Age-Related Changes of the Avian Brain Measured by FDG-PET Imaging

by

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Abstract

Functional asymmetries of the brain's hemispheres play an important role in the completion of cognitive tasks by human and non-human vertebrates. For pigeons, the left hemisphere is more important for discrimination of local cues, such as discriminating pieces of grain scattered among grit, whereas the right hemisphere is more important for processing global cues, such as a predatory bird flying overhead. Brain functionality declines with age, and the hemispheres age at different rates. We investigated the age-related differences in metabolic rate of each hemisphere by using PET imaging. Adult, old and very old pigeons searched for grains scattered among grit using both eyes, only the right eye or only the left eye. Overall, the activity of both hemispheres decreased significantly in old and very old subjects compared to the adult group, which resulted in significantly poorer performance by very old subjects when the left hemisphere was not available (using only the left eye). However, in spite of the reduction in the brain activity of the old subjects, they could still perform the task significantly better than the very old group. Also, Lateralization Index differed significantly among all there eye conditions only in adult pigeons. In conclusion, coupling behavioural study with a neuroimaging technique, we showed that the avian

brain, similar to the mammalian brain, can compensate for the effects of aging to a limited extent and until a certain age.

Contents

Abstract ii
Table of Contents vii
List of Figures viii
List of Tables xi
Acknowledgments xiii
Dedication xiv

1 Introduction **3**

2 Background **6**
2.1 Age-Related changes in cognitive abilities 6
2.2 Theoretical models of hemispheric asymmetries and aging in human . 8
2.3 The Importance of animal studies for human studies 12
2.4 Effects of aging on cognitive abilities in animals 13
2.5 Avian cognition 14
2.6 Pigeons as a model for the study of aging 15
2.7 Brain lateralization in birds 17
2.8 Basics of PET imaging 19
2.9 18F-FDG PET imaging 20
2.10 FDG uptake and quantification 23
2.11 Factors affecting the FDG uptake and SUV 23
2.11.1 Biological factors 24
2.11.2 Technical factors 25
2.12 Small animal PET imaging system 27
2.13 Applications of FDG-PET imaging in small animals 28
2.13.1 Epilepsy 29
2.13.2 Alzheimer Disease 30
2.13.3 Behavioural and Cognition studies 31

3 Methods **34**
3.1 Subjects 34

3.2 Behavioural Apparatus . . . 35
3.3 General Procedure . . . 35
3.3.1 Familiarization Phase . . . 36
3.3.2 Habituation Phase . . . 37
3.3.3 Testing phase . . . 39
3.4 Imaging Protocol . . . 42
3.4.1 18F-FDG PET image acquisition . . . 42
3.4.2 System Calibration . . . 42
3.4.3 Normalization . . . 44

4 Data Analysis **45**
4.1 Data Analysis . . . 45
4.1.1 Behavioural Assessment: Grit-grain task . . . 45
4.2 PET Data Analysis . . . 46
4.2.1 Correction for subject motion and image reconstruction . . . 46
4.2.2 SPM Toolbox . . . 47
4.2.3 Spatial pre-processing and PET template creation . . . 47
4.2.4 SUV calculation . . . 49
4.2.5 Measuring Lateralization Index . . . 50

5 Results **52**
5.1 Behavioural results . . . 52
5.1.1 Total number of pecks: Differences between age groups . . . 52
5.1.2 Total number of pecks: Differences between eye conditions in each age group . . . 53
5.1.3 Correlation between age and total number of pecks . . . 53
5.1.4 Success rate: Differences between age groups . . . 54
5.1.5 Success rate: Differences between eye conditions for each age group . . . 56
5.1.6 Correlation between age and success rate . . . 57
5.1.7 Summary: Behavioural assessment . . . 57
5.2 PET data results . . . 58
5.2.1 Left hemisphere SUV: Differences between age groups . . . 58
5.2.2 Left hemisphere SUV: Differences between eye conditions for each age group . . . 59
5.2.3 Right hemisphere SUV: Differences between age groups . . . 60
5.2.4 Right hemisphere SUV: Differences between eye conditions for each age group . . . 62
5.2.5 Correlation between age and SUV of each hemisphere . . . 63
5.2.6 Lateralization Index: Differences between age groups . . . 66
5.2.7 Lateralization Index: Differences between eye conditions for each age group . . . 67

5.2.8 Correlation between Lateralization Index and age 68
5.2.9 Summary: PET data analyses 68

6 Discussion **69**
6.1 Discussion 69
6.1.1 Age and reduction of cognitive performance in grit-grain discrimination task 70
6.1.2 Reduction in 18F-FDG uptake of the avian brain with normal aging 72
SUV of the left hemisphere in the grit-grain task 73
SUV of the right hemisphere in the grit-grain task 74
6.1.3 Positive Lateralization Index in grit-grain task 75
6.1.4 Relation between behavioural data and PET data 75
6.1.5 Limitations 77

7 Conclusion and Future directions **78**
7.1 Conclusion 78
7.2 Future directions 79
7.2.1 To what extent can the old group perform a cognitive task as well as the adult group? 80
7.2.2 Longitudinal grit-grain discrimination study 81
7.2.3 Studying healthy aging in pigeons in a spatial cognition task . 81

A Behavioural and PET data analyses **83**
A.0.1 Individual differences: Relation between age and total number of pecks 84
A.0.2 Individual differences: Ratio of eaten grains to total number of pecks 86
A.0.3 Individual differences: Relation between age and success rate . 89
A.0.4 Individual differences: The left hemisphere SUV 91
A.0.5 Individual differences: The right hemisphere SUV 92
A.0.6 Individual differences: Relation between age and LI 96
A.1 Analyses without the cerebellum included 97
A.1.1 Left cerebrum SUV: Differences between age groups 98
A.1.2 Left cerebrum SUV: Differences between eye conditions for each age group 99
A.1.3 Right cerebrum SUV: Differences between age groups 99
A.1.4 Right cerebrum SUV: Differences between eye conditions for each age group 101
A.1.5 Correlation between age and SUV of right and left cerebrum . 101
A.1.6 Lateralization Index of the cerebrum: differences between age groups 107

A.1.7 Lateralization Index of the cerebrum: differences between eye condition for each age group 109
A.1.8 Correlation between Lateralization Index and age 110
A.1.9 Lateralization Index of the cerebrum 110
A.1.10 Summary of comparing PET results of whole hemisphere and results of excluding cerebellum 111

Bibliography **135**

List of Figures

2.1 Generation of two high energy annihilation photon as a result of annihilation after positron emission by the positron-emitting radionuclide. 21
2.2 Detectors of a PET scanner arranged in a circle. Annihilation occurs within the circle. Detectors detect photons that arrive simultaneously. A photon's origin can be found by using the line of response (LOR) between each detector pair that detected the photons. 22

3.1 *(a)* The set up inside the apparatus with a view of the metal tray containing grains mixed with grit. A video camera on a tripod was located inside the arena in front of the metal tray. A GoPro Hero5 camera was attached in the middle of one side of the metal tray for test sessions; *(b)* a view of the out side of the apparatus. 36
3.2 (a) shows the image of eye ring around the eye of a pigeon. (b) shows the eye cap covered the left eye of the pigeon 38
3.3 Time line of the test session. The time (t) is based on minutes. 39
3.4 Pigeons performed the grit-grain task under three different eye condition from left to right: right eye capped, binocular, left eye capped conditions. 40
3.5 A pigeon performing the grit-grain task in monocular eye condition . 40
3.6 Image of Concorde Microsystems/Siemens microPET R4 PET system used for small animal imaging 43

4.1 To create a PET template, the PET data were reoriented into the MRI atlas and the all the reoriented PET data were realigned and averaged. The averaged PET images was co-registered into the MRI atlas. The brain mask was used to remove non-brain area. The result was the PET template. 48
4.2 Normalization of reoriented PET images of each subject into the PET template to decrease the variation of brain size and shape of each subject 49

4.3 *a)* The left hemisphere mask used as a ROI to extract the activation of the left hemisphere of the brain in grit-grain task; *b)* The right hemisphere mask used as a ROI to extract the activation of the right hemisphere of the brain in grit-grain task. 51

5.1 Success rate for each eye condition (BIN - binocular, CR - capped right eye, CL - capped left eye) and age group. Error bars represent standard errors. * denotes significant pairwise comparisons between age groups within a given eye condition at $p < 0.05$. 55
5.2 A negative relation between success rate and age in CR - capped right eye condition. Each dot represents the success rate of one subject. The shaded area represents 95% confidence interval. 57
5.3 Average of SUV of the left hemisphere for each eye condition (BIN - binocular, CL - capped left eye, CR - capped right eye) and age group. Error bars represent standard errors. ** denotes significant pairwise comparisons between age groups within a given eye condition at $p < 0.01$. 60
5.4 Average of SUV of the right hemisphere for each eye condition (BIN - binocular, CL - capped left eye, CR - capped right eye) and age group. Error bars represent standard errors. * and ** denote significant pairwise comparisons between age groups between age groups within a given eye condition at $p < 0.05$ and $p < 0.01$ respectively. 62
5.5 BIN eye condition: *(a)* relation between SUV of the left hemisphere and age; *(b)* relation between SUV of the right hemisphere and age). The shaded area represents 95% confidence interval. 63
5.6 CL eye condition: *(a)* relation between SUV of the left hemisphere and age; *(b)* relation between SUV of the right hemisphere and age).The shaded area represents 95% confidence interval. 64
5.7 CR eye condition: *(a)* relation between SUV of the left hemisphere and age; *(b)* relation between SUV of the right hemisphere and age). The shaded area represents 95% confidence interval. 65
5.8 Lateralization Index in different eye condition (BIN - binocular, CL - capped left eye, CR - capped right eye) and age groups. Error bars represent standard errors. 66

A.1 Individual differences in total number of pecks for each age group under different eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available) 84

A.2 Comparing the ratio of eaten grains out of the total number of pecks by each subject in each age group in each eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available). Trend-lines are calculated based on the least squares method. 88
A.3 Individual differences in success rate for each age group under different eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available) 89
A.4 Individual left hemisphere SUV and average of the three age groups in each eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available). 91
A.5 Individual and left hemisphere SUV and average of the three age groups in each eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (right hemisphere is available), *(c)* CR - capped right eye (left hemisphere is available). 92
A.6 Individual differences in LI in each age group in different eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available) 96
A.7 BIN eye condition: *(a)* relation between SUV of the left cerebrum and age; *(b)* relation between SUV of the right cerebrum and age). The shaded area represents 95% confidence interval. 102
A.8 CL eye condition: *(a)* relation between SUV of the left cerebrum and age; *(b)* relation between SUV of the right cerebrum and age).The shaded area represents 95% confidence interval. 103
A.9 CR eye condition: *(a)* relation between SUV of the left cerebrum and age; *(b)* relation between SUV of the right cerebrum and age). The shaded area represents 95% confidence interval. 104
A.10 Lateralization Index were calculated by considering only the cerebrum regions and excluding the cerebellum in different eye condition (BIN - binocular, CL - capped left eye, CR - capped right eye) and age groups. Error bars represent standard errors. 108

List of Tables

5.1 Mean and Standard Error (SE) of total number of pecks in each eye condition for each age group. 53
5.2 Mean (%) and Standard Error (SE) of success rate in each eye condition for each age group 55
5.3 Mean (%) and Standard Error (SE) of success rate in the adult group from the first test session to the third test session 56
5.4 Mean and Standard Error (SE) of the left hemisphere SUV in each eye condition for each age group 59
5.5 Mean and Standard Error (SE) of the right hemisphere SUV in each eye condition for each age group 61
5.6 Mean and Standard Error (SE) of Lateralization Index in each eye condition for each age group 67

A.1 Number of total peck of each subject in three different eye conditions (BIN, CL, CR). 85
A.2 Trendline equation and R^2 value of each age group in BIN eye condition 86
A.3 Trendline equation and R^2 value of each age group in CL eye condition 87
A.4 Trendline equation and R^2 value of each age group in CR eye condition 87
A.5 Success rate of each subject in three different eye conditions (BIN, CL, CR). 90
A.6 SUV of each subject in BIN eye conditions. 93
A.7 SUV of each subject in CL eye conditions. 94
A.8 SUV of each subject in CR eye conditions. 95
A.9 Lateralization Index (LI) of each subject in three different eye conditions (BIN, CL, CR). 97
A.10 Mean and Standard Error (SE) of the left cerebrum SUV in each eye condition for each age group 99
A.11 Mean and Standard Error (SE) of the right cerebrum SUV in each eye condition for each age group 100
A.12 SUV of cerebrum of each subject in BIN eye conditions. 105

A.13 SUV of cerebrum of each subject in CL eye conditions. 106
A.14 SUV of cerebrum of each subject in CR eye conditions. 107
A.15 Mean and Standard Error (SE) of Lateralization Index of cerebrum region in each eye condition for each age group 109
A.16 Lateralization Index (LI) of cerebrum of each subject in three different eye conditions (BIN, CL, CR). 112

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List of Abbreviations

18F-FDG 18F-2-deoxy-2- uoro-D-glucose, or 18F-labelled fluorodeoxyglucose

CF Calibration Factor

CRUNCH Compensation-Related Utilization of Neural Circuits Hypothesis

CT Computed Tomography

DLPFC dorsolateral prefrontal cortex

FOV Field Of View

HAROLD Hemispheric Asymmetry Reduction in Older Adults

IP Intraperitoneal

IV Intravenous

KA Kainic Acid

LI Lateralization Index

LOR Line Of Response

MAP Maximum A Posteriori

MRI Magnetic Resonance Imaging

MWT Morris Water Task

PET Positron Emission Tomography

PFC prefronal cortex

ROI Region Of Interest

SE Standard Error

SPM Statistical Parametric Mapping

SUV Standardized Uptake Value

VOI Volume Of Interest

WT Wild-Type

Chapter 1

Introduction

Brain lateralization refers to the functional specialization of each brain hemisphere and plays an integral role in almost all cognitive tasks [1]. As the brain ages, it experiences anatomical and functional changes that are linked to decline in cognitive performance. In other words, aging affects the brain laterality and the function of each hemisphere when it is engaged in a cognitive task. Interestingly, brain lateralization is observed not only in humans but also in many non-human vertebrates. There is a considerable literature on brain lateralization in birds [2, 3, 4, 5], which indicates that birds are an excellent animal model to study brain lateralization. Moreover, birds have a unique brain structure in which their optic nerves are completely crossed [6], which allows us to study the function of each hemisphere separately by occluding one eye and restricting visual information to the ipsilateral hemisphere. Studies on brain lateralization in birds have shown that for pigeons, the left hemisphere is more important for the discrimination of local cues, such as discriminating pieces of grain scattered among grit. The right hemisphere is important for processing global cues,

such as a predatory bird flying overhead [7, 5, 8, 4, 9]. Furthermore, pigeons present an important animal model to further our understanding of age-related functional changes and the underlying neural mechanisms as they are long-lived and slowly aging animals, similar to humans [10]. Moreover, the function of each hemisphere can be studied by covering one eye. The use of advanced non-invasive imaging methods plays and important role to further our understanding of the function of each hemisphere and age-related functional changes of the each hemisphere. Positron Emission Tomography (PET) imaging using the glucose analogue 18F-fluorodeoxyglucose (FDG) is considered as one of the most useful tools for brain imaging in humans and also in animals. This nuclear medicine imaging technique, which involves the injection of positron emitting radiotracer into the subject's body, allows mapping of glucose utilization within the brain. We used an advanced neuroimaging method to non-invasively study the brain function in a pigeon model of aging by using FDG-PET imaging. The aim of this study was to assess the effects of aging on the success rate of discrimination performance in the pigeon and investigate the effect of aging on metabolic rate of each hemisphere and cerebral asymmetry. In this study, 15 pigeons were used as subjects, separated by age into three groups (adult, old and very old groups). Pigeons performed a food foraging discrimination task (grit-grain task). They underwent three sessions involving a FDG-PET scan, during which the pigeon experienced one of three eye conditions: binocular (BIN) (no eye capped), left eye capped (CL), during which the left hemisphere processed almost all visual information; right eye capped (CR), during which the right hemisphere processed almost all visual information. Studying the brain functionality in a long-lived and slowly

aging animal model could give insight into the mechanisms underlying healthy aging. In addition, many different experiential factors such as education level or life style, which might affect the healthy process of aging, can be controlled by using an animal model. The rest of this thesis is organized as follows:

In Chapter 2, the background of this work is described including the importance of animal studies for human studies, avian cognition, the importance of pigeons as a model of aging and brain lateralization in birds, basics of PET imaging, FDG uptake and quantification, factors affecting the FDG uptake, application of FDG-PET imaging in small animals.

In Chapter 3, the method of study, general procedures including familiarization phase, habituation phase and test session as well as imaging protocols are described in detail.

In Chapter 4, the methods of data analysis including behavioural assessment and PET data analysis are covered.

In Chapter 5, the behavioural results and PET data results are described in detail.

In Chapter 6, the observed behavioural and PET data results, relation between the behavioural results and PET data as well as limitations in this study are discussed.

Finally, Chapter 7 presents the conclusion and future directions for the current study.

Chapter 2

Background

2.1 Age-Related changes in cognitive abilities

Advancements in medical science and significant improvements in healthy life style have resulted in large increases in quality of life and life expectancy for many people across the world, especially in developed nations. According to data from World Population Prospects, the 2015 Revision,

> The number of older persons aged 60 years or over has increased considerably in recent years in most countries and regions. Between 2015 and 2030, the number of people in the world aged 60 years or over is projected to grow by 56 percent, from 901 million to 1.4 billion, and by 2050, the global population of older persons is projected to more than double its size in 2015, reaching nearly 2.1 billion [11] .

Aging brings many visible changes, such as gray hair, wrinkles, tooth loss, muscle shrinkage and physical changes over time. These changes are not the only evidence of aging because, as we age, our brain ages too. During aging, our brain experiences detrimental changes including structural changes, reduction in blood flow, synaptic

and functional degeneration and changes in the brain's functional connectivity. In consequence, aging is linked to decline in a wide range of cognitive processes, including; processing speed, attention, episodic memory, visual memory, and learning [12, 13, 14, 15]. Age-related decline can occur in different regions of the brain in different forms. For example, the prefrontal cortex, which is specialized in complex cognitive functions such as attention [16, 17, 18], might experience a volume decline, whereas another region of the brain, the hippocampus, which governs memory tasks [19, 20, 21], shows poor functionality as a result of decline in neuronal activation [22, 23]. A review by Hedden and Gabrieli [24] indicated that aging affects cognitive abilities differently. Although some of our cognitive abilities such as general knowledge and verbal abilities increase at a very slow rate across the lifespan or remain steady, others such as memory and orientation decrease slowly. Studies have revealed that for both humans [25] and rhesus monkeys [26], older adult individuals showed an obvious reduction in working memory performance, compared to younger adult individuals. Another study with human participants between 8 and 75 years of age, studied the ability to remember the location of objects using a visual working memory task, showing that aging affects the memory of object locations [27]. Certain spatial-navigation strategies are also affected by aging [28]. For instance, elderly individuals may show a stronger reliance on important visual features or landmarks instead of using distance, length or geometric aspects of the environment for orientation. However, young individuals can easily navigate by using such geometric features [29]. Although older individuals may perform a cognitive task more poorly than younger individuals, some older individuals can perform the task at the same level or even

better than younger individuals [30]. Difference in cognitive performance for elderly individuals may be related to different factors such as genetics [31], social involvement [32], education level [33] and compensatory mechanisms [30]. Functional brain imaging studies have revealed that older individuals, who showed higher performance in a verbal working memory task, activated more areas in both hemispheres of the brain than young subjects, who showed activity mainly in the left hemisphere. Recruiting more areas of the brain might allow old individuals to compensate for the losses which come with age [34, 35].

2.2 Theoretical models of hemispheric asymmetries and aging in human

Brain lateralization (also referred to as functional specialization) refers to the functional dominance of one hemisphere for a certain task and is observed for many cognitive functions [1]. Lateralization was investigated for the first time by a French surgeon called Broca [1] in 1861. He had a patient who could not produce any language, except for a single syllable which was "tan". Further analysis revealed that the left side of the brain is mainly involved in language processing and production, and as the patient had lesions in the left hemisphere, specifically in the area associated with speech production, he could not produce any language [1]. Studies of cognitive function and aging studies coupled with neuroimaging methods have led to the proposal of different models of how hemispheric asymmetries might be affected by aging. One of these models is the right-hemisphere aging model [36]. This model suggests

that the right hemisphere of the brain shows more functional decline because of aging than the left hemisphere, which means aging affects the function of the right hemisphere more than the left hemisphere [36]. It is important to mention that this model is primarily based on behavioural analysis rather than functional and neuroimaging evidence. A cross-sectional study about the effect of aging on verbal and spatial abilities [37] revealed evidence for right-hemisphere aging. Their results showed that the right hemisphere, which is more involved in spatial processing, of aged participants was more impaired compared to the left hemisphere, which is more involved in verbal tasks. In another study, age-related changes have been studied in younger and older adults performing physical and name identity letter-matching tasks and the findings also showed more decrease in the functionality of the right hemisphere for older adults [38]. The right hemisphere aging hypothesis has also been supported by other studies[39, 40, 41, 42].However, the results from several studies are not consistent [43, 44, 45].

