UNIVERSITEIT GENT

FACULTY OF VETERINARY MEDICINE

FUNCTIONAL BRAIN IMAGING IN THE DOG

SINGLE PHOTON EMISSION TOMOGRAPHY AS A RESEARCH AND CLINICAL TOOL FOR THE INVESTIGATION OF CANINE BRAIN PHYSIOLOGY AND PATHOPHYSIOLOGY

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LIST OF ABBREVIATIONS

Alzheimer's disease: AD
Beta-carboxymethoxy-iodophenyl-tropane: β-CIT
Binding index: BI
Blood brain barrier: BBB
Brain registration and automated SPECT semiquantification: BRASS
Brain uptake index: BUI
Cerebrospinal fluid: CSF
Computed tomography: CT
Dopamine transporter: DAT
Diethylenetriaminepentaacetic acid: DTPA
Equilibrum dissociation constant: K_d
N,N"-1,2-ethylene-diylbis-L-cysteine diethylester dihydrochloride: ECD
Full width at half maximum: FWHM
Gamma-aminobutyric acid receptor: GABA receptor
Glucoheptonate: GHA
d,l-hexamethylpropyleneamine-oxime: HMPAO
Iodobenzamide: IBZM
d,l-N-isopropyl-p-iodoamphetamine hydrochloride: IMP
Magnetic resonance imaging : MRI
Magnetic resonance spectroscopy: MRS
Maximal specific binding: B_{max}
Mega becquerel: MBq
Multiple-time graphical analysis: MTGA
N-methyl-D-aspartate receptor: NMDA receptor
Non uniform attenuation coefficient: NUAC
Parkinson's disease: PD
Partial volume effect: PVE
Positron emission tomography: PET
Regional cerebral blood flow: rCBF
Region of interest: ROI
Selective serotonin reuptake inhibitor: SSRI
Single photon emission tomography: SPET
Volume of interest: VOI
5-hydroxytryptamine (serotonin): 5-HT
5-hydroxytryptamine-2A: 5-HT2A
^{99m}Tc: metastable Technetium
^{123}I: radioiodine

INTRODUCTION

"The notion of watching the brain think, both in health and disease, has become reality"

S. Lewis, N. Higgins from "Brain imaging in psychiatry", 1996

INTRODUCTION

1. General introduction

This thesis evaluates the use of single photon emission tomography (SPET) as functional imaging modality in the study of canine brain and its relation to behavioural pathology. First, the use of SPET in the study of *in vivo* brain perfusion was assessed.
Second, *in vivo* measurement of the serotonin-2A (5-HT2A) receptor binding in the dog brain was evaluated with SPET. The clinical applications of these techniques in aged and in impulsive, aggressive dogs were evaluated.

This introduction will serve to define the methodological context of the study.

First, since the use of nuclear medicine functional imaging modalities is not as wide-spread in veterinary medicine as it is in human medicine, the first section of the introductory remarks present some basic and general principles of nuclear medicine. A short historical overview will be given, together with some notes on the current implementation of radiation in diagnostic imaging and radiation protection in veterinary nuclear medicine.

A second section will narrow the focus of nuclear medicine to "brain" imaging and its application in the study of pathological brain-behaviour relationships. First concerning the functioning brain, there is a long tradition in the study of brain-behaviour interactions via *post-mortem* or surgical and pharmacological interventional studies. Hence, a short overview on human and animal studies from the "pre-imaging era" will be presented. This is followed by a short overview of the principles of nuclear brain imaging, both perfusion or metabolism and receptor imaging, in physiological and pathophysiological conditions.

A third section will narrow the focus even more to two conditions, old age and impulsive behaviour, that will be evaluated with perfusion and serotonin-2A receptor studies, using SPET imaging techniques and that will constitute the clinical application of this thesis. First, based on *in vitro* and *post-mortem* neuropathological and biochemical studies, the rationale behind perfusion imaging and serotonin-2A imaging in aged dogs is given. Second, impulsive behaviour is defined and its relationship to brain abnormalities, both structurally and functionally, is given. The link of this pathological condition and abnormalities in regional blood perfusion and serotonin-2A receptor is explained.

2. Principles of nuclear medicine

A. Historical overview

Only a few months after Röntgen discovered X-rays in 1895, Henri Becquerel demonstrated the natural radiation properties of uranium salt crystals. Shortly thereafter, Pierre and Marie Curie separated and identified radium, thorium and polonium, all three elements emitting radiation. Radiation therapy was soon introduced for medical use by applying radium and later radon as local implants to tuberculosis skin lesions. In the beginning of the twentieth century, radiation became a popular health cure and was soon good for all ailments. Radioactive water was sold under several forms as a drinkable tonicum, oil containing radium was used to cure arthritis and skin disorders and radium containing electric blankets were claimed to combine the natural healing forces of electricity and radiation. Although, at that time, virtually nothing was known about radiobiology, it was amazing that the concept of radiation hormesis was already adopted. Indeed, high doses of radium were used for therapy, utilizing its destructive properties, while small doses of radium were believed to have stimulating properties, re-energizing weak and inactive cells.[1] In the 1930's an important step in the introduction of artificial radioactivity was set by Enrico Fermi who discovered that chemical elements became radioactive when irradiated with neutrons. This period was also important for the development of equipment that measured radioactivity, thus resulting in the start of "radiobiology", a science that investigates the biological effects of radiation.

Half way the 20th century it was found that the uptake of strontium in pathological bone tissue was increased before radiographic changes could be detected, paving the way for the diagnostic use of radiation. Later, the improvement of specific detection systems, such as the Anger gamma camera in 1958, and the development of radionuclides, such as Technetium-99m, fastened the evolution of this diagnostic imaging modality. The fact that the applicability of this technique kept growing can be illustrated by the recognition of nuclear medicine as a separate speciality in human medicine, separate from radiotherapy, internal medicine and radiology. In recent years progression has been made towards the field of molecular imaging, consisting of establishing receptor imaging, the use of labelled monoclonal antibodies and peptides and the introduction of labelled markers in gene therapy.

In 2002, this evolution in the field of nuclear medicine interestingly resulted, for example, in the extension of the name of "European Journal of Nuclear Medicine" to "European Journal of Nuclear Medicine and Molecular Imaging".

B. Current implementation of radiation in diagnostic imaging

Radiation used for diagnostic purposes consists of three types of radiation: X-rays, gamma-rays (γ-rays) and beta+ (β+) rays. Radiography and related computed tomography, using X-rays, is generally used for morphological imaging purposes while β+ and γ-rays are used for functional diagnostic imaging, visualizing metabolism and measuring time related activity in selected organs. In this study y emitting radio nuclides were used and will therefore be further discussed in this paragraph. The major difference between X-rays and γ-rays lies in their origin and not their nature, since both are electromagnetic rays. X-rays are generated outside the nucleus in the electron cloud, while gamma rays develop in the nucleus. Another major difference is that X-rays are generated by the X-ray machine, emitting radiation through the subject, causing a transmission or attenuation map of the subject, registered on photographic film. Gamma-rays, generated from the injected radionuclide inside the patient, are emitted from the subject itself and are detected by dedicated devices, forming an emission map. This detecting device is the Anger gamma camera, equipped with a collimator. The gamma camera is illustrated in Figure 1.

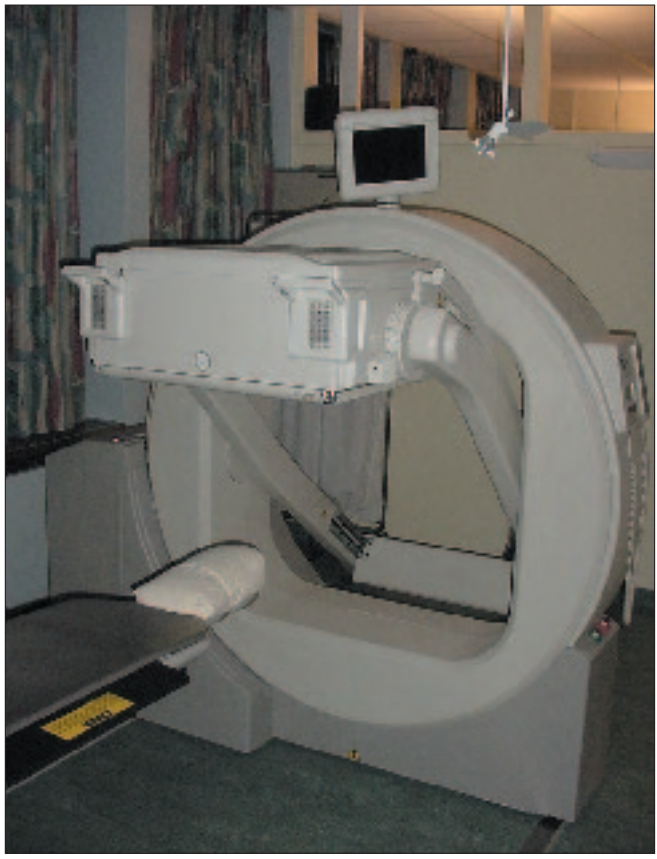

Fig 1: Gamma camera, mounted in gantry

As was mentioned before, Single Photon Emission (Computed) Tomography (SPE(C)T) was used in this study. This acquisition mode consists of a computer assisted rotation of one or multiple detector-heads in a 360° circular or elliptical way round the patient, focussing the target organ and registering emitted photons. The advantage of multiple heads is that the examination time is shortened. Further, dedicated computer software will filter the obtained data and reconstruct them to a three dimensional image. The advantage of this tomographic imaging is that the ratio of target/background (= signal/noise) will be improved, since noise can be eliminated on the separate slices. Also, localization of the lesion is facilitated since other structures, or other parts of the organ, will not superimpose the region-of-interest. Principles of SPET imaging are illustrated in Figure 2A and 2B. It is important to note the difference in nomenclature of the slice orientation used in this veterinary work, compared to the nomenclature generally used in human nuclear medicine (Fig. 2B).

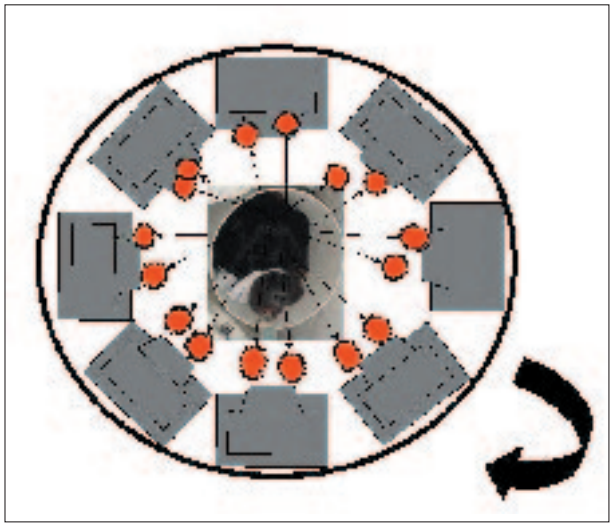

Fig 2A: The SPET acquisition mode consists of a computer assisted rotation of one or multiple detector-heads, equipped with dedicated collimators, in a 360° circular or elliptical way round the patient, focussing the target organ and registering emitted photons (represented by the red dots).

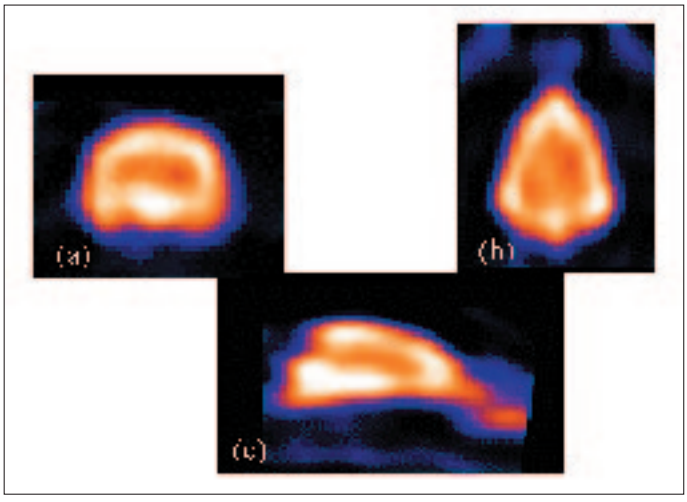

Fig 2B: Transversal (in human nuvlear medecine = coronal) (a), horizontal (in human nuclear medecine = transversal) (b) and sagittal images (c) of a perfusion study obtained with SPECT. Three-dimensional evaluation of brain structures is facilitated using this method.

Selection of the radionuclide and its tracer are of major importance because they will determine uptake in the target organ under investigation. As an example diphosphonates will be subject to adsorption to the bone dependent on bone activity and therefore "isotope labelled diphosphonates" can be used for investigating bone pathology involving increased bone metabolism. (Fig 3). Since the application of brain imaging in veterinary nuclear medicine is new, the first part of this thesis is dedicated to the investigation of radioligands, suitable for neuroimaging in dogs. In particular, the 99mTechnetium labelled N,N"-1,2-ethylene-diylbis-L-cysteine diethyl ester dihydrochloride (ECD) will be evaluated in a group of 10 normal dogs in Chapter 1 and the applicability of the specific 5-HT2A receptor ligand 123I labelled R91150 for canine brain studies, will be determined in Chapter 3 and 4.

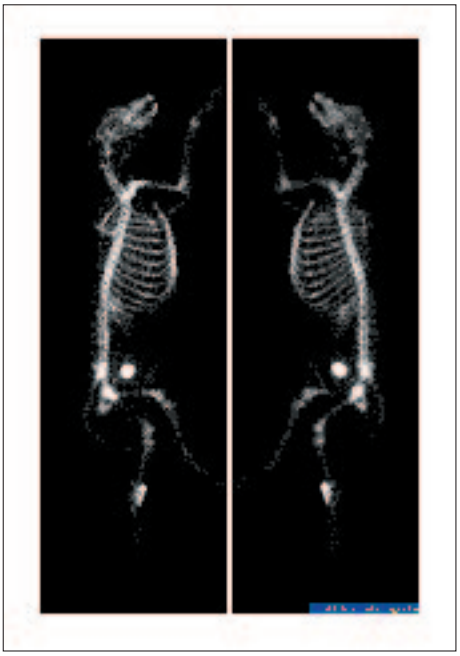

Fig 3: Example of a total body bone scan of a dog, using the ^{99m}Technetium labelled methyl-diphosphonate tracer with captation of radioactivity in the skeleton. Note the accumulation of radioactivity in the bladder (large arrow) and the site of the IV injection of the tracer (small arrow) and the presence of only one foreleg (the other was amputated).

C. Radioprotection: matter of concern

Radioprotection is an important issue to consider in both human as veterinary nuclear medicine. As far as the animal is concerned, radiation hazards should not be overemphasized, since life expectation precludes development of long term radiation effects. The most important measures are aimed towards protection of the handler, owner and clinician. Hence, it is of importance that the veterinary nuclear medicine physician informs staff and owners on these issues. First, since most radiopharmaceuticals are eliminated by the liver and the kidneys, attention has to be paid to contamination with faeces and urine. Hence, radioprotective measures are mainly directed to prevent direct contact with contaminated fluids and therefore, gloves are worn. Second, distance from the source, in this case the animal patient, is indicated, since the energy of radiation is inversely correlated with distance to its source. If these radioprotective measures can not be guaranteed, the animal must be kept in isolation in the medical imaging unit of the veterinary hospital until radiation hazards are minimized. Concerning this matter, no general rules on a time-frame can be given since the half-life (biological and physical) of the tracer and the injected dose have to be taken into account in determining the amount of radiation and possible contamination in time.[2] In this thesis the isotope Technetium-99m (140keV) with a physical half-life of 6 hours was used for the perfusion studies. The ligand used for the 5-HT2A receptor imaging was labelled with iodine-123 (160keV) which has a half-life of 13 hours.

Fig 4: Three views of a sculpture demonstrating the different modules of the working brain according to Gall.

3. Functional brain imaging

"MAPPING THE FUNCTIONING BRAIN"

A. History of brain-behaviour research

The brain is constructed of, and organized by a highly refined network of neurons and synapses and is fueled by various neurotransmitters and neurohormones, in order to achieve three major goals: basic survival, social interaction and the cognitive organization of living. The search to unravel brain functions started early in man's history. Medical case reports on brain pathology, written in 1700 BC, were discovered in Luxor in 1862 by Edwin Smith. Hence, the knowledge that the "brain governed the body", was already introduced by the Egyptians. For a very long time, this statement formed the basis of empirical neurosurgical strategies in behavioural pathologies that were mostly cruel and ineffective at the same time.

In the realm of the emergence of criminology in the 19th century, and in an attempt to understand the biological basis of deviant behaviour, "phrenology" became a separate science under guidance of Franz Gall. His brain and skull maps were largely incorrect, but were the first attempt to consider the brain as functionally inhomogeneous and consisting of functional "modules". The original phrenological concepts by Gall are depicted in Figure 4. By the end of the 19th century, beginning of the 20th, more "sophisticated" investigational methods were used to map brain functions, making an end to "phrenology". First, case reports in humans on acquired brain lesions, accidentally or disease-related, were the basis of a more correct definition of brain-behaviour relationship. In this period, based on the post-mortem analysis of brains of patients who had cerebro-vascular accidents, Broca and Wernicke were the first to correctly localize the language area in the brain, located in a complete different area as was determined by the phrenologists. Further, with experiments such as electrical stimulation in both man and animals, ablation studies and regional brain cooling in animals, researchers tried to prove links between behavioural patterns and anatomical brain regions. This research resulted in a rather premature introduction of this knowledge in the treatment of human patients with behavioural disorders. Under impulse of Egas Moniz, frontal lobotomy was widely used for patients with uncontrollable aggression. Paradoxically, Moniz was killed by one of his own lobotomized patients. In that era, the neurosurgeon travelling between mental institutions and "treating fourteen patients in one morning", was a reality.

Only in the 1960s, with the emergence and the introduction of the first brain-dedicated pharmaceuticals, a reasonable alternative was offered for lobotomy and electro-convulsion therapy. Initially, serendipity lead to the awareness that certain neurotransmitters were involved in behavioural and psychiatric disorders. Huge scientific efforts, often supported by pharmaceutical industry, lead to the development of neuroleptics such as chlorpromazine, haloperidol and risperidone and anti-

depressants such as imipramine and fluoxetine. Paradoxically, it was the application of these psychotropics in the treatment of behavioural disorders, that finally offered a better insight in neuroreceptor functioning and pathophysiology of behavioural and psychiatric disorders.

Recently, a tendency is set to turn away from the phenomenological approach, based on nosological, i.e. syndromal clusters, towards a transnosological approach that is based on symptoms that are apparent across nosological diagnostic categories. This approach proved to be more successful in linking neuropathological and biochemical dysfunctions to behavioural symptoms and disorders.[3] In this context, "impulsivity" can be found as a common symptom in several psychiatric disorders, such as personality disorders, eating disorders, impulsive behaviour disorders, suicide, and is consistently brought in connection with a dysfunctional serotonergic system, throughout the diagnostic nosological categories.[4-8]

B. Functional brain imaging with PET and SPET modalities

Since functional brain imaging allows the investigation of perfusion or metabolism and of brain receptor status *in vivo*, a tendency to examine patients with behavioural disorders with positron emission tomography (PET) and SPET modalities developed. Differences between these two modalities will be discussed in Chapter 3. In this thesis, SPET is used for all investigation procedures. First, to evaluate brain perfusion with SPET, two major radiopharmaceuticals are used: ^{99m}Tc-d,l-hexamethyl-propylene-amine-oxime (HMPAO) and ^{99m}Tc-ECD. Due to their lipophilicity, both tracers pass the blood brain barrier (BBB). They are incorporated into the brain cells and trapped by conversion to hydrophilic compounds.[9] With these compounds it is not only possible to evaluate and delineate brain regions under resting conditions but also when activated during task performance in normal and abnormal states, thus visualizing the "working" brain. Recently, the Ghent Molecular Imaging Group developed a split-dose imaging paradigm to investigate patients before (rest condition) and while (activation) undergoing cognitive challenge of the frontal cortex.[10] Moreover, enhanced or decreased perfusion can be demonstrated after pharmacological intervention.[11,12] The important advantage is that the functional state of the brain at the time of injection is "frozen" for some hours, providing the possibility to perform the acquisition some time after injection, an advantage that is exploited for visualization of epileptic foci during an epileptic fit.[13,14] Second, research of the last 20 years has focussed on the development of specific radioligands to visualize and quantify different neurotransmitter receptor systems in physiological and pathological conditions. Virtually all neuroreceptor systems can be studied through a specific and receptor-dedicated radiolabelled tracer.[15] Although the aetiology of disease is not addressed, functional imaging provides a mean to elucidate the pathophysiology of behavioural disorders, to develop itself as a possible marker of a pathological condition and to evaluate biochemical and physical receptor changes during treatment.

4. Nuclear imaging and behavioural disorders

As described previously, functional imaging offers the opportunity to investigate functional disease of the brain linked to perfusion alterations or neurotransmitter system dysfunction and reflected as behavioural disorders.

Among the different neurotransmitter systems, the serotonergic system has been implicated in depression, anxiety, out-and inward directed aggression and eating disorders in man and in aggression and domestication in animals. Much of the complexity of the serotonergic system is resulting from its interactions with other neurotransmitter pathways and from the diversity of different 5-HT receptor subtypes in the brain. Seven serotonergic receptor families are recognised, comprising several subtypes which differ in molecular structure, in signal transducing mechanisms, anatomical distribution and responses to different agonists and antagonists. Among these, the 5-HT1A, 5-HT2A and 2C receptor subtypes have become a topic of intensive research and have been increasingly firmly connected with behaviour.[16]

A. Perfusion and serotonin-2A receptor alterations related with age

Aging effects are found to change perfusion and metabolism as well as binding properties of the serotonin-2A radioligand in humans, thus leading to age related depression, dementia and Alzheimer disease.[17-19] These neuropathological events consist of a complicated and yet not completely elucidated cascade of intra- and extracellular mechanisms.

