSSGEPPKSGDRSGYSSPGSPGTPGSRSRTPSLPT  534
                               APPGQKGQANATRIPAKT P+PKTPP +G+  KSGDRSGYSSPGSPGTPGSRSRTPSLPT
A0A337SA80 A0A337SA80_FELCA 477  APPGQKGQANATRIPAKTTPSPKTPPGTGDSGKSGDRSGYSSPGSPGTPGSRSRTPSLPT  536

P10636 TAU_HUMAN          535  PPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTENLKHQPGGGKVQI  594
                               PPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNV+SKIGSTENLKHQPGGGKVQI
A0A337SA80 A0A337SA80_FELCA 537  PPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNVRSKIGSTENLKHQPGGGKVQI  596

P10636 TAU_HUMAN          595  INKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQV  654
                               INKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQV
A0A337SA80 A0A337SA80_FELCA 597  INKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQV  656

P10636 TAU_HUMAN          655  EVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSP  714
                               EVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSP
A0A337SA80 A0A337SA80_FELCA 657  EVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSP  716

P10636 TAU_HUMAN          715  VVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL                758
                               VVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL

A0A337SA80 A0A337SA80_FELCA 717  VVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL                760
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Comparison of the amino acid sequences for tau between humans and mice.

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P10636 TAU_HUMAN          1    MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG   60
                               MA+PRQEF+ MEDHAG Y           T+ QDQEGD D GLKESP Q P +DG+EEPG
A2A5Y6 A2A5Y6_MOUSE       1    MADPRQEFDTMEDHAGDY-----------TLLQDQEGDMDHGLKESPPQPPADDGAEEPG   49

P10636 TAU_HUMAN         61    SETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG   120
                               SETSDAKSTPTAEDVTAPLVDE AP KQAAAQPHTEIPEG TAEEAGIGDTP+ ED+AAG
A2A5Y6 A2A5Y6_MOUSE      50    SETSDAKSTPTAEDVTAPLVDERAPDKQAAAQPHTEIPEGITAEEAGIGDTPNQEDQAAG   109

P10636 TAU_HUMAN        121    HVTQEPESGKVVQEGFLREPG-----PPGL-----SHQLMSGMPGAPLLPEGPREATRQP   170
                               HVTQEPE  ++  +  L EPG      P L     + Q +S M GAPLLP+G REAT QP
A2A5Y6 A2A5Y6_MOUSE      110    HVTQEPEKVEIFSQSLLVEPGRREGQAPDLGTSDWTRQQVSSMSGAPLLPQGLREATCQP   169

P10636 TAU_HUMAN        171    SGTGPEDTEGGRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQ   230
                               SGT PED E    A ELL+          GPP K   G++R GS+EEVDED  VDESS Q
A2A5Y6 A2A5Y6_MOUSE      170    SGTRPEDIEKSHPASELLRR---------GPPQKEGWGQDRLGSEEEVDEDLTVDESS-Q   219

P10636 TAU_HUMAN        231    DSPPSKASPAQDGRPPQTAAR----EATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDG   286
                               DSPPS+AS       PQ  +     E  S+PG P EG++PLP DF SKVS E  AS+P+G
A2A5Y6 A2A5Y6_MOUSE      220    DSPPSQASLTPGRAAPQAGSGSVCGETASVPGLPTEGSVPLPADFFSKVSAETQASQPEG   279

P10636 TAU_HUMAN        287    PSVG-RAKGQDAPLEFTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEAR--GPSLG   343
                               P  G   +G +A  EFTFHVEI  +  KEQ      L  A   G PGE  +A+  GPS+G
A2A5Y6 A2A5Y6_MOUSE      280    PGTGPMEEGHEAAPEFTFHVEIKASTPKEQ-----DLEGATVVGVPGEEQKAQTQGPSVG   334

P10636 TAU_HUMAN        344    EDTKEADLPEPSEKQPAAAPRGKPVSRVPQLKARMVSKSKDGTGSDDKKAKTSTRSSAKT   403
                               + TKEA L EP  KQPAA   G+PVSRVPQLKAR+ SK  D TG+D+KKAKTST S AK
A2A5Y6 A2A5Y6_MOUSE      335    KGTKEASLQEPPGKQPAAGLPGRPVSRVPQLKARVASK--DRTGNDEKKAKTSTPSCAKA   392

P10636 TAU_HUMAN        404    LKNRPCLSPKHPTPGSSDPLIQPSSPAVCPEPPSSPKYVSSVTSRTGSSGAKEMKLKGAD   463
                                 +RPCLSP  PT GSSDPLI+PSSPAVCPEP +SPK+VSSVT R GS G K+MKLKGAD
A2A5Y6 A2A5Y6_MOUSE      393    PSHRPCLSPTRPTLGSSDPLIKPSSPAVCPEPATSPKHVSSVTPRNGSPGTKQMKLKGAD   452

P10636 TAU_HUMAN        464    GKT--KIATPRGAAPPGQKGQANATRIPAKTPPAPKTPPSSGEPPKSGDRSGYSSPGSPG   521
                               GKT  KIATPRGAA P QKG +NATRIPAKT P+PKTPP SGEPPKSG+RSGYSSPGSPG
A2A5Y6 A2A5Y6_MOUSE      453    GKTGAKIATPRGAASPAQKGTSNATRIPAKTTPSPKTPPGSGEPPKSGERSGYSSPGSPG   512

P10636 TAU_HUMAN        522    TPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTE   581
                               TPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPS++KSRLQTAPVPMPDLKNV+SKIGSTE
A2A5Y6 A2A5Y6_MOUSE      513    TPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSASKSRLQTAPVPMPDLKNVRSKIGSTE   572

P10636 TAU_HUMAN        582    NLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGS   641
                               NLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGS
A2A5Y6 A2A5Y6_MOUSE      573    NLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGS   632

P10636 TAU_HUMAN        642    LGNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKA   701
                               LGNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKA
A2A5Y6 A2A5Y6_MOUSE      633    LGNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKA   692

P10636 TAU_HUMAN        702    KTDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL      758
                               KTDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
A2A5Y6 A2A5Y6_MOUSE      693    KTDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL      749
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