It has been suggested that the variable results in the above studies might be a result of different levels of complexity of the cognitive tasks used to assess age effects [46]. In fact, differences of brain activation in different age groups are more likely to be found with more complex tasks. Another possibility is that prefrontal areas are the primary brain regions that show reduction in functional and neural hemispheric asymmetries [46, 34] and it might not generalize to posterior parietal brain regions [47]. In addition, aging effects on the right hemisphere of the brain might simply not be found in all tasks. It depends on the specific areas of the brain involved.

A different model, the "hemispheric asymmetry reduction in older adults" (HAROLD)

model [36], proposes that the functional activation of the prefronal cortex (PFC) are less lateralized for older individuals compared to younger individuals [36]. Studies have shown that for young adult participants the right PFC was more activated than the left during episodic memory retrieval. However, for older adults, both PFCs were active in the same cognitive task [48]. Many other studies using different episodic memory retrieval tasks have shown bilateral activity only in aged adults [49]. In other domains such as working memory, Reuter-Lorenz et al. [34] found bilateral PFC neuronal activity in aged adults in both verbal and spatial tasks. Both behavioural and neuroimaging studies in this area supported the argument that bilateral activation and recruitment of both hemispheres allow aged adults to compensate for age-related losses and keep their performance in cognitive tasks [48]. A number of researchers have shown [50] that recruiting more regions of the brain to perform a cognitive task in older adults caused worse task performance instead of better performance [51, 52]. Therefore, the relationship between brain activation on one side and task responses and behaviour in older adults on the other side is complicated, and the idea that more areas in the brain are recruited to act as compensatory mechanisms is likely an over-simplification.

The "Compensation-Related Utilization of Neural Circuits Hypothesis" (CRUNCH) [53] is a model that explains both performance improvement and deficiency associated with the recruitment of additional brain resources in aged adults. This model states that the aged brain has already recruit additional neural resources as a compensatory recruitment even when the task complexity is low and easy to solve. As task demands increases, there are no neural resources left to recruit. This may cause

the poor performance in complex tasks for older adults. However, younger adults can activate other neural resources during difficult tasks because those neural resources are not necessarily recruited [53]. Using event related fMRI, a study by Cappell et al. [54] showed an over-activation of the dorsolateral prefrontal cortex (DLPFC) when working memory load was low which was defined as retrieving 4 letters. Moreover, it demonstrated the under-activation of the DLPFC when working memory load was high (retrieving 7 letters) in older individuals which is consistent with the CRUNCH model. Interestingly, as the memory load increased, young individuals engaged the same additional regions of the brain engaged by the old individuals at a low memory load demand [54]. In conclusion, various theoretical models of hemispheric asymmetries was discussed in this section. Although these models are well studied and mainly used in human studies, due to many factors, studying cognitive changes as a result of healthy aging is difficult. These factors include, individual differences, level of complexity of the cognitive tasks, genetic differences, different experiential background and etc. Due to the mentioned factors, it is hard to reject or support any of these models and it is not simple to answer why some studies did not support any of the models. Moreover, a very large sample size is needed to generalize an observation. It is not easy to control these factors in human studies especially in a large sample size. Animal models can play an important role in the study of affects of aging on cognitive abilities by minimizing some of these factors.

2.3 The Importance of animal studies for human studies

Animal models are not only used for discovering and understanding the function of organs in a living body but also they are used for many other purposes. Results from animal studies can be used in studies of human bodies because the majority of general cell processes are very similar in non-human animals and humans. Of course, differences between species of non-human animals and humans exist but their similarities are not negligible. For example, although there are genetic differences between mice and humans, they share more than 95% similar genes [55]. Another reason that we use animal research is to understand how a normal complex biological and chemical process in the body can be changed as a result of diseases and disorders. Animal models can be critical when there is no other alternative methods. Furthermore, not all the techniques and invasive methods can be applied to humans because of ethical restrictions but animal models can advance our understanding. Animal models of disease can be used to study a wide range of neurodegenerative disorders such as Alzheimer's disease. These models serve as predictive models which can also be used to investigate new drugs and treatments to cure a disease. Animal models can play an important role in comparative psychology and cognitive psychology [56]. In fact, physiology and neurology can be linked by studying animal behaviours. For studies such as those on cognition or healthy aging, several factors including different experiential background, nutrition can be controlled by using animals. In the study of healthy aging, healthy subjects are needed which might be difficult in humans.

There might be individuals with undiagnosed cognitive impairments and disorders in human samples, which affects the results of a healthy aging study [57]. Considering the aforementioned factors, animal studies are a valuable addition to studies with human participants. Animal models offer a reliable experimental control from diets to experiential factors and can pave the way to study age-related cognitive decline.

2.4 Effects of aging on cognitive abilities in animals

Aging is not restricted to humans but also non-human vertebrates experience it. In fact, no one can escape the deleterious effects of aging. There is evidence that aging affects the function of an animal's brain and cognitive abilities. For example, aged rhesus monkeys were considerably impaired compared to younger rhesus monkeys in assessments of age-related short-term memory [58, 59]. Other studies have shown that prefrontal cortical neural changes come with aging in different species [60, 61, 62]. For example, similar to humans, the learning rate and memory ability of dogs decreases with age [63]. Fish such as zebrafish, goldfish and Nothobranchius furzeri have been used as a model for the study of aging. They showed a decline in learning during an operant conditioning task due to age [64]. During this task, the fish was placed in a shuttle box. A red color light was presented on one part of the shuttle box and served as a visual cue. The other side of the box served as an unconditioned stimulus. The fish had to move to the unconditioned stimulus part within 15 seconds from light onset and otherwise, a punishment was delivered. During this study young fish of about 5 weeks old learned the task faster than old fish of an age of 9 weeks. This study showed that learning rate decreases with aging [64]. The effect of aging on spatial cognition

has also been studied for rats. The common methods to study the effect of aging and spatial cognition is through the use of the Morris Water Task (MWT) [65] and maze learning tasks [66]. In the MWT, the animal is placed in a pool of opaque water and needs to swim to find an invisible escape platform. It has to rely only on visual cues provided by the environment or displayed on the walls surrounding the pool to locate the platform. As the animal learns the task across several trails, it usually finds the platform faster and uses a shorter path length to the platform with successive trials. Studies on rats in a circular platform [67], a water maze [66] and MWT [65] showed that aged rats demonstrated poorer performance than young rats. Furthurmore, age-related decline in spatial reference memory abilities are related to impairments of the hippocampus, which is involved in spatial cognition tasks [67, 66]. In conclusion, aging affects the cognitive abilities of non-human animals. Comparative cognition and behavioural studies have evaluated the effects of aging on the cognitive abilities of a wide range of animals. These studies have shown that aging affects many of cognitive abilities including the short-term memory, learning rate and spatial cognition tasks in non-human animals.

2.5 Avian cognition

Studies on avian cognition have revealed that birds have many cognitive abilities similar to mammals and they have shown the capability of performing complex cognition [68, 69]. For instance, Innovative tool manufacturing [70], episodic-like memory [71], planning for future needs [72] and spatial memory [73] are examples of complex cognition abilities in birds. In general, a complex cognition is defined as "all men-

tal processes that are used by individuals for deriving new information out of given information, with the intention to solve problems, make decision, and plan actions" [74]. From the anatomical perspective, it was believed for a long time that birds are not able to perform highly cognitive tasks [75]. It was assumed that the anatomical foundation is responsible for higher cognitive abilities. Moreover, the higher cognition power of mammals was a result of the expansion of neural processing in the neocortex. Since birds do not have a neocortex, this suggested that they lacked complex cognition compared to mammals [76]. Further research into neuroanatomical aspects has revealed that in spite of structural difference of the pallium in the avian brain and the neocortex in the mammalian brain, the connectivity of the avian pallium is very similar to the mammalian neocortex [75]. The pallium is not layered like the cerebral cortex in mammals but it shows a nuclear structure [75] and closely compacted neurons in the brain might result in a high speed of processing and increased cognitive abilities[77].

2.6 Pigeons as a model for the study of aging

Birds might be a good study species for understanding age-related changes. Birds (class of Aves) show a wide range of aging patterns and life spans. For example, the shortest lived bird, the common quail, can live up to 8 years[78]. In contrast, macaws can live up to almost 100 years[78]. Interestingly, several bird species have longer life spans compared to other animals such as rats and mice [79]. Birds and in particular pigeons, present an important and excellent animal model to further our understanding of age-related cognitive degeneration and the underlying neural

mechanisms, especially in regard to hemispheric asymmetry. Pigeons have been shown to perform transitive inference [80], long-term visual memory tasks [81] and numerical discrimination [82]. Visual long-term memory in pigeons has been shown to recall about 1200 images and they kept memory for these pictures over several years [81]. They have an excellent navigational skills which allow them to find their way back to the nest from thousands of miles away [83]. In addition, they have been used as a model for understanding the procedure of learning, visual discrimination [5], spatial memory [73], and navigation in the field of neuroscience [69]. Like most birds, pigeons are warm-blooded and undergo a very slow aging process [10], more similar to humans than other rodent models. They may live between 20 to 25 years (thought to approximate 80-90 human years) [10]. In fact, the longevity of pigeons and effects of aging on the cognitive changes in pigeon [73] may allow us to use pigeons as a model to study age-related changes of the brain [10]. Studies of age-related changes in brain function or cognitive abilities in birds have primarily focused on the encoding of spatial or feature information. In one study [73], young pigeons (2–3 years of age) and old pigeons (11-14 years of age) needed to locate a goal feeder which was characterized by its geometric location and visual features within a circular array of eight feeders. Younger pigeons learned the task sooner and were more accurate in their first choice than the older pigeons [73]. A study has investigated the effects of aging on spatial working memory in pigeons [84]. The result of this study showed that old pigeons were less accurate in the task compared to young pigeons because of an impairment of spatial working memory as a result of aging [84]. In addition, impairments in spatial memory are as a result of changes in the hippocampus [85].

A study on spatial memory decline in pigeons has shown that, as pigeons age, the size of the hippocampal formation in the brain increases as a result of accumulation of old cells [85]. Therefore, behavioural and cortical similarities between pigeons and mammalian brains, which were discussed in the previous section, and similarities in the aging process between pigeons and humans has made the pigeon an important animal model for the study of the deleterious effects of aging.

2.7 Brain lateralization in birds

Brain lateralization is not unique to humans but is present in many non-human vertebrates, including mammals [86], birds [2, 3], reptiles [87, 88], and fish [89, 90]. For instance, the right hemisphere of the brain is associated with social aspects and responses including the fear related responses, perception of fear, gaze perception, in several species such as chicks [91, 92] and sheep [93]. Birds have a well-developed visual system [4]. The bird's visual system, in particular the pigeon and the domestic chick, has been studied for further understanding of hemispheric asymmetries and visual cognition [94, 4]. In birds, the optic nerves are virtually completely crossed (unlike in mammals) which causes most visual information coming into an eye to be directed to the contralateral hemisphere [6]. It is because birds lack a corpus callosum which provides the connection of the left and right side of the brain in mammals [95]. This anatomical arrangement results in a "natural split-brain" like situation; visual information coming into the right eye is almost exclusively processed by the left-brain hemisphere and vice versa [96, 95, 5]. As a consequence, covering one eye allows us to study the hemisphere ipsilateral to the covered eye [95, 5, 97]. There

are several studies which have used eye covering technique for birds. Their results have shown a number of hemispheric lateralizations in the avian brain [98, 8, 99, 100, 101, 102]. Among birds, the pigeon's brain lateralization has been well studied and documented [97, 5, 103, 104, 105, 6]. Studies on avian brain lateralization revealed the superiority of the left hemisphere in pigeons in pattern discrimination tasks, rule learning, and categorization of visual stimuli [7, 5]. However, the right hemisphere is more important in processing of global cues and social cognition and detection of a predatory bird flying overhead [8, 4, 9]. In a study with adult pigeons [5], the type of asymmetry under investigation has been studied in a food foraging discrimination task ("grit-grain task") in which pigeons needed to consume 30 grains mixed with 30 grams of grit within 30 seconds after the first peck under three eye conditions: both eyes seeing (both hemispheres available), right eye seeing (the left hemisphere only) and left eye seeing (right hemisphere only). The discrimination performance was defined as the percentage of grains eaten to the total number of pecks. Results showed that pigeons performed the task better in the both eyes seeing condition compared to the two monocular seeing conditions. Also, discrimination performance of most of the pigeons was higher in the right eye seeing (left hemisphere) than the left eye seeing (right hemisphere) condition. Interestingly, there was a positive correlation between the asymmetry index (absolute difference in performance between right eye seeing and left eye seeing) and discrimination performance in the both eyes seeing condition. This indicates that more lateralized pigeons showed higher performance in the right eye seeing condition. In line with this approach and considering that aging might affect the function of each hemisphere differently, in my thesis the effects

of aging on the success rate of discrimination performance in pigeons were assessed. Specifically, the aim of my research was to investigate the effect of aging on metabolic rate of each hemisphere and cerebral asymmetry of three groups of pigeons (adults, old, and very old pigeons) when performing a grit-grain discrimination task by using the fluorodeoxyglucose Positron Emission Tomography (FDG-PET) technique. FDG-PET imaging technique has potential to give a better understanding of the avian brain function. Studying the age-related changes of brain functionality in an animal model can be extrapolated to the effects of aging in humans brain functionality and give insight into the healthy process of aging.

2.8 Basics of PET imaging

Positron emission tomography (PET) is a non-invasive nuclear medicine imaging method which is used to study the function of organs in humans and in animals [106]. PET neuroimaging can be used for cancer and tumor diagnosis, investigation of neurodegenerative disorders, movement disorders, or cardiovascular disease. The assessment of metabolism rates and protein accumulation through PET imaging can reveal impairment and abnormalities in organs which might be helpful in the diagnosis of disease and disorders at very early stages. PET utilizes the unique decay characteristics of radionuclides (isotopes) that undergo positron emission decay [106]. The isotope (produced in a cyclotron) is unstable because of excess nuclear energy. The isotope is labeled into a biological molecule which plays a carrier role. In fact, the carrier molecule and isotope form the radiotracer that is administrated into the subject's body through intravenous injection. The radiotracer can interact with spe-

cific proteins in the body and thus distributed into body tissue [106]. When the radionuclide decays, it produces a positron ($\beta+$). The positrons interact with the surrounding electrons. When a positron meets an electron, annihilation occurs which ultimately produces two high-energy photons (annihilation photon with 511 keV energy) being emitted simultaneously in opposite directions (at 180 degree from each other [106], see Figure2.1). A PET scanner consists of a set of detectors arranged in a circle where annihilation occurs, see Figure2.2. Detectors detect photons that arrive simultaneously. A photon's origin can be found by using the line of response (LOR) between each detector pair that detected the photons. LORs that present the information of detected photons (annihilations) are grouped together and stored as a sinogram. The sinogram is the raw PET data. Each row in the sinogram shows the parallel line integrals that correspond to a projection of the activity at a specific angle. These sinograms are reconstructed into cross-sectional images that represent the radioactivity distribution in the body [106].

2.9 18F-FDG PET imaging

Fluorodeoxyglucose F18 (F18-FDG), chemically known as 2-[18F]-fluoro-2-deoxy-D-glucose, is the most used PET radiotracer in nuclear medicine. FDG is a glucose analog. 18F-FDG is produced by substituting the normal hydroxyl group at the C-2 position in the glucose molecule with fluorine-18, a positron-emitting isotope. Following the injection of 18F-FDG into the body, 18F-FDG moves inside the body through blood circulation and is absorbed into cells through glucose transporters. It is then trapped within cells where it undergoes the first step of glucose metabolism,

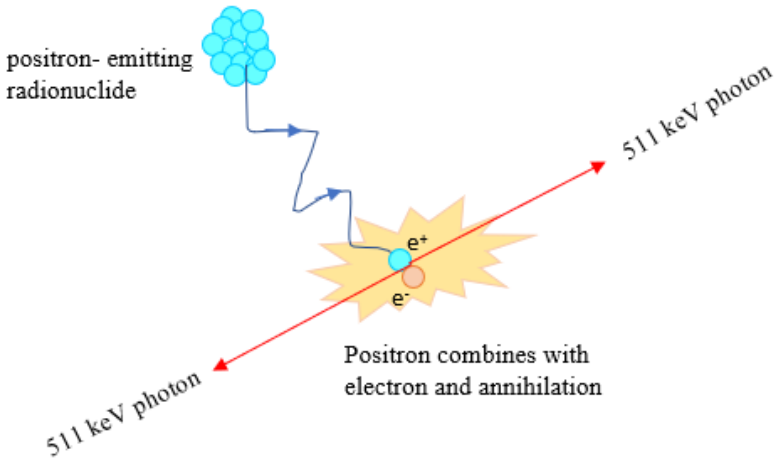


Figure 2.1: Generation of two high energy annihilation photon as a result of annihilation after positron emission by the positron-emitting radionuclide.

namely phosphorylation (as the hydroxyl is replaced with 18F, 18F-FDG can not proceed further in the process of glycolysis, the breakdown of glucose to extract energy for cellular metabolism). This leads to an accumulation of the radiotracer in the cell where its radioactivity decays with a half-life of 109.7 minutes. Therefore, FDG-PET can be used to provide a measurement of cellular glucose metabolism and allows the mapping of glucose utilization within an organ like the brain, or heart, as well as cancerous cells [107]. If 18F-FDG is administered while the subject is engaged in a task, the region of the brain processing the task will show increased accumulation of 18F-FDG due to an increase in metabolic activity in this region [108], which allows

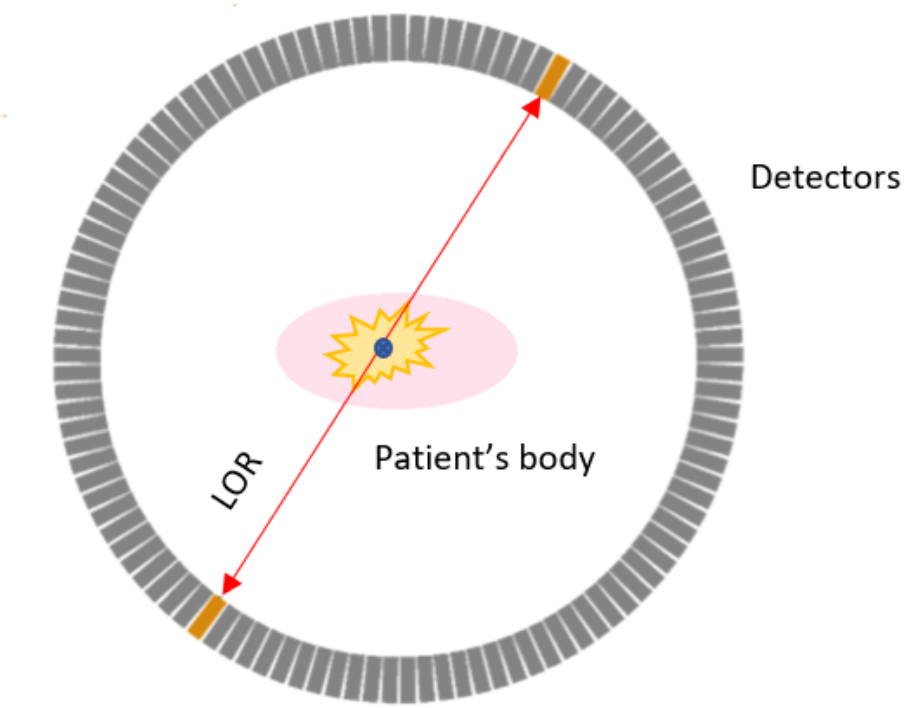


Figure 2.2: Detectors of a PET scanner arranged in a circle. Annihilation occurs within the circle. Detectors detect photons that arrive simultaneously. A photon's origin can be found by using the line of response (LOR) between each detector pair that detected the photons.

us to measure the neuronal and functional activity of the brain. The half-life of 18F-FDG makes it possible to study a subject's brain function and metabolism rate while performing a cognitive task outside of the scanner and scanning the brain afterwards. With this method, we can examine brain function in tasks that require the subject to stand or move around, or that require the subject to look at or interact with certain stimuli. In order to test brain function, 18F-FDG needs to be injected before the

cognitive task is started. After the injection, the subject performs the cognitive task outside of the scanner and is then scanned. Another advantage of this method is that the animals can also be scanned while under anesthetization and the effect of anesthesia is small because FDG uptake has happened already[109].