First, aging in brain was found to be accompanied by structural changes, resulting from an imbalance in neuroprotection and neurodegradation in both animals and humans. Neuronal survival is determined by the presence of neuroprotective proteins and the activation of compensatory mechanisms provoked by certain toxic factors. Oxidative damage, causing oxidation of lipids, proteins, enzymes, DNA and RNA, is thought to be one of the initiating toxic events occurring with aging. Compensatory mechanisms are activated but when failing, these toxic events will inevitably lead to cell apoptosis, representing programmed cell death with absence of inflammatory responses as opposed to cellular necrosis, and neuronal loss.[20,21] Furthermore, deposition of senile plaques, composed of β-amyloid, a toxic peptide derived from misprocessing of an amyloid precursor protein, is found mainly in the cortical structures of aging canine brain, comparable to findings in humans.[22] In dogs, β-amyloid will increase from the age of 10 years in an age dependent manner and will result in synapse loss.[20] Moreover, amyloid angiopathy, resulting from vascular deposition of β-amyloid, damaging the endothelialcells and the smooth muscle cells, provokes decreased vascular distensibility and reactivity and as a consequence causes perfusion alterations in the brain.[23-25]

Perfusion alterations may therefore be detected in the aging canine brain with functional imaging.

Second, aging is also accompanied by reduction of functioning of several neurotransmitter systems, including the dopaminergic and the serotonergic system in animals and humans.[18,26-36] In humans decreased functioning of the serotonergic system is thought to be responsible for age-related depression.[17] Moreover, since the serotonergic system has an important role in the regulation of brain microcirculation, dysfunction might lead to deficits in brain perfusion.[37] Recently, the Ghent Molecular Imaging Group demonstrated a reduction in brain serotonin-2A radioligand binding index in old healthy volunteers and, to a larger extent, in patients with Alzheimer's disease.[38]

Hence, concerning serotonine-2A receptor binding, one can expect that binding index alterations may also be detected in the aging canine brain.

B. Impulsive behaviour

Impulsive behaviour, for animals defined as "incapacity to wait or to delay response"[39] and for humans as "acts related to inadequate self-control, impaired impulse control"[39], result in reactions that are "sudden" and "unpremeditated"[40]. This impulsive behaviour is often aggressive in nature and therefore, has large impact on the victim and on the perpetrator.

It is important to define this "impulsive" behaviour in contrast to "normal" aggressive behaviour in animals. Indeed, several types of aggression, such as maternal, intermale, territorial and predatory aggression are described in animals, depending on different neuronal circuits and hormonal mechanisms.[41,42] These behavioural patterns are in-born and instinctive in nature and as such, considered as normal coping activities to a set of environmental stimulus conditions. Therefore, they are not pathological, although they can become beyond control, influenced by previous experiences or situational determinants. Most of these reactions have been accepted by humans during domestication of animals, as long as these reactions are appropriate in relation to the stimuli and as long as they can be foreseen.[43]

Moreover, impulsive, aggressive behaviour can be of advantage under certain circumstances, such as in a dangerous environment where quick reactions and high levels of arousal coupled to a high degree of aggressivity are necessary as coping mechanisms. But, in safer habitats this behaviour will result in unnecessary attacks leading to injury.[44] Extensive research has been performed on impulsivity, pointing at the frontal cortex and subcortical structures as the structural anatomical substrate and at lowered activity of the serotonergic system as the biochemical substrate for impulsive behaviour.

First, studies on the anatomical and structural substrate of these impulsive disinhibited disorders were based on acquired brain damage in humans and on ablation studies in animals. Phineas Gage was one of the first clinical proves that the frontal cortex played an important, if not major role in the expression of this abnormal, disinhibited behaviour.[45] This previously conscientious railway worker turned into an impulsive, aggressive drunk after an accident with a metal rod that perforated his

left fronto-cortical lobe. This was later confirmed with frontal ablation studies in several animal species, demonstrating that the frontal cortex exerted control over limbic drives of hunger and aggression.[46] Summarized, the frontal cortex will generate an adequate, premeditated response based on the association of the limbic and sensoric information guided by possible previous experiences, thereby permitting acceptable or "in-context" necessary reactions or inhibiting certain inappropriate reactions.

In humans disturbed frontal perfusion and metabolism was demonstrated in murderers and aggressive individuals with SPET[47,48] and altered metabolism was found with PET[49-51]. It is important to notice that in these cases disturbed frontal perfusion patterns were present without concurring structural anatomical brain abnormalities, as was proven with CT or MRI and therefore represented true functional perfusion and metabolism deficits.

No comparable functional imaging studies in animals are present at this moment. It can be hypothesized that comparable functional deficits in the frontal brain structures of dogs with impulsive behavioural disorder exist.

Second, studies on the functional biochemical deficits in the brain of impulsive subjects pointed at the involvement of a deficient serotonergic system in impulsive behaviour.[6,39,52-66] This is one of the most replicated findings in biological psychiatry in human medicine. But also studies in other species demonstrated a prominent role of serotonin in social adequate and inadequate behaviour.[67-75] The cell bodies of the serotonergic neurons are located in the raphe nucleus, with widespread projections to the limbic system, hypothalamus and the cortical regions, explaining their link with mood and behavioural disorders. The influence of the serotonergic system on behaviour in animals and man has been investigated with direct and indirect studies. A decreased metabolism, demonstrated by decreased amounts of 5-hydroxy indolic acetic acid (5-HIAA, the principal metabolite of 5-HT) in cerebrospinal fluid (CSF) was found both in humans as in animals with impulsive, aggressive and self-injurious behaviour.[6,60,61,69-72,77-80] Dietary tryptophan depletion increased aggressiveness and tryptophan supplementation decreased aggressiveness in both animals and humans.[57,63,68,81] Using pharmacological probes, affecting the serotonin transporter mechanism and/or the receptor, aggressive behaviour both in animals and humans could be influenced.[67,82-88] More specific studies on the 5-HT2A receptor, using measurements of 5-HT2A receptors density on platelets, demonstrated increases in binding index in suicidal patients and in patients with aggressive personality disorders.[54,66,89-92] Direct autoradiographic studies showed increased density of 5-HT2A receptors in patients, committed suicide.[73,93,94]

Since the development of specific receptor radioligands for functional imaging, using SPET or PET, these methods have received increasing attention in research on the serotonergic system *in vivo*, under physiological and pathological conditions, with and without pharmacological interventions. Concerning SPET imaging, a relatively new radioligand ^{123}I-5-I-R91160 has been used to investigate the serotonin-2A receptor status in normal volunteers.[26,95] Recently, the Ghent Molecular Imaging Group investigated human patients with recent suicide attempts[8] and patients with eating disorders[7]

and found a decreased cortical serotonin-2a binding index. No comparable functional imaging studies on the serotonin-2A receptor in animals are present at this moment. One could expect comparable functional deficits in the cortical brain structures of dogs with impulsive behavioural disorder.

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SCIENTIFIC AIMS

Research on the pathophysiology of abnormal canine behaviour in vivo is scarce due to the fact that objective measurement of behaviour is difficult. The only available way to investigate the disordered animal is by means of behavioural testing and elaborated questionnaires, which are no doubt of significant importance but remain subjective (operator/owner dependent). It was the aim of this study to evaluate the applicability of functional imaging of the brain in the dog, i.e. to evaluate this technique as a tool to visualize and measure perfusion and the 5-HT2A receptor in normal dogs, aging dogs and in dogs showing impulsive aggressive behaviour.

In this work we tried to address the following questions:

1. **Can we measure canine brain perfusion with functional brain imaging, using the SPET modality, and what is the normal distribution pattern in dogs without neurological or behavioural disorders?**
2. **What are the technical issues to consider when performing receptor radioligand imaging studies, using the SPET modality?**
3. **Is it possible to use the radioligand, ^{123}I-5-I-R91150 to image and quantify the 5-HT2A receptor in canine brain?**
4. **What is the normal distribution pattern of this receptor in the brain of dogs without neurological or behavioural disorders?**
5. **What is the influence of age on brain perfusion and binding characteristics of the specific serotonin-2A radioligand ^{123}I-5-I-R91150 in normal aging canine brain?**
6. **Can we include impulsive aggressive behaviour in dogs as a clinical behavioural disorder to investigation with this imaging modality?**
7. **Is it possible to demonstrate differences in brain perfusion and/or serotonin-2A radioligand binding using SPET, between normal dogs and dogs showing impulsive, aggressive behaviour?**

Because information on the use of this modality for brain examinations in dogs is scarce, the first part of this study consisted of the acquirement of normal databases of both perfusion and 5-HT2A receptor ligand in normal adult individuals.

To measure brain perfusion, ^{99m}Tc-ECD was chosen as radiopharmaceutical. First, it is ^{99m}-Technetium labelled, a label generally preferred in nuclear medicine because of its excellent physical characteristics for imaging (140 keV; T1/2 = 6 hours) and since it is relatively cheap and readily available from “on site” ^{99}Mo/^{99m}Tc generators. It also has superior imaging qualities compared to ^{99m}Tc-HMPAO. Concerning the use of the radiopharmaceutical ^{99m}Tc-ECD in dogs, we did not perform elaborate biodistribution studies, because it was already shown in a previous preclinical feasibility study concerning the use of ECD in several species, that this tracer achieved low, but sufficient brain

penetration in the dog (1.2% ID) lasting 2.5 hours. However, we determined the time to achieve a stable plateau for brain concentration after injection of the radiopharmaceutical in two dogs and found it was as fast as 2 minutes (Fig.1) and a maximal brain concentration of 1,5% ID was registered.

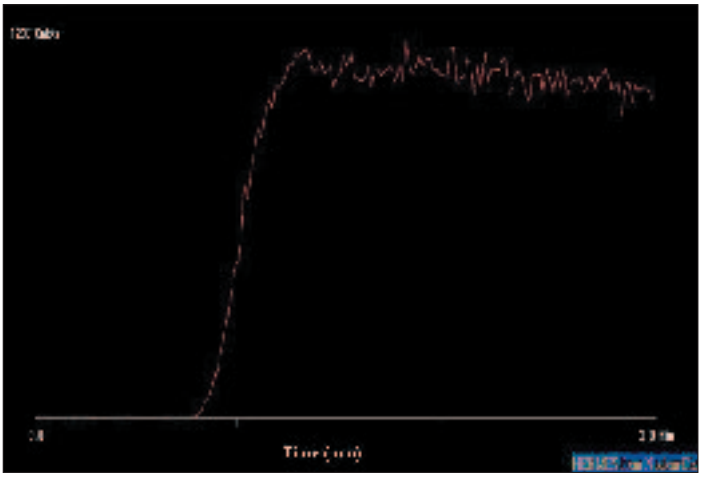

Fig 1: ^{99m}Tc-ECD cerebral uptake curve in function of time in a normal dog. Dynamic frames of 6 seconds were acquired for a period of 8 minutes. Brain uptake of the radiopharmaceutical stabilizes before 2 minutes post-injection.

The regional brain perfusion, using ^{99m}Tc-ECD and SPET, in 10 normal dogs, aged between 1 and 9 years old, including 5 males and 5 females, is determined in Chapter 1. In the next study, described in brief in Chapter 2, a template is generated with accompanying region map, from the brain perfusion data of 12 normal dogs (6 males and 6 females), aged younger than 8 years. This computer operated procedure facilitates the fitting procedure of the individual patient data to the normal data base and eliminates operator bias when determining regional activity with manually drawn VOI's. In Chapter 3 an overview is given on the imaging techniques and characteristics of radioligands, used for neuroreceptor imaging. The differences between positon emission tomography (PET) and single photon emission tomography (SPET) are briefly outlined in this chapter. The essential properties of the radioligands and the major difficulties encountered in their development are discussed. This review on the technical aspects is followed by a literature overview on the application of neuroreceptor imaging in research on animal models.

Concerning the particular serotonin-2A receptor radioligand, ^{123}I-5-I-R91150, a biodistribution study had to be performed, because although the tracer has been used in humans, primates and rats, nothing is known about its applicability in the dog. The specificity and reversibility of radioligand binding and the optimal scanning time are evaluated in Chapter 4. In Chapter 5, the regional distribution pattern of this serotonin-2A radioligand is evaluated in 10 normal dogs, 5 males and 5 females, age ranging from 1 to 9 years. Ample evidence exists from literature that age plays a significant role in both perfusion and serotonin-2A receptor density. Therefore we investigate age influences in a larger group of animals, in Chapter 6. The subjects are divided into two age categories. One group consists of dogs younger than 96 months (N=12, 6 males and 6 females) and the other includes individuals aged 96 months and older (N=12, 5 males and 7 females).

Since persuasive evidence is provided in literature that impulsive, aggressive behaviour is associated with altered brain functioning and involvement of the serotonergic system (Chapter 7), brain perfusion and binding properties of the serotonin-2A radioligand are estimated in impulsive aggressive dogs and compared with the data obtained from normal reference dogs (Chapter 8). The selection of these dogs is based on a questionnaire, compiled from information found in literature and further adapted towards recognition of impulsive individuals.

CHAPTER 1

Regional brain perfusion in 10 normal dogs measured using Technetium-99m-ethyl cysteinate dimer SPECT

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Summary

Single photon emission computed tomography (SPECT) of the brain using perfusion tracers allows estimation of regional brain perfusion. This allows in vivo examination of brain function in the setting of neuropsychological and pathophysiological changes. However functional imaging data on brain perfusion in dogs are limited.

Hence, the aim of this study was to determine the scintigraphic regional perfusion pattern of the normal canine brain. Ten healthy shepherd type dogs were injected with 925 MBq Technetium-99m ethyl cysteinate (ECD) 20 minutes prior to the examination. Acquisition was performed using a triple head gamma camera equipped with fanbeam collimators. Uniform attenuation correction and triple energy window correction were applied. Computed tomographic images were obtained from the same dogs, reoriented along the orbito-meatal axis and SPECT perfusion data were coregistered to the CT-volume data. Based on a morphological and suggested brain divisions, regions-of-interest (ROIs) were defined for the bilateral frontocerebral, temporocerebral, parietocerebral, occipitocerebral, cerebellar, and subcortical area. Regional count density was normalized on total counts. All dogs had the highest uptake in the thalamic/striatal area compared to a rather homogeneous cerebral uptake. No significant left/ right count differences were found, but a rostro-caudal gradient (+ 12-13%) was present. In this group, age and gender did not influence the perfusion pattern.

Introduction

Computed tomography (CT) and magnetic resonance imaging (MR) are the diagnostic imaging techniques for evaluating structural brain pathology, while single photon emission computed tomography (SPECT) and PET (positron emission tomography) enable three-dimensional visualisation of functional parameters reflecting cerebral blood flow, brain metabolism and binding potential of receptor ligands. SPECT perfusion imaging using ^{99m}Technetium labelled radiopharmaceuticals has become a routine procedure in human nuclear medicine, enabling visualisation of regional blood flow and under normal coupling also of brain metabolism. It is interesting to note that the first study that demonstrated the coupling of cerebral perfusion and metabolism in the dog was already published in 1890.[1]

While ^{99m}Tc-HMPAO (hexamethylpropylene amine oxime) was the first ^{99m}Tc labelled tracer to be commercialised for evaluation of rCBF, ^{99m}Tc-ECD (ethylcysteinate dimer) is a relatively new lipophilic tracer with a faster blood clearance and chemically more stable than ^{99m}Tc-HMPAO. In the human brain, the lipophilic ^{99m}Tc-ECD crosses the intact blood brain barrier (BBB) and is trapped intracellular within 2 minutes in proportion to the cerebral blood flow, probably by de-esterification to polar complexes.[2,3] The optimal imaging time is between 30-120 min. post injection (p.i.) in humans, but acquisition may be started as early as 10 min.[4] No redistribution of the tracer occurs and the uptake and distribution, which are proportional to rCBF, remain unchanged for two hours. Regional grey-to-white matter contrast begins to decrease after 2 hours. Redistribution (difference in grey matter activity pattern) starts from 4 hours onwards and is region dependent.[3]

Currently accepted clinical indications for SPECT functional brain imaging in humans are diagnosis and follow-up of various neurological disorders, such as dementia, epilepsy and cerebrovascular disorders. In psychiatry, brain SPECT especially using receptor-ligands, may be considered a promising tool.[5-7] In addition to studies in basal conditions, activation studies using specific paradigms may be applied.[8,9] These activation tasks generate detectable and reproducible regional CBF changes during task performance.[10]

The radiopharmaceuticals used for conventional functional brain imaging of small animals remain confined to the blood pool in the presence of an intact BBB, only diffusing into the parenchyma when pathology disrupts this barrier. Classically ^{99m}Tc-DTPA (diethylenetriaminepentaacetic acid) and ^{99m}Tc-GHA (glucoheptonate) were used. Planar brain imaging using these radioligands has been applied in the diagnosis of infectious, vascular and tumoral lesions.[11]

Limited information is available on imaging using planar imaging or single photon emission computed tomography (SPECT) in dogs with radiopharmaceuticals crossing the intact BBB. In one study brain imaging is reported in 8 beagles with ^{99m}Tc-HMPAO and ^{123}I-IMP (d,l-N-isopropyl-p-iodoamphetamine hydrochloride). The authors describe the uptake, distribution and clearance of both radiopharmaceuticals and quantify the effects of small doses of cocaine on the kinetics and localization

of the tracers.[12] In another study on the retention mechanism of ^{99m}Tc-ECD (ethyl cysteinate dimer) in multiple species, including monkeys (6), ferrets (2), rabbit (1), miniswine (1) and dogs (2), a species-specific brain retention of this lipophilic tracer is found. Retention was found to be good in human and non-human primates, average in dogs and poor in rats, rabbits, ferrets and miniswine. These authors find a brain uptake index (BUI) of 1.2% of the injected dose in dogs and a biological half-life (T1/2) of 2.5h compared to 4.8-6.8% and 4h in humans.[2]

The purpose of the present study was to assess the feasibility of canine brain imaging with ^{99m}Tc-ECD and high-resolution SPECT and to define its regional distribution pattern in the normal dog. This information could be used as a reference atlas of brain perfusion in subsequent pathophysiologic studies. The study constitutes the basis of a local project aiming at elucidating functional brain changes in aggressive dogs using SPECT perfusion tracers and receptor ligands.

Materials and methods

Ten healthy shepherd type dogs, 5 males and 5 females, aged between 1 and 9 years (mean age = 5.5; SD = 2.9), with a body weight between 23 and 41 kg (mean weight= 31.9; SD= 6.4) were studied. The dogs had no history of neurological disorders or behavioural abnormalities. These dogs were used to being handled for intravenously injections and imaging procedures. The examination procedures never resulted in excitation or aggression and were performed according to good animal practice.

^{99m}Tc-ECD (Neurolite®, Dupont Pharmaceuticals Ltd., Brussels, Belgium) was injected intravenously (925 MBq (25mCi)) after sedation and prior to general anaesthesia. None of the dogs experienced an adverse reaction to the radiopharmaceutical. Sedation was obtained with 10-30µg/kg medetomidin hydrochloride IM (Domitor®, Pfizer) and general anaesthesia was induced with 2-3mg/kg iso-propylphenol IV (Rapinovet®, Mallinckrodt) and maintained with halothane (Fluothane®, Zeneca) to effect. All dogs were positioned in ventral recumbency. SPECT was performed with a triple head gamma camera (Toshiba GCA-9300A, Dutoit Medical, Antwerp, Belgium), equipped with high resolution fanbeam collimators (FWHM 7.8 mm). Acquisition was started 15 to 25 minutes after injection of the tracer. Total acquisition time was 40 minutes. For each acquisition, 90 projection images were obtained on a 128x128 matrix using a continuous scan mode by rotating each head 120°. The images were reconstructed with filtered back projection after rebinning to parallel data and a Butterworth-filter (cut-off 0.16 cycli/pixel, order 8). Pixel size was 1.72 mm. Sorensen attenuation correction with a uniform attenuation coefficient of 0.12 /cm and triple-energy window scatter correction were applied according to standard clinical settings, as described previously.[13] In addition to the perfusion study, computed tomographic brain imaging (scanner pace plus, GE Medical Systems, Wisconsin, USA) was performed within 2 days. The dogs were positioned in dorsal recumbency. Contiguous 5 mm transverse and dorsal scans were obtained using acquisition parameters of 120 kV and 100 mA. The CT slices were formatted with a soft tissue window setting (WW = 150; WL = 50). After the scan, images were reconstructed in the sagittal plane. Six consecutive dorsal plane images were used corresponding to anatomic levels of a stereotactic atlas of the dog's brain[14]. (fig 1). The SPECT perfusion data were manually fitted to the CT data set on a medical workstation using the Multimodality software of Nuclear Diagnostics (Hägersten, Stockholm, Sweden). (fig 2).

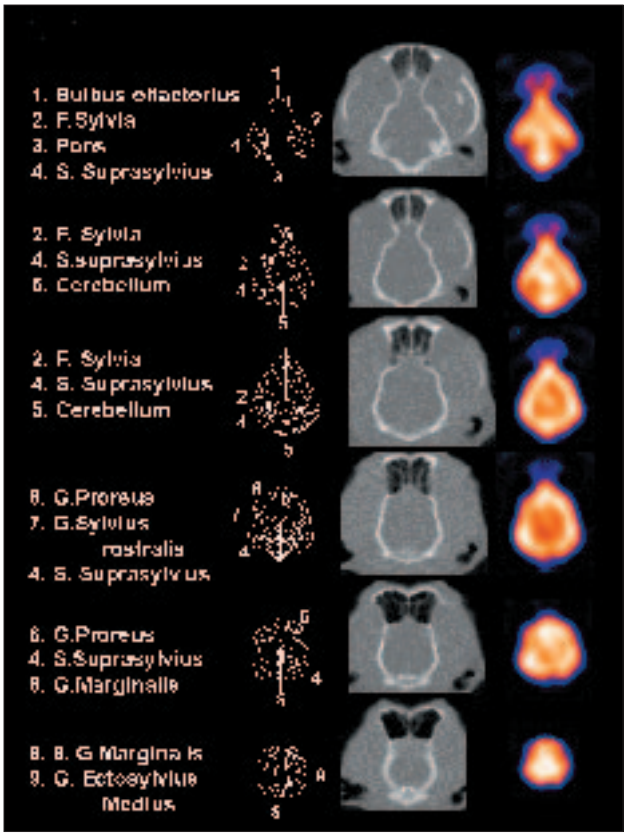

Figure 1: Horizontal slices at different brain levels: anatomic, CT data, and emission images. From top to bottom slices are shown from the base of the brain to the dorsal side. Significant brain structures are indicated.