2.10 FDG uptake and quantification

Quantitative and semi-quantitative measurements play an important role in medicine. The Standardized Uptake Value (SUV) is an unitless nuclear medicine term used as a semiquantitative method in preclinical and clinical image analyses of FDG-PET images. SUV represents the radiotracer uptake in a Region Of Interest (ROI) related to the average uptake throughout the body, as described by the following equation:

$$SUV = \frac{\text{RC}}{(\text{ID} \ / \ \text{W})} \tag{2.1}$$

where RC is the radioactivity concentration within a ROI, which can be the pixel intensities of a calibrated PET image [kBq/ml], corrected to the time of injection. ID is the injected dose into the subject's body, corrected to the time of injection in kBq, and W is the subject's weight in grams [110].

2.11 Factors affecting the FDG uptake and SUV

Several biological and technical factors affect the uptake of 18F-FDG and PET quantification (SUV). Despite the existence of all these factors, measuring SUV is a common and adequate method for many clinical and research applications. Factors

that might affect SUV have been studied and reviewed [111, 112] and tried to provide ideas and strategies to minimize the effect of these factors on SUV. Here, we briefly review the important factors affecting the FDG uptake and suggested strategies for minimizing the effects of these factors on SUV.

2.11.1 Biological factors

One of the most important biological factors that affect the uptake of the radiotracer is the subject's blood glucose level [112]. After absorption of 18F-FDG by a cell, the hexokinase enzyme starts to phosphorylate the 18F-FDG and to produce 18F-FDG-6-phosphate, which is captured in the cell. There is a competition between 18F-FDG and glucose for phosphorylation as hexokinase also phosphorylates glucose [113]. Due to this competition, a high level of blood glucose before PET scanning decreases the uptake of 18F-FDG and affects the SUV. The effect of blood glucose on PET imaging has been studied for example by Sprinz et al. [114], who investigated the effect of blood glucose on SUV in different organs in the body. They reported a significant impact of the glucose level on the 18F-FDG uptake in the brain. However, the effect of blood glucose on SUV in other organs was minimal. Thus, it has been recommended by several studies [115, 116, 117] that subjects be fasted for at least 6 hours prior to the injection of 18F-FDG to achieve accurate measurements.

Movement of the subject during scanning and abnormal breathing patterns (e.g., from lung lesions) can cause image artifacts and result in a low resolution image, which might impede correct SUV measurements. In hybrid imaging methods, which is the combination of structural and functional images such as PET/CT, artifacts

from the subject's body movement and ultimately low resolution image may results in a mismatch between the structural image (such as Computed Tomography) and the functional image (such as PET) [112]. For animal studies, anesthesia is a common method to minimize movement. Also, the subject's respiration rate needs to be monitored to make sure that the breathing pattern is normal. Another important factor is the subject's comfort. In clinical applications and human studies, long delays between patient or subject preparation to imaging because of poor coordination or lack of knowledge about the imaging process might lead to a high stress level that can affect the uptake of FDG. To deal with this issue, a good coordination of the staff involved in the imaging, providing a sham experiment and explanation of each step of the imaging procedure prior to imaging are recommended. In order to reduce the stress level in animal studies, animals can be familiarized with the imaging room, and ideally the imaging would occur in the same room as other aspects of the experiment. Additionally, animals should be blindfolded during the injection and handled with appropriate care.

2.11.2 Technical factors

Technical factors are linked to errors in system calibration, measurement of activity in the syringe prior to injection and residual activity after the injection, incorrect timing, as well as to errors in the paravenous administration of 18F-FDG and anesthesia induction. To solve these issues, it is necessary to use a calibrated system, by performing a system calibration in each imaging session, that is used for SUV calculation. Furthermore, a run-sheet containing all the information related to the syringe

activity and residual activity can prevent the researcher from entering of wrong information or from forgetting to enter them. The correct administration of the FDG is a critical factor, especially in small animal PET imaging studies. There are many different methods for animal injections such as using catheters inserted into the blood vessels, needles and oral gavage [118]. In animal PET studies, the radiotracer is usually administered through intraperitoneal (IP) or intravenous (IV) injection. An IP injection is the injection of the radiotracer into the peritoneum (body cavity), whereas an IV injection is the injection directly into a vein. The tail vein IV injection is the most common method for lab animals such as rodents but both IP and IV injection routes have been used in animal studies [119, 120]. Although the IP injection is more convenient and more common for animal studies, it could lead to an unsuccessful injection if an injection occurs into the bowel, urinary bladder, or subcutaneously [120]. The IV injection is a very quick and effective way to inject the tracer into the body, because it is administrated into the vein directly and the blood circulation carries the tracer. In contrast, the initial distribution of a tracer injected through the IP route is slower than through the IV route, as the tracer needs to spread out across the peritoneal membrane into the vessels [121]. Also, the speed of radiotracer delivery can sometimes be an important factor in a PET imaging study. It is recommended to administer via IV injection. Normally, removing the needle does not cause a significant blood loss.

Anesthesia is another factor that needs to be considered in small animal PET imaging studies. Generally, anesthesia is used for preclinical and research studies rather than for clinical purposes. Anesthesia not only affects the animal preparation but also

influences the uptake and obtained signal. Anesthesia is normally used to minimize movements of the animal to obtain a high quality image. Anesthesia is administered by using either gas (inhalant) or an injection. Gas anesthesia by using isoflurane is the most common way to restrict movement in animal studies. The advantage of this method is having a constant level of anesthesia as long as needed. In addition, recovery from gas anesthesia is very easy and quick and has minimal physiological side effects [118]. One way to reduce movement of the animal and at the same time eliminate the impact of anesthesia on the animal's physiology and PET data, is by performing the FDG injection (and allowing for its uptake) in the conscious subject followed by imaging in the unconscious subject.

2.12 Small animal PET imaging system

Historically, animals have played a significant role in scientific and clinical research. Without animal research, millions of people would be victim of diseases such as smallpox, measles, mumps, and tetanus. Many drugs and therapy methods, as well as diagnosis techniques that have been used for treatment of human disease could not be achievable without animal models. Undoubtedly, there are many challenges and limitations in conducting animal studies that rely on technology, especially regarding imaging systems. The spatial resolution of PET systems, which is mainly designed for human subjects, does not allow the imaging of small regions and tissue of small animals such as rats [122]. As a result, the noticeable interest from academia and industrial companies in animal model research has led to the development of small animal PET imaging systems. Small animal PET imaging systems (microPET) are

designed mainly for mice and rodents. Since the mid 1990s, when the first PET image of a rat brain was published and the first version of this imaging system was introduced [123], small animal PET imaging has been developed and used extensively in biology, neurology and biomedical research. The spatial resolution of small animal PET systems is in the range of 1- to 2-mm [124]. More information about small animal microPET can be found in [125]. Recently, small animal PET has been combined with structural imaging systems such as CT and Magnetic Resonance Imaging (MRI) thus providing both functional and anatomical information simultaneously which has paved the way for a better understanding of disease and disorders.

2.13 Applications of FDG-PET imaging in small animals

Advances in PET imaging systems and the development of systems with high spatial resolution that can identify the structures within small animals have created new opportunities for the applications of PET imaging for animal studies. Along with behavioural observations, neuroimaging techniques can be implemented with animal models such as primates, rodents and also birds to further our understanding of many different diseases and disorders. In addition, many new clinical therapies and diagnosis methods are first evaluated in small animals. Also, longitudinal studies can be performed in a single animal by using PET imaging, eliminating the need to sacrifice an animal to gain access to its brain. In fact, using PET imaging may allow us to investigate in vivo molecular changes from the early stages of biological

development to different stages of disease progression. Recently the PET imaging technique has been applied on small animals for a wide range of purposes in oncology, heart disease, drug development, neuroscience, neurological disease and brain studies [126]. In the following section, I will give a summary of the applications of FDG-PET imaging in selected studies of neurological diseases.

2.13.1 Epilepsy

Epilepsy is one of the common neurological disorders. There are several different types of seizures such as generalized, focal, or tonic-clonic seizures [127]. PET imaging might pave the way to study differences in terms of synaptic and brain activation in different types of epilepsy and help with the classification of seizures. Animal models can be used to evaluate new drugs and treatments. The utilization of the FDG metabolism to visualize seizures in rats was first done by Kornblum et al. [108]. In this study, rats underwent an initial FDG-PET scanning to establish baseline uptake in the control condition. One week later, the rats were injected with Kainic Acid (KA) to induce seizures and then another FDG-PET scan was performed. They were scanned with a microPET system after a 45 minute uptake period. The results showed a significant FDG uptake and metabolism rate in the dorsal and ventral hippocampus and the entorhinal cortex during seizures. In addition, Mirrione at al. [128] used pilocarpine to induce seizures in mice and showed an increased uptake of FDG in the hippocampus, as large as 33.2%.

2.13.2 Alzheimer Disease

FDG-PET imaging has been used for the assessment of changes in brain neuropathology and motor functions as a result of Alzheimer disease (AD). The FDG-PET technique has been used on a wide range of mouse models. Several studies have shown an increase in cerebral glucose metabolism [129, 130]. However, others revealed a decrease in FDG uptake[131, 132]. Bouter et al. [133] used Tg4-42 mice as an animal model to study changes in the brain glucose metabolism rate as a biomarker for AD by using 18F-FDG/MRI. In this study, two age groups (young and aged) of Tg4-42 and wild-type (WT) mice performed the Morris water maze task to assess of spatial reference memory. During this task, mice needed to find a hidden platform in a circular swimming pool. Then, they were tested in a probe trial in which the platform was removed, and they were placed into the pool from a new entry point. They had to swim for 1 minutes while their swimming path was recorded. Results showed that aged Tg4-42 needed a longer time to find the platform than the other age group and both WT groups. Also, by comparing the whole cerebral SUV in each age group, the researchers found that aged Tg4-42 mice showed a notably lower SUV compared to young Tg4-42 mice and both WT groups. Young Tg4-42 and the WT groups did not show any significant difference in FDG uptake. When measuring SUV of different brain regions for these groups, significant differences were found in the hippocampus, forebrain and midbrain areas.

2.13.3 Behavioural and Cognition studies

Recently, FDG-PET imaging has been used to evaluate brain activity and synaptic responses during the performance of behavioural tasks in animal models from birds to primates. Two studies have used FDG-PET imaging to investigate the effect of cocaine on the brain of nonhuman primates while they were tested in a cognitive task with low and high cognitive load [134, 135]. The results of these studies were important as they assessed how brain and its cognitive function are affected in the addicted state. One recent study used FDG-PET to study avian brain activation. Wild crows were captured and FDG was administered. Then the crows were exposed to handlers wearing either a "threatening" mask (a mask worn when capturing the bird) or "friendly" mask (worn when feeding and caring for the bird). Comparison of the two groups showed increased FDG uptake for the "threatened" crows in regions of the brain known to be associated with perception, attention, fear and escape behaviour [117].

PET imaging has also been used to investigate the release of dopamine in the striatum, a region of the forebrain that controls social and sexual behaviours, as a response to song stimulation in female and male zebra finches [119]. The activity in the brain regions involved during flight have been studied in flying starlings by using FDG-PET imaging [136].

An FDG-PET imaging study has investigated the relation between glucose metabolism and attention on the rat brain in an attention task [137]. In this study, rats performed the task in an operant chamber that had five aperture. The task started when the rat poked its nose (nose poking) into the the food magazine pellet while the house

light was on. After that the light was turned off and then one of the five holes was randomly illuminated as a visual stimulus. The rat needed to monitor the five apertures for the light stimulus and chose the correct aperture by performing a nose-poke within 5 seconds. If the rat made a nose-poke before the appearance of a stimulus or chose an incorrect aperture, the lights turned off for 5 seconds and then a new trial started. The rats were scanned in a baseline condition (the 5 chambers' lights were on and the rat received food after making a nose-poke in any of them), during a learning condition (when learning the task) and an attention condition (when rats had completely learned the task and their attention was tested) which contained two different stages, namely low attention and high attention. Comparing the FDG uptake during low attention and high attention showed that there was a significant increase in FDG uptake in the right retrosplenial cortex and a significant decrease in the right medial geniculate nucleus compared to the learning condition. The behavioural data showed that there was a significant difference in the latencies of correct trials between high attention and low attention condition, which showed that rats were quicker to respond to the task correctly during the high attention condition [137]. Overall, small animal studies using FDG-PET imaging are receiving more attention which provide a better understanding in different applications and purposes in medicine and research. In fact, this method provides an opportunity to study brain metabolism and function in animal models of human disease and disorders.

PET imaging has only been recently used with bird, and never yet pigeons. Recognizing the unique insights that studying the avian brain offers for the understanding of age-related changes in cognitive functioning, we used advanced neuroimaging meth-

ods to non-invasively study brain function and age-related changes of metabolic rate in each hemisphere using a pigeon model of aging for the first time. The main objective of this research is to understand the behavioural and neural effects of aging on hemispheric asymmetry.

Chapter 3

Methods

3.1 Subjects

15 pigeons (Columba livia) were used as subjects. They were grouped into three age groups: adult (n=5, 2 males between 1-2 years of age), old (n=5, 3 males between 11-14 years of age) and very old (n=5, 3 males between 17-21 years of age) individuals. The pigeons were housed in individual home cages (60 cm length x 60 cm width x 38 cm height) in a colony room at the University of Manitoba. The colony room was kept on a 12h light-dark cycle, with lights coming on at 7:00 am. All subjects had ad libitum access to grit and water and were maintained between 85%-90% of *adlib* weight by controlled feeding of mixed grain and seeds. All methods and procedures complied with the institutional ethical standards of the University of Manitoba's Animal Care Committee.

3.2 Behavioural Apparatus

All sessions were conducted in a uniformly white, enclosed rectangular environment with a wooden base (200 cm L x 100 cm W) and frame (213 cm H), see Figure3.1. Wooden walls (60 cm H) were mounted around the frame and lined on the inside with white plastic boards. White opaque plastic sheets were suspended from the top of the frame to block out external visual cues but also allow the experimenter to enter the apparatus. Downward sliding doors were located in the bottom center of each wall. The floor of the apparatus was covered with black rubber flooring. Lighting was provided by four identical fluorescent bulbs mounted above the ceiling. Inside the arena (apparatus), there was a metal tray (66cm L x 63cm W) with a rim with a height of 6 cm. A video camera (EverFocus) on a tripod was located inside the arena in front of the metal tray. This permitted a view of the metal tray and its surroundings were in the frame. The video camera was connected to a computer running BiOserve which was used to monitor and record all experimental trials. A GoPro Hero5 camera was attached in the middle of one side of the metal tray and captured the view of inside the metal tray and provided a high-definition view of the tray during test sessions.

3.3 General Procedure

The experiment consisted of three phases including familiarization phase, habituation phase and testing phase, which are discussed in the next section.



(a)



(b)

Figure 3.1: *(a)* The set up inside the apparatus with a view of the metal tray containing grains mixed with grit. A video camera on a tripod was located inside the arena in front of the metal tray. A GoPro Hero5 camera was attached in the middle of one side of the metal tray for test sessions; *(b)* a view of the out side of the apparatus.

3.3.1 Familiarization Phase

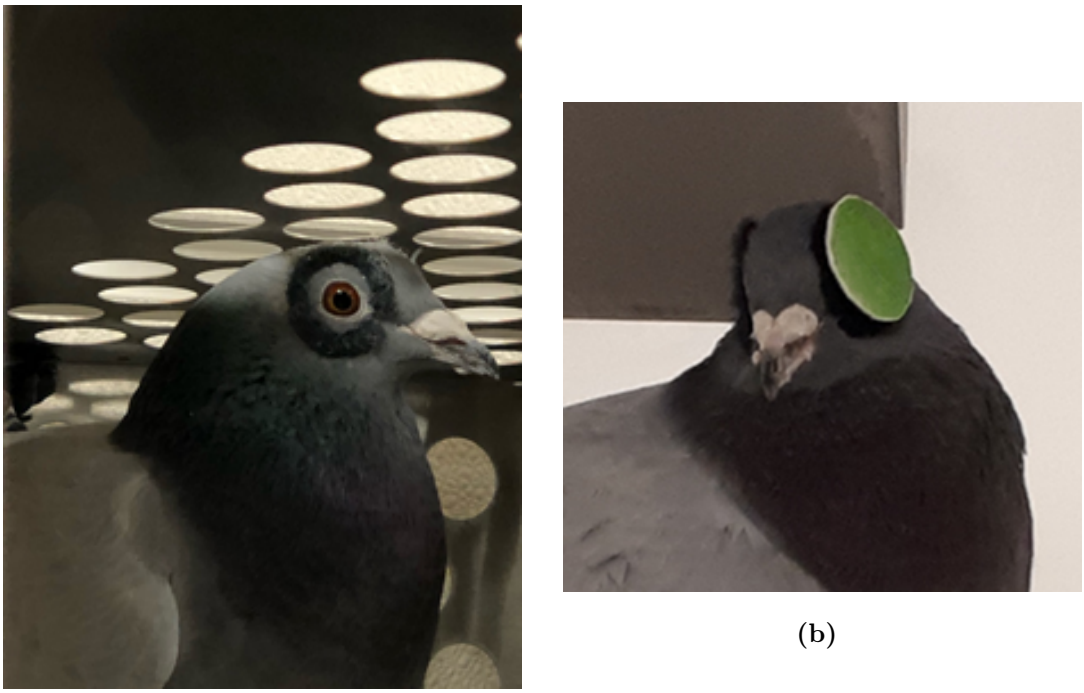
An initial arena familiarization phase was administrated for pigeons that did not have any experience in the arena within the previous year to familiarize them with the arena and the experimental environment. During this phase, a pigeon's daily food ration was placed on a white plastic tray (42 cm L x 29 cm W) in the center of the metal tray. All the lights in the room and above the apparatus were switched off. The pigeon was brought from the colony room into the experimental room in

a white plastic transport box, which was placed on the ground near the apparatus. The pigeon was removed from the box and placed into the arena through the white opaque plastic sheets, after which the apparatus lights were switched on. The subject had to approach and consume the food in the tray within 30 minutes which was considered as a successful session. If a pigeon did not consume any grains within the 30 minutes, the pigeon was given an additional 30 minutes to consume the food inside the apparatus. Regardless of either eating or not eating during the additional 30 minutes, the session was considered as an unsuccessful session, as the criteria was consumption of food within the first 30 minutes. The trial ended approximately one minute after the pigeon completely stopped eating, or one hour, whichever occurred first. Any leftover food was provided to the pigeons in their home cages during the night feeding. To pass this phase, the pigeon had to peck for 30 seconds continuously during at least two successful sessions in the last three familiarization sessions.

3.3.2 Habituation Phase

Following the familiarization phase, pigeons were habituated to wearing an eye cap. First, the feathers around the pigeon's eye were carefully trimmed. Then, to occlude each eye, a ring made from the loop part of Velcro (approx. 25 mm diameter and 5 mm thick) was attached around the eye with non-toxic eyelash adhesive. To create an eye cap, the hook of Velcro, equivalent in size to the eye ring, was glued onto a round piece of tetra pack carton. The eye rings remained attached for the duration of the experiment, whereas the cap was only attached to one eye during experimental sessions. Figure 3.2 shows the eye ring around the eye of a pigeon and the eye cap

covering the eye.



(a) **(b)**

Figure 3.2: (a) shows the image of eye ring around the eye of a pigeon. (b) shows the eye cap covered the left eye of the pigeon

During the habituation phase, the pigeon first received its daily ration of food in its home cage with one eye capped, alternating daily between capping the left or the right eye. Pigeons were given 30 minutes during the evening feedings in the home cage with eye-cap. Once a pigeon started to eat within 30 seconds after receiving the food while one eye was covered inside the home cage, it was ready to receive its daily food ration inside the arena in the same way as described in the familiarization phase section, but now with one eye capped, alternating daily between the left and the right eye. If a pigeon did not eat while wearing an eye-cap, we removed any food

that might have been left over from the previous night from their home cages in the morning of each session to make them motivated to eat during the training session. Two of the subjects did not habituate to eat their food while wearing an eye cap after 14 sessions of training. Therefore, they were replaced by two other pigeons. To move to the test session, pigeons had to experience at least two consecutive session of capped left eye and capped right eye in the arena. The habituation was continued until the scheduled test session.

3.3.3 Testing phase

Figure 3.3 shows the order of procedure and the duration of each step for the completion of a test session.

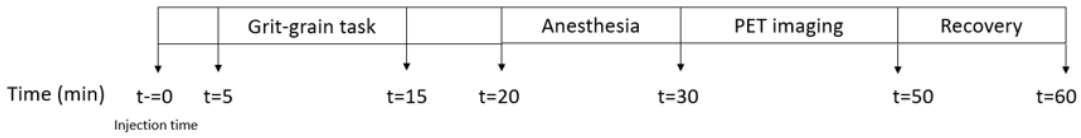


Figure 3.3: Time line of the test session. The time (t) is based on minutes.

Each bird underwent three test sessions involving a fluorodeoxyglucose-positron emission tomography (FDG-PET) scan, during which the pigeon experienced one of three eye conditions: **binocular** (no eye capped), during which both hemispheres were available to process visual information; **left eye capped**, during which the left hemisphere processed almost all visual information; **right eye capped**, during which the right hemisphere processed almost all visual information. Figure 3.4 shows each eye condition. The three sessions were at least one week apart to allow pigeons to recover from the anesthesia and from the radiotracer injection. The order in which

the three sessions were administered was counterbalanced across age groups and sex.



Figure 3.4: Pigeons performed the grit-grain task under three different eye condition from left to right: right eye capped, binocular, left eye capped conditions.



Figure 3.5: A pigeon performing the grit-grain task in monocular eye condition

In order to reduce the blood glucose level and enhance FDG uptake, pigeons were fasted for at least 6 hours prior to the testing sessions. In the test sessions, pigeons performed the grit-grain discrimination task. This task required the pigeon to peck from the metal tray containing 300 wheat grains mixed with 300 gram of grit similar in color, shape and size within 10 minutes [5]. Figure 3.5 shows a pigeon performing the

test session while the right eye of the pigeon is covered by using an eye cap. Subjects were individually moved from the home cages in the colony room to the experimental room using a transport box. The metal tray containing 300 wheat grains mixed with 300 grams of grit was placed inside the apparatus. Unlike during the familiarization and training sessions, there was no plastic tray present inside the arena.

At the beginning of a test session, the pigeon received an intravenous injection of up to 35 MBq FDG diluted in saline solution. One of the subjects experienced intraperitoneal injection due to excessive hemorrhage from the wing arteries. The pigeon was returned to the transport box for two minutes of rest. Then, an eye cap was fitted according to the current eye capping condition.

The lights in the arena and the experimental room were turned off and the cameras were turned on. The subject was removed from the transport box and placed into the arena onto the metal tray. The arena lights were turned on to signal the start of the trial. The pigeon was allowed to peck at and consume any grains from the tray within 10 minutes. After that, the trial ended by turning the arena lights off and moving the pigeon from the arena into the transport box.

Following the behavioural task, the bird was anesthetized using 4% isoflurane in oxygen with a flow rate of 200-400 mL/min. Then imaging started 30 minutes after injection and the bird was imaged for 20 minutes. An animal warming pad with heated water was available if needed to keep the subject warm during the scanning period. The respiration rate of the subject was monitored by a pad sensor connected to a BIOPAC monitoring system. Following completion of the PET imaging, the pigeon was recovered from anesthesia and held in an individual cage (60 cm length x

60 cm width x 38 cm height) inside the experimental room for 24 hours to allow for the physical decay of the radioactive tracer. In the cases where a pigeon did not perform any pecks within the 10-minute test session, it was given an additional 5 minutes. If the pigeon still had not performed any pecks at the end of the additional time, the session was aborted and repeated after a minimum of seven days later. Pigeons that did not complete all three conditions within five sessions were dropped and replaced by another pigeon. This was the case for two pigeons.

3.4 Imaging Protocol

3.4.1 18F-FDG PET image acquisition

High-resolution FDG-PET images were acquired using a Concorde Microsystems/Siemens microPET R4 PET system [138, 139] for 20 minutes, approximately 30 minutes after tracer injection. Figure 3.6 shows the small animal microPET R4 system.

3.4.2 System Calibration

PET imaging allows us to measure the activity concentration of the radio-tracer within a volume of interest. In order to convert the image count density into an activity concentration, the system needed to be calibrated accurately. Generally, one of the common ways of system calibration is scanning a uniform phantom or source with a known activity concentration (Bq/ml), such as a uniform cylinder. Then, the cylinder images are reconstructed with the same parameters used for the study. In order to measure the activity concentration in a PET image, the Volume Of Interest

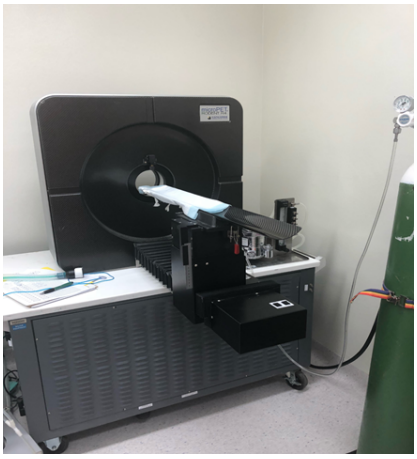


Figure 3.6: Image of Concorde Microsystems/Siemens microPET R4 PET system used for small animal imaging

(VOI) was defined in PET images by using an image analysis software package which allowed us to calculate the average voxel value within the VOI. Then Equation 3.1 was used to calculate the Calibration Factor (CF). It is important to note that the activity concentration of the phantom needed to be corrected to the scan acquisition time for decay [106].

$$CF = \frac{\text{Activity concentration corrected to the scan acquisition time}}{\text{Average count density}} \tag{3.1}$$

In this study, a plastic water-filled bottle with volume of 609 ml and 66 mm diameter, approximates the pigeon size, was used as a cylinder phantom. At the end of each session, the bottle was scanned for five minutes. Then, the ASIPro software was used to draw a circle as a VOI in seven consecutive slices of PET image from the bottle scans and the average count density was calculated.

3.4.3 Normalization

Normalization in PET imaging is an important step and necessary to produce a uniform image. Non-uniform detector efficiency responses and variations in geometrical and detector electronics result in a non-uniform image which requires correction. A normalization file corrected individual sinograms and differences in efficiency of coincidences in different planes [106]. The normalization file can be generated using the same plastic water-filled bottle used for the system calibration[106]. The bottle must be centered in the system field of view (FOV). In this study, right after the scanning of the water bottle, the normalization file was acquired by choosing 200,000,000 counts in microPET manager software.

Chapter 4

Data Analysis

4.1 Data Analysis

4.1.1 Behavioural Assessment: Grit-grain task

All the videos were analyzed to count the total number of pecks a pigeon made during each testing session. In addition, the remaining grains after each session of the experiment were collected and counted to calculate the number of grains eaten. The success rate was measured as shown in Equation 4.1:

$$successrate = \frac{\text{number of grains eaten}}{\text{total number of pecks}} \times {}^{100} \tag{4.1}$$

Kruskal-wallis H test were used to test overall differences between age groups for each eye condition separately. Friedman tests were used to assess differences between each eye condition for each age group separately. Values below p = 0.05 were considered statistically significant. All analyses were conducted using the jamovi project (2019) Computer Software (Jamovi version 09.2.8)[1]. The Spearman's rank-order correla-

[1] `https://www.jamovi.org`

tion which is the nonparametric version of the Pearson product-moment correlation. Spearman's correlation coefficient was used to measure the strength and direction of association between two ranked variables.

4.2 PET Data Analysis

4.2.1 Correction for subject motion and image reconstruction

According to Phelps [106] "the goal of image reconstruction is to provide quantitatively accurate cross sectional images of the distribution of positron-emitting radiopharmaceuticals in the object that is being scanned, using the externally detected radiation along with the mathematical algorithms of computed tomography" (p.70). The images were reconstructed by using a Maximum A Posteriori (MAP) [140] algorithm with a zoom size of 2.113. PET images can be viewed as a dynamic sequence for detecting head motion and movements. If the subject moved during the scanning, the frames with motion are discarded and those frames in which the subject remained stationary were summed to be a single frame and used for further analysis. To discard the frames with motion, the 20 minutes PET data was divided into 20 single frames (i.e. 20×60 seconds) by using microPET Manager software. Then, the MAP reconstruction algorithm applied on all frames to reconstruct the image. Then, the frames with motion can be detected by visualization in ASIPro software and approximating the head position from the first frame to the last frame. Then, frames with motion were chosen to be discarded and frames in which the subject remained stationary were summed. Then, the PET data were reconstructed. The result was

two single frames. One frame contained the sum of frames with motion and the other contained the sum of frames in which the subject remained stationary, which can be easily selected and saved as a single stationary frame and used for further analysis.

4.2.2 SPM Toolbox

All spatial pre-processing and PET analysis on the reconstructed images were conducted by using the Statistical Parametric Mapping toolbox (SPM 12)[2][141] using on MATLAB 2018b[3].

4.2.3 Spatial pre-processing and PET template creation

Figure 4.1 shows all the steps that were completed on raw PET data in SPM to create a PET template. A 3-dimensional Magnetic Resonance Imaging (MRI) atlas of the pigeon's brain that references common brain areas [142] was used as a pigeon's brain atlas. All of the reconstructed PET images were converted to NIFTI format by using Amide software [143]. All subjects' PET data were manually reoriented to the origin of the MRI template using 3-dimensional rigid body transformation which is defined by three translations in the X,Y and Z directions and three rotations (pitch, roll, yaw) [144]. All of the reoriented images were realigned. The realignment happens in two steps: first, all images were realigned to the first selected image which did not need to be the first acquired in time. secondly, a mean image was computed to which all images were realigned in a second pass. A dilated brain mask [142] was used to remove the non-brain regions. The mean image from the realignment section was used

[2]`https://www.fil.ion.ucl.ac.uk/spm/software/spm12/`
[3]`http://www.mathworks.com`

in the next step, which is co-registration. Co-registration is used to align scans from different modalities, for instance, alignment of functional image (PET) to structural image (MRI). The source image (the mean of all PET images) was matched to the reference image (the MRI atlas). The result was the PET template which was used for the analysis.

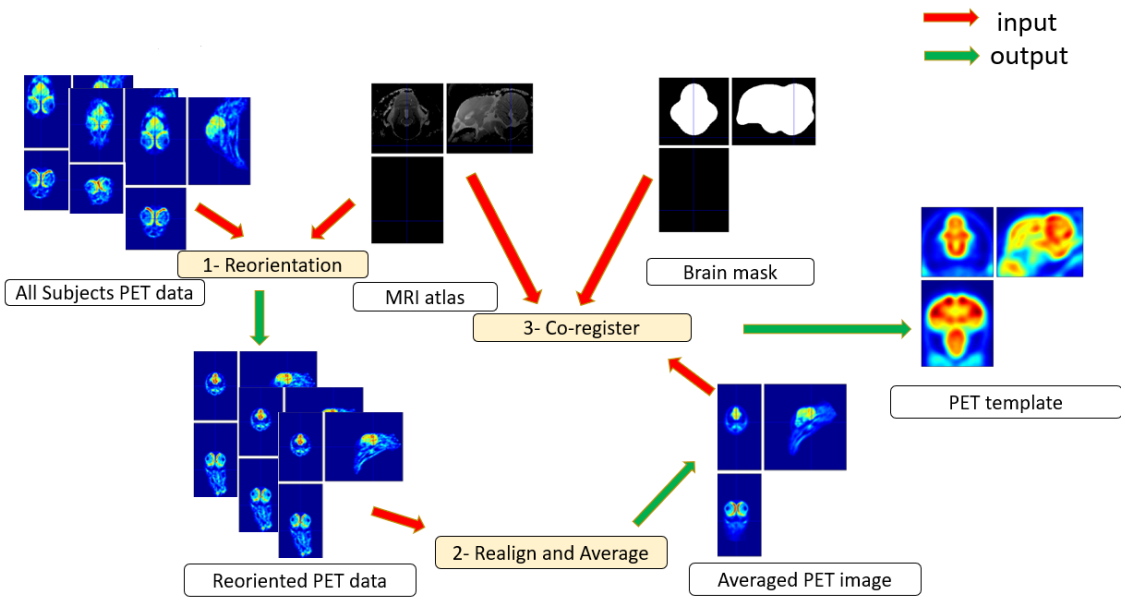


Figure 4.1: To create a PET template, the PET data were reoriented into the MRI atlas and the all the reoriented PET data were realigned and averaged. The averaged PET images was co-registered into the MRI atlas. The brain mask was used to remove non-brain area. The result was the PET template.

The next step was normalization which mapped each subject's reoriented PET image onto the template. It applied transformation and translations to minimize the variability between subjects scans in terms of brain size and shape. All the PET images were normalized on the PET template by using old normalization in the

SPM toolbox. The dilated brain mask was used as a template weighting. Figure 4.2 shows the step of the normalization of one of the reoriented PET images on the PET template in SPM.

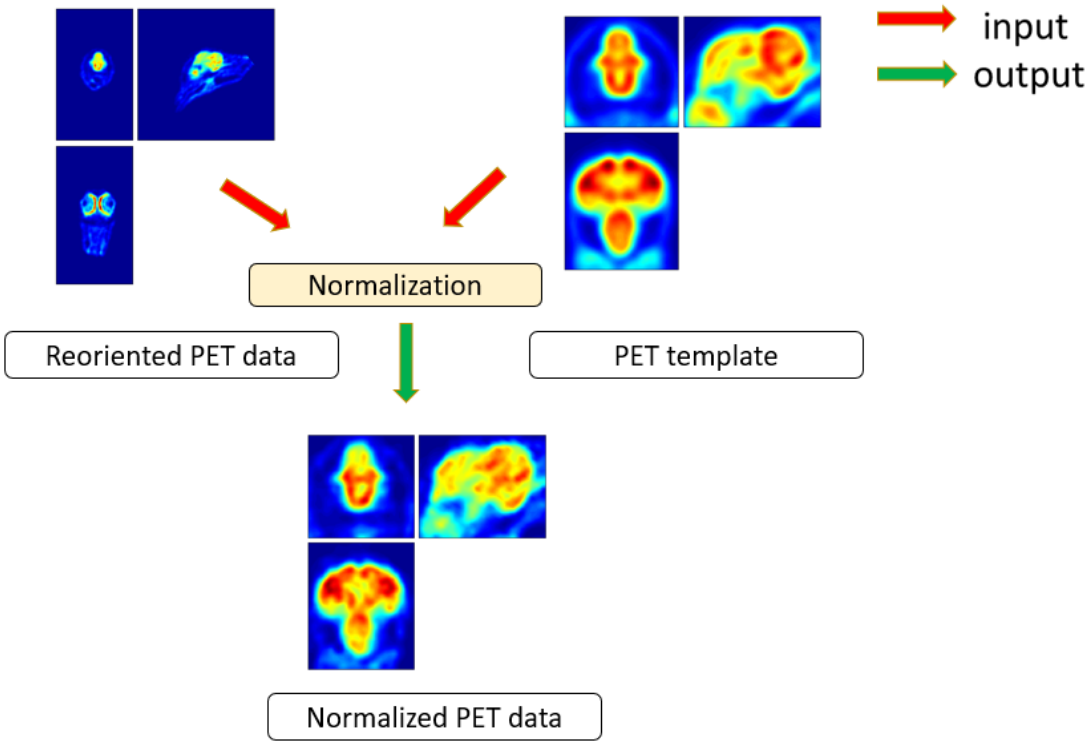


Figure 4.2: Normalization of reoriented PET images of each subject into the PET template to decrease the variation of brain size and shape of each subject

4.2.4 SUV calculation

The Standard Uptake value (SUV) of the left and right brain hemispheres were calculated for each pigeon in each eye condition by using left and right brain hemisphere masks using the SPM toolbox. To do this, each PET image of each subject was uploaded as a source image. The brain mask was uploaded as a region of inter-

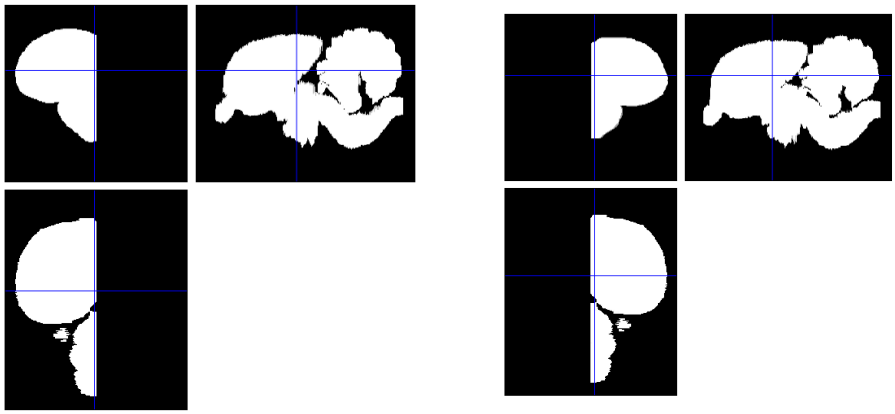
est file and the mean of activity concentration of each hemisphere were calculated. then, the activity concentration was corrected to the time of injection. By entering the injected dose and the weight of each subject, SUV was calculated. Spearman's correlation was used to evaluate if there was a correlation between age and SUV of each brain hemisphere in each eye condition.

4.2.5 Measuring Lateralization Index

The Lateralization Index (LI) for each subject in each eye condition was calculated as shown in Equation4.2.5:

$$LI = \frac{\text{SUV of the left hemisphere - SUV of the right hemisphere}}{\text{SUV of the left hemisphere + SUV of the right hemisphere}} \tag{4.2}$$

The LI is a useful measurement that shows the contribution of the hemisphere which is more responsible for a cognitive task compared to the other. This comparison is an important means of examining whether aging might affect the laterality of the brain in a cognitive task. Positive LI shows that, based on the formula, the SUV of the left hemisphere is higher than the right hemisphere. Also, the negative LI value showed lower SUV of the left hemisphere compared to the right hemisphere. To measure the SUV of each hemisphere, a mask of each hemisphere was used as a Region Of Interest (ROI). Figure 4.3 shows the mask of left and right hemisphere.



(a) **(b)**

Figure 4.3: *a)* The left hemisphere mask used as a ROI to extract the activation of the left hemisphere of the brain in grit-grain task; *b)* The right hemisphere mask used as a ROI to extract the activation of the right hemisphere of the brain in grit-grain task.

Chapter 5

Results

5.1 Behavioural results

5.1.1 Total number of pecks: Differences between age groups

The total number of pecks were counted to see if there is any significant difference in the total number of pecks between adult, old and very old groups. The mean and standard error of each age group in each eye condition are listed in Table 5.1. Kruskal-Wallis H test showed that there was no significant difference in the total number of pecks between age groups in the BIN condition (both hemispheres were available; $X^2 = 1.040$, $p = 0.595$, $df = 2$), in CL condition (only left hemisphere was available; $X^2 = 0.740$, $p = 0.691$, $df = 2$) or the CR condition (only right hemisphere was available; $X^2 = 1.548$, $p = 0.461$, $df = 2$), see Figure A.1 and Table A.1 in Appendix A for each subject total pecks.

Table 5.1: Mean and Standard Error (SE) of total number of pecks in each eye condition for each age group.

Age group	BIN eye condition	CL eye condition	CR eye condition
Adult	Mean: 286.6	Mean: 212.4	Mean: 304.8
	SE: 88.09	SE: 91.72	SE: 92.65
Old	Mean: 292.6	Mean: 308.8	Mean: 229.4
	SE: 132.01	SE: 167.68	SE: 150.12
Very old	Mean: Mean: 435	Mean: Mean: 295	Mean: 307.4
	SE: 136.29	SE: 126.64	SE: 219.80

5.1.2 Total number of pecks: Differences between eye conditions in each age group

Friedman non-parametric test showed that there was no significant differences among eye conditions (BIN, CL, CR) in the adult group ($X^2 = 0.400$ $p = 0.819$, $df = 2$), in the old group, ($X^2 = 0.400$, $p = 0.819$, $df = 2$), and in the very old group ($X^2 = 0.400$, $p = 0.819$, $df = 2$).

5.1.3 Correlation between age and total number of pecks

Spearman's correlation showed that there was no significant correlation between the total number of pecks and each individual birds age in the BIN eye condition ($r_s = 0.278$, $p = 0.31$), in the CL eye condition ($r_s = 0.246$, $p = 0.38$), and in the CR

eye condition (r_s = -0.248, p = 0.37).

5.1.4 Success rate: Differences between age groups

To evaluate the accuracy of each subject in discrimination and compare the difference between age groups, success rate were measured. Shown in Figure 5.1 and Table 5.2, Kruskal-Wallis H test showed that there was no significant difference in success rate among age groups in the BIN eye condition (X^2 = 3.86, p = 0.15, df = 2) and in the CL eye condition (X^2 = 0.56, p = 0.76, df = 2). However, the success rate significantly differed between age groups in the CR eye condition (X^2 = 6.031, p = 0.049, df = 2). Post-hoc pairwise comparisons demonstrated that there was a significant difference between the very old group and the old group (W = 3.111, p = 0.028), and the very old group and the adult group in the CR eye condition, see Figures A.2, A.3, and Tables A.2, A.3, A.4 and A.5 in Appendix A for each individual success rate in each eye condition.