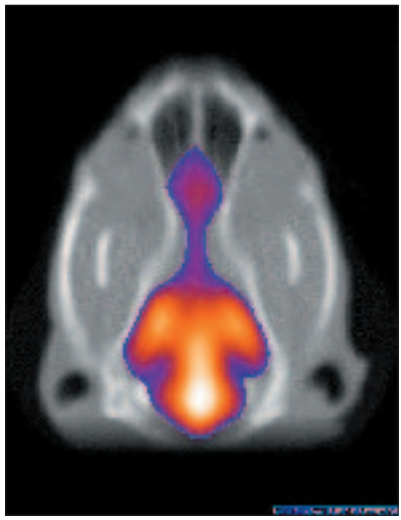

Figure 2: Example of ^{99m}Tc-ECD flow image, fitted on a corresponding CT image. This is a horizontal slice, located at the base of the brain.

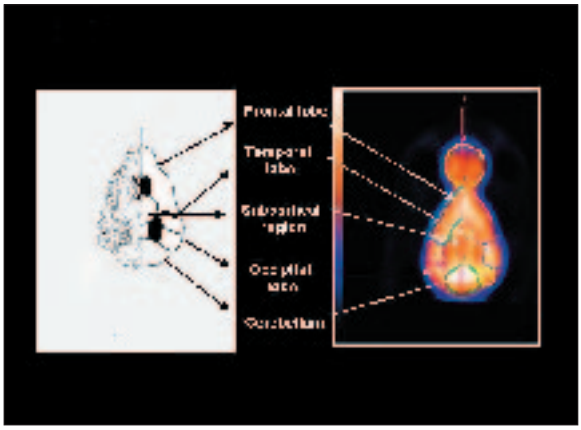

Figure 3: Example of regions of interest (ROI) map drawn on brain slices. Irregular regions of interest are manually drawn. This horizontal slice is taken at the lower middle part of the brain.

For co-registration a mutual information cost function, minimalized by a down-hill simplex algorithm, was used. The mutual information registration criterion allows fully automated, highly robust affine registration of multimodal images without the need for pre-processing or user interaction.[15]

Irregular regions of interest (ROI) were drawn manually on the CT images for further quantitative analysis. Consensus ROI's were placed by two investigators (KP and PDB) on frontocerebral, temporocerebral, parietocerebral, occipitocerebral, subcortical area and the cerebellum based on the description by Redding (1978)[25]. (Fig 3). Further differentiation was not possible because of the small size of the individual structures and the limited contrast of the CT imaging system and limited resolution of the SPECT system.

From the SPECT data, average counts per pixel were calculated for all regions. A perfusion index was obtained by normalising the average regional counts to total counts of all ROI's. A rostro-caudal gradient was defined as (R-C/R+C)*100, where R is the bilateral rostral (frontal) ROI count and C the caudal (occipital) ROI count.

Non-parametric statistical analysis of the data was performed by means of the SPSS package (Statistical Software Package for the Social Sciences, v9.0, SPSS Inc, USA). Correlations between age, weight and regional cerebral blood flow were calculated with Spearman's correlation test. Left to right differences in frontocerebral, temporocerebral, parietocerebral and occipitocerebral cortex and rostro-caudal differences between frontocerebral al and occipitocerebral perfusion were evaluated through the related samples Wilcoxon Signed Rank Test. Differences in age, weight and regional cerebral blood flow versus gender were evaluated with the independent sample Mann-Whitney U test. Differences in standard deviation for the different regions were evaluated with the Mann-Whitney U test. Spearman's correlation coeficients were calculated for ROI size (pixels per ROI) and the measured activity (counts per pixel). Level of significance was set at $p =< 0.05$

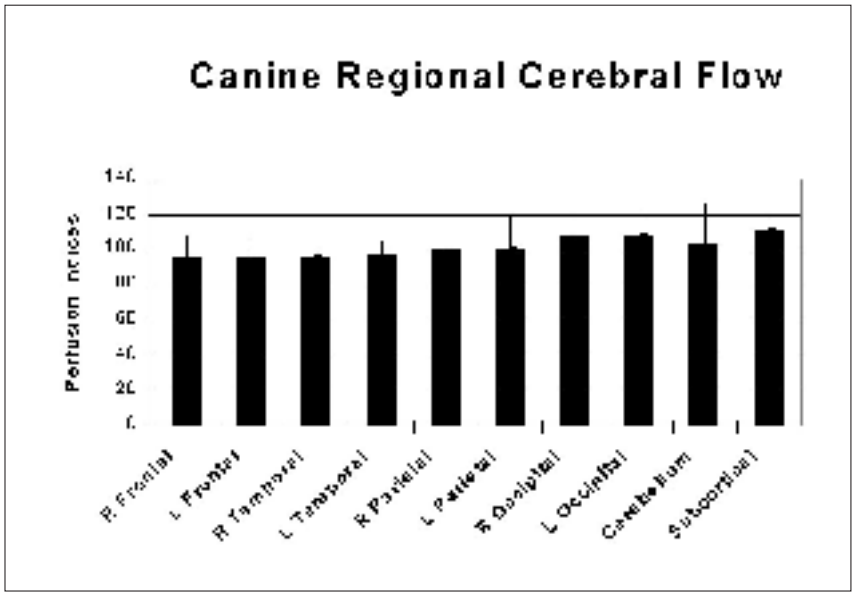

Figure 4: Canine regional cerebral bloodflow, expressed as relative regional ^{99m}Tc-ECD uptake values, calculated as the ratio between regional brain counts to total brain counts expressed as %, are shown in this table.

	RF	LF	RT	LT	RP	LP	RO	LO	Cer	SC
Mean										
PI	0.95	0.95	0.95	0.97	0.99	1.00	1.07	1.07	1.02	1.11
sd	0.09	0.09	0.04	0.07	0.08	0.08	0.06	0.07	0.11	0.09

Table 1: Canine regional perfusion indices (PI): mean and standard deviations (sd).
Legend: RF: right frontal lobe; LF: left frontal lobe; RT: right temporal lobe; LT: left temporal lobe; RP: right parietal lobe; LP: left parietal lobe; RO: right occipital lobe; LO: left occipital lobe; Cer: cerebellum; SC:

Results

Characteristic examples of the brain perfusion pattern and the fitted ROI's are shown in figure 1 and 3. The mean perfusion index for the separate regions is outlined in figure 4 and table 1. The highest perfusion index was found in the thalamic region and the lowest in the frontocerebral cortex. When comparing left versus right regional uptake in fronto-, temporo-, parieto- and occipito- cerebral region, no statistically significant differences were found. A significant perfusion gradient was present between right frontocerebral (rostral) and occipitocerebral (caudal) (+ 13%) perfusion (Wilcoxon's $Z= 2.70$; $p<0.01$) and left frontal and occipital (+ 12%) perfusion ($Z= 2.29$; $p<0.05$).

In this sample, there were no significant relationships between age or weight and any of the regional cerebral uptake measurements. Also, when comparing male and female dogs, no significant differences for gender concerning age, weight and regional cerebral uptake were found.

Discussion

This study is, to our knowledge, the first report of regional cerebral blood flow in dogs using ^{99m}Tc-ECD. In addition to the regional distribution pattern, the normal database is based on structural correlation and provides semiquantitative data. In summary, it was found that regional brain uptake is characterised by a rostro-caudal gradient, with highest uptake of the thalamic/striatal and occipitocerebral regions and absence of demonstrable significant left-right perfusion differences. Age and gender were not found to affect the results.

A significant rostro-caudal gradient was found in this series of adult dogs which is in comparison with the results of SPECT examinations in adult humans.[4,16,17] This pattern was observed both with the eyes open and closed, and is probably related to specific metabolism of 99mTc-ECD in the brain, although the intrinsic reason for such pattern is to date unexplained. In this series the most active areas were the subcortical area and the occipitocerebral region. The standard deviation of the perfusion index for the individual ROI's varied between 0.04 and 0.09 for all regions except for the cerebellum (0.11). (table 1). The higher, although not significant different, standard deviation for the cerebellum may result from variable attenuation through the overlying tympanic bullae and occipital bone for which the uniform attenuation does not account accurately. The higher uptake in the subcortical area is in agreement with the results of rCBF studies with ^{123}I-IMP in beagles[12] where the greatest regional uptake was found in the thalamus. No significant left-right differences were found. In humans, lateralization is mainly found in the male, especially in the frontal area in relation to age.[17]

No gender difference was found in this group of dogs. No other data are available for animals to compare our results with. In human studies, gender dimorphism in brain perfusion or metabolism has been under debate.[17,18] However, recent studies using advanced voxel-based statistical analysis techniques have shown neocortical and cerebellar differences under resting conditions.[17,20] With regard to the influence of age, the statistical power of this feasibility study was limited to demonstrate significant findings. In humans, a gradual decrease in rCBF with increasing age is found with a specific frontotemporal pattern.[17,18,21] Therefore, larger groups may need to be investigated before conclusions can be drawn on aging and gender effects on canine brain perfusion.

Methodological study limitations:

General anaesthesia can influence the rCBF by reduction of blood pressure and heart rate after halothane administration. Inhibition of CBF autoregulation in response to blood pressure changes, is responsible for the vasodilatory effects of halothane and changed blood flow to various organs, with specifically decreased vascular resistance in the brain, resulting in increased CBF.[22] Since all dogs were anaesthetised 10-15 min after the injection of the tracer, which at that stage is already trapped

intracellular, it was not expected that this would influence rCBF. Medetomidine produces no significant effect on brain metabolism but reduces cerebral blood flow in general. This suggests uncoupling of cerebral metabolism and flow due to decreases in central catecholamine turnover.[23] The effects of the sedative given 40 minutes before anaesthesia could theoretically influence global perfusion, but as in this study semiquantification was used with normalisation to total counts of the individual brain, any global effect on brain perfusion would therefore be minimalized.

Since limited anatomic information is obtained with the flow images, these emission images were co-registered on the individual CT images[14]. Stereotactic atlas was used to identify anatomical landmarks to identify the different regions. Although in human functional brain mapping a stereotactic atlas where brain images are resized into a defined stereotactic space is used[24], such a system is not available for dogs. The use of a corresponding three-dimensional coordinate system would allow a more accurate group analysis of specific areas.

No consensus is found on the anatomic localisation of the different canine brain regions. We used a classification[25] which agrees largely with a published anatomic division[26]. The frontal lobe (motor area, behaviour regulation and executive functions) is delineated caudally by the sulcus cruciatus. Posterior to this sulcus is the parietal lobe (sensory cortex). The temporal lobe (memory and auditory function) is situated ventral to the parietal and caudal to the frontal lobe. The occipital lobe (visual reception and interpretation) lies caudal to the parietal lobe and caudodorsal to the temporal lobe.

The anatomic delineation of the different cerebral regions used for SPECT coregistration and analysis might be improved by the higher contrast and resolution power of magnetic resonance imaging[27]. This technique may allow a more detailed study of the canine perfusion, corrected for partial volume effects that originate from the limited SPECT resolution in comparison to the dimensions of the objects under investigations.

PRACTICAL CONSIDERATIONS:

In this study a ^{99m}Tc labelled agent was used as it is generally preferred as a label for nuclear medicine in humans because its physical characteristics for imaging are excellent (140 keV, physical T1/2= 6.02 h) and because it is readily available from ^{99}Mo/^{99m}Tc generators. ^{99m}Tc-ECD has a higher brain-blood partition coefficient as compared to ^{99m}Tc-HMPAO because of its lower rate constant of back-diffusion from brain to blood. It is also superior in its sensitivity to lesion detection and lesion contrast because less protein binding in the blood is noted for ^{99m}Tc-ECD than for ^{99m}Tc-HMPAO.[28,29] ECD was also chosen as radiopharmaceutical in this study because it is more stable on the shelf and therefore more convenient to handle than HMPAO (in vitro stability is 6 hours after reconstitution

compared to 30 min for HMPAO). On the other hand, HMPAO is more stable in the brain and has a slightly higher extraction fraction, and stabilized forms have been commercialized.

In humans, a delay of at least 10 minutes between injection and acquisition is recommended.[4] In our series the time interval was between 15 and 25 min because time, elapsing from inducing anaesthesia and intubation of the animal, varied slightly. Injection of the tracer was performed in two dogs in dynamic projections and adequate uptake was noted in the early stages of the acquisition.

The injected dose also varied slightly between dogs since all dogs were given 925 MBq (25mCi) intravenously and weight variation (23-41 kg) was not taken into consideration. No influence of weight on regional perfusion was noted. In humans the recommended dose varies between 10-25 MBq/ kg. In this study dose injected ranged between 16 and 32MBq/kg, which was higher since the BUI is only 1.2% in the dog compared to 4.8% in humans. For personnel involved in acquiring the SPECT study, a safe distance can be maintained because of general anaesthesia.

In human studies on rCBF either the cerebellum or the whole brain activity are the more common references chosen to obtain region ratios.[18] The cerebellum has advantages of higher sensitivity for detection of abnormalities and relative stability in a number of diseases such as Alzheimer's disease in humans. The lack of exact anatomic delineation and appropriate scatter correction in the dog may increase regional variability and therefore a decrease in sensitivity for detection of pathologic uptake values may result. The uniform attenuation coefficient of 0.12/cm corresponds to an estimation of the theoretical broad-beam attenuation value in the absence of non-uniform attenuation measurements. The influence of surrounding bone structures and air-filled cavities may necessitate an optimization of this value since it has been shown a paradoxical skull effect may lower the effective coefficient to be used in the correction algorithm.[30] This phenomenon may be in part responsible for the high activities found in the central brain areas (subcortical area) as compared to cortical uptake.

Also partial volume effects may be responsible for this radially decreasing uptake, since small region-of-interest size, influx from surrounding peripheral regions and the inclusion of white matter in the cortical regions may artificially heighten the relative central values.

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CHAPTER 2

Use of an automatic registration procedure for standardisation of canine brain perfusion data obtained with single photon emission tomography

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Introduction

Interpretation of brain images obtained with SPET demands reliable and reproducible quantification of the data. This requires an accurate registration method of the individual patient's data to a standardized image, on which the cortical and subcortical regions are delineated. Manual registration is commonly implemented with special software (i.e. the multimodality registration program from Hermes, Nuclear Diagnostics), enabling the alignment of the patient's data manually to a standardized image by reorientating data in three dimensions. Unfortunately this procedure is operator dependent and as such can introduce errors in the final result. In addition, due to size and shape differences between different canine breed and due to positional artefacts, manual registration can introduce variability in the results of semi-quantification of the flow data. Also, manual registration is time-consuming. More, the accuracy of the VOI's placement largely depend on a reproducible and objective registration of the patient's data. Automated registration of the individual patient's perfusion data to a template combined with an automatically applied region map, consisting of semi-quantitative "volume of interest" (VOI) analyses and a comparison of the obtained results to normal values (mean and standard deviation) result in a more reliable analysis of brain perfusion images, as compared to manual registration and visual interpretation.[1]

The aim of the study was to explore the value of automatic registration to a reference template and to include a predefined VOI map on canine brain perfusion data, using the methodology of the Brain Registration and Automated SPECT Semiquantification (BRASS) program from Nuclear Diagnostics.

Materials and methods

A ^{99m}Tc-ECD image template was created including 12 normal dogs (6 males and 6 females), aged between 12-84 months (mean: 49 months, SD: 29). The included individuals were free of neurological disease or behavioural disorders. ^{99m}Tc-ECD (Neurolite®, Dupont Pharmaceuticals Ltd., Brussels, Belgium) was injected intravenously (925 MBq (25mCi)) after sedation and prior to general anesthesia, in a quiet room with dimmed lights. Care was taken not to provoke anxiety. Sedation was obtained with 10-30µg/kg medetomidin hydrochloride IM (Domitor®, Pfizer) and general anesthesia was induced with 2-3mg/kg iso-propylphenol IV (Rapinovet®, Mallinckrodt) and maintained with halothane (Fluothane®, Zeneca) to effect. All dogs were positioned in ventral recumbency with the head placed in a special moulage. SPECT was performed with a triple head gamma camera (Toshiba GCA-9300A, Dutoit Medical, Antwerp, Belgium), equipped with high resolution fanbeam collimators (FWHM 7.8 mm). Acquisition was started 15 to 25 minutes after injection of the tracer depending on the progress of induction of the anaesthesia. Total acquisition time was 20 minutes. For each acquisition, 90 projection images were obtained on a 128x128 matrix using a continuous scan mode by rotating each head 120°. Data were acquired for one main energy window and two scatter windows, with a Toshiba GCA9300 triple headed gamma camera, equipped with low energy, high resolution fan-beam collimators. All data were filtered, scatter corrected, re-binned to parallel data and reconstructed with filtered back-projection (0.16 cycles/pixel; order 8), as described previously.[2] Uniform Sorensen attenuation (0.12/cm) was applied. Pixel size and slice thickness were 1.72 mm.

For the first part of the study, each reconstructed image was manually adjusted by two experienced persons to one arbitrary chosen image, which was reoriented to a standardized position along the X,Y and Z axis. (Fig 1A and B) and centred in the matrix. To register the individual image to this “reference” image the multi modality software allowing for manual scaling, rotation and shifting and thus adjusting 9 transformation parameters (X scale, Y scale, Z scale, X shift, Y shift, Z shift, XY tilt, XZ tilt and YZ tilt) was used. This procedure was repeated twice to refine registration.

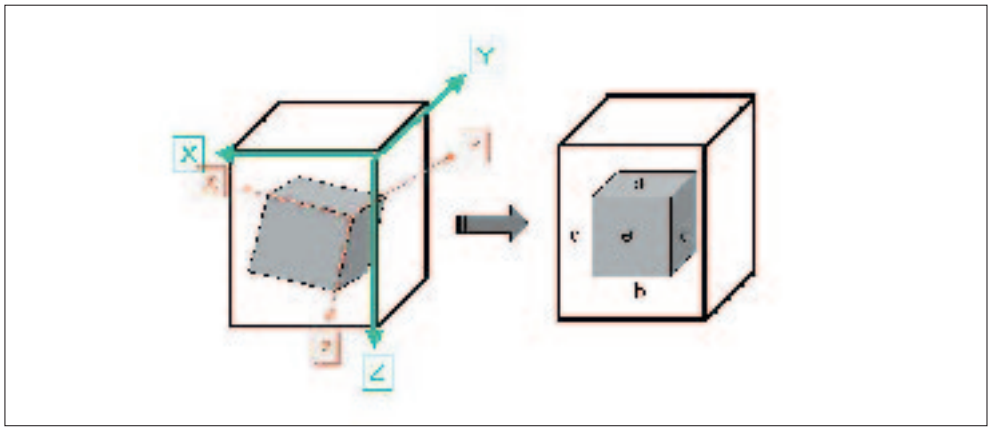

Fig 1: Spatial reorientation of the image data is performed by adjusting X, Y, Z parameters by shifting and rotating the data. Top (a), base (b), right side (c), left side (d), rostral side (e) of the brain are readjusted to obtain a standard image.

A standardized "volumes of interest map" (VOI) was applied to each image, supplying values for regional activity. (Fig 2). This VOI approach is used to obtain quantitative information on regional radioactivity values. The standardized VOI map consisted of 10 cortical and 1 subcortical regions with inclusion of all grey matter. The division of the anatomical brain regions was chosen according to the proposal of Redding.[3,2] Average counts per pixel were obtained for all regions and regional average counts were normalised to average counts per pixel from all predefined regions, expressed as percentage, representing the perfusion index.

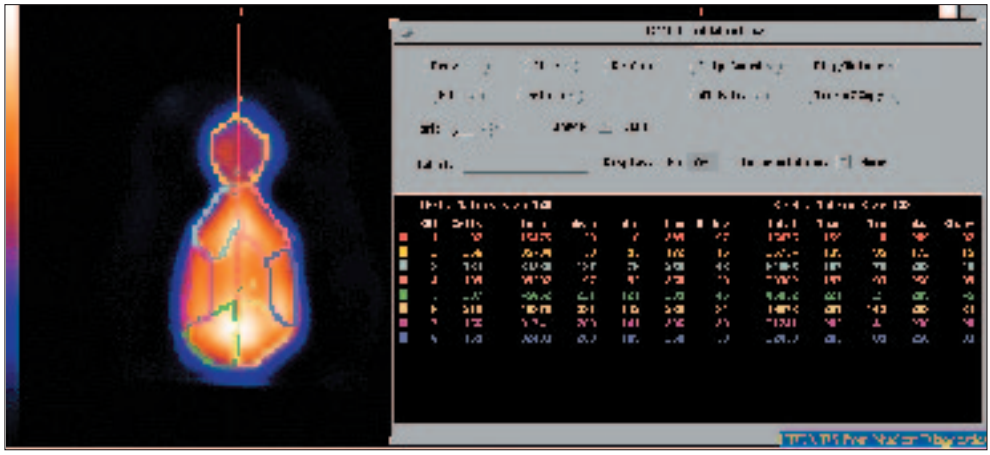

Fig 2: Manually drawn regions delineate the different brain areas (left image). Total counts per region and average counts per pixel are automatically registered and shown in the ROI tool window.

For the second part of the study a mean image was created by sequentially adding all centred images voxel by voxel to each other. (Fig 3). This composite template represents an average, normalized distribution of counts in the brain for the three planes, creating a three dimensional mean image. More, a coefficient of variance template is generated at the same time, representing the standard deviation divided by the mean count distribution. (Fig 4). All individual images were then re-registered on this mean image with an automatic registering algorithm, allowing shifting, scaling and rotation in all three directions as described in the first part. The automatic registration procedure is based on the principal axes transformation, resulting in an approximate fit, and on the downhill simplex minimization algorithm, using the count difference method, to refine the fit.[4] (Fig 5). A regionmap, defined on the template image and using the same anatomically predefined regions (10 cortical and 1 subcortical region) as used in the previous part, was automatically applied on the individual image data, generating the perfusion index for all regions. (Fig 6).

Standard deviations of normalized average radioactivity in the different brain regions were obtained for both groups.