Table 5.2: Mean (%) and Standard Error (SE) of success rate in each eye condition for each age group

Age group	BIN eye condition (%)	CL eye condition (%)	CR eye condition (%)
Adult	Mean: 33.6 SE: 2.929	Mean: 23.0 SE: 9.141	Mean: 31.6 SE: 8.857
Old	Mean: 36.0 SE: 10.789	Mean: 28.0 SE:11.276	Mean: 28.0 SE: 5.617
Very old	Mean: 16.7 SE: 5.582	Mean: 11.9 SE: 3.979	Mean: 6.5 SE: 3.191

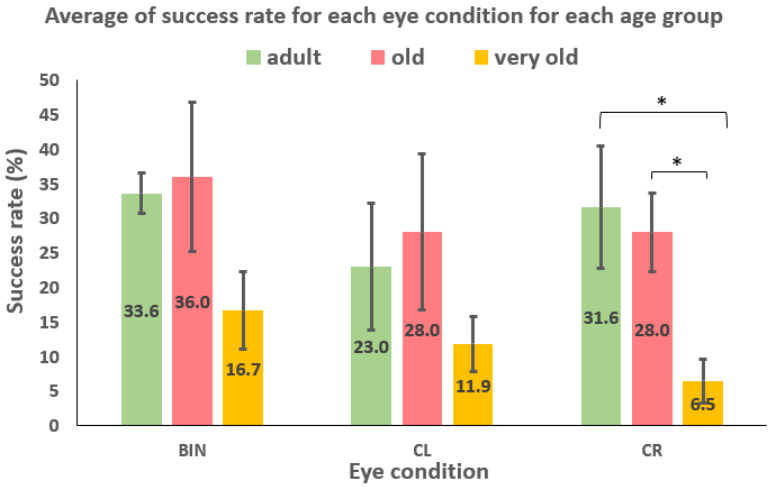


Figure 5.1: Success rate for each eye condition (BIN - binocular, CR - capped right eye, CL - capped left eye) and age group. Error bars represent standard errors. * denotes significant pairwise comparisons between age groups within a given eye condition at $p < 0.05$.

Statistical analyses showed that the performance of the adult pigeons were affected by the order of test session ($X^2 = 7.60$, $p = 0.022$, $df = 2$). Pairwise comparisons showed that there was a significant overall decrease in performance from the first test session to the second test session ($W = 4.619$, $p = 0.002$). Also, there was a significant decrease from the first to the third test session ($W = 4.041$, $p = 0.004$). However, as the order of session in which the three eye conditions were administered was counterbalanced across age groups and sex, there is little concern that the decrease in the pigeon's willingness to perform across subsequent sessions affected the validity of the data.

Table 5.3: Mean (%) and Standard Error (SE) of success rate in the adult group from the first test session to the third test session

Age group	First test session (%)	Second test session (%)	Third test session (%)
Adult	Mean: 43.29	Mean: 22.81	Mean: 22.00
	SE: 3.13	SE: 8.82	SE: 5.03

5.1.5 Success rate: Differences between eye conditions for each age group

Friedman non-parametric tests showed that there was no significant difference in success rate among eye conditions (BIN, CL, CR) in the adult group ($X^2 = 0.400$, $p = 0.82$, $df = 2$), in the old group ($X^2 = 2.80$ $p = 0.25$, $df = 2$), and in the very old group ($X^2 = 1.20$ $p = 0.55$, $df = 2$).

5.1.6 Correlation between age and success rate

Spearman's correlation showed that there was no significant correlation between success rate and age in the BIN eye condition (r_s = -0.40, p = 0.135) and in the CL eye condition (r_s = -0.217, p = 0.44). However, results indicated a significant negative relationship between success rate and age in the CR eye condition (r_s = -0.557, p = 0.03), see Figure 5.2. This shows that as age increased, success rate decreased significantly in the CR eye condition.

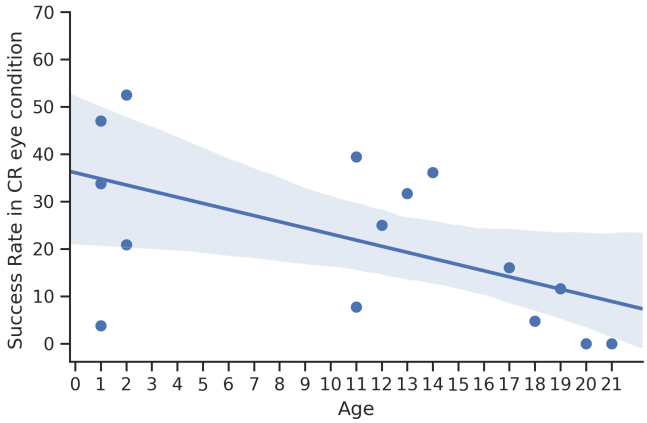


Figure 5.2: A negative relation between success rate and age in CR - capped right eye condition. Each dot represents the success rate of one subject. The shaded area represents 95% confidence interval.

5.1.7 Summary: Behavioural assessment

Overall, adult and old pigeons showed very similar performance. As we expected, very old pigeons showed the worst performance. The success rate of the adult significantly differed from that of the old and very old groups in the CR eye condition (only

right hemisphere was available). A significant negative relationship between success rate and age was only observed in the CR eye condition. There was no significant difference between age groups in the total number of pecks.

5.2 PET data results

5.2.1 Left hemisphere SUV: Differences between age groups

Shown in Figure 5.3 and Table 5.4, Kruskal-Wallis H test showed that the SUV of the left hemisphere differed significantly between age groups in the BIN eye condition (both hemisphere were available; $X^2 = 6.18$, $p = 0.046$, $df = 2$). Post-hoc pairwise comparisons showed that there was a significant difference between the adult group and the very old group, ($W = 3.693$, $p = 0.009$). However, there was no significant difference in SUV of the left hemisphere between age groups in the CL eye condition (only left hemisphere was available; $X^2 = 3.92$, $p = 0.141$, $df = 2$) or the CR eye condition (only right hemisphere was available; $X^2 = 5.42$, $p = 0.067$, $df = 2$). See Figure A.4 and Table A.16, Table A.7 and Table A.8 in Appendix to see the SUV of left hemisphere for each individual per age group. Also, see Appendix A.1, A.1.1 and Tables A.10, A.12, A.13 and A.14 for results of analyses without the cerebellum included.

On average, the adult group showed higher FDG uptake in the left hemisphere compared to the old and the very old group in all eye conditions.

Table 5.4: Mean and Standard Error (SE) of the left hemisphere SUV in each eye condition for each age group

Age group	BIN eye condition	CL eye condition	CR eye condition
Adult	Mean: 4.56	Mean: 4.09	Mean: 4.12
	SE: 0.260	SE: 0.424	SE: 0.291
Old	Mean: 3.23	Mean: 3.08	Mean: 3.01
	SE: 0.564	SE: 0.312	SE: 0.318
Very old	Mean: 2.90	Mean: 3.02	Mean: 3.08
	SE: 0.296	SE: 0.334	SE: 0.204

5.2.2 Left hemisphere SUV: Differences between eye conditions for each age group

Friedman non-parametric test showed that there was no significant difference in left hemisphere SUV between eye conditions (BIN, CL, CR) in the adult group (X^2 = 2.80, p = 0.247, df= 2), in the old group (X^2 = 0.00 p = 1.000 df= 2) and in the very old group (X^2 = 1.60, p = 0.449, df = 2). See Appendix A.1.2 for results of analyses without the cerebellum included.

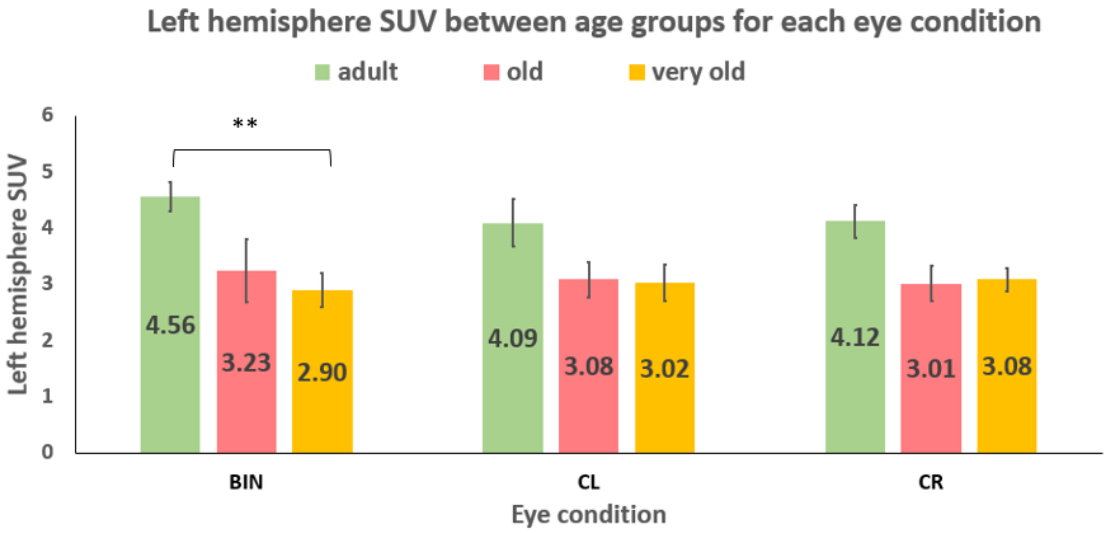


Figure 5.3: Average of SUV of the left hemisphere for each eye condition (BIN - binocular, CL - capped left eye, CR - capped right eye) and age group. Error bars represent standard errors. ** denotes significant pairwise comparisons between age groups within a given eye condition at $p < 0.01$.

5.2.3 Right hemisphere SUV: Differences between age groups

Shown in Figure 5.4 and Table 5.5, Kruskal-Wallis H test showed that SUV of the right hemisphere differed significantly between age groups in the BIN eye condition (both hemisphere were available; $X^2 = 6.18$, $p = 0.046$, $df = 2$). Post-hoc pairwise comparison showed that the SUV of the right hemisphere in the BIN condition significantly differed between very old and adult groups ($W = 3.693$, $p = 0.009$). There was no significant difference in SUV of the right hemisphere between age groups in the CL eye condition (only left hemisphere was available; $X^2 = 3.42$, $p = 0.181$, $df = 2$). Additionally, SUV of the right hemisphere in the CR eye condition significantly

differed between age groups in the CR eye condition ($X^2 = 6.62$, $p = 0.037$, $df = 2$). Post-hoc pairwise comparison showed that it was significantly different between very old and adult groups ($W = 3.102$, $p = 0.028$), and between old and adult groups ($W = 3.102$, $p = 0.028$). See Figure A.5 and Tables A.16, A.7 and A.8 in Appendix to see the SUV of right hemisphere for each individual per age group. Overall, the adult group showed higher FDG uptake in the right hemisphere compared to the old and the very old group in all eye conditions. Also, see Appendix A.1, A.1.3 and Tables A.11, A.12, A.13 and A.14 for results of analyses without the cerebellum included.

Table 5.5: Mean and Standard Error (SE) of the right hemisphere SUV in each eye condition for each age group

Age group	BIN eye condition	CL eye condition	CR eye condition
Adult	Mean: 4.33 SE: 0.235	Mean: 3.78 SE: 0.405	Mean: 4.06 SE: 0.274
Old	Mean: 3.07 SE: 0.550	Mean: 2.97 SE:0.273	Mean: 2.92 SE: 0.291
Very old	Mean: 2.78 SE: 0.281	Mean: 2.88 SE: 0.342	Mean: 3.00 SE: 0.227

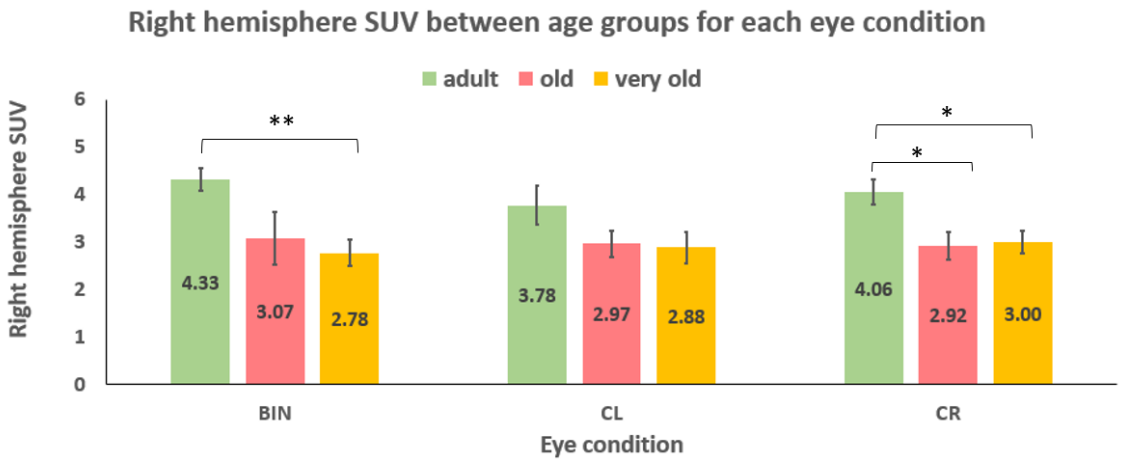


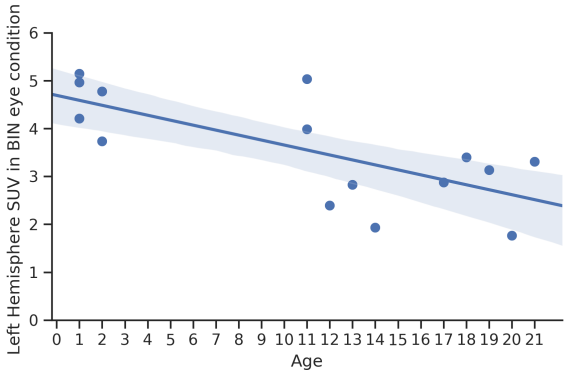
Figure 5.4: Average of SUV of the right hemisphere for each eye condition (BIN - binocular, CL - capped left eye, CR - capped right eye) and age group. Error bars represent standard errors. * and ** denote significant pairwise comparisons between age groups between age groups within a given eye condition at $p < 0.05$ and $p < 0.01$ respectively.

5.2.4 Right hemisphere SUV: Differences between eye conditions for each age group

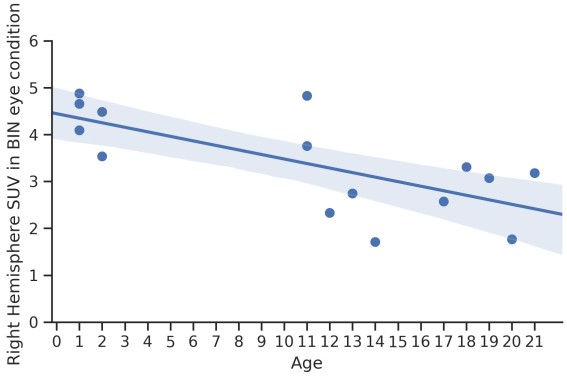
Friedman non-parametric test showed that there was no significant difference in right hemisphere SUV between eye conditions (BIN, CL, CR) in the adult group ($X^2 = 1.20$, $p = 0.549$, $df = 2$), in the old group ($X^2 = 0.400$ $p = 0.819$ $df = 2$) and in the very old group, ($X^2 = 1.20$, $p = 0.549$, $df = 2$). Also, see Appendix A.1.4 for results of analyses without the cerebellum included.

5.2.5 Correlation between age and SUV of each hemisphere

For the BIN eye condition (see Figure 5.5), there was a significant negative correlation between the SUV in the left hemisphere and age (r_s = -0.731, p = 0.002), and also between the SUV in the right hemisphere and age (r_s = -0.724, p = 0.002). Also, See Appendix A.1.5 for the correlation between age and SUV of right and left cerebrum.



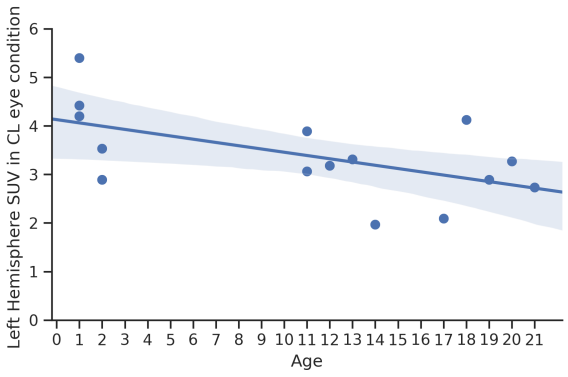
(a)



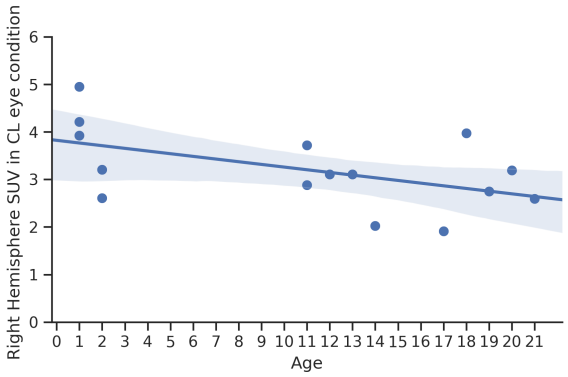
(b)

Figure 5.5: BIN eye condition: *(a)* relation between SUV of the left hemisphere and age; *(b)* relation between SUV of the right hemisphere and age). The shaded area represents 95% confidence interval.

For the CL eye condition (see Figure 5.6), there was a significant negative correlation between the SUV in the left hemisphere and age (r_s = -0.601, p = 0.018), and also between the SUV in the right hemisphere and age (r_s = -0.551, p = 0.033). Also, See Appendix A.1.5 for the correlation between age and SUV of right and left cerebrum.



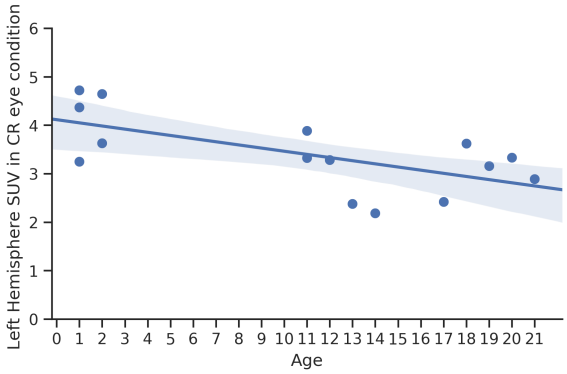
(a)



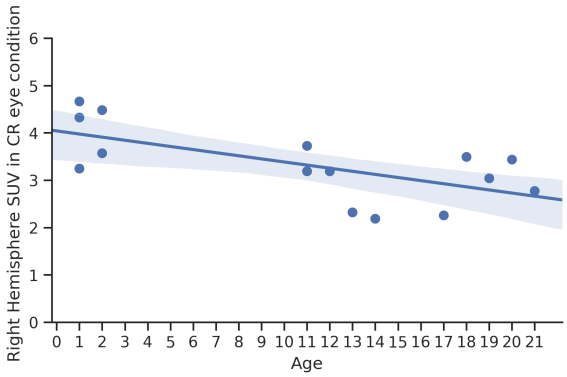
(b)

Figure 5.6: CL eye condition: *(a)* relation between SUV of the left hemisphere and age; *(b)* relation between SUV of the right hemisphere and age).The shaded area represents 95% confidence interval.

For the CR eye condition (see Figure 5.7), there was a significant negative correlation between the SUV in left hemisphere and age (r_s = -0.580, p = 0.023), and also between the SUV in the right hemisphere and age (r_s = -0.619, p = 0.014). Also, See Appendix A.1.5 for the correlation between age and SUV of right and left cerebrum.



(a)



(b)

Figure 5.7: CR eye condition: *(a)* relation between SUV of the left hemisphere and age; *(b)* relation between SUV of the right hemisphere and age). The shaded area represents 95% confidence interval.

5.2.6 Lateralization Index: Differences between age groups

Shown in Figure 5.8 and Table 5.6, Kruskal-Wallis H test showed that there is no significant difference in Lateralization Index (LI) between age groups in the BIN eye condition (both hemispheres available; $X^2 = 2.62$, $p = 0.270$, $df = 2$), in the CL eye condition (only left hemisphere available; $X^2 = 5.18$, $p = 0.075$, $df = 2$), or in the CR eye condition (only right hemisphere is available; $X^2 = 2.56$, $p = 0.277$, $df = 2$), see Figure A.6 and Table A.9 to compare each individual LI. Also, see sections A.1.6 and A.1.9 in Appendix for results of Lateralization Index of the cerebrum.

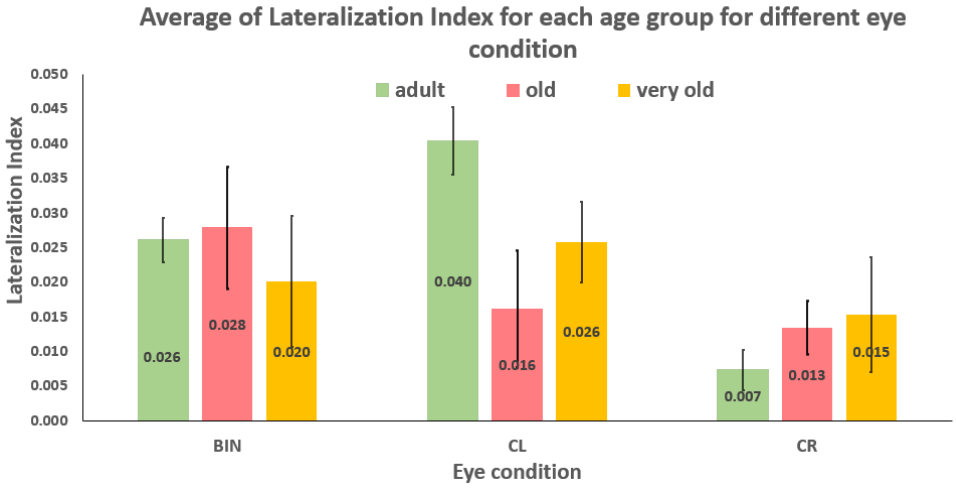


Figure 5.8: Lateralization Index in different eye condition (BIN - binocular, CL - capped left eye, CR - capped right eye) and age groups. Error bars represent standard errors.

Table 5.6: Mean and Standard Error (SE) of Lateralization Index in each eye condition for each age group

Age group	BIN eye condition	CL eye condition	CR eye condition
Adult	Mean: 0.026	Mean: 0.040	Mean: 0.007
	SE: 0.003	SE: 0.005	SE: 0.003
Old	Mean: 0.028	Mean: 0.016	Mean: 0.013
	SE: 0.009	SE: 0.008	SE: 0.004
Very old	Mean: 0.020	Mean: 0.026	Mean: 0.015
	SE: 0.009	SE: 0.006	SE: 0.008

5.2.7 Lateralization Index: Differences between eye conditions for each age group

Friedman non-parametric test showed that there was no significant difference in LI between eye conditions (BIN, CL, CR) in the very old group ($X^2 = 4.80$, $p = 0.091$, $df = 2$) and in the old group ($X^2 = 1.20$ $p= 0.549$, $df = 2$). However, there was a significant difference between eye conditions in the adult group ($X^2 = 10.0$, $p = 0.007$, $df = 2$). Pairwise post-hoc comparisons revealed that the LI in adult pigeons differed significantly between all three eye conditions, all $p < 0.001$. Also, see sections A.1.7 and A.1.9 in Appendix for results of Lateralization Index of the cerebrum.

5.2.8 Correlation between Lateralization Index and age

Spearman's correlation showed that there was no significant correlation between LI and age in the BIN eye condition (r_s = -0.403, p = 0.137), in the CL eye condition (r_s = -0.433, p = 0.107) and in the CR eye condition (r_s = 0.307, p = 0.266). Also, see sections A.1.8 in Appendix for results of Lateralization Index of the cerebrum.

5.2.9 Summary: PET data analyses

All age groups showed more SUV in the left hemisphere compared to the right hemisphere in all three eye conditions. The SUV of both the left hemisphere and the right hemisphere differed significantly between the adult and the very old group in the BIN eye condition. Interestingly, SUV of the right hemisphere was significantly different between the adult group and other age groups when only the right hemisphere was available (i.e. in the CR eye condition). There was no significant difference in SUV of the right hemisphere and SUV of the left hemisphere between age groups when the left hemisphere was available (i.e. in the CL eye condition). There was a significant negative relation between SUV of each hemisphere and age for each eye condition. Lateralization Index differed significantly among all there eye conditions only in adult pigeons.

Chapter 6

Discussion

6.1 Discussion

During this study, we assessed the effects of normal aging on 18F-FDG uptake of each brain hemisphere. Three age groups of pigeons (adult pigeons, old pigeons, and very old pigeons) were used as subjects. Pigeons performed a grit-grain discrimination task under three eye conditions (Binocular, Capped Left eye and Capped Right eye) and right after the task fluorodeoxyglucose Positron Emission Tomography (FDG-PET) imaging technique was used to measure the standard uptake value and compare the 18F-FDG brain uptake of each brain hemisphere. It was assumed that adult pigeons would show higher performance in the grit-grain discrimination task than old and very old pigeons. More brain activity in the left hemisphere was anticipated as the left hemisphere of a pigeon's brain is more involved in this task [7, 5], whereas the right hemisphere is more important for processing global cues, such as a predatory bird flying overhead [8, 4, 9]. Extensive statistical analyses were conducted to evaluate

differences between age groups and within age groups in success rate and FDG uptake of each hemisphere.

6.1.1 Age and reduction of cognitive performance in grit-grain discrimination task

As predicted, the very old group showed the poorest discrimination performance during both binocular and monocular eye conditions compared to adult and old groups. In the BIN eye condition (both hemispheres were available), there was no significant difference in the success rate among age groups. This might be because both hemispheres were involved in performing the task. Having both hemispheres available might help very old and old groups to perform the task and conceal the effect of aging on the grit-grain discrimination task. Also, in the CL eye condition (left hemisphere was available), there was not any significant difference in the success rate between age groups. One possible reason might be that the effect of aging on discrimination performance could not be easily revealed when the left hemisphere, which is more involved in this task, was available. Interestingly, the effect of aging and differences between age groups was observed mainly in the CR eye condition, when the left hemisphere, the more dominant hemisphere in the grit-grain task, was not available. In addition, the success rate significantly differed between the adult group and both older groups. This may suggest that the effects of aging can be observed when the left hemisphere was not available, as our data supported that with age, success rate decreases significantly only in the CR eye condition, especially for the very old age group with subjects aged 17 to 21. It is possible that the discrimina-

tion performance of very old pigeons was affected as a result of aging. Interestingly, the adult and the old groups did not differ significantly in terms of success rate in any eye conditions, even in the CR eye condition. This may suggest that due to the slow process of aging in pigeons, the old group is not yet being affected to the same degree as the very old group.

A study of visual lateralization in pigeons [5] showed that adult pigeons performed the grit-grain discrimination task better under the binocular (both hemispheres available) condition than either monocular condition. Also, pigeons showed significantly higher success rates under the CL eye condition than the CR eye condition, though this study did not consider the age of pigeons. In line with this study, the pigeons in the current study in all three age groups showed higher performance in the binocular eye condition (BIN) compared to the monocular eye conditions. However, we did not find any significant difference between eye conditions in any age group. The reason that we did not find the same result in our subjects might be because of several reasons. The first reason was that our sample size was too low. In the previous study [5], one hundred and eight adult pigeons were used to study visual lateralization in pigeons. However, in our study, only fifteen pigeons separated by age into three different groups were used to assess the effects of normal aging on 18F-FDG brain uptake of each hemisphere. The number of subjects in each age group were limited in our study which results in a lower power to find statistical significance. The second reason was that in the previous study [5], pigeons were given only 30 seconds after their first peck at 30 grains mixed with 30 grams of grit. Also, ten sessions for each eye condition (thirty sessions in total) were conducted. However, in

our study, pigeons were required to peck at 300 grains mixed with 300 grams of grit for 10 minutes as we were interested to conduct imaging, it needed to keep pigeons involved in the discrimination task for a longer period of time. Also, one session for each eye condition (three sessions in total) was conducted. It might be possible that either increasing the time for performing the grit-grain task or repeating a session several time might change the success rate. Thirdly, the previous study [5], was a pure behavioural study. However, in our research, behavioural study was coupled with PET imaging. It is possible that the memory of previous anesthesia during this experiment in combination with the presence of the veterinarian or being handled with gloves in the testing sessions may have affected the behavioural performance compared to the normal situation (i.e. during a pure behavioural experiment).

6.1.2 Reduction in 18F-FDG uptake of the avian brain with normal aging

Our result showed that 18F-FDG uptake was decreased in both the left and the right hemisphere of older subjects. This finding is consistent with human studies that showed a general reduced F18-FDG uptake of older people as a process of aging [145, 146]. Although the healthy subjects were used in both studies, in one study [146] it is mentioned that some factors such as smoking, years of alcohol consumption, which was not considered in their sample size, might affect the health of older individuals compared to young individuals. Furthermore, we found a significant negative relationship between 18F-FDG uptake and age in all three eye conditions, which indicates that age was associated with an overall decline in 18F-FDG uptake, not so

much the specific eye capping condition. In the following subsections, the effects of aging on the SUV of each hemisphere separately are discussed.

SUV of the left hemisphere in the grit-grain task

Our results showed that there was a significant difference between adult and very old groups in the BIN eye condition, which represents the reduction of the left hemisphere SUV in advanced age.

As the left hemisphere is more involved in performing the grit-grain task, the different age groups did not show any significantly different FDG uptake in the CL eye condition in which the left hemisphere is available. In addition, the SUV of the left hemisphere did not differ significantly between age groups in the CR eye condition. Also, in this eye condition, the left hemisphere was still highly active, though the right eye was occluded and mainly the right hemisphere was processing the visual information.

None of the age groups showed a significant difference in the left hemisphere SUV between eye conditions (BIN, CL, CR) which may suggest that the SUV of the left hemisphere did not associate so much with the specific eye capping condition.

In conclusion, the SUV of the left hemisphere, which is primarily responsible for local discrimination, did not differ significantly not only between age groups in monocular eye conditions but also between eye conditions in each age group. However, the SUV of the left hemisphere decreased with age as a result of aging.

SUV of the right hemisphere in the grit-grain task

Comparing the SUV of the right hemisphere between age groups showed that there was a significant difference in the BIN eye condition between adult and very old groups, which represents the reduction of the right hemisphere SUV in advanced age.

There was not any significant difference in SUV of the right hemisphere between age groups in the CL eye condition. In this eye condition, it was mainly the left hemisphere which processed the discrimination process.

We found a significant difference in the SUV of the right hemisphere between the adult and the old groups, and the adult and the very old groups in the CR eye condition. This may suggest that when the left hemisphere was not available and mainly the right hemisphere was processing visual information (i.e. in the CR eye condition), differences in 18F-FDG uptake of right hemisphere between age groups appeared. None of the age groups showed a significant difference in the SUV of right hemisphere between eye conditions (BIN, CL, CR), which shows SUV of the right hemisphere did not associate so much with the specific eye capping conditions.

In conclusion, when the dominant hemisphere in the grit-grain task (i.e., the left hemisphere) is not available, differences between age groups can be found. Furthermore, the right hemisphere of the old and the very old subjects were less active compared with the adult group in the grit-grain task. In addition, SUV of the right hemisphere decreased with age in all eye conditions which indicates the effect of aging on the right hemisphere.

6.1.3 Positive Lateralization Index in grit-grain task

Regardless of the eye capping condition, the Lateralization Index (LI) was positive, which indicated that the left hemisphere showed higher 18F-FDG uptake compared to the right hemisphere even in the CR eye condition in which the right hemisphere is more involved in processing visual information though there were two exceptions. One of them was an old subject that showed higher SUV in the right hemisphere only in CL eye condition and another one was a very old subject that showed higher SUV in the right hemisphere only in CR eye condition. The positive LI suggests two important points. First, the behaviour studies that showed the left hemisphere is more involved in the discrimination grit-grain task is now supported by neuroimaging technique. Second, regardless of eye condition, the more specialized hemisphere for the cognitive task showed more activation. Also, there was no significant difference in the LI between age groups in our study. The LI significantly differed between eye conditions only in the adult group.

6.1.4 Relation between behavioural data and PET data

This study showed that the SUV of both hemispheres decreased for the oldest subjects, which was linked to the cognitive decline in very old subjects compared to adult subjects. Moreover, our results showed that when the left hemisphere was available (i.e. in the CL eye condition) in the girt-grain task, differences between age groups as a result of aging were not observed either in the SUV of both hemispheres or the success rate. Whereas, when the left hemisphere, the dominant hemisphere in the grit-grain task, was not available, differences between age groups was found. In

this condition, the left hemisphere was not available to compensate for the aging right hemisphere. Also, the right hemisphere of the very old group could not deal with the deleterious effects of aging. Consequently, very old subjects could not perform the grit-grain task even as well as old subjects. Interestingly, when the left hemisphere was not available, there was not only a significant difference in the SUV of both hemispheres between the old and very old groups but also SUV of both hemispheres were numerically similar. However, the success rate in the old group was still significantly better than the very old group. This might suggest that the normal reduction in FDG uptake of both hemispheres in the old group notwithstanding, their brains was still active in an efficient way to perform the grit-grain discrimination task significantly better than the very old group. It can be concluded that, like the human brain, the avian brain can compensate for the effects of aging to some limited extent and probably until a certain age range. This study showed that neither SUV of the left hemisphere nor the SUV of the right hemisphere differed significantly between eye conditions. Indeed, the more specialized hemisphere for the grit-grain task showed more activation for all age groups.

To our knowledge, this is the first investigation of age-related changes of the function of each hemisphere in the avian brain by using FDG-PET imaging. In conclusion, similar to humans, the SUV of both hemispheres decreased slowly in older subjects which results in cognitive impairments in terms of visual discrimination in advanced age. This study supports that pigeons could be a good animal model for the study of healthy aging and further our understanding of age-related changes of the brain and the underlying neural mechanisms.

6.1.5 Limitations

There were some limitations to this study. First, this was a pilot project to study aging on pigeons by using FDG-PET imaging. Therefore, a small sample size was used in each age group. A larger sample size would be needed for further studies. Second, statistical analyses showed that the performance of the adult pigeons were affected by the order of test session. There was a significant decrease in performance from the first test session to the second test session. Also, there was a significant decrease from the first to the third test session. However, as the order of session in which the three eye conditions were administered was counterbalanced across age groups and sex, there is little concern that the decrease in the pigeons' willingness to perform across subsequent sessions affected the validity of our data. The reason that it was observed only for the adult group might be because the adult subjects, that were between 1 and 2 years of age, had not yet participated in many lab experiments and were thus probably more sensitive to factors such as injection and anesthesia.

Chapter 7

Conclusion and Future directions

7.1 Conclusion

During this project, we used the pigeon as an animal model for the study of healthy aging. We aimed to investigate the effects of aging on success rate in a grit-grain discrimination task and study changes in the metabolic rate of each brain hemisphere and brain lateralization. Fifteen pigeons, which were grouped into three age groups: adult (between 1-2 years of age), old (between 11-14 years of age) and very old (between 17-21 years of age), were used as subjects. Each bird underwent three test sessions involving a fluorodeoxyglucose-positron emission tomography (FDG-PET) scan, during which the pigeon performed the grit-grain discrimination task under each of three eye conditions: binocular, left eye capped, and right eye capped. Indeed, due to the avian brain structure, the function of each brain hemisphere can be studied relatively independently by covering one eye of the pigeon. Our result showed that the standard uptake value (SUV) of both hemispheres decreased with age which results

in a very poor success rate in very old subjects compared to the adult subjects. In addition, the effect of aging and differences between age groups was mainly observed when the left hemisphere, which is more specialized for the type of local discrimination that is required by the grit-grain task, was not available. In this condition, the right hemisphere could not compensate for the cognitive losses that come with aging in very old subjects. Consequently, very old subjects could not perform the grit-grain task even as well as old subjects. However, in spite of the normal reduction in both hemispheres FDG uptake in the old group, they can still activate their brain in a way to perform the grit-grain discrimination task significantly better than the very old group. Moreover, Lateralization Index showed that regardless of the eye condition, the SUV of the left hemisphere was more than the SUV of the right hemisphere in this cognitive task. We did not find any significant difference in the Lateralization Index between age groups. Lateralization Index was different significantly among all three eye conditions only in adult pigeons. This might suggest that the laterality of the avian brain changed with age. Overall this study suggested that, age was associated with an overall decline in 18F-FDG uptake. Like the human brain, the avian brain can compensate for the effects of aging to some limited extent and probably until a certain age.

7.2 Future directions

This study makes a number of contributions to the literature on aging and functional hemispheric asymmetries, especially the literature concerning using pigeons as an animal model for the study of healthy aging. However, there are still areas for

improvement through future research, described in the following subsections.

7.2.1 To what extent can the old group perform a cognitive task as well as the adult group?

In this study, the deleterious effects of aging in the grit-grain discrimination task was mainly observed in the very old group with the age of 17 to 21. There was a significant difference in success rate between the old group and the very old group, but not between the adult group and the old group. A future behavioral study should be conducted on the old group and the adult group with a more difficult cognitive task such as a spatial disorientation task, requiring birds to encode featural cues (e.g., distinctive objects with different color in each corner of a parallelogram-shaped arena) and geometric cues (e.g., relative length of walls and angular amplitude) in order to locate a hidden target. Changing the type of information during testing sessions would allow one to assess which cues the birds encode during training. Coupling this approach with monocular occlusion techniques, the function of right and left hemispheres in encoding the featural and geometric cues may be examined. Such a study would allow one to discriminate age-related changes to the right and left hemisphere. Changes in encoding the features would show whether pigeons use feature cues or the geometric cues to locate the hidden target. As the left hemisphere of the brain is more involved in the processing of visual features and local landmarks, the failure of encoding the features would suggest the effects of aging on the left hemisphere. Whereas changes in encoding the wall length (global geometric cue) compared to angular amplitude (a local geometric cue) would show the ability of the

pigeon to encode the environment based on the global geometric features. Failure in encoding the global geometric cues would be as a result of effects of aging on the right brain hemisphere. Results from such a study, could be compared to the grit-grain discrimination task used here to provide supporting evidence for the differential age-related changes in the hemispheres by group and adult groups of pigeons.

7.2.2 Longitudinal grit-grain discrimination study

Future research should focus on continuing the current cross-sectional study to a longitudinal study (repeating the same study on the same subjects over time). The advantage of longitudinal study would be assessment of cognitive changes with aging at both the group and the individual level.

7.2.3 Studying healthy aging in pigeons in a spatial cognition task

In this study we focused on a grit-grain discrimination task because it is a task that examines local discrimination abilities in which the left hemisphere was more involved and the function of each hemisphere was studied through covering one eye. In addition, the deleterious effects of aging were observed mainly in the right hemisphere of the brain. Future research should focus to study healthy aging in pigeons while they were involved in a cognitive task in which the right hemisphere is more involved, such as a spatial cognition task and the function of each hemisphere should be studied through covering one eye. Spatial cognition, which enables us to use knowledge about the environment to determine where we are, and how to navigate

from one location to another, declines by increasing age and this can be an early sign of age-related deleterious effects. The pigeon navigation system also affords a parallel model of human spatial memory. Studies examining the use of spatial information during orientation have shown that humans and pigeons readily learn about the featural properties (e.g., color or pattern) of objects in their environment, and these features are integrated with the geometric information (e.g., distances or directions and length) [147, 148]. Similar to humans, pigeons rely on visual cues to guide orientation in environments; they follow roads [149] and navigate by landmarks [150]. The pigeon navigation system has received considerable study for short and long distance navigation at behavioural, cognitive and neurological levels [151, 152], using laboratory and outdoor real-world environments [151]. Featural information and geometric information, which are the main properties of a spatial world, are differentially encoded by the left and right hemisphere, respectively [153]. So, it could be proposed that aging may differentially affect how featural and geometric information is encoded, and this might be due to relative changes in the function of the two brain hemispheres.

Appendix A

Behavioural and PET data analyses

A.0.1 Individual differences: Relation between age and total number of pecks

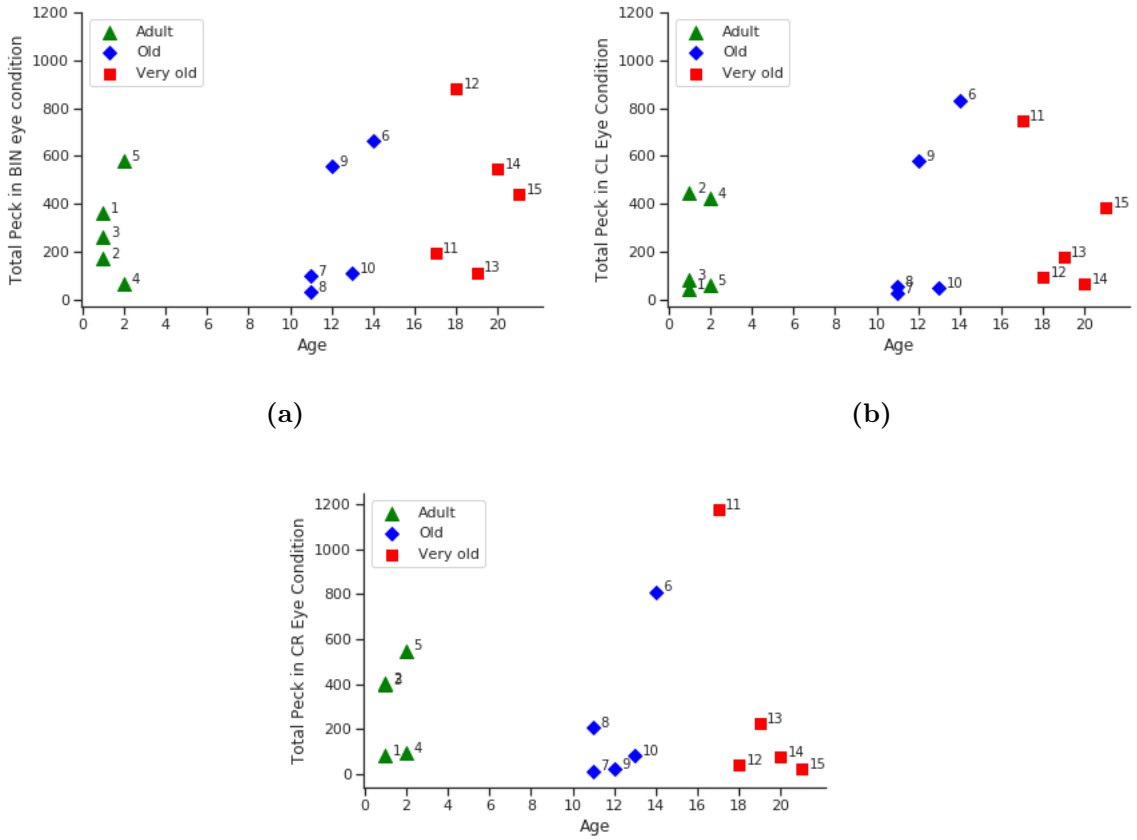


Figure A.1: Individual differences in total number of pecks for each age group under different eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available)

Table A.1: Number of total peck of each subject in three different eye conditions (BIN, CL, CR).