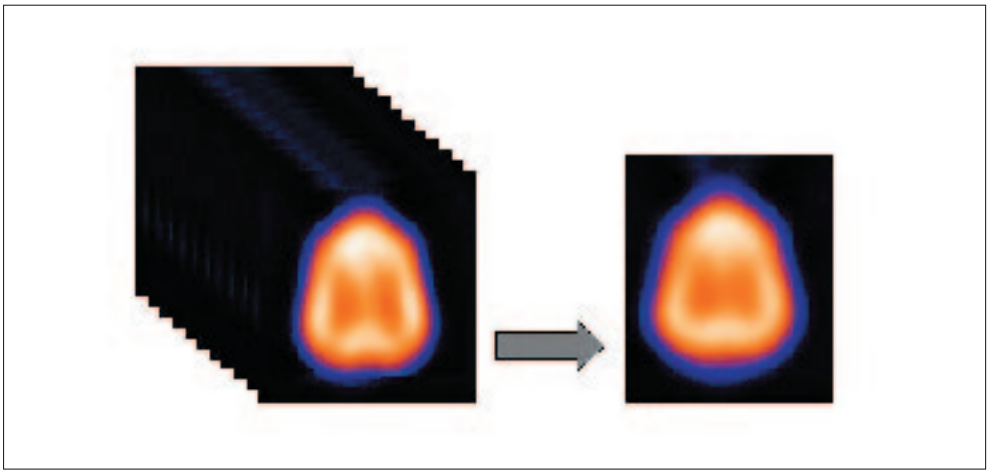

Fig 3: All individual image data for all slices are added and averaged (left image), creating a mean image (template) (right image).

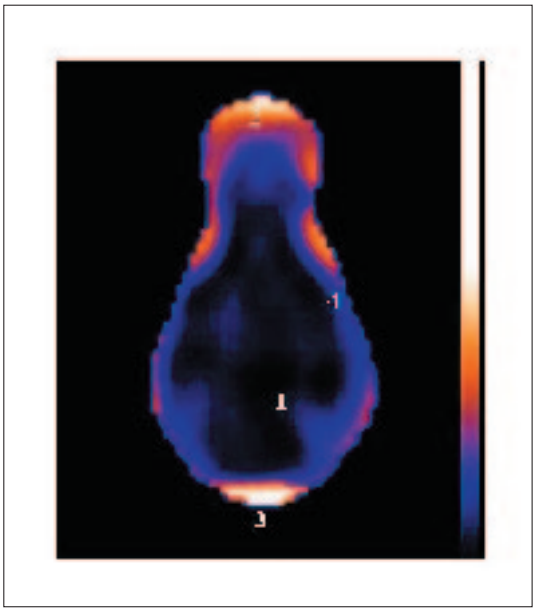

Fig 4: Template of variance. Highest variance is noted near the fissure between the temporal region and the cerebellum (1), in the olfactory bulb region (2), at the posterior edge of the cerebellar region (3) and in the superficial cortical regions (4).

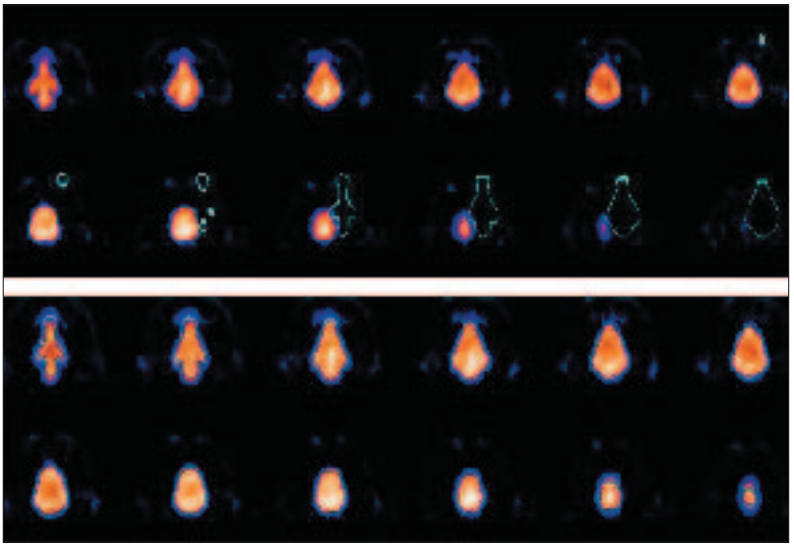

Fig 5: The top row of images represent the data before automatic alignment with BRASS software. The faint blue line demarcates the spatial orientation of the template slices. The bottom row shows the images aligned to the template data (blue line).

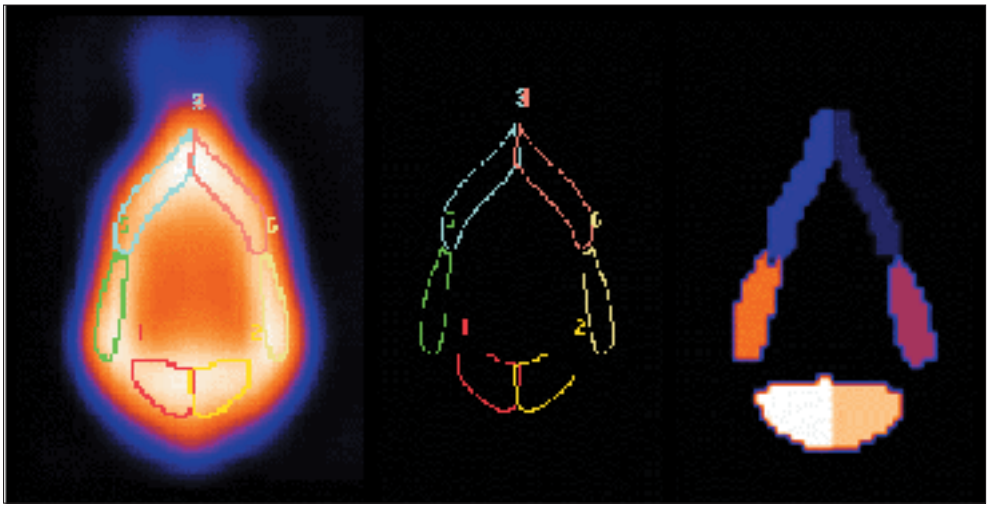

Fig 6: The left image demonstrates the manually drawn ROI's. A region specific value (1-11), linked to a particular colour pattern, is attributed to all predefined brain regions. The computer program will automatically apply this regionmap to newly acquired image data.

Results

No visual discrepancies were noted after the manual and the automatical fitting procedure, although the standard deviation of perfusion index for the different cerebral regions was highest after manual fit compared to the automatic registration. (Fig 7). Loss of resolution of the template image due to amalgamating patient data is minimal and barely noted visually.

The average time spent by each operator to align the 12 image sets to the arbitrary choosen image was 10 minutes per set, compared to a mean of 6 minutes for the automatic registration procedure.

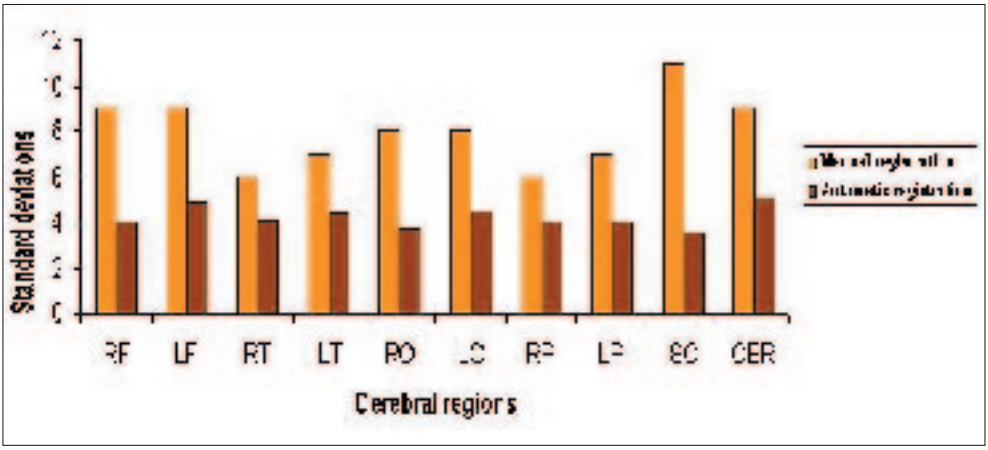

Fig 7: Standard deviations of regional perfusion data are given for the different brain regions. Highest values are found for the manual fit.
Legend: RF: right frontocortical region; LF: left frontocortical region; RT: right temporocortical region; LT: left temporocortical region; RO: right occipitocortical region; LO: left occipitocortical region; RP: right parietocortical region; LP: left parietocortical region; SC: subcortical area; Cer: cerebellar area.

Conclusion

The template based automatic fit, using BRASS software from Nuclear Diagnostics, can be used for registration of canine brain flow data and has the advantage that semi-quantification can be performed more accurate and less subjectively. The higher standard deviations found with manual fitting results from the fact that parameters for shifting, scaling and rotation are sequentially changed. Since these parameters are dependent on each other, changing one influences the position of the others and hampers optimal alignment.

On visual inspection virtually no difference was noted in positioning of the reoriented images between both methods. Quality of the template appeared similar to the individual images although on the template image background noise was reduced and the edges were slightly blurred and smoothed. Overall, resolution was comparable with the original individual images.

The template of the coefficient of variance showed high values in the superficial cortical layers and in the caudal border of the cerebellum, probably due to the variable negative skull effect which is prominent in these regions. Difference in skull thickness could explain this phenomena. These findings are similar to the regional variances found in a human study, using the same methodology.[1] Registered regional activity in the olfactory bulb is prone to variety, presumably depending on the activation state of this very important sensory region, which is beyond control.

This template based automated registration method provides a mean to compensate for size and shape differences between different breeds, a major concern in canine brain imaging and enables to adjust positional differences.

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CHAPTER 3

Evaluation of cerebral neurotransmitter physiology and pathophysiology with PET and SPET imaging modalities in animal models: a review

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Summary

Since positron emission tomography (PET) and single photon emission tomography (SPET) have become widely available as imaging modalities, studies have been performed on neuroreceptor physiology and pathophysiology in humans and animals. Several neurological and psychiatric diseases related to neurotransmitters and receptor functioning have been investigated.

In order to investigate the biological effects of new compounds, this imaging modality is becoming more and more popular in the pharmaceutical industry, since pharmacokinetics and pharmacodynamics of new drugs can now be evaluated *in vivo*, omitting extensive *in vitro* examinations. The response to pharmaceuticals can be evaluated more objectively as compared to the potentially subjective evaluation of the condition of the patient, based on clinical examination and interpretation of neuropsychological tests.

This review focuses on the technique of this imaging modality and the nature of radioligands used for neuroreceptor imaging. A brief overview of the research on neuroreceptor physiology, neuropathology, drug evaluation and substance abuse, with the emphasis on animal models will be given. Future prospects for implementation in veterinary medicine are discussed.

Introduction

Already in the 19th century, Langley formulated the concept of "receptive substances", involving the interaction of drugs with tissue.[1] Later, the Nobel prize winner Ehrlich proposed that interaction with toxic substances was caused by the presence of certain cell surface groups (receptors) showing binding properties with these toxins (ligands). This binding was due to high complementarity between the stereochemical configuration of the ligand and the receptor with which it interacts.[2] With the formulation of the receptor concept, an explanation was found for the specific effects of tracer amounts of substrate on a target organ.

Classification of receptors is based on their chemical specificity (e.g. the α- and β- adrenergic receptors) or on their anatomical localisation (e.g. pre- and postsynaptic receptors, intra-and extracellular receptors)[3], while ligands are classified according to their ability to generate a response. Full agonists will generate a maximal receptor response while partial agonists will never produce a maximal activation of the receptor no matter how high a concentration is applied. Whether a ligand is a full or partial agonist is a tissue- and drug- dependent phenomenon, although endogenous agonists for a receptor type usually behave as full agonists. Antagonists block the response in a reversible, irreversible, competitive, non-competitive or mixed way. Distribution and kinetics of the ligand (drug or endogenous substrate) can be quantified with *in vitro*, *ex vivo* (injection of the ligand *in vivo*, measuring tissue activity after death) and *in vivo* imaging techniques using radioactive labelled molecules.[4] *In vitro* techniques have been used to evaluate ligand-receptor responses and to quantify receptors. In recent years, functional imaging techniques with positron emission tomography (PET) and single photon emission tomography (SPET) are gradually gaining importance as ligand-receptor interactions which can be evaluated in their natural environment in the living subject.[4,5]

This technology is now more commonly applied in drug development. Functional imaging is also used as a research and diagnostic tool for studying neurologic and neuropsychiatric diseases in humans, providing insight into complex receptor interactions. In this context, animal models were introduced to investigate neuropathologic disorders, usually created by genetic, surgical or pharmacological manipulation of the animal. In most cases rodents (*ex vivo* and *in vitro*) and non-human primates are used, although it has been suggested that dogs would provide a valid alternative model because they are widely available, rather cheap and easy to keep and handle, compared to non-human primates. This has lead to the validation of different models of natural canine neuropathologic entities for human disease.[6,7] But, also from a veterinary point of view, this technique may facilitate research on the mechanisms of neurologic and behavioral disorders in dogs and may monitor the effects of therapy.

The aim of this article is to give a review of receptor imaging techniques and their implementation, with emphasis on the *in vivo* imaging of neuroreceptors with PET and SPET modalities. Special attention will be given to neuroreceptor research in which animal models are used to illustrate the clinical potential of this modality for the investigation of causes and treatment of behavioural diseases in animals.

Methodological considerations

IMAGING METHODS:

1. Receptor imaging *in vitro*

Neurotransmitter receptors in frozen tissue sections can be visualized and quantified using autoradiography. In order to achieve high spatial resolution, radionuclides with low β-emission energies, such as ^{3}H, ^{125}I and ^{14}C, are used. These radionuclides are bound to highly specific probes, hereby providing a radiolabelled ligand (e.g. ^{3}H-spiperone). These examinations can be performed *in vitro* or *ex vivo*. Specific binding, which is binding of the radiolabeled ligand with the receptor, and non-specific binding, being the activity persisting in the presence of an excess of a specific non radioactive displacer, can be quantified, providing an estimation of receptor density (B_{max}) and affinity ($1/K_d$) (K_d= dissociation constant at equilibrum).[5,8] Another method to evaluate the effects of receptor binding is the measurement of the cellular responses elicited by the ligand-receptor interaction. Changes in cyclic adenosine monophosphate (cAMP) concentration, glucose production or electrical and mechanical responses enable biochemical characterisation of the receptor.[4,9]

Immunohistochemistry offers an alternative method to visualize receptors in vitro . However, this technique does not fall under the scope of this article and will therefore not be discussed.

Since the natural environment of pre-and postsynaptic membranes is not accounted for *in vitro*, assumptions on drug interaction or ligand-receptor behavior from *in vitro* experiments should be regarded with caution when adopted to receptor estimation *in vivo*. Ex vivo techniques require the death of the animal under investigation, since the β-particles, emitted during ^{3}H,^{14}C or ^{125}I decay, are strongly attenuated by tissue and therefore cannot be measured with external detecting devices.

2. Receptor imaging *in vivo*

In vivo techniques offer a more realistic representation of receptor behavior, because receptor characteristics can be altered due to interaction with its intra-and extracellular regulatory mechanisms.[10] The functional imaging modalities, PET and SPET enable external imaging of the receptor with ligands labelled with γ and β^{+} emittors, thus permitting evaluation of biological processes *in vivo* in a safe and non-invasive way. Due to the high affinity and specificity of the radioligand for the receptor and because of the sensitivity of the imaging system, only tracer amounts, usually in the picomolar range, of this radiolabeled ligand are applied, thus avoiding physiologic or toxic effects.[4] *In vivo* sequential studies are possible and time/activity curves in region of interests can be generated.

Unfortunately, some of these interactions can give misleading receptor occupancy estimation. As an example, cellular response to agonistic stimulation may desensitise or even internalize the G-protein coupled receptor, a mechanism by which the receptor is translocated intracellular. This may result in variability in visualization of the receptor.[11] In addition, processes such as perfusion and, as a consequence, delivery of the radioligand may influence receptor binding.[12]

PET IMAGING

In PET studies, positron emitting radionuclides are used. Following emission, these positrons travel a few millimetres in matter before they interact with an electron and annihilation energy is produced in the form of two opposite photons with an energy of 511 keV each. Both photons are detected by external detectors. The most commonly used radionuclides are ^{15}O ($T_{1/2}$=2 min), ^{13}N ($T_{1/2}$=10 min), ^{11}C ($T_{1/2}$=20 min), ^{18}F ($T_{1/2}$=110 min) and ^{76}Br ($T_{1/2}$=16 hours). The short half-lives of ^{11}C and ^{18}F enable repetitive investigations within a short time interval in the same individual, using the same or different radioligands, in identical or different test conditions.[5] The advantage of ^{18}F is that the emitted positrons travel a very short distance before annihilation occurs, due to their lower kinetic energy, hereby providing more exact localization of the emitted positrons.[5]

With PET, absolute quantification is possible and high spatial resolution (3-4 mm) can be achieved. Disadvantage is that the radionuclide must be generated by a cyclotron which must be in the immediate vicinity of the camera due to the short half life of most of these elements.[5,9]

SPECT IMAGING

SPECT is based on the emission of photons, registered by external detectors, so-called gamma-cameras. Mostly Technetium (^{99m}Tc, $T_{1/2}$=6h) or iodine (^{123}I, $T_{1/2}$=13h) are used. The longer half life of these radionuclides precludes repetitive investigations on the same individual on the same day but can be an advantage for studies on receptor occupancy requiring prolonged scanning times.[8] Spatial resolution depends on the collimator and camera configuration, but is in general lower than PET (7-10 mm vs 3-4 mm). Due to inherent physical problems such as attenuation, scatter and partial volume effects, photon registration is not with certainty coming from the region of interest, therefore specific binding is measured semi-quantitative.

LIGAND PROPERTIES

In order to determine the receptor kinetics *in vivo*, the characteristics of the ligand are of crucial importance.

1. Labelling of the ligand

The labelling of the ligand is not a simple procedure and it can alter its biological activity, rendering it unsuitable for binding with the receptor. It can increase the lipophilicity of the ligand and can enhance non-specific binding with plasma proteins and blood cells.[10] Labelling with ^{11}C usually does not cause alteration of the physicochemical properties of the compound, but labelling with ^{18}F or ^{76}Br may alter its properties. Labelling with either ^{99m}Tc or ^{123}I usually has an important impact on the physicochemical properties of the ligand.[5,10] Another important factor in the labelling procedure is the duration of radiosynthesis, since the half-life of the radionuclide has to be considered. Therefore, with regard to PET tracers, this procedure should be kept as short as possible and the label should be introduced as late in the synthetic procedure as possible.[5] Specific radioactivity (MBq/mole) of the synthetised radioligand has to be high, since low specific radioactivity results in need for higher ligand concentrations, resulting in increasing risk for biological effects and in substantial occupation of binding sites by unlabeled ligand.[5]

Exogenous antagonists are preferred because dilution with endogenous agonists is less likely to happen, affinity for the receptor is usually higher and receptor responses interfering with the binding estimation are less likely to occur.[9,10]

2. Selectivity and affinity:

Affinity is the chemical strength of the binding, selectivity refers to the strength of ligand interaction with one receptor compared to interactions with other receptors (the ability to recognize receptor molecules). Biological substrates frequently lack selectivity and high affinity. Since affinity is formulated as the ratio of association and dissociation, high affinity would be a negative quality for endogenous neurotransmitters since the receptors need the ability to react fast to stimuli. The opposite is true for receptor probes used in research which must have high affinity and selectivity to make visualization possible.[13] Selectivity and affinity are closely related since high affinity for a receptor results in high selectivity. Lack of selectivity may only be acceptable when the targeted receptors are clearly separated anatomically from the other imaged receptors or receptor subtypes.[5,8,9]

3. Biodistribution and metabolism:

During its way to the brain the radioligand might be trapped or bound to cells, plasma proteins and other molecules. Lipophilicity, measured as log P, the value of the octanol-water partition coefficient expressed in log units, is an essential property of the radiopharmaceutical in order to pass the blood brain barrier but increases aspecific binding at the same time. Depending on the methods used for measuring log P, values should be between 1 and 3.5 for neuroimaging tracers to be useful.[14] Furthermore, tracer metabolism in vivo may result in both labelled and non-labelled metabolites. In addition, labelled metabolites might bind to receptors or might be trapped in cells. Since PET and SPET imaging detects only radioactivity it is not possible to distinguish between the signal produced by the labelled parent molecule and the labelled metabolite. Therefore, ideally, metabolism should be away from the investigated organ and metabolites should be non labelled. In case metabolites are labelled, they should not cross the blood brain barrier or, if they do, should not be retained in the target area. The degree of activity due to labelled metabolites can be estimated and corrective binding curves generated, by measuring the metabolites in plasma during the study.[5,8-10,12,15-35]

4. Quantification of receptor binding:

PET and SPECT data can be analysed at three levels of sophistication. The simplest way is visual inspection, determining areas of increased or decreased binding. This method is of limited value since it is subjective and biased by the skills of the interpreter.

The next level of analyzing data is the semiquantitative or “reference region” approach on condition that binding with the receptor is reversible and that a steady state between uptake and washout of the radioligand after a single bolus injection, can be achieved. The ratio of binding in regions with high density of receptors, and thus high specific binding, to that in low density areas, representing the reference region, is calculated. This a priori knowledge of regional receptor density and distribution, can be derived from autoradiographic or immunohistochemic studies.[4,9] The main advantage of the ratio method is that it provides a simple and quick semi-quantification for clinical use, not requiring blood samples and not dependent on models and rate constants.[36] However, the technique is unreliable when a substantial amount of metabolites of the radioligand, with affinity for the receptor, are formed that pass the blood brain barrier. Another possible confounder for this approach is that a differential rate of wash out between receptor-rich areas and areas with non-specific binding may be present or that there are heterogeneous non specific binding sites.[8,36] In humans, but especially in veterinary nuclear medicine, bloodflow changes, induced by exogenous administered compounds, such as anaesthetics or sedatives, resulting in modulation of the capillary surface, can provoke changes in the equilibral state.[37]

Mathematical methods have been developed to quantify dynamic data in order to calculate K_d (equilibrium dissociation constant) and B_{max} (maximal specific binding which is proportional to receptor density) more accurately.[9] The compartmental model is based on different compartments in which the tracer can pass or accumulate. Mostly, a three compartmental model that localizes the tracer in different localizations, namely plasma (the input function), free or non specifically bound, and specifically bound is used.[9] Rate constants are introduced to describe the fractional transfer of ligand along these compartments.[8,9] Multiple-time graphical analysis (MTGA) is an alternative technique used to quantify data over time as the radioactive tracer accumulates in tissue. This method is model independent and as a consequence facilitates the processing of the data. Two different methods are developed: the Patlak plot is used for a tracer that will be trapped for the length of the experiment in one compartiment and that is considered essentially irreversibly bound[38] and the Logan plot which is applicable for reversible tracers[39].