ID	Group	Age	Total peck in BIN	Total peck in CL	Total peck in CR	Order of test session
Subject 1	adult	1	359	46	80	BIN, CR, CL
Subject 2	adult	1	172	447	402	CR, CL, BIN
Subject 3	adult	1	259	82	397	BIN, CL, CR
Subject 4	adult	2	63	426	96	CL, CR, BIN
Subject 5	adult	2	580	61	549	CR, BIN, CL
Subject 6	old	14	665	834	811	BIN, CR, CL
Subject 7	old	11	100	26	13	CL, BIN, CR
Subject 8	old	11	30	55	208	CR, CL, BIN
Subject 9	old	12	558	581	20	BIN, CL, CR
Subject 10	old	13	110	48	82	CR, BIN, CL
Subject 11	very old	17	194	749	1175	CR, CL, BIN
Subject 12	very old	18	879	96	42	BIN, CR, CL
Subject 13	very old	19	113	178	224	CL, CR, BIN
Subject 14	very old	20	547	65	76	BIN, CL, CR
Subject 15	very old	21	442	387	20	CL, BIN, CR

A.0.2 Individual differences: Ratio of eaten grains to total number of pecks

The ratio of eaten grains to total number of pecks of each subject in three eye conditions are visualized in Figure A.2. In these graphs, each dot represents the ratio of eaten grains to total number of pecks of one subject. Trendlines are the line of best fit calculated by using least squares method. They represent the general pattern of eaten grains to total number of pecks in each age group. Comparing the trendline of each age group showed that the adult group and the old group performed similarly in all three eye conditions. However, very old subjects showed the worst performance in all three eye conditions especially in the CR eye condition (only left hemisphere was available). In this eye condition, the left hemisphere was not available and the effect of aging in the very old group was more visible, see Tables A.2 and A.3 and A.4.

Table A.2: Trendline equation and R^2 value of each age group in BIN eye condition

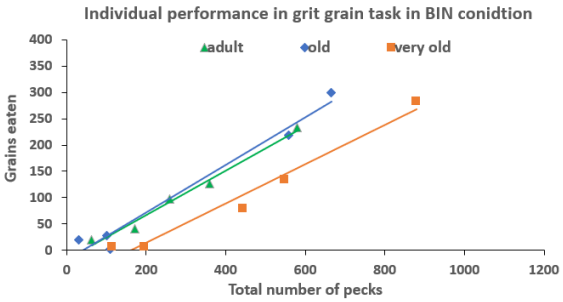
Age group	Trendline equation	R^2 value
Adult	Y= 0.424 X - 18.14	0.984
Old	Y= 0.455 X - 19.88	0.972
Very old	Y= 0.372 X - 60.049	0.969

Table A.3: Trendline equation and R^2 value of each age group in CL eye condition

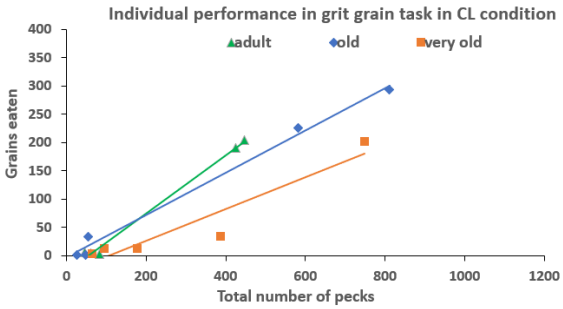
Age group	Trendline equation	R^2 value
Adult	Y= 0.513 X - 27.32	0.995
Old	Y= 0.375 X - 3.113	0.991
Very old	Y= 0.282 X - 30.49	0.895

Table A.4: Trendline equation and R^2 value of each age group in CR eye condition

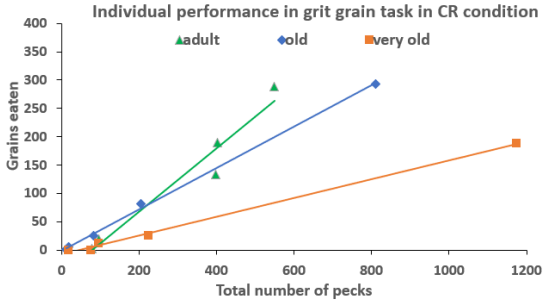
Age group	Trendline linear equation	R^2 value
Adult	Y= 0.560 X - 43.96	0.952
Old	Y= 0.365 X - 1.366	0.998
Very old	Y= 0.166 X - 7.590	0.997



(a)



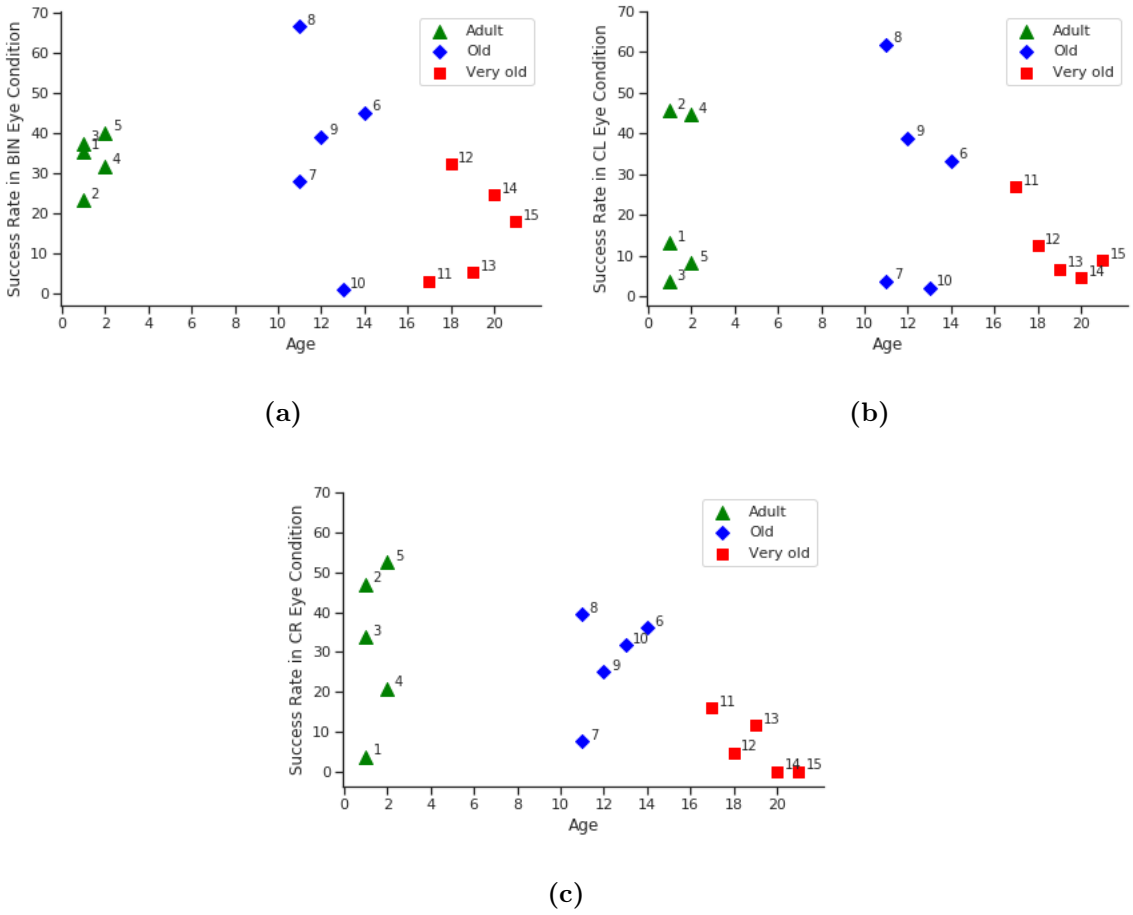
(b)



(c)

Figure A.2: Comparing the ratio of eaten grains out of the total number of pecks by each subject in each age group in each eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available). Trend-lines are calculated based on the least squares method.

A.0.3 Individual differences: Relation between age and success rate



(a) **(b)**

(c)

Figure A.3: Individual differences in success rate for each age group under different eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available)

Table A.5: Success rate of each subject in three different eye conditions (BIN, CL, CR).

ID	Group	Age	Success rate in BIN	Success rate in CL	Success rate in CR	Order of test session
Subject 1	adult	1	35.376	13.043	3.750	BIN,CR,CL
Subject 2	adult	1	23.256	45.637	47.015	CR, CL, BIN
Subject 3	adult	1	37.452	3.658	33.753	BIN, CL, CR
Subject 4	adult	2	31.746	44.601	20.833	CL, CR, BIN
Subject 5	adult	2	40.172	8.197	52.459	CR, BIN, CL
Subject 6	old	14	45.100	33.330	36.100	BIN, CR, CL
Subject 7	old	11	28.000	3.846	7.692	CL, BIN, CR
Subject 8	old	11	66.667	61.818	39.423	CR, CL, BIN
Subject 9	old	12	39.068	38.898	25.000	BIN, CL, CR
Subject 10	old	13	0.909	2.0833	31.707	CR, BIN, CL
Subject 11	very old	17	3.093	26.969	16.000	CR, CL, BIN
Subject 12	very old	18	32.309	12.5000	4.762	BIN, CR, CL
Subject 13	very old	19	5.310	6.741	11.607	CL, CR, BIN
Subject 14	very old	20	24.680	4.615	0.000	BIN, CL, CR
Subject 15	very old	21	17.873	8.785	0.000	CL, BIN, CR

A.0.4 Individual differences: The left hemisphere SUV

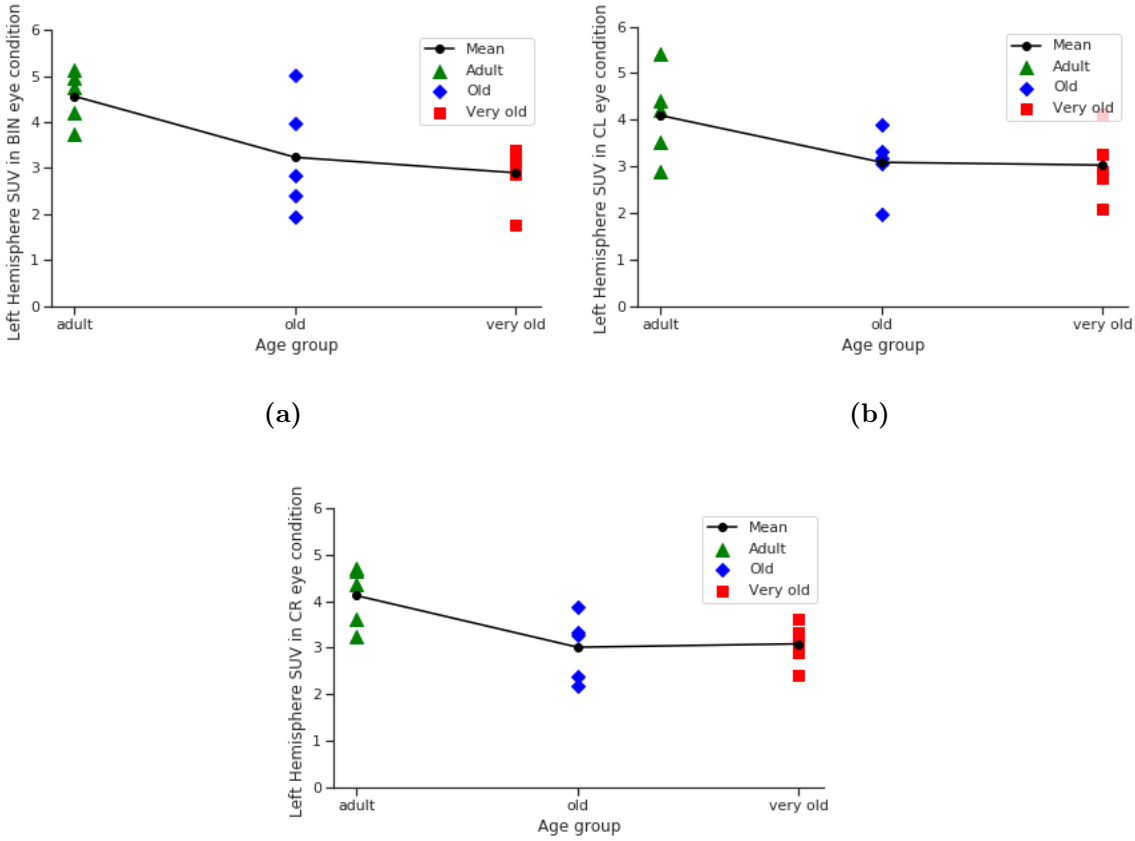


Figure A.4: Individual left hemisphere SUV and average of the three age groups in each eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available).

A.0.5 Individual differences: The right hemisphere SUV

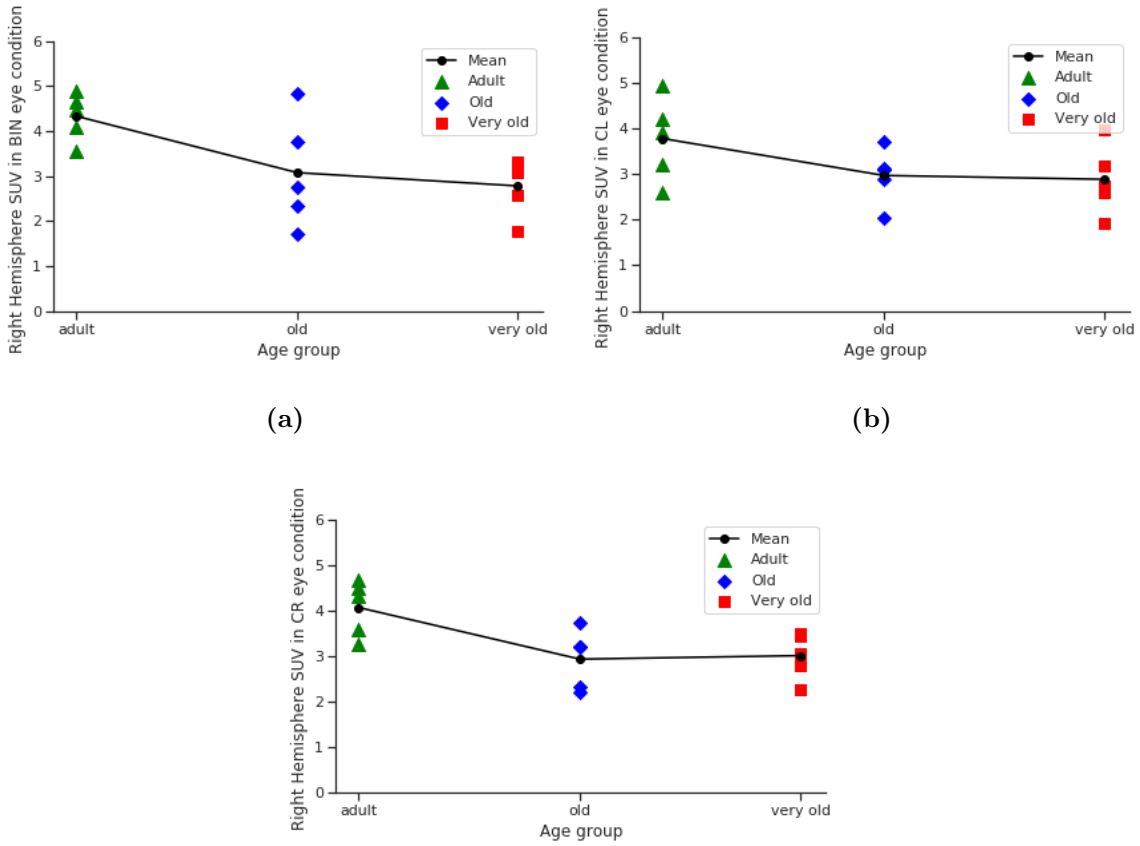


Figure A.5: Individual and left hemisphere SUV and average of the three age groups in each eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (right hemisphere is available), *(c)* CR - capped right eye (left hemisphere is available).

Table A.6: SUV of each subject in BIN eye conditions.

ID	Group	Age	Left-Hem SUV (BIN)	Right-Hem SUV (BIN)
subject 1	adult	1	4.961	4.655
subject 2	adult	1	5.144	4.876
subject 3	adult	1	4.208	4.091
subject 4	adult	2	3.731	3.538
subject 5	adult	2	4.773	4.482
subject 6	old	14	1.930	1.707
subject 7	old	11	3.986	3.761
subject 8	old	11	5.031	4.825
subject 9	old	12	2.396	2.329
subject 10	old	13	2.828	2.748
subject 11	very old	17	2.872	2.570
subject 12	very old	18	3.399	3.307
subject 13	very old	19	3.136	3.071
subject 14	very old	20	1.767	1.767
subject 15	very old	21	3.312	3.177

Table A.7: SUV of each subject in CL eye conditions.

ID	Group	Age	Left-Hem SUV (CL)	Right-Hem SUV (CL)
subject 1	adult	1	5.401	4.951
subject 2	adult	1	4.202	3.922
subject 3	adult	1	4.421	4.210
subject 4	adult	2	3.530	3.203
subject 5	adult	2	2.891	2.609
subject 6	old	14	1.969	2.026
subject 7	old	11	3.066	2.886
subject 8	old	11	3.888	3.719
subject 9	old	12	3.180	3.108
subject 10	old	13	3.313	3.111
subject 11	very old	17	2.095	1.910
subject 12	very old	18	4.126	3.977
subject 13	very old	19	2.894	2.750
subject 14	very old	20	3.272	3.195
subject 15	very old	21	2.735	2.591

Table A.8: SUV of each subject in CR eye conditions.

ID	Group	Age	Left-Hem SUV (CR)	Right-Hem SUV (CR)
subject 1	adult	1	4.372	4.326
subject 2	adult	1	4.721	4.664
subject 3	adult	1	3.251	3.251
subject 4	adult	2	3.629	3.575
subject 5	adult	2	4.644	4.480
subject 6	old	14	2.186	2.186
subject 7	old	11	3.324	3.191
subject 8	old	11	3.888	3.726
subject 9	old	12	3.282	3.193
subject 10	old	13	2.376	2.319
subject 11	very old	17	2.420	2.259
subject 12	very old	18	3.620	3.495
subject 13	very old	19	3.157	3.040
subject 14	very old	20	3.333	3.437
subject 15	very old	21	2.892	2.771

A.0.6 Individual differences: Relation between age and LI

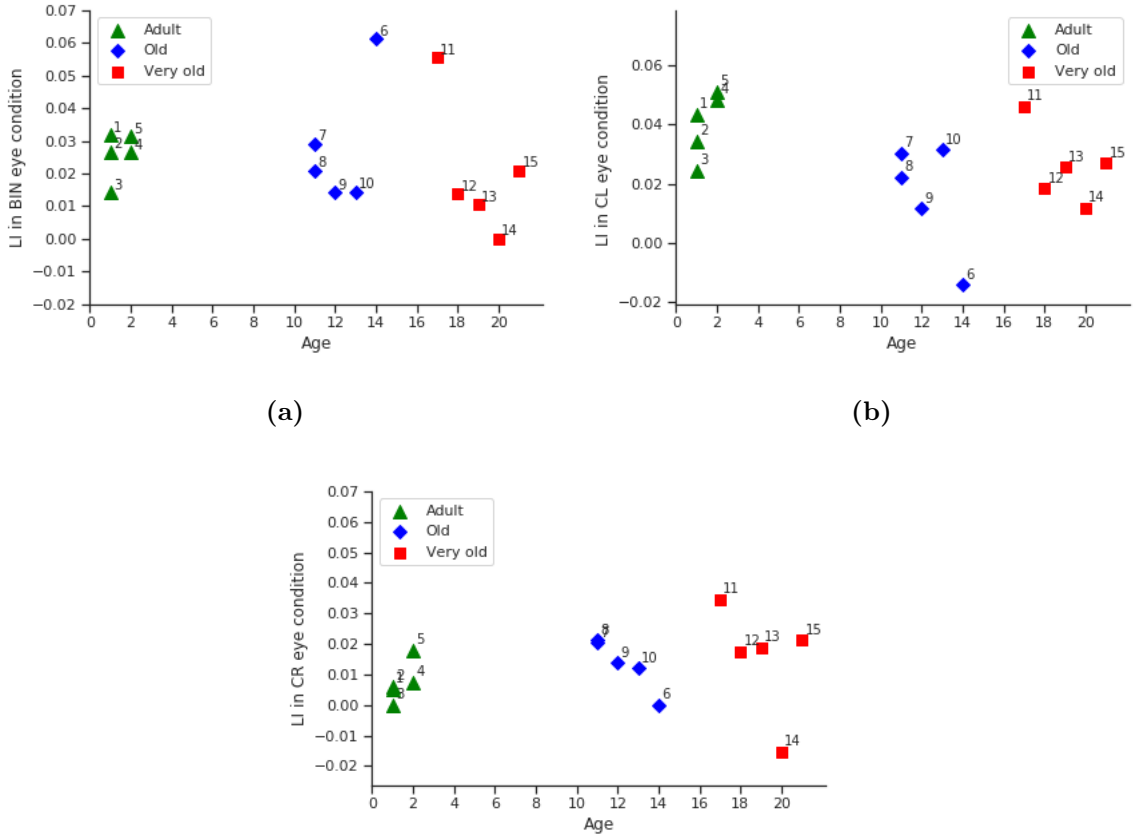


Figure A.6: Individual differences in LI in each age group in different eye condition; *(a)* BIN - binocular (both hemispheres are available), *(b)* CL - capped left eye (left hemisphere is available), *(c)* CR - capped right eye (right hemisphere is available)

Table A.9: Lateralization Index (LI) of each subject in three different eye conditions (BIN, CL, CR).

ID	Group	Age	LI in BIN	LI in CL	LI in CR
Subject 1	adult	1	0.0318	0.0435	0.005
Subject 2	adult	1	0.027	0.034	0.006
Subject 3	adult	1	0.014	0.024	0.000
Subject 4	adult	2	0.026	0.048	0.007
Subject 5	adult	2	0.031	0.051	0.0180
Subject 6	old	14	0.0612	-0.014	0.000
Subject 7	old	11	0.029	0.030	0.020
Subject 8	old	11	0.021	0.022	0.021
Subject 9	old	12	0.014	0.011	0.0137
Subject 10	old	13	0.014	0.031	0.012
Subject 11	very old	17	0.055	0.046	0.034
Subject 12	very old	18	0.0137	0.018	0.017
Subject 13	very old	19	0.010	0.026	0.018
Subject 14	very old	20	0.000	0.012	-0.015
Subject 15	very old	21	0.021	0.027	0.021

A.1 Analyses without the cerebellum included

Functional hemispheric asymmetries in avian brain were mainly studied only at the cerebrum areas and did not discuss the brain laterality and visual processing in

cerebellum of avian brain. The cerebellum is involved in the timing, coordination, and modulation of the motor system with incoming visual, vestibular, auditory, and somatosensory information in all vertebrates[154]. Also, the cerebellum plays an important role in muscular coordination. The cerebellum in birds is highly developed and is important for flight as the complex body movements are required for flying [155].

As we were not interested in the cerebellum region of the brain, it was excluded from the mask. Results of the analyses without the cerebellum included are discussed in next subsections.

A.1.1 Left cerebrum SUV: Differences between age groups

Shown in Table A.10, Kruskal-Wallis H test showed that the SUV of the left cerebrum did not differ between age groups in the BIN eye condition (both hemisphere were available; $X^2 = 5.66$, $p = 0.059$, $df = 2$). In addition, there was no significant difference in SUV of the left cerebrum between age groups in the CL eye condition (only left hemisphere was available; $X^2 = 3.92$, $p = 0.141$, $df = 2$) or the CR eye condition (only right hemisphere was available; $X^2 = 4.88$, $p = 0.087$, $df = 2$). See Table A.12, Table A.13 and Table A.14 to see the SUV of left cerebrum for each individual per age group. On average, the adult group showed higher FDG uptake in the left cerebrum compared to the old and the very old group in all eye conditions.

Table A.10: Mean and Standard Error (SE) of the left cerebrum SUV in each eye condition for each age group

Age group	BIN eye condition	CL eye condition	CR eye condition
Adult	Mean: 4.73	Mean: 4.20	Mean: 4.22
	SE: 0.246	SE: 0.459	SE: 0.294
Old	Mean: 3.44	Mean: 3.26	Mean: 3.06
	SE: 0.605	SE: 0.289	SE: 0.318
Very old	Mean: 3.01	Mean: 3.16	Mean: 3.19
	SE: 0.310	SE: 0.380	SE: 0.215

A.1.2 Left cerebrum SUV: Differences between eye conditions for each age group

Friedman non-parametric test showed that there was no significant difference in left cerebrum SUV between eye conditions (BIN, CL, CR) in the adult group ($X^2 = 2.80$, $p = 0.247$, $df = 2$), in the old group ($X^2 = 0.400$ $p = 0.819$ $df = 2$) and in the very old group ($X^2 = 1.60$, $p = 0.449$, $df = 2$).

A.1.3 Right cerebrum SUV: Differences between age groups

Shown in Table A.11, Kruskal-Wallis H test showed that SUV of the right cerebrum did not differ significantly between age groups in the BIN eye condition (both hemisphere were available; $X^2 = 5.66$, $p = 0.059$, $df = 2$). Also, There was no sig-

nificant difference in SUV of the right cerebrum between age groups in the CL eye condition (only left hemisphere was available; $X^2 = 2.96$, $p = 0.228$, $df = 2$). Additionally, SUV of the right cerebrum in the CR eye condition significantly differed between age groups in the CR eye condition ($X^2 = 6.62$, $p = 0.037$, $df = 2$). Post-hoc pairwise comparison showed that it was significantly different between very old and adult groups ($W = 3.693$, $p = 0.037$). See Tables A.12, A.13 and A.14 to see the SUV of right cerebrum for each individual per age group. Overall, the adult group showed higher FDG uptake in the right cerebrum compared to the old and the very old group in all eye conditions.

Table A.11: Mean and Standard Error (SE) of the right cerebrum SUV in each eye condition for each age group

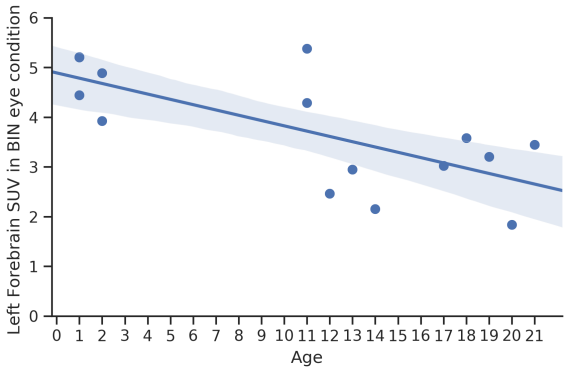
Age group	BIN eye condition	CL eye condition	CR eye condition
Adult	Mean: 4.56	Mean: 3.98	Mean: 4.28
	SE: 0.244	SE: 0.428	SE: 0.295
Old	Mean: 3.29	Mean: 3.16	Mean: 3.06
	SE: 0.591	SE:0.242	SE: 0.268
Very old	Mean: 2.93	Mean: 3.05	Mean: 3.18
	SE: 0.291	SE: 0.384	SE: 0.254

A.1.4 Right cerebrum SUV: Differences between eye conditions for each age group

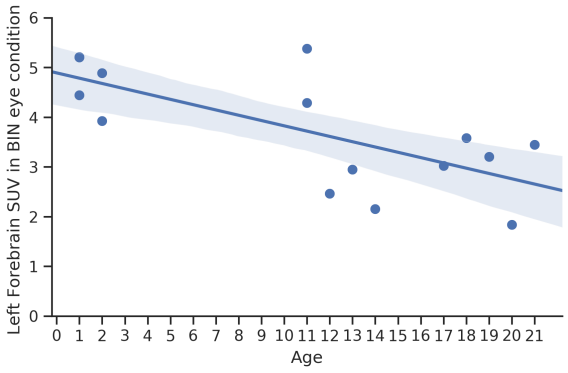
Friedman non-parametric test showed that there was no significant difference in right cerebrum SUV between eye conditions (BIN, CL, CR) in the adult group (X^2 = 1.20, p = 0.549, df = 2), in the old group (X^2 = 0.000 p = 1.000 df = 2) and in the very old group, (X^2 = 1.60, p = 0.449, df = 2).

A.1.5 Correlation between age and SUV of right and left cerebrum

For the BIN eye condition, shown in Figure A.7, there was a significant negative correlation between the SUV in the left cerebrum and age (r_s = -0.715, p = 0.003), and also between the SUV in the right cerebrum and age (r_s = -0.707, p = 0.003).



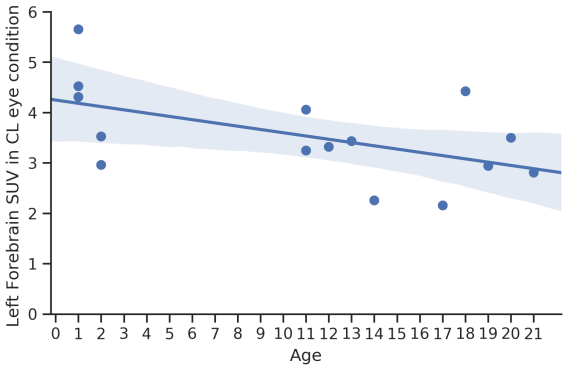
(a)



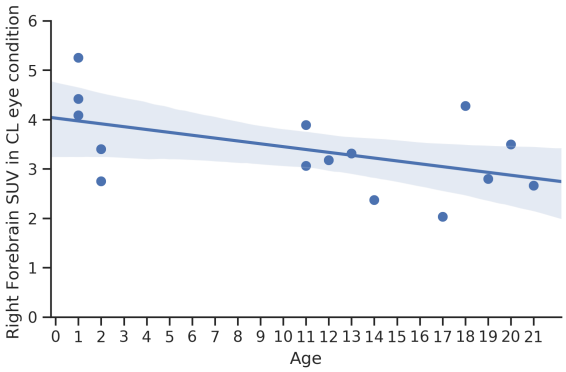
(b)

Figure A.7: BIN eye condition: *(a)* relation between SUV of the left cerebrum and age; *(b)* relation between SUV of the right cerebrum and age). The shaded area represents 95% confidence interval.

For the CL eye condition, shown in Figure A.8, there was a significant negative correlation between the SUV in the left cerebrum and age (r_s = -0.582, p = 0.023), and also between the SUV in the right cerebrum and age (r_s = -0.517, p = 0.048).



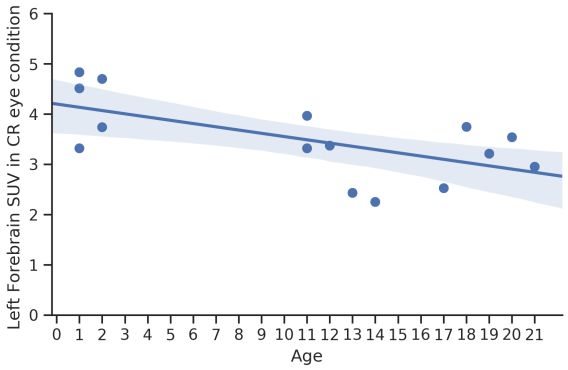
(a)



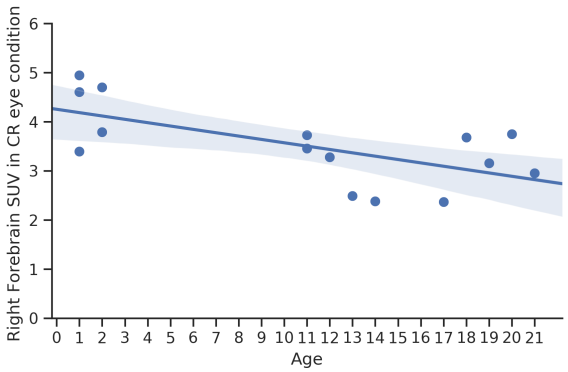
(b)

Figure A.8: CL eye condition: *(a)* relation between SUV of the left cerebrum and age; *(b)* relation between SUV of the right cerebrum and age).The shaded area represents 95% confidence interval.

For the CR eye condition, shown in FigureA.9), there was a significant negative correlation between the SUV in left cerebrum and age (r_s = -0.5480, p = 0.035), and also between the SUV in the right cerebrum and age (r_s = -0.585, p = 0.022).



(a)



(b)

Figure A.9: CR eye condition: *(a)* relation between SUV of the left cerebrum and age; *(b)* relation between SUV of the right cerebrum and age). The shaded area represents 95% confidence interval.

Table A.12: SUV of cerebrum of each subject in BIN eye conditions.

ID	Group	Age	Left cerebrum SUV (BIN)	Right cerebrum SUV (BIN)
subject 1	adult	1	5.205	4.960
subject 2	adult	1	5.210	5.076
subject 3	adult	1	4.442	4.325
subject 4	adult	2	3.924	3.731
subject 5	adult	2	4.889	4.714
subject 6	old	14	2.152	1.855
subject 7	old	11	4.287	4.061
subject 8	old	11	5.375	5.169
subject 9	old	12	2.462	2.462
subject 10	old	13	2.947	2.947
subject 11	very old	17	3.023	2.796
subject 12	very old	18	3.582	3.490
subject 13	very old	19	3.201	3.136
subject 14	very old	20	1.836	1.8714
subject 15	very old	21	3.447	3.379

Table A.13: SUV of cerebrum of each subject in CL eye conditions.

ID	Group	Age	Left cerebrum SUV (CL)	Right cerebrum SUV (CL)
subject 1	adult	1	5.651	4.251
subject 2	adult	1	4.313	4.089
subject 3	adult	1	4.525	4.422
subject 4	adult	2	3.529	3.399
subject 5	adult	2	2.961	2.749
subject 6	old	14	2.258	2.373
subject 7	old	11	3.246	3.066
subject 8	old	11	4.057	3.888
subject 9	old	12	3.324	3.180
subject 10	old	13	3.433	3.312
subject 11	very old	17	2.156	2.033
subject 12	very old	18	4.424	4.279
subject 13	very old	19	2.942	2.797
subject 14	very old	20	3.499	3.499
subject 15	very old	21	2.807	2.663

Table A.14: SUV of cerebrum of each subject in CR eye conditions.

ID	Group	Age	Left cerebrum SUV (CR)	Right cerebrum SUV (CR)
subject 1	adult	1	4.511	4.605
subject 2	adult	1	4.834	4.948
subject 3	adult	1	3.323	3.395
subject 4	adult	2	3.737	3.791
subject 5	adult	2	4.698	4.698
subject 6	old	14	2.250	2.379
subject 7	old	11	3.323	3.456
subject 8	old	11	3.968	3.725
subject 9	old	12	3.370	3.282
subject 10	old	13	2.432	2.488
subject 11	very old	17	2.527	2.366
subject 12	very old	18	3.744	3.682
subject 13	very old	19	3.215	3.157
subject 14	very old	20	3.540	3.749
subject 15	very old	21	2.951	2.951

A.1.6 Lateralization Index of the cerebrum: differences between age groups

As shown in Figure A.10 and Table A.15, Kruskal-Wallis H test showed that there is no significant difference in Lateralization Index (LI) of the cerebrum region of the

brain between age groups in the BIN eye condition (both hemispheres were available; $X^2 = 1.26$, $p = 0.532$, $df = 2$), in the CL eye condition (only left hemisphere available; $X^2 = 1.34$, $p = 0.512$, $df = 2$), or in the CR eye condition (only right hemisphere is available; $X^2 = 1.12$, $p = 0.572$, $df = 2$), see Table A.16 to compare the LI each individual.

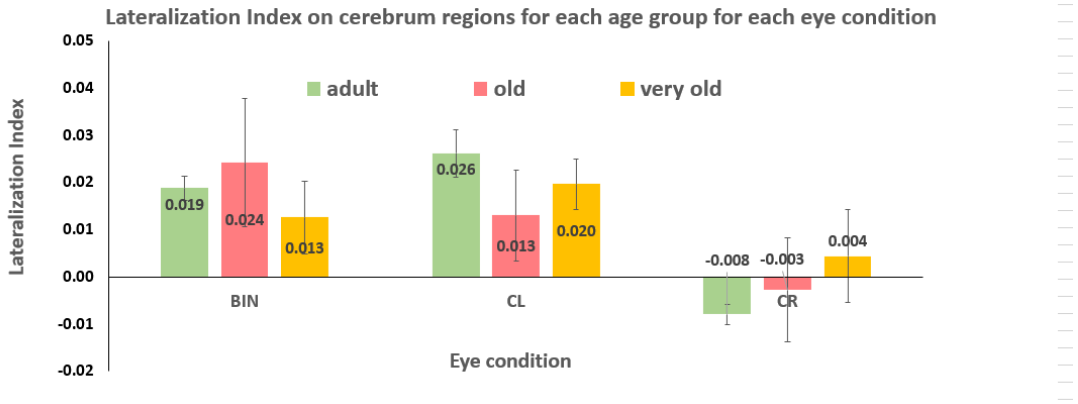


Figure A.10: Lateralization Index were calculated by considering only the cerebrum regions and excluding the cerebellum in different eye condition (BIN - binocular, CL - capped left eye, CR - capped right eye) and age groups. Error bars represent standard errors.

Table A.15: Mean and Standard Error (SE) of Lateralization Index of cerebrum region in each eye condition for each age group

Age group	BIN eye condition	CL eye condition	CR eye condition
Adult	Mean: 0.019	Mean: 0.026	Mean: -0.008
	SE: 0.003	SE: 0.005	SE: 0.002
Old	Mean: 0.024	Mean: 0.013	Mean: -0.003
	SE: 0.014	SE: 0.010	SE: 0.011
Very old	Mean: 0.013	Mean: 0.020	Mean: 0.004
	SE: 0.008	SE: 0.005	SE: 0.010

A.1.7 Lateralization Index of the cerebrum: differences between eye condition for each age group

Friedman non-parametric test showed that there was no significant difference in LI between eye conditions (BIN, CL, CR) in the very old group ($X^2 = 5.20$, $p = 0.074$, $df = 2$) and in the old group ($X^2 = 2.80$ $p= 0.247$, $df = 2$). However, there was a significant difference between eye conditions in the adult group ($X^2 = 7.60$, $p = 0.022$, $df = 2$). Post-hoc pairwise comparisons demonstrated that the LI in adult group significantly differed between BIN and CR eye conditions ($W = 4.041$, $p = 0.004$). Also, it significantly differd between CL and CR eye condition ($W = 4.619$, $p = 0.002$).

A.1.8 Correlation between Lateralization Index and age

Spearman's correlation showed that there was no significant correlation between LI and age in the BIN eye condition (r_s = -0.327, p = 0.205), in the CL eye condition (r_s = -0.291, p = 0.293) and in the CR eye condition (r_s = 0.150, p = 0.594).

A.1.9 Lateralization Index of the cerebrum

All three age groups showed positive Lateralization Index in BIN and CL eye condition which showed that the left side of cerebrum was more involved in the discrimination grit-grain task. The behaviour studies that showed the left hemisphere is more involved in the discrimination grit-grain task is now supported by neuroimaging technique. For the CR eye condition, the adult and old subjects showed negative LI which showed that the SUV of the right sode of cerebrum was higher than the SUV of the left side of cerebrum. In other word, the right cerebrum mainly processed the visual information. However, for the very old subjects, the left side of cerebrum was more involved compared to the right side of cerebrum as the LI was positive in even CR eye condition. Three out of five subjects in very old group showed positive LI in CR eye condition. Also, there was no significant difference in the LI between age groups in our study. The LI significantly differed between eye conditions only in the adult group.

A.1.10 Summary of comparing PET results of whole hemisphere and results of excluding cerebellum

In both analysis (using the whole right and left hemisphere mask and using the mask without cerebellum included), results showed that the SUV decreased in older pigeons compared to younger pigeons. In addition, results of both analysis showed that there was a significant negative correlation between age and SUV in all three eye conditions. There was no significant difference in LI by using whole hemisphere mask or excluding cerebellum from the mask between age groups in any eye condition. The LI significantly differed between eye conditions only in the adult group.

Table A.16: Lateralization Index (LI) of cerebrum of each subject in three different eye conditions (BIN, CL, CR).

ID	Group	Age	LI in BIN	LI in CL	LI in CR
Subject 1	adult	1	0.024	0.037	-0.010
Subject 2	adult	1	0.013	0.027	-0.012
Subject 3	adult	1	0.013	0.012	-0.011
Subject 4	adult	2	0.025	0.019	-0.007
Subject 5	adult	2	0.018	0.037	0.000
Subject 6	old	14	0.074	-0.025	-0.028
Subject 7	old	11	0.027	0.029	-0.020
Subject 8	old	11	0.020	0.021	0.032
Subject 9	old	12	0.000	0.022	0.013
Subject 10	old	13	0.000	0.018	-0.011
Subject 11	very old	17	0.039	0.029	0.033
Subject 12	very old	18	0.013	0.017	0.008
Subject 13	very old	19	0.010	0.025	0.009
Subject 14	very old	20	-0.009	0.000	-0.029
Subject 15	very old	21	0.010	0.026	0.000

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