5. Testing new ligands

Preclinical testing of new ligands is usually performed both *in vitro* and *in vivo* studies. Autoradiographic studies can provide information concerning affinity, selectivity and non-specific binding but no data can be obtained concerning the biodistribution unless ex vivo or in vivo experiments are conducted.

The advantage of SPET and PET is that the distribution of the radiotracer and its selectivity and reversibility in relation to the receptor can be determined in the living subject in the so-called biodistribution studies. Estimation of the specific binding can be evaluated with displacement ("chaser" studies) or pre-treatment ("blocking" studies) with cold competitor reducing the specific binding in tissue and leaving the non-specific bound fraction.[5,8-10,17-20,22,24-29,31,34,35,40-73] Also, the optimal scanning time, the time interval where a pseudo-equilibral state is achieved, can be determined, which is essential when semi-quantification of receptor binding will be performed.

One of the great advantages of using animal models lies in the possibility of repeated intra-subject scanning in order to compare tracers and to monitor therapeutic interventions.

Practical applications

1. Confounding factors

Pathological changes in receptor occupancy may be due to altered synthesis or release of neurotransmitters, or may be a consequence of altered blood flow (e.g. ischemia) or degenerative processes.[74] However, in order to evaluate pathology, one must also take physiological alterations in receptor behavior into account.

• Age and gender:

Functional brain imaging studies in humans, in non-human primates and in rats demonstrated that the binding of several neurotransmitter systems is influenced by age and gender and that modulation of receptor binding parameters can occur due to hormonal influences such as during the normal menstrual cycle and menopauze.[9,75,76,77-78,79-90,91,92]

An age related decline of the cerebral 5-HT2A receptor has been demonstrated with ^{123}I-5-I R91150 in dogs[93], using SPET, and with ((R)-(+)-[3-OCH3-^{11}C]-MDL 100907 in primates, using PET.[82] Regional decreases of the 5-HT1A receptor were observed in primates using the PET tracer, carbon-11 labelled N-(2-(4-(2-methoxy-phenyl)-1-piperazin-1-yl)ethyl)-N-(2-pyridyl)cyclohexane-carboxamide (WAY-100635). Decreases of the serotonin transporter (SERT) were registered in aged primates with PET using the difference in radioactivity between the active compound [^{11}C]trans-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio)phenyl-pyrrolo-[2,1-a]isoquinoline ([^{11}C]+McN5652) and the inactive enantiomer [^{11}C]-McN5652 during sequential scanning as a measure for specific binding.[94] Age related decrease of the dopamine-1 (D1) and D2 receptor binding, dopamine transporter (DAT), phosphodiesterase-IV activity and muscarine cholinergic receptors were demonstrated with respectively, carbon-11 labeled ((R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol) (SCH23390), ^{11}C-raclopride, [^{11}C](2beta-carbomethoxy-3beta-(4-fluorophenyl)-tropane) (beta-CFT), [^{11}C] rolipram and (+)N-[^{11}C]-methyl piperidyl benzilate ((+)3-MPB and 4-MPB) PET tracers in primates.[75,86,95-97] In one study, binding alterations of the vesicular acetylcholine transporter in aging rhesus monkeys, imaged with the PET tracer [^{18}F] (+)-4-fluorobezyltrozamicol ([^{18}F] FBT), showed individual differences, suggesting that the cholinergic system may undergo a differential susceptibility to the aging process.[98]

• Influence of anaesthetic drugs and cerebral perfusion:

Sedation and anaesthesia are prerequisites for animal research on receptor imaging. The effects of several anaesthetics are twofold. They can act on the receptor directly or indirectly through other receptor systems, such as the anaesthetic ketamine, an antagonist of the N-methyl-D-aspartate (NMDA) receptor, which interferes also indirectly with the dopaminergic and serotonergic system.[99-103] They can also produce cerebral metabolic effects, blood pressure and perfusion changes.[104-107] In one

study different kinetics of the radiotracer [^{11}C]-methamphetamine (MAP) were observed comparing halothane and pentobarbital anaesthesia in rhesus monkeys.[108] In another monkey study, isoflurane anaesthesia enhanced the direct and indirect effects of the DAT inhibitors cocaine and GBR12909.[109] On the other hand, low variability in radioligand binding with the D2 receptor was registered in a study with primates, using the PET tracer [^{18}F]-fluoroclebopride (FCP) between induction with ketamine or with fluothane by mask induction.[110] Nevertheless, it remains important to select the anaesthetic combination that provokes lowest disturbances in the equilibrum reached between the receptor and its radioligand and to stick to one anaesthetic protocol for this type of research.

• Influence of cellular responses and manipulation of other neurotransmitter systems

As mentioned previously, under the influence of agonists and in some cases also of antagonists, receptor availability on the cellular membrane might change because of desensitisation and internalisation mechanisms.[11,111-113] It is hypothesized for the challenged (administration of an exogenous chemical compound will provoke the release of endogenous neurotransmitter) dopaminergic system, that internalisation might influence the binding parameters of the radioligands, depending on their biochemical properties. The intracellular shift might result in lipophilic radioligands entering the cell and low lipophilic not, resulting in a different binding profile. Also, the intracellular environment (e.g. sodium concentration) might inhibit binding to the receptor for some and not for other radioligands.[11] Furthermore, endocytosis of receptors after internalization may result in "down-regulation" of receptors by lysosomal degradation.[111]

Another important topic in research is the interaction of the different neurotransmitter systems hereby using modulating pharmacological probes. These experiments contribute to a large extent to the understanding of neuropsychiatric diseases and towards elucidating the efficacy of medication used for these disorders. To enable visualization of endogenous fluctuations, the employed radioligands should be sensitive to changes in neurotransmitter concentrations.[114-117] The interaction of the dopaminergic D2 system and the muscarinic cholinergic system was evaluated by sequential pretreatment with anticholinergic drugs and dopaminergic antagonists, demonstrating respectively reduced D2 receptor ([^{18}F]N-methylspiroperidol, NMSP) and muscarine receptor radioligand ([N-^{11}C-methyl]benztropine) binding.[118] Administration of scopolamine (a muscarine cholinergic antagonist) resulted in increased dopamine synthesis, measured with [^{11}C]-1-3,4-dihydrophenylalanine (DOPA), an increased binding of the DAT radioligand 2beta-[^{11}C]carbomethoxy-3beta-(4-fluorophenyl)tropane (CFT) and decreased binding of the D2 ligand [^{11}C]-raclopride. The extracellular level of dopamine, measured with microdialysis, remained static despite the increased synthesis, due to enhanced DAT availability but the affinity of the D2 receptor decreased.[119] The dopaminergic regulation of the striatal cholinergic activity was measured using the nicotinic cholinergic receptor PET tracer norchloro [^{18}F]-fluoroepibatidine (NFEP). It was demonstrated that pretreatment of the D2 receptor with agonists and antagonists could respectively increase and decrease the binding of the cholinergic receptor radioligand.[114] The interaction of the serotonergic and dopaminergic system was examined with a

serotonergic antagonist (altanserin) and the selective serotonin reuptake inhibitor (SSRI) citalopram. Blocking the 5-HT2 receptor resulted in increased binding of the D2 receptor radioligand ^{11}C-raclopride in the extrastriatal region and in decreases in the striatal area, while, when enhancing the serotonergic concentration in the synaptic cleft with SSRI's, opposite effects were noted.[120] In another study, the effects of pharmacologic manipulation of gamma-aminobutyric acid (GABA) and serotonergic receptors on the central cholinergic system were marked.[121]

2. Clinical applications

• Drug industry:

These imaging modalities have become a valuable tool in the development of new drugs. Degree and duration of receptor occupancy and the effect of different drug dose regimes can be evaluated. The advantage of functional imaging is that the doses of the labeled compounds are so low that pharmacologic and toxicologic effects mean no risk to the individual. Therefore, information on distribution and receptor binding can be obtained very early in the development of the drug.

Measurements of the labelled compound in plasma may provide information on metabolism.[122] Two main strategies are followed for evaluation of pharmacologic receptor occupancy. Firstly, direct studies in which the drug itself is labelled and secondly, *indirect studies*, in which the direct or indirect effects of the drug on the receptor are investigated by the use of receptor specific radioligands.[4,120-150]

Receptor occupancy studies can be used also in the determination of the dose at which drugs are therapeutically effective and have the slightest side effects.[151,152] Using the radioligand ^{11}C raclopride, selective for D2 dopamine receptors, it was demonstrated that above 70% of receptor binding corresponded to the "anti-psychotic" effect while above 80% occupancy of the receptors resulted in catalepsy-extrapyramidal symptoms in animals.[153,154] D2 receptor occupancy was evaluated for different doses of 3 neuroleptic drugs (clozapine, risperidone and haloperidol) with the dopamine D2 selective ligand ^{18}F fallypride. Displacement of the ligand was demonstrated in rodent and primate brain with decreasing potency observed for haloperidol > risperidone > clozapine and with D2 receptor occupancy in the striatal as well as the extrastriatal region at therapeutic doses.[150]

• The influence of toxic substances on brain receptors

Insight in the pathophysiological changes, caused by neurotoxic substances at receptor level, can be provided by functional imaging studies. This has been proved useful in the evaluation of toxic effects of frequently abused drugs such as ketamine, amphetamines, cocaine and ecstasy.

It was demonstrated with PET that ketamine had an impact on both the dopamine receptor, imaged with ^{11}C-raclopride, and the DAT, with ^{11}C labelled cocaine analogs beta-CIT-FE (N-(2-fluoroethyl)-2beta-carbomethoxy 3beta-(4-iodophenyl)tropane) and beta-CFT, in monkeys.[99,103] In primates acute administration of amphetamine lead to a decrease in striatal D2 receptors, imaged with ^{18}F-fallipride and of extrastriatal D2 receptors, measured with ^{11}C-FLB457.[117,155] A prolonged reduction in binding of raioiodinated benzamide SPET tracers, (S)(-)-N-[(1-ethyl-2-pyrrolidinyl)methyl] 2-hydroxy-3-[I-123]iodo-6-methoxy-benzamide (iodobenzamide, IBZM) and (S)-5-iodo-7-((1-ethyl-2-pyrrolidinyl)methyl) carboxamido-3-dihydrobenzofuran (iodobenzofuran, IBF) SPET tracers was found after amphetamine injection, with an excellent correlation between reduction of D2 receptor binding measured with SPET and peak dopamine release measured with microdialysis after various doses.[156] Chronic administration (10-14 days) showed long-term decreased striatal dopamine function with the PET tracer 6-[^{18}F]fluoro-L-DOPA and decreased binding of 11C-raclopride, suggesting down-regulation of the D2 receptor.[57,157,158] In another primate study the difference in wash-out rate of the D2 radioligand [^{18}F]FCP under influence of various psychostimulating drugs was compared and more pronounced effects were detected after methamphetamine and amphetamine relative to cocaine and methylphenidate, a result that is consistent with the ability of each drug to elevate synaptic dopamine levels.[159] A dog model demonstrated the effect of chronic metamphetamine intake on the cerebral dopamine transporters showing long-term nigrostriatal dopaminergic deficits.[160] A decrease in DAT was demonstrated in primates with the PET tracer [^{11}C]2 beta-carbomethoxy-3 beta-(4-fluorophenyl)-tropane (WIN35428).[161,162]

In animal and human PET studies, using the serotonin transporter ligand ((+)-[^{11}C]-McN5652), repeated administration of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) was shown to induce neurotoxic lesions to serotonergic neurons.[163-165]

Research has not been limited to illegal drugs but has also provided interesting information concerning receptor behavior under influence of substances such as nicotine, alcohol and benzodiazepines. The effect of chronic nicotine administration, in differing doses, on neuronal acetylcholine (AcCh) receptors was evaluated *in vivo* with SPECT, with the ^{123}I-A85380 ligand in baboons, showing, at a dose equivalent to 20 cigarettes per day, an upregulation of this receptor.[166] The effect of nicotine on the dopaminergic system was shown to be insufficient to displace [^{11}C]raclopride in monkey brain.[167]

Animal experiments showed an *in vivo* association between alcohol intoxication, aggression and serotonin transporter availability, measured with the SPET tracer [^{123}I]methyl3 beta-(4-iodophenyl)tropane-2-carboxylate (beta-CIT) in nonhuman primates.[168]

• Characterisation neuropsychiatric disorders:

Dysregulation of one or more neurotransmitter systems is implicated in the pathophysiology of many neuropsychiatric disorders, such as Parkinson's and Huntington's disease, schizophrenia and mood disorders.[4,9]

Functional brain imaging with ligands can offer important information on the biochemistry of neuropsychiatric diseases. Since animal models are used more frequently in research on human neuropsychiatric disorders, functional imaging of the animal brain becomes increasingly important. Also, recently, the advantage of using a natural occurring (in absence of genetic, surgical or pharmacological manipulation) canine model for certain psychiatric disease entities such as obsessive compulsive disease (OCD), cognitive dysfunction, anxiety and impulse control disorders, has been emphasized, because of important similarities in pathophysiology.[6]

Ischemia

Brain ischemia, resulting from thrombotic or embolitic processes constitutes an increasing problem in the elderly population. An animal model, using experimentally induced hypoxic ischemic insults by unilateral arteria cerebri media occlusion in rats, showed lesions with change in cerebral blood flow and an accompanying significant reduction in binding potential of ^{123}I-iomazenil, a central benzodiazepine receptor antagonist. The iomazenil distribution correlated well with neuronal distribution which finding could have both diagnostic and therapeutic implications in human neurology.[169] A similar study in baboons, using the 11C-flumazenil tracer, reported a marked, early and sustained decrease of binding reflecting synaptic damage.[170] Changes in striatal D2 receptor binding assessed with SPECT ligands, could be used to evaluate hypoxic-ischemic brain damage that occurs in the absence of anatomical lesions.[171]

Degenerative dementia

The most common form of dementia in humans is Alzheimer's disease (AD). This is a neurodegenerative disease with a reduction in brain acetylcholine content in predelicted brain regions.

The iodine-^{123} labeled E-(R,R)-1-azabicyclo(2.2.2)oct-3-yl-alpha-hydroxy-alpha-(1-iodo-1-propen-3-yl)-alha-phenylacetate (E-IQNP, M1 receptor) and Z-(R,R)-1-azabicyclo(2.2.2)oct-3-yl-alpha-hydroxy-alpha-(1-iodo-1-propen-3-yl)-alha-phenylacetate (Z-IQNP, M2 receptor) tracers designed for SPECT imaging of muscarinic acetylcholine receptors, first tested in monkeys and later in humans, may be promising as a diagnostic tool in Alzheimer's disease.[172] Studies with the cholinergic PET ligand ^{11}C (+)3-MPB in monkeys, demonstrated an age related reduction of cholinergic receptor density in frontal and temporal cortices, the regions affected initially in Alzheimer's disease patients.[86] Moreover, current pharmacotherapeutic strategies with choline-esterase inhibitors in the treatment of AD can be evaluated through serial scanning before and during treatment.

Parkinson's disease and Parkinsonism

Parkinson's disease (PD) is an extrapyramidal movement disorder which is associated with degeneration of dopamine containing neurones in the nigrostriatal pathway. A parkinsonian animal model for the disease is created by the injection of 1-methyl-4-1,2,3,6-tetrahydropyridine (MPTP), a

toxic substance that destroys the substantia nigra containing dopaminergic neurons. It was applied in monkeys[174] and in minipigs[175]. PET imaging with the ligand ^{11}C NS 2214 showed a reduced number of catecholamine uptake sites in the MPTP treated minipig. An increase in postsynaptic dopamine D2 receptor binding, imaged with ^{11}C-raclopride, suggest an upregulation of D2 receptors due to reduced presynaptic dopamine availability[9,89], in MPTP treated monkeys comparable to the findings in patients with Parkinson's disease.[176] A decrease of DAT was found in MPTP treated primates, imaged with a novel tracer for PET, ^{11}C-PE2I[177] and with the SPET tracer ^{99m}Tc-2beta-((N,N'-bis(2-mercaptoethyl)ethylenediamino)methyl-3beta-(4-chlorophenyl) tropane) (TRODAT)[178]. Moreover, a correlation was found in a primate study between DAT density, imaged with ^{123}I beta-CIT for SPET, and clinical staging of PD.[179] A reduced binding of the 6-deoxy-6-beta-[^{18}F]-fluoronaltrexone (cyclofoxy) to the opioid receptors was found in MPTP lesioned monkeys, suggesting involvement of this system in the physiopathology of PD.[180]

Schizophrenia

Alterations in neurotransmitter biochemistry have been examined extensively in schizophrenia, a disease that has been found associated with an overactive neurotransmitter system.[181,182] Research has been focussed on the dopaminergic and serotonergic system, evaluating the pre- and postsynaptic neurotransmitters (D2, 5-HT2A and DAT).[183] In an animal model, using primates with temporal limbic damage, the D2 receptor and DAT were imaged with the SPET tracers ^{123}I-IBZM and the ^{123}I-beta-CIT, respectively.[184,185] Further research on schizophrenia, concerning functional imaging and animal models, has focussed on evaluation of therapy with neuroleptics, more specifically in elucidating the extrapyramidal side effects (see above).

Aggressive and impulsive behavior

One of the most replicated findings in biological psychiatry is the reduction of the serotonin metabolite 5-HIAA in the cerebrospinal fluid of violent suicide attempters[186]. Over years, it became evident that dysfunctions of the serotonergic system play a role in impulsive behavior, such as impulsive aggression, pyromania, kleptomania etc. A first study in dogs showed a significant increase in cortical binding potential of the selective 123I-5-I-R91150 ligand in impulsive aggressive dogs.[187]

Future prospects

Neurotransmitter receptor imaging may serve several purposes. First, it is used to study neuropsychiatric diseases in order to elucidate the pathophysiology of disease *in vivo* and to clarify clinical diagnostic and prognostic uncertainties. Instead of utilizing trial and error to see whether a drug might help, this imaging technique may identify receptor abnormalities, with topographic localization in the brain, giving guidance to instituting and monitoring effective therapy. Due to the radiation burden, examination of young people, or repeated examinations at different ages of the same individual cannot be carried out. Since radiation burden is not a constraint in animal studies, repeated scanning of the same animal can be performed, starting from very young age to senescence. This could offer important information on receptor development at very young age, before full maturation of the central nervous system. From a veterinary point of view, the pathophysiology of behavioral problems, such as certain forms of canine aggression and impulsivity, and the effect of medical treatment can be investigated. Also, changes in neurotransmitter systems appear to be involved in age related cognitive decline and in mood disorders, which currently are important research topics not only in humans, but also in veterinary medicine.

Neurotransmitter imaging serves an important role in monitoring neurologic and neuropsychiatric drug therapy. Pre- and post-therapy imaging can visualize effects of drugs and can make a link with clinical progress or can explain unresponsiveness to therapy. It is an important and indispensable tool for the pharmaceutical industry. It provides a means to in vivo evaluate the working mechanism of the drug under investigation, to estimate dosage, to monitor side effects and to evaluate the therapeutic effects of newly developed drugs. Since serial scanning is not a problem in animal studies, drug-receptor interaction can also be monitored over time and related to clinical effects.

However, future research should also be directed towards resolving considerable methodological problems. Most probably not all subtypes of the receptor families are yet known so that interactions of the radioligand with a known receptor might not be so specific for that particular receptor. A second limiting factor is the availability of ligands for different receptors, since the stringent requirements of their design limits the variety of available probes. When implementation will be considered in veterinary medicine, radiopharmaceuticals will have to be economical favourable. Therefore, radiolabelled tracers for SPET will be most welcomed, as will pharmaceuticals labelled with ^{99m}-Tc. A third possible confounder is the receptor quantification method. Up to now, routinely measurements of receptor binding are based on normalisation to a reference region. These methods preclude differentiation between change in number and change in affinity of the receptor. This issue will become important when both parameters change or when affinity changes and the number of receptors remains the same. The latter situation implies a differential expression of certain subunits of

the receptor rather than altered levels of endogenous transmitter, a situation with consequences for therapeutic intervention. Another important issue is the response of the receptor to agonistic influences *in vivo*. The effects can be unexpected, as is seen with the challenged dopaminergic system, using different radioligands. Recent evidence suggested that besides simple binding competition between the endogenous neurotransmitter and the radioligand, other mechanisms might underly the interactions.

In summary, neuroreceptor imaging provides important insights in pathophysiology of disease resulting in more efficient treatment protocols. This technique may potentially be adopted by veterinary medicine when appropriate and economical advantageous radiopharmaceuticals are available. This imaging technique can be implemented in the research of behavioral disorders in the dog; it can be used to evaluate the kinetics and dynamics of therapeutic drugs and to monitor outcome of therapy in clinical cases. More, the use of larger animal models can be of relevance for understanding human cognitive, emotional and behavioural characteristics and deviations.

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CHAPTER 4

Biodistribution and displacement of the selective 5-HT2A receptor antagonist ^{123}I-5-I-R91150 in the normal dog

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Summary

Increasing interest evolves in mapping receptors in vivo with functional imaging modalities such as SPET (single photon emission tomography) and PET (positron emission tomography). Since SPET is a more accessible functional imaging modality than PET and overall more economical, radioligands suitable for this technique are more demanded.

Recently, ^{123}I-5-I-R91150, a radioligand with high selectivity and affinity for 5-HT2A receptors in the brain, has been introduced for SPET.

This study reports on the whole-body distribution and brain uptake of the selective ^{123}I-5-I-R91150 ligand in four normal dogs. The frontal to cerebellar ratio of uptake in time was determined in three dogs. Time-activity curve of venous blood was determined in one dog. Maximal global brain uptake was found at 10-60 minutes post-injection. Higher brain uptake was noted in the frontal cortical areas compared to the cerebellum. The frontal-cerebellar ratio reached highest values at 90-180 minutes. Reversibility and pharmacological selectivity of ligand binding was demonstrated through displacement and blocking studies with the 5-HT2A receptor antagonist ketanserin.

This study demonstrates that the specific 5-HT2A iodinated ligand can be used for imaging and semi-quantification of the 5-HT2A receptors in the canine brain in vivo with SPET.

Introduction

The serotonergic system has been brought under attention in human and veterinary medicine literature because of its importance in mood disorders such as depression, in psychotic disorders such as schizophrenia and disorders or conditions wherein impulsive behaviour is the prominent feature.[1-3] Recent studies in man demonstrated a correlation between altered 5-HT2A receptor binding and neuropsychiatric disorders.[4] Dysfunctions of serotonin turnover have already been related to aggressive behaviour in several animal species.[5-14] In dogs a reduction of 5-hydroxy-indol-acetic acid (5-HIAA), a metabolite of serotonin, was found in the cerebrospinal fluid in aggressive compared to non-aggressive dogs.[15] Also pharmacological modifications, based on the regulation of the serotonergic system, are widely applied in the therapy of behavioural disorders in dogs.[16]

Initially the involvement of serotonin was demonstrated in man through indirect estimations of serotonin turnover by measuring (5-HIAA), in cerebro-spinal fluid.[17] Direct estimation of serotonergic receptors was accomplished through post-mortem radioligand-based autoradiographic studies.[18] In recent years, nuclear imaging techniques with radiolabelled serotonin receptor antagonists have been introduced.[19] Several ligands were introduced for PET examinations such as [11C] labelled ketanserin[20], methylspiperone[21], methylketanserine[22] and bromine-LSD (MBL)[23], [18F] labelled fluoroethylaltanserin[24], fluoroethylketanserin[25] and setoperone[26]. The main problems encountered with these ligands were that affinity for D2 receptors resulted in poor selectivity in some of them and that in others the specific/ non specific ratios were low and therefore not ideal for imaging studies.[27] Since SPET is a more accessible and more economical imaging modality, increasing effort has been directed towards the development of radioligands for this type of examination. Recently, ^{123}I-5-I-R91150, a highly selective iodinated antagonist of the 5-HT2A receptor with high affinity, was introduced for SPET examinations. In vitro selectivity with regard to other 5-HT, α1, α2, D2 and D1 receptors was at least a factor 50. Inhibition of binding of the radioligand with ketanserin and ritanserin and displacement with ritanserin proved that the binding was reversible and that the compound was stable in vitro.[28]

In vivo uptake of this radioligand has been measured in rodents, primates and humans.[29,30] Reversibility of binding and selectivity was demonstrated in humans and primates with displacement studies.[27,31] Research and clinical studies in human populations with behavioural disorders have been published using this radioligand.[3,30,32-34]

The aim of this study was to investigate ^{123}I-5-I-R91150 binding in healthy dogs, to assess the dynamic aspects of uptake and wash-out of brain activity. Reversibility of binding and the existence of a pseudo-equilibral state was investigated, a necessary requirement for future semi-quantification, as was the pharmacological selectivity of the ligand.

Materials and methods

Four dogs (dog 1, German shepherd, male, 2 years; dog 2, Rottweiler, male, 2 years; dog 3, German shepherd, male, 4 years; dog 4, Kaukasian shepherd, male, 4 years) were included in the study. Their average weight was 37 kg (SD: 11). None of the dogs had a history of previous major disease or neurological disorder. The study protocol was approved by the local ethical committee. The whole body studies, SPET examinations and displacement studies were all performed on separate days for logistical and practical reasons.

Tracer

^{123}I-5-I-R91150 is synthesised by electrophilic substitution on the 5-position of the methoxybenzamide group of R91150, followed by purification with high-performance liquid chromatography. The product has a radiochemical purity of more than 99 % and is sterile and pyrogen free. The specific activity of the injected tracer is 10 Ci/µmol. The tracer is a 5-HT2A antagonist with high affinity (Kd = 0.11 nM). The selectivity of the ligand for 5-HT2A receptors with regard to other neurotransmitter receptors such as other 5-HT receptors, including 5-HT2C and 5-HT1A, dopamine receptors, adrenergic receptors and histamine receptors is at least a factor of 50.[28,29] The tracer is displaceable with ketanserin.[31]

Blood radioactivity curve

Venous blood samples (1ml) were taken from one dog (dog 1) at different time points (t= 15, 30, 45, 60, 75, 90 sec and 2, 3, 5, 7.5, 10, 12.5, 15, 20, 30, 60, 120 and 180 min) post-injection (p.i.). These samples were used to calculate the total radioactivity in blood as a function of time.

Whole body scintigraphy

A whole body scintigraphy was performed in three dogs (dog 1, 2, 3) immediately after the injection of 4.7-7.7 MBq/kg bodyweight. Fig.1. The acquisition was performed with a triple head gamma camera (Irix, Marconi, Ohio, Cleveland). The dogs were premedicated with detomidine (10-30 µg/kg IV) (Domitor®, Pfizer, Orion, Orion corporation, Espoo, Finland), anesthesia was induced with propofol (2-3 mg/kg IV) (Rapinovet®, Schering Plough Animal Health, Brussels, Belgium) and maintained with halothane (Fluothane® Zeneca, Destelbergen, Belgium) to effect. Anterior and

posterior images were registered simultaneous by using two heads of the camera. On each dog, five whole body acquisitions of 20 minutes were performed over a time period of 3 hours, obtaining data at 30, 6', 100, 130 and at 160 min. p.i. For quantification of radioactivity uptake after injection of the tracer, regions of interest (ROI) were drawn manually over the total body and around different organs (brain, ocular region, lung, liver, gallbladder, caudal abdomen, defined as the region between the caudal delineation of the liver and the cranial border of the pelvis, and total body) on both anterior and posterior whole body images on a medical workstation using the Multimodality software of Nuclear Diagnostics (Hägersten, Stockholm, Sweden) on the earliest images. Shapes and sizes, i.e. number of pixels, were kept constant over all subsequent images. For each ROI, i.e. each organ, the geometric mean activity, corrected for physical decay, of total anterior and posterior counts was calculated. The total body geometric mean activity calculated on the first image was taken as total injected activity, considering that no urine was excreted prior to the first whole body scan. The activity in the total body and different organs was expressed as the percentage of the injected activity (%IA) calculated by the following equation: (geometric mean net counts in organ and total body)/(geometric mean counts in first total body)x 100.

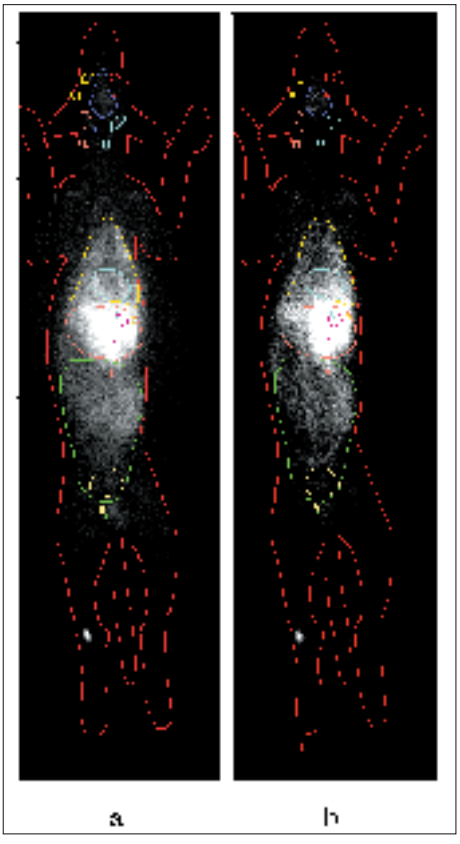

Figure 1: Total body image at 60 minutes: ventral (a) and dorsal (b) view.

SPET brain imaging

SPET examinations of the brain were performed in three dogs (dog 1, 2, 4) using a triple head gamma camera ((Toshiba GCA-9300A, Dutoit Medical, Antwerp, Belgium) up to 180 minutes p.i. of ^{123}I-5-I-R91150 at a dosage of 4.7-7.7 MBq/kg bodyweight. Each dog underwent 13 acquisitions initially performed at a rate of one every 10 minutes (duration 6 minutes). The last 3 acquisitions were performed at a rate of one every 35 minutes (duration 20 minutes). SPET data were reconstructed using Ordered Subset Expectation Maximization (OSEM, 2 iterations, 4 subsets), a commercially available software algorithm. Post-filtering was performed with a Butterworth filter (0,650 cycles/pixel, order 8). Images were analysed with ROI's, kept constant and manually drawn around the frontal cortex, the cerebellum and around the total brain of the SPET images. Regional radioactivity uptake was expressed as activity per voxel normalized to the decay corrected injected activity (defined at the different acquisition time intervals). The frontal region of interest was measured as the sum of 2 slices with highest frontal activity. The cerebellar region of interest was delineated on two consecutive slices. The anatomic localisation of both frontal cortex and cerebellum was verified with the help of computed tomography (CT) images, obtained from each dog, using commercially available software based on a mutual information cost function, minimalized by a down-hill simplex algorithm. The mutual information registration criterion allows fully automated, highly robust affine registration of multimodal images without the need for pre-processing or user interaction. [35] The ratio of frontal to cerebellar uptake was used as a relative measure of specific binding. The cerebellar uptake was used as a reference for non-specific binding since this is a region void of 5-HT2A receptors. [36-39]

Displacement study

One dog (dog 4) was injected with two different doses, on two separate days, of the 5-HT2A antagonist ketanserin (1mg/kg and 5mg/kg bodyweight respectively) after two baseline SPET acquisitions of 20 minutes duration, started at 140 minutes p.i. of ^{123}I-5-I-R91150 (6.2 MBq/kg body weight). Acquisitions of 10 minutes were obtained for 75 minutes after injection of ketanserin. The acquisitions were performed with a triple head high resolution, fanbeam collimated gamma camera (Toshiba GCA-9300A, Dutoit Medical, Antwerp, Belgium). An additional study was performed in this same dog, three weeks after the last displacement study, with cold ketanserin, administered at a dose rate of two times 30mg I.V. (1.5 mg/kg), one hour and one hour and a half prior to the administration of the labelled tracer. One acquisition of 20 minutes was started 90 minutes after the tracer injection.

RESULTS

Neither adverse effects were noted at or after injection of the radiopharmaceutical nor were any side effects noted after injection of ketanserin.

Blood radioactivity curve

Blood clearance curve demonstrated a sharp decrease of radioactivity 3 minutes p.i. At 20 minutes a decrease of radioactivity to 2% of the injected dose was noted. The registered activity in the blood remained at a stable plateau from 20-40 minutes onwards (1%). (Fig 2).

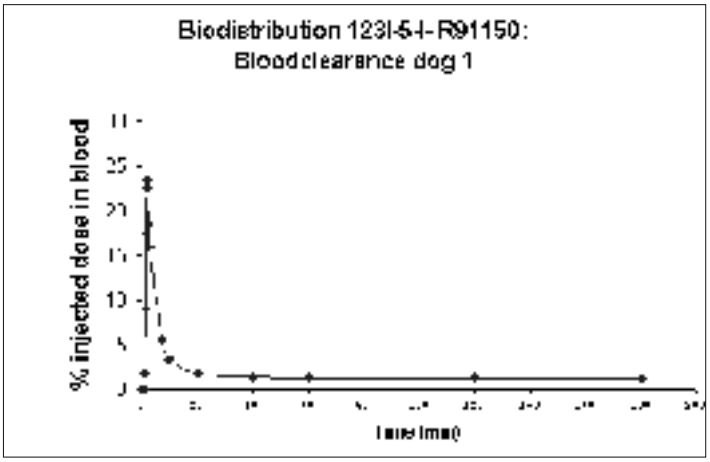

Figure 2: Radioactivity measured in time in blood as % injected dose

Whole body scintigraphy:

At 30 minutes, lungs showed the highest uptake (20-29%) followed by the liver area (10-22%) and the caudal abdomen (6-9%).(table 1) At 60 minutes, the uptake in the lungs decreased and the uptake in the liver and bile bladder increased. The heart was visible. Brain uptake was highest between 30 and 60 minutes (1.0-1.6%). At 100 minutes lung uptake further decreased while uptake in the liver (18-21%) and the bile bladder (0.7-1.7%) increased. Uptake in the liver and bile bladder reached a plateau at 100-130 minutes. No kidney nor urinary bladder activity was visualized at any time during the examination. Periocular uptake remained at a low steady level throughout the procedure (0.1-0.2%).

	30 min			60 min			100 min			130 min			160 min		
	Subj 1	Subj 2	Subj 3	Subj 1	Subj 2	Subj 3	Subj 1	Subj 2	Subj 3	Subj 1	Subj 2	Subj 3	Subj 1	Subj 2	Subj 3
Blood	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2
Lung	25.0	20.5	29.0	9.5	13.9	8.7	8.1	15.1	8.0	7.5	13.7	7.5	7.0	12.4	7.0
Heart	4.8	3.5	3.9	3.9	2.8	2.9	3.2	2.5	2.8	2.7	2.5	2.6	2.6	2.0	2.4
Liver	17.1	10.7	22.1	18.7	12.5	22.3	18.2	20.0	21.0	18.2	19.8	20,5	17.9	19.7	20.5
Bile bladder	0.6	1.4	0.1	1.1	0.9	0.4	1.7	0.7	0.8	1.8	0.4	0.6	2.0	0.4	0.5
Eye	0.1	0.1	0.1	0.1	0.2	0.1	0.1	1.0	0.1	0.1	0.2	0.1	0.1	0.2	0.1
Brain	1.2	1.5	1.5	1.0	1.6	1.1	0.9	1.4	1.1	0.8	1.2	1.1	0.8	1.1	1.0

Table 1: Data obtained from total body acquisitions: tracer uptake (% of total body radioactivity) in different regions

SPET brain imaging

SPET images provided good anatomical definition of the frontal regions and showed low uptake in the cerebellar area. (Fig 3). Peak uptake in the cerebellar area occurred slightly before peak uptake in the frontal area, but never reached the same level of activity as the frontal area.

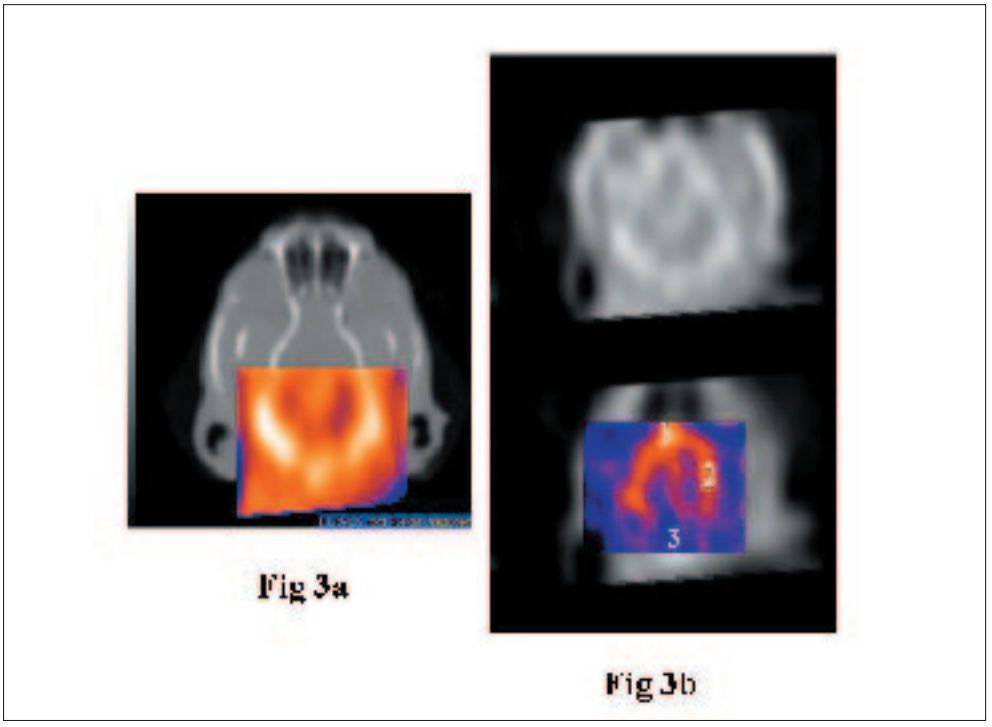

Figure 3: a. Horizontal slice of transmission data fitted to the CT slice taken from the same dog, facilitating topographic orientation on the emission data. b. On the emission images fronto-cortical (1) and temporo-cortical (2) uptake of radioactivity is visualized. Low uptake is noted in the cerebellar area (3).

Specific binding of the ligand to the receptors could be estimated from the counts registered in the fronto-cortical region (total binding) and the cerebellum (free and specific bound ligand). The frontal to cerebellar ratio increased to a maximum of 1.85-2.39 at 90-100 minutes p.i. A constant ratio was found between 90 and 180 minutes p.i. The peak ratio was consistently higher in dog 4. This dog showed more anxiety and was less cooperative than the two other animals. (Fig 4).

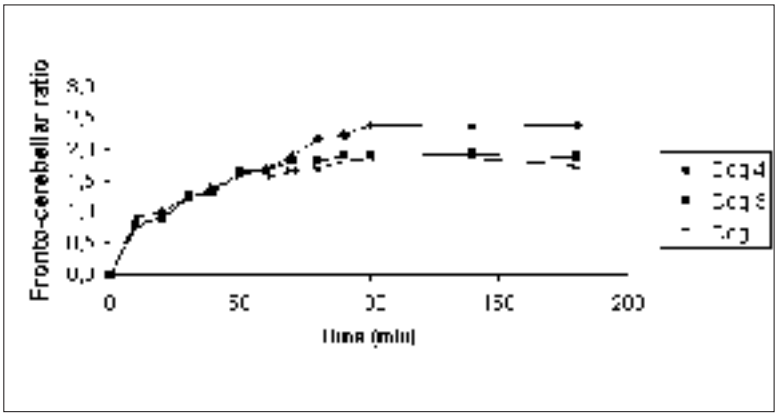

Figure 4: Graphic illustration of fronto-cerebellar ratio in function of time, generated from the SPECT data.

Displacement study

Intravenous administration of the receptor blocking agent ketanserin after administration of ^{123}I-5-I-R91150 resulted in rapid decrease of the radioactivity in the cortical areas. The ratio of fronto-cortical/cerebellar activity (specific/non-specific binding) was 2.2-2.4 in the baseline study. After the administration of cold (not labelled with ^{123}I) ketanserin binding decreased to 1.5-1.7 with 1 mg/kg and 1.0-1.09 with 5mg/kg, indicating displacement of the radioligand from the receptors. (Fig 5). Pretreatment with ketanserin led to uniform low binding of the ^{123}I-5-I-R91150. (Fig 6).

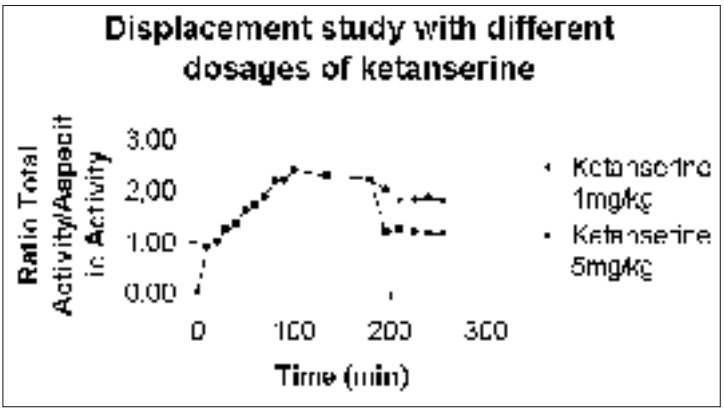

Figure 5: Evaluation of total and specific binding in time (measured as activity/ pixel), after displacement with 1mg/kg and 5 mg/kg ketanserin injected IV at 180 minutes after administration of labelled R 91150.

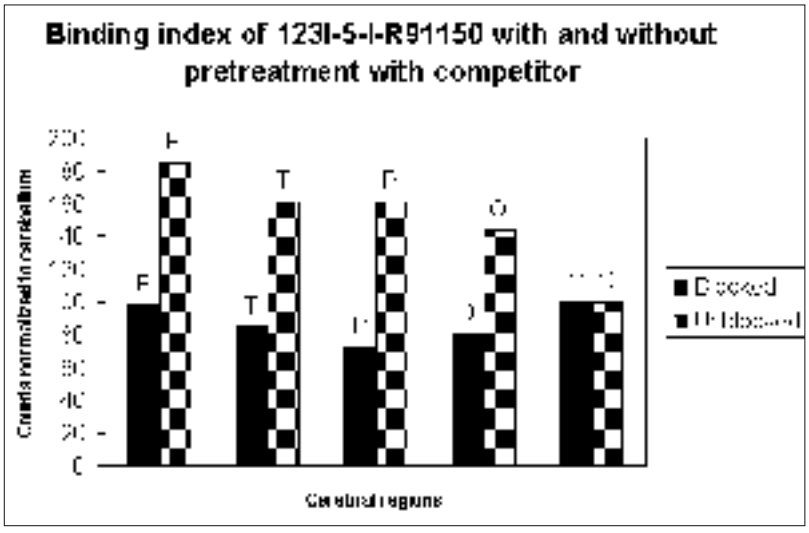

Figure 6: Binding of 123I-5-I-R91150: uptake in the different brain regions with (blocked) and without (unblocked) pretreatment with cold ketanserine.
F= frontal cortex - T= temporal cortex - P= parietal cortex - O= occipital cortex - C= cerebellum

Discussion

The tracer under investigation crossed the canine blood brain barrier and concentrated mainly in the cortical regions, showing specific and reversible binding. Radioactivity in blood decreased fast and remained stable thereafter, indicating a fast distribution to and no redistribution from various organs. Redistribution would result in disruption of the steady state and as a consequence, would preclude semi-quantification of receptor binding using the "ratio" method. On the total body scans it was found that, in the early phase, highest activity was present in the lungs. Activity in the liver predominated from 60 minutes onwards.

At no time the bladder shadow, nor the kidneys were visualized in our series. In humans, highest activity in the liver and bowel is already evident on the early images and although the bladder shadow is visualized at 180-210 minutes, the activity registered in this organ is low.[30] This finding may implicate that elimination of the tracer and its radioactive metabolites mainly occur by the intestinal system rather than by the urinary system, probably as a result of plasma protein binding.

The maximal ligand uptake (1.0-1.6% of total body activity) in the canine brain is lower than what is found in humans (2%)[30]. In general, low brain uptake is thought not to be caused by low permeability through the blood brain barrier (BBB) but rather by low extractability due to high plasma protein binding.[31] More specifically, mass difference relative to bodyweight between the brain of the examined dogs (100-120g) and the average human brain (1200g) has also to be considered. Despite this low uptake, SPET imaging allows localization of the 5-HT2A receptors in the brain and gives information regarding the dynamic binding of these receptors. Relative high uptake is noted in the ocular area (0.1-0.2%). In one study performed in primates, this activity precluded accurate measurement of the activity in the frontal cortex.[31] This was not a problem in our study because of the more rostral position of the eyes in the dog. Ketanserin did not affect the periocular activity in primate studies nor did it in our displacement study, suggesting that this activity is caused by non specific binding rather than by the presence of receptors in this area.[31] This non specific binding could involve melanin, since it is known that a variety of substances (amphetamine, (nor)ephedrine, prostaglandines, adrenoreceptor agonists and antagonists, chloroquine, rifampicine, chlorpromazine, bezodiazepines..) exhibit melanin binding properties.[40-44]

The maximal cortico-cerebellar ratio found in these dogs (ratio 1.85-2.39) between 90 and 180 minutes p.i. was similar but slightly higher than the ratios found in normal human (ratio 1.4 at 100 minutes) and non-human primates (ratio 1.5 at 60 minutes).[30,31] In rats, the ratio of uptake in the frontal cortex relative to the cerebellum (a region free from 5-HT2A receptors [45]) was 10 at 60 minutes p.i. [29] The density of 5-HT2A receptors in the cortex is about three times higher than in the primate cortex, thus explaining the higher cortico-cerebellar ratio in this species.[31]

Cerebellar peak radioactivity was reached between 0-10 minutes in primate brain and at 5 minutes in humans.[30,31] In our dogs, peak uptake of cerebellum and fronto-cortical area was reached on the first image, at 6 minutes, as a result of vascular activity. After this peak, activity decreased over time with a higher fronto-cortical activity compared to the cerebellar activity. The fronto-cortical to cerebellar activity (specific/ non specific binding ratio) reached its maximal value at approximately 90 minutes p.i., remaining relatively stable until 180 minutes, indicating this to be the optimal scanning time interval. These findings are similar to the findings in man and non-human primates. In healthy humans peak activity in the frontal cortex was reached at approximately 100-120 minutes p.i. and remained stable from approximately 100 minutes p.i. onwards[30]. In non-human primates a stable plateau is reached at 150 minutes.[31]

Displacement with ketanserin resulted in a reduced radioactivity in the cortical regions of non-human and human primates.[27,31] In our study, a decrease of activity in all cortical areas was noted on the first image at 10 minutes p.i., to the level observed in the cerebellum, remaining constant during the following 60 minutes indicating displacement of the ligand from the receptors. Uniform low uptake in the brain was noted after pre-treatment with ketanserin suggesting blockade of the receptors. These findings indicate reversibility and specificity of binding, essential prerequisites for quantification of receptors.

This study suggests that the radiolabelled 5-HT2A receptor antagonist R-91150 is a suitable tracer to map and (semi)quantify receptor binding in the canine brain in order to investigate abnormalities related to this receptor.

Conclusive remarks

First, the use of dogs in research on new potential tracers is of practical interest. Since housing, maintenance and handling of dogs is less tedious than primates and since dogs are also easy to obtain and less expensive, it is worth the effort to investigate the use of dogs in this type of research. Moreover, it has been proposed that certain canine behavioural disorders show resemblance to human brain disease. Alterations in aging brain of dogs and humans show similar neuropathological changes, including apoptosis, beta-amyloidal plaque formation and amyloidal angiopathy.[46-48] Since the serotonergic system has been brought in connection with mood disorders in aging, it would be of scientific interest to evaluate the binding parameters of the 5-HT2A radioligand in canine aging brain. Also, impulsive aggression in both man and dogs seems to be based on similar biological grounds, as was shown with studies measuring cerebrospinal fluid concentrations of 5-hydroxyindoleacetic acid (5-HIAA) in inward or outward directed aggression in man and aggressive behaviour in dogs, inversely correlating with the unmediated character of the violent acts in man and with the unpredictability of biting incidents in dogs.[15,49-52] Also, recent research in veterinary science has focussed on the evaluation of behaviour and cognitive status of dogs with tests and goal oriented questionnaires, making assessment of canine behaviour more objective.[53,54]

Second, concerning the evaluation of the 5-HT2 receptor in particular, this receptor has been brought in connection with depression, aggression, suicide and eating disorders in man.[3,34,55,56] The contribution of SPET in evaluating the status of this receptor is principal of practical value, since this imaging modality is more economical and more accessible for most nuclear facilities. The availability of the specific antagonist R91150, labelled with radioiodine, has already contributed to the in vivo investigation of the binding properties of the 5-HT2A receptor in aging, suicidal behaviour, eating disorders and therapeutic intervention with atypical neuroleptics in schizophrenic patients.[3,32-34]

It would therefore be interesting to evaluate the 5-HT2A receptor status with this radioligand for SPET, in selected canine brain disease that show similarities in biological substrate to human disorders. To evaluate the advantage of a canine model, future studies are necessary to examine the binding parameters of this particular radioligand in pathologic states. This research modality could have the additional advantage in that it may determine the impact of certain drugs on the 5-HT2A receptor in canine brain.

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CHAPTER 5

Regional binding index of the selective 5-HT2A radiolabelled antagonist 123 I-5-I-R91150 in the normal canine brain imaged with single photon emission tomography

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Summary

The pattern of the specific 5-HT2A (5-hydroxytryptamine 2A receptor) antagonist ^{123}I-5-I R91150 was measured in 10 healthy dogs without neurologic and behavioural abnormalities. Eight cortical regions (left and right fronto-, temporo-, parieto-, and occipito-cortical area), and one global subcortical region (including the thalamic system) were compared with a reference region lacking receptors, i.e. the cerebellum. The ^{123}I labelled radioligand was injected intravenously 100-120 minutes prior to acquisition. Both transmission and emission data were obtained with a triple head gamma-camera equipped with high resolution fanbeam collimators. The emission data were corrected for scatter and attenuation. To delineate different cerebral regions more accurately, the regions of interest (ROI) defined in a former study on brain perfusion measured with ^{99m}Tc-ethyl cysteinate dimer (ECD) in the same dogs were used. The co-registration of the ^{99m}Tc-ECD and the ^{123}I-5-I-R91150, obtained from each dog, was realized with the help of corresponding transmission maps. By normalizing each regional cerebral activity to the activity observed in the cerebellum, the regional radioactivity (binding index) could be relatively quantified. Highest brain uptake was noted in the fronto-cortical brain areas (right: 1.85, left: 1.89), followed by the temporo-cortical region (right: 1.58, left: 1.56). Least uptake was noted in the more caudal and middle brain regions (occipito-(right: 1.46, left: 1.41), parieto-cortical (right: 1.30, left: 1.26) and striatal region (1.19)). Neither gender nor age influence was noted in this series.

The ^{123}I labelled serotonin-2A receptor ligand appears to have similar cortical binding in the normal canine brain as shown in humans and other animal species. A frontocortical to occipitocortical (rostro-caudal) binding index gradient was identified within the dog that has not been seen in imaging studies from humans and other animal species. The significance of these results will need further investigation. This normative data can be used to compare regional brain uptake of the 123I-radioligand to dogs with behavioral disorders related to the serotonergic system, in future studies.

Introduction

Serotonin (5-HT, 5-hydroxytryptamine) is a central and a peripheral neurotransmitter. It is synthesized from the amino acid L-tryptophan by sequential hydroxylation and decarboxylation. It is stored in pre-synaptic vesicles, released from central and peripheral nerve-terminals during neuronal firing and metabolized by the mono-amino-oxidase (MAO) enzyme to 5-hydroxy indole acetic acid (5-HIAA).[1] Impairments of the serotonergic neurotransmitter have been associated with a variety of human mental disorders, including psychotic disorders, mood and anxiety disorders, eating disorders and dementia.[2]

A lower 5-HIAA concentration in cerebrospinal fluid (CSF) is thought to be an indication of reduced serotonergic turnover. This reduced 5-HIAA in CSF is found in subjects that attempt suicide or harm themselves, in violent offenders including murderers, in impulsive patients such as arsonists and aggressive alcoholics, and in children with conduct disorders, e.g. children injuring animals frequently and deliberately.[3-6] Low 5-HIAA in CSF is also found in mammals and non-human primates. In non-human primates a lower 5-HIAA in CSF is detected in violent individuals.[7-9] In aggressive, impulsive dogs, defined as sudden, unpredictable, poorly inhibited aggressive behavior, a decrease in 5-HIAA is detected in the cerebrospinal fluid, a result maintained after controlling for breed and irrespective of age and gender.[10] It is concluded from these studies that the serotonergic system plays an important role in impaired impulse control and modulates aggressive behaviour throughout a large variety of animal species.[8,10]

Within the serotonergic system several 5-HT receptor types and subtypes have been recognized[1,11,12], each with a particular function. Because of its involvement in neuro-psychiatric conditions such as mood and anxiety disorders, in aggression and impulsivity, attention has been focused on the 5-HT2A receptor. Distribution of the 5-HT2A receptors was initially investigated post-mortem with autoradiography[13-16] or with immuno-histochemistry[17] in both animals and humans. In humans, primates and rats, these receptors are found predominantly in the rostral cerebral cortical areas, to a somewhat lesser extent in the caudal regions and absent in the cerebellum.[17-19] Based on post-mortem brain receptor studies in violent suicide victims, increased 5-HT2A receptor densities were found in cortical areas, predominantly in the frontal cortex.[20,21] However, fast and unpredictable post-mortem alterations in receptor density and affinity are an important methodologic weakness of post-mortem brain research.

Nuclear medicine techniques enable in vivo examination of neuro-psychiatric disorders and results of treatment[18]. Radioligands with selective in vivo binding affinity to the 5-HT2A receptor have been developed for positron emission tomography (PET)[22-24] and single photon emission computed tomography (SPECT).[19] SPECT makes use of radionuclides with a longer half life and therefore does not require a cyclotron in the vicinity of the camera and it is therefore a more accessible technique. ^{123}I-5-I-R91150 (R93274), a highly selective radioiodinated antagonist of the 5-HT2A receptors was

synthesized for SPECT imaging.[25,26] The feasibility of this tracer was tested in rats, non-human primates and humans.[19,27] The specificity of cortical binding of this radioligand to 5-HT2A receptors was investigated in primates and dogs through displacement studies with ketanserin, a 5-HT2A antagonist.[27,32] In rats, the ratio of radioactivity in the frontal cortex relative to the cerebellum, a region free from 5-HT2A receptors, is 10:1 at 60 minutes. In non-human primates, the cortex-cerebellar ratio is 1.5 at 60 minutes.[27] In sequential SPECT brain scans in humans the cortico-cerebellar ratio (1.5- 1.8) reaches a plateau between 90 and 110 minutes, remaining stable from then onwards, thus reflecting a pseudoequilibrium.[19] Since these pilot studies, several studies have been carried out in normal humans and in pathologic states.[28-31]

Since nothing was found concerning the characteristics of this radioligand in dogs, we performed preliminary studies and demonstrated that the binding characteristics of the selective ^{123}I-5-I-R91150 radioligand for the 5-HT2A receptor fulfilled the requirements for semi-quantification in the canine brain, using the ratio of activity in a region to a reference region. Binding was reversible and a steady state (pseudo-equilibrum) was reached from 90 minutes onwards.[32]

The aim of this study was to investigate the distribution pattern of the regional binding of ^{123}I-5-I-R91150 radioligand for 5-HT2A receptor imaging in the canine brain and consequently determine the distribution pattern of 5-HT2A receptors within the brain in a group of 10 normal dogs using SPECT attenuated and scatter corrected images. Knowledge of the normal distribution pattern will enable studies of brain pathology associated with canine behavioral abnormalities caused by altered binding properties of the 5-HT2A receptor.

Materials and methods

Subjects

Ten normal healthy shepherd type dogs were studied. There were 5 intact males and 5 intact females. Age varied between 1 and 7 years (mean: 4.1; standard deviation (SD): 2.3) and weight between 13 and 41 kg (mean: 30.0 kg; SD: 9.0). This study was approved by the local ethical committee.

Tracer

^{123}I-5-I-R91150 was synthesized by electrophilic substitution on the 5-position of the methoxybenzamide group of R91150, followed by purification with high-performance liquid chromatography. The product had a radiochemical purity of more than 99 % and was sterile and pyrogen free. A specific activity of 370 GBq/µmol was obtained.

The tracer is a 5-HT2A antagonist with high affinity (Kd = 0.11 nM) and selectivity for 5-HT2A receptors. The selectivity of the ligand for 5-HT2A receptors with regard to other neurotransmitter receptors such as other 5-HT receptors, including 5-HT2C and 5-HT1A, dopamine receptors, adrenoreceptors and histamine receptors is at least a factor of 50. The tracer is displaceable with ketanserin, a competitor for receptor binding.[25,26 27]

In this study, the decay corrected injected activity ranged from 4.07-7.77 MBq/kg (mean: 5.55MBq/kg; SD: 0.04) IV. The interval between injection and acquisition ranged from 100 to 120 minutes (mean: 113 minutes; SD: 8.6).[32]

SPECT acquisition

The SPECT acquisitions were performed under general anesthesia. Premedication to anesthesia was obtained with 10-30µg/kg IV medetomidin hydrochloride (Domitor®, Pfizer, Orion, Orion corporation, Espoo, Finland). General anesthesia was induced with 2-3 mg/kg iso-propylphenol (Rapinovet®, Schering Plough Animal Health, Brussels, Belgium) intravenously and maintained with halothane (Fluothane®, Zeneca, Destelbergen, Belgium) to effect. The dogs were positioned in ventral recumbency. The SPECT acquisition was performed with a triple head gamma camera (Toshiba GCA-9300A, Dutoit Medical, Antwerp, Belgium), equipped with high resolution fanbeam collimators (full width at half maximum (FWHM) of 7.8 mm). A single photon transmission computed tomography scan (TCT-scan) of 4 minutes was obtained prior to the emission scan, using three 153-Gadolinium

(Gd) rod sources. This TCT images, having sufficient contrast to recognize bone and sinuses, were obtained for later co-registration of serotonin-2A receptor emission data with ^{99m}Tc-ECD perfusion emission data, obtained in a former study.[33]

Following the TCT scan, emission images were acquired over 20 minutes, thus obtaining the whole brain volume within the single scanning session. For each acquisition, 90 projection images were obtained on a 128x128 matrix using a continuous scan mode by rotating each head twice over 120°. The emission data sets were reconstructed with filtered back projection after rebinning to parallel data and applying a Butterworth-filter (cut-off 0.16 cycles/pixel, order 8). Pixel size was 1.72 mm. Uniform Sorensen attenuation correction with linear attenuation coefficient of 0.12/cm and triple-energy window scatter correction were applied.

After reconstruction, data were transferred to another computer equipped with Hermes software from Nuclear Diagnostics (Hägersten, Stockholm, Sweden) for processing. Each receptor radioligand image was co-registered with a formerly obtained perfusion image from the same dog. Because of the poor anatomic definition of neuroreceptor imaging studies, the simultaneously acquired transmission data were used to optimise co-registration of both data sets. (Fig 1).

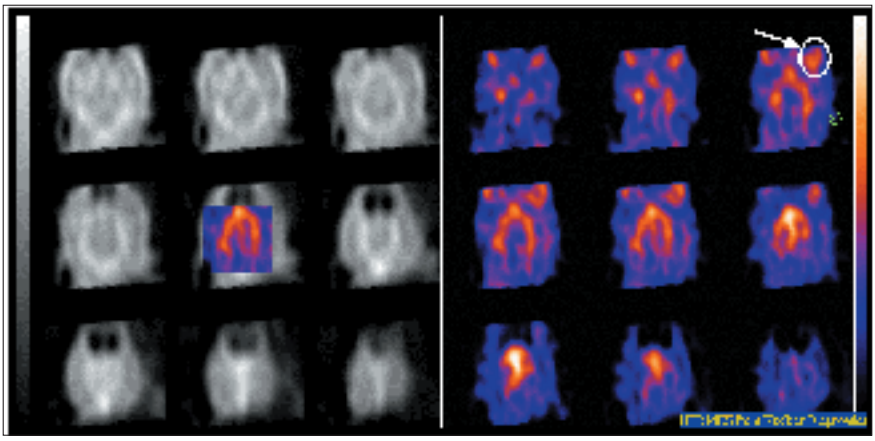

Figure 1: The emission data of the ^{123}I-5-I-R91150 study (right set of images) are automatically fitted to their transmission data (TCT data) (left set of images), provided that the dog's head did not move between both acquisitions. The bone structures of the skull are recognizable on the TCT images. Note the high uptake in the ocular region (circular marking).

Co-registration of the TCT data was performed with the multimodality software of Nuclear Diagnostics. This computer program, displaying images in a dual window setting, allows for manual co-registration by providing tools for rotation and translation images in all three dimensions. The parameter values used to register the TCT map of the receptor radioligand image to the TCT map of the perfusion image were then applied to the emission image of the receptor radioligand, thus co-registering this image with the perfusion image. This allowed us to use the regions of interest defined on the perfusion images for the serotonin-2A receptor images. (Fig 2a, b, c, d). The predefined and standardized regions of interest consisted of eight cortical brain regions (left and right fronto-, temporo-, parieto- and occipitocortical), one global subcortical region and the cerebellum. The regional classification was performed according to prior definitions (1978) and was also described in more detail in a former study on brain perfusion in the dog.[33,34]

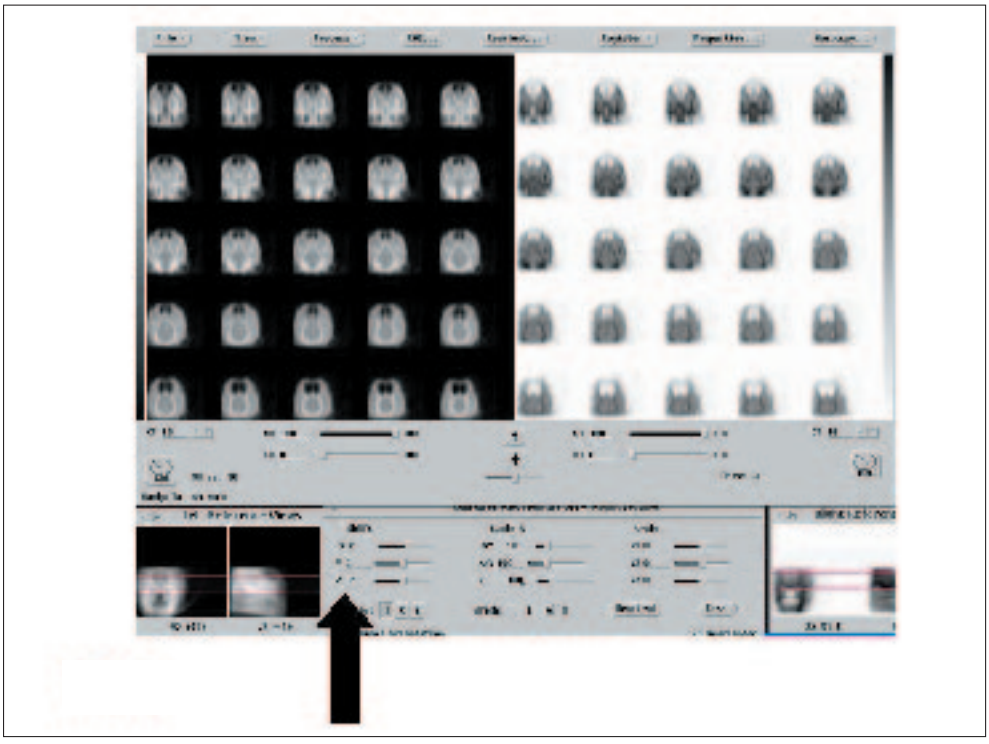

Figure 2: a. Overview of horizontal TCT slices from the skull. Manual co-registration of the TCT data of the receptor radioligand studies (right images) to the TCT images of the perfusion study (left images) is performed by rotation, shifting and scaling of the TCT data of the receptor radioligand studies, using the Multi-Modality software from Hermes (Nuclear Diagnostics).

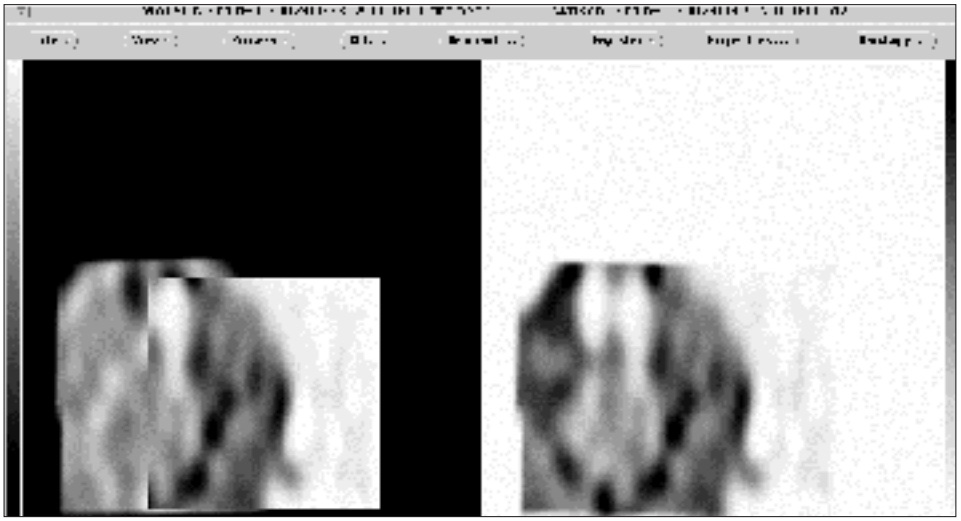

Figure 2: b. Detail of a TCT image from a perfusion study (left image) and from the receptor radioligand study (right image). Using the sliding window facility, the TCT image of the receptor radioligand study will be superimposed on the TCT image of the perfusion study, allowing visual inspection of the fitting procedure. The use of a different color scale simplifies this procedure.

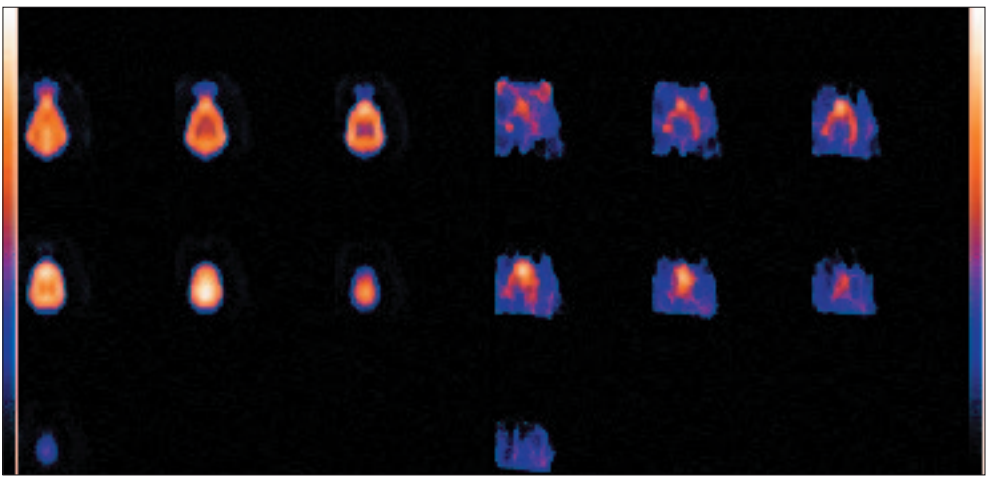

Figure 2:c. The parameters (rotation, shift and scale) used for co-registration of the TCT data of both studies are extended to the emission data of the receptor radioligand images. By this procedure the emission data of both studies are adjusted to each other.

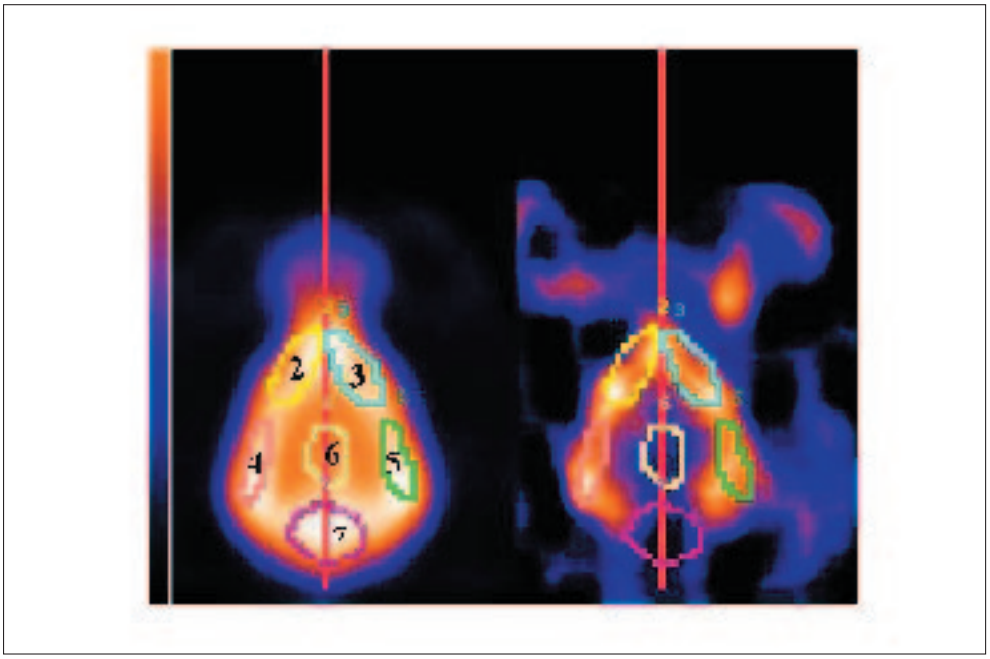

Figure 2:d. Horizontal slices taken at the level of the lower midbrain. The predefined, standardized regions of interest on the perfusion image are automatically extended to the emission image of the ^{123}I-5-I-R91150 study. This procedure is performed for all slices. 2: left fronto-cortical region, 3: right fronto-cortical region, 4: left temporo-cortical region, 5: right temporo-cortical region, 6: subcortical region, 7: cerebellum

The binding index (BI) was operationally estimated as (counts/pixel in regional cortex) / (counts/pixel in reference region). This BI is proportional to the concentration of available receptors under near equilibral conditions. To apply this semi-quantification method using a BI as a measure for specific binding (binding to the receptor under investigation), a reference region is needed. This region must be void of the concerned receptor and must have similar concentration of free and non-specific bound radioligand as the region under examination. The cerebellum satisfies these conditions and is therefore selected as a reference region for studies involving the 5-HT2A receptor.

Statistics

Correlations between age, weight, injected dose/kg bodyweight and regional activity were calculated using the Spearman's correlation. Differences in age, weight, dose and regional activity versus gender were evaluated with the independent samples Mann-Whitney U test. Left to right BI in fronto-, parieto-, temporo- and occipito-cortical regions and rostro-caudal gradient, were evaluated through the related samples Wilcoxon Signed Rank Test. At first, the null hypotheses on laterality was that there were no differences between left and right sided binding indices. Secondly, the null hypothesis on rostro-caudal serotonin-2A binding was that no difference between frontal and occipital binding index was present. Level of significance was set at $p < 0.05$.

Results

Highest uptake was noted in the fronto-cortical cerebral region (right fronto-cortical region (RF): 1.85, SD: 0.07; left fronto-cortical region (LF): 1.89, SD: 0.09) followed by the temporo-cortical region (right temporo-cortical region (RT): 1.58, SD: 0.15; left temporo-cortical region (LT): 1.56, SD: 0.11). The parieto-cortical (right parieto-cortical region (RP): 1.30, SD: 0.39; left parieto-cortical region (LP): 1.26, SD: 0.35), occipito-cortical (right occipito-cortical region (RO): 1.46, SD: 0.21; left occipito-cortical region (LO): 1.41, SD: 0.21) and subcortical regions (1.19, SD: 0.40) had a lower BI. The lowest activity was, as expected, noted in the cerebellar region set at 1.0. (Fig 3). There was no correlation between injected dose and regional activity. There were no significant differences between male and female subjects concerning age, weight, injected dose/kg bodyweight and regional activity, in any of the regions.

When comparing bilateral regional activity of the fronto-, temporo- and occipito-cortical region, no significant difference was found. There was a significant correlation between right and left parietal binding index (_=0.67; p=0.02). There was a significant fronto-occipito-cortical gradient (left and right; Z=-2.67; p=0.008).

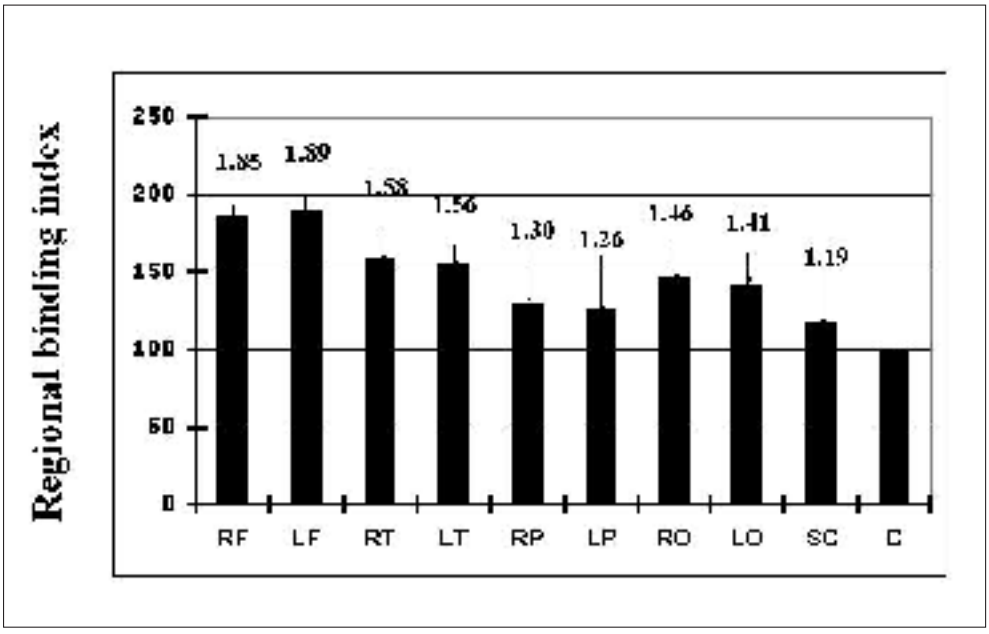

Figure 3: Graphical representation of the mean registered activity normalized to the cerebellum in the different brain regions.
Legend: RF: right fronto-cortical region, LF: left fronto-cortical region, RT: right temporo-cortical region, LT: left temporo-cortical region, RP: right parieto-cortical region, LP: left parieto-cortical region, RO: right occipital region, LO: left occipital region, SC: subcortical region, C: cerebellum

Discussion

Results are in agreement with previously published human and animal studies on the in vivo distribution pattern of ^{123}I-5-R91150.[19,27] Despite the low ligand uptake (1-1.6%) the high resolution, fanbeam collimated SPECT provides useful information about receptor binding in different brain structures.

In rats, the density of 5-HT2A receptors in the cortex is about three times higher than in the primate cortex. This contributes to the higher cortico-cerebellar ratio in this species. In our series, highest relative uptake was found in the cerebral cortex (fronto-cortical region having highest values), followed by the subcortical region and least activity, as expected, was found in the cerebellum. In humans, a similar pattern of regional variation was found with highest binding in the cerebral cortex, followed by the striatal area, and lowest in the cerebellum, compatible with data from autoradiographic studies.[15,19] The rostro-caudal cortical gradient recognized in this group is not replicated in the few existing studies using this ligand. The regional binding index of the cortical regions is also more homogenous in human studies. However, a small variability in highest and lowest regional binding index is apparent between existing human studies.[19,31] More, in some studies not all cortical regions are evaluated[29,31]. This was also the situation in one primate study in which fronto-cortical activity was not measured due to technical reasons, and parieto-cortical binding index was not mentioned.[27] A possible explanation for this higher BI in the fronto-cortical brain regions in our series could be the species dependent distribution of these receptors.[14] Larger studies are needed, to verify the presence of this gradient. In primates, a high uptake was noted in the ocular and periocular region, contaminating the region of interest of the frontal cortical area.[27] A similar high uptake in the ocular area was noted in our series. Since ocular uptake in the study with primates[27] and in our preliminary study on dogs[32], was not influenced by ketanserin, this activity is most probably caused by non-specific binding to undefined substances (proteins, lipids, other receptors...).

In humans, an age related decline of 5-HT2A receptor density was found both with autoradiographic studies and with in vivo studies, using positron emission tomography (PET) or SPET.[28,35-37] In this study only a weak, non significant correlation was found with age and right fronto-cortical binding index, but the number of subjects is too small to draw definite conclusions regarding this matter. Gender difference was not found in a human study including 26 normal volunteers[28] nor was this present in this study.

Methodologic considerations and limitations:

General anesthesia is necessary in order to perform these studies. It is therefore important to consider the effects of the anaesthetics used. Drugs having an effect on the serotonergic system, directly or indirectly, have to be excluded. However, global cerebral blood flow (CBF) alterations as result of the anaesthetic drugs, are inevitable.

Medetomidine is an α_2 agonist and produces no direct effect on the 5-HT2A receptors, or on brain metabolism, but reduces cerebral blood flow in general, suggesting uncoupling of cerebral metabolism and flow due to decreases in central catecholamine turnover.[38] Induction of general anesthesia obtained with iso-propylphenol also decreases blood flow. Halothane, isoflurane and sevoflurane cause cerebral vasodilation and decreased vascular resistance in the brain.[39] In one study it was demonstrated that the use of dexmedetomidine prior to isoflurane and sevoflurane significantly attenuated dilation of cerebral arterioles induced by these volatile anaesthetics.[40] It is unlikely that the regional variation of radioactivity is due to global flow alterations in the brain. Because all dogs received the same anaesthetic protocol, individual blood flow pattern changes would be minimalized.

Another possible confounding factor is the partial volume effect, which increases as the size of the object and resolution (FWHM) of the system decrease. It may be assumed that the activity due to specific binding, registered in the cortical regions, will be more affected by the partial volume effect than the activity registered in the area chosen as a reference for non-specific binding.

Conclusions:

This study presents data from canine brain uptake patterns of the 5-HT2A receptor radioligand, ^{123}I-5-I-R91150 using SPECT imaging. The findings in this study agree with previous studies in normal human and non-human primates. Recognition of the normal distribution pattern of the 5-HT2A receptor will enable evaluation of pathologic states of this receptor. The diagnostic value of this technique will be tested in future investigations on canine neuropsychological disease involving the serotonergic system. Also, this technique has the potential to become an attractive tool to image the influence of pharmacologic interventions on receptor binding status and to correlate alterations in binding index with therapeutic outcome.

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CHAPTER 6

Effects of aging on brain perfusion and serotonin-2A receptor binding in the normal canine brain measured with single photon emission tomography

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Summary

Normal aging is associated with a decrease in number and size of neurons, loss of synapses and neuronal branching and with reduced functioning neurotransmitter systems, such as the serotonergic system. These structural and functional alterations have important impact on the behavioural, cognitive and affective status of the individual. With the introduction of functional brain imaging in veterinary medicine, the canine brain can be examined in vivo, evaluating changes in perfusion, metabolism and neurotransmitter systems. Since cognitive decline is recognised in the aging dog, it was our aim to investigate whether age related changes concerning cerebral perfusion and binding index of the selective 5-HT2A receptor ligand ^{123}I-5-I-R91150 could be found in the canine brain. A group of twelve normal, aging dogs, aged 96 months or older, was compared to a normal reference group (N=12), younger than 96 months. SPET images were obtained, using the radiopharmaceutical ^{99m}Tc-N,N"-1,2-ethylene-diylbis-L-cysteine diethylester dihydrochloride (^{99m}Tc-ECD) for evaluation of the regional perfusion and the selective radioligand ^{123}I-5-I-R91150 for visualization of the 5-HT2A receptor. Regional decrease of cerebral blood-flow was noted in the fronto- and temporocortical area and in the subcortical region. Age was negatively correlated with perfusion in the left and right fronto-cortical region. The binding index of the neuroreceptor radioligand was decreased in the fronto-cortical region, with a significant negative correlation with age in the right fronto-cortical area. No correlation was found between alteration of perfusion and binding index of the receptor ligand, suggesting that age related perfusion differences do not influence the binding of this radioligand. These results suggest that age related effects should be considered in functional canine brain imaging.

Introduction

Increasing interest has evolved on the pathophysiology of aging processes in the canine brain, not only to serve the dogs' own benefit but also to create a model for research on the human aging brain. Natural similarities with humans in symptomatology and neuropathologic findings[1] compared to rodents, and the ease to maintain and handle dogs compared to primates makes this model more favourable. Dogs are also more easy to obtain and are less expensive than primates.[2,3] The most obvious histological alterations in the aging canine brain are cell loss and accumulation of β-amyloid in brain tissue and cerebral vessels[4,5], comparable to the alterations found in the aging human brain[1,2,6]. Brain cell apoptosis has been observed in dogs, showing a relationship with the amount of amyloid deposition[7] and correlating well with a dementia index, according to Uchino[8]. Similar apoptotic alterations of brain cells have been found to a larger extent in Alzheimer's disease patients, in comparison to their age matched controls.[9]

Behavioural and cognitive observations in the aging dog, including the evaluation of the effect of environmental stimuli, pharmacological probes and nutritional interventions, pointed at a sociobiological substrate of canine age-related behaviour.[1,10,11] Cortical atrophy and an increase in cerebrospinal fluid containing ventricular spaces was demonstrated with post-mortem investigations and with morphologic imaging techniques, such as magnetic resonance imaging (MRI) and computed tomography (CT).[12,13] Unfortunately, the structural changes in age-associated brain pathology occur later than functional disturbances and show a broad overlap with normal age related changes as is found in human brain.[14,15] Furthermore, structural changes do not correlate with observed neurological disease, as was demonstrated in a study on canine brain ventricular size in normal and pathologic conditions.[16]

The introduction of functional brain imaging in veterinary medicine recently led to the evaluation of the normal canine regional cerebral blood perfusion (rCBF) pattern with ^{99m}Tc-N,N"-1,2-ethylene-diylbis-L-cysteine diethylester dihydrochloride (ECD)[17], enabling also the evaluation of brain metabolism, coupled with cerebral perfusion (already demonstrated in 1890 in dogs)[18], and to the evaluation of neurotransmitter receptor binding with specific radioligands (e.g. ^{123}I-5I-R91150)[19,20], using SPET. Concerning brain perfusion or metabolism in aged subjects, controversy exists about the presence of a global decrease of human[21-24] and canine[13] brain perfusion and metabolism, but evidence for regional decrease is consistent in human literature. Most often, in human studies, reduction in frontal and temporal perfusion or metabolism is demonstrated in aged, but not cognitive disturbed, subjects.[22-28] Moreover, regional decreased metabolism has been demonstrated in aging non-anaesthetised dogs.[29] In general, research concerning *in vivo* SPET and PET receptor imaging with selective ligands could demonstrate age related reduced binding to serotonergic and dopaminergic receptors in the human brain.[30-34] An age dependent decrease in dopaminergic, serotonergic and adrenergic receptor density was also found in rats and primates.[35-40] Increasing evidence emerges that

the serotonergic system, involved in the regulation of mood and behaviour, plays a major role in the behavioural changes seen in age related disorders.[21,41] *In vivo* SPET imaging of the serotonin-2A receptor, was already carried out in a reference population of healthy volunteers and in some clinical populations in human neuropsychiatric studies. These studies demonstrated an age-dependent reduction in serotonin-2A binding index. Up to now, animal studies were only limited to small, preclinical, rodent and primate studies. Recently, we demonstrated the feasibility of the technique in healthy canine subjects.[19,20] In analogy with human studies in old aged subjects, one could expect a reduction in serotonin-2A binding index in old aged but cognitively intact dogs.

A possible relation between changes in brain perfusion and brain serotonin-2a binding index could exist through a mechanism by which the presence of serotonin decreases perfusion by altering vascular tone of the cerebral vasculature.[42] Changes in the permeability of the blood brain barrier (BBB) have been observed when brainstem serotonin raphe neurons are manipulated[43], an interesting finding in view of the age related BBB alterations found recently on MRI in dogs[13]. After all, the serotonergic system plays a role in the innervation of blood vessels and in the modulation of brain perfusion (and metabolism).[43,44] Also, decreased perfusion on itself, may alter the availability of neurotransmitter precursors and in the same line of thinking, altered metabolism may decrease neurotransmitter production.

The aim of this study was to investigate the effects of aging on regional cerebral blood flow and on the binding parameters of the 5-hydroxytryptamine-2A (5-HT2A) ligand. For clinical purposes, knowledge of normal variations and alterations in the distribution pattern of flow tracers in aging brain is necessary when investigating pathological states. In future, knowledge on the perfusion and serotonin-2A status can help in the diagnosis of age-related cognitive decline versus dementia in dogs and can serve as a model for pathophysiological and pharmaceutical intervention studies on age-related cognitive decline and dementia in human subjects.

Methods

SUBJECTS

Twelve "aged" dogs, five males and seven females, all aged 96 months and older, and a reference group (6 males, 6 females), aged less than 96 months, were included. Table 1. All these dogs were privately owned and housed since young age. None of the dogs in both groups had a history of neurological disorders or behavioural abnormalities. No obvious cognitive decline was present in the "old age" group. None of the dogs were on medication or received a specific diet. The Uchino dementia index [8], based on a questionnaire, attributing a value to the cognitive status of the animal, was used to rule out dementia in the aging animals. This questionnaire evaluated cognitive functions such as eating behaviour, social cognition and recognition, housetraining, sensoric and motoric activity and changes in sleep-wake cycle. In addition, computed tomographic brain imaging was performed to rule out brain pathology (scanner Pace Plus, GE Medical Systems, Wisconsin, USA)

The examination procedures never resulted in excitation, anxiety or aggression and were performed according to good animal practice. The two examinations, brain perfusion SPECT and brain 5-HT2A receptor binding SPECT, were performed on separate occasions to account for the half-life of the radionuclides and as a safety precaution, related to the higher sensitivity of older individuals for general anaesthesia.[45]

	Gender	Age (months)	Race	Weight (kg)
Old aged group				
1	F	120	German shepherd	30
2	F	144	Bobtail	26
3	F	108	German shepherd	36
4	F	96	Rotweiler	48
5	F	120	German shepherd	36
6	M	144	Tibetan Terrier	14
7	F	96	Rotweiler	40
8	M	144	Bouvier	38
9	M	108	German shepherd	32
10	M	108	German shepherd	45
11	F	132	Border collie	20
12	M	108	Belgian shepherd	36
Reference group				
1	F	36	German shepherd	26
2	F	84	Belgian shepherd	34
3	M	84	Cross-bred shepherd	33
4	F	12	Border collie	16
5	M	84	Cross-bred shepherd	40
6	M	48	German shepherd	38
7	M	12	German shepherd	30
8	F	72	Belgian shepherd	28
9	M	48	German shepherd	30
10	F	24	German shepherd	25
11	M	12	Cross-bred shepherd	23
12	F	72	German shepherd	29

Table 1: Demographical data of the included dogs

Radiopharmaceuticals

SEROTONIN-2A RECEPTOR BINDING TRACER

The receptor binding studies were performed with ^{123}I-5-I-R91150 as radiotracer. ^{123}I-5-I-R91150 is synthesized by electrophilic substitution on the 5-position of the methoxybenzamide group of R91150, followed by purification with high-performance liquid chromatography. The product has a radiochemical purity of more than 99 % and is sterile and pyrogen free. A specific activity of 370 MBq/µmol is obtained. The tracer is a 5-HT2A antagonist with high affinity (K_d = 0.11 nM) and selectivity for 5-HT2A receptors. The selectivity of the ligand for 5-HT2A receptors with regard to other neurotransmitter receptors such as other 5-HT receptors, including 5-HT2C and 5-HT1A, dopamine receptors (D1 and D2), α1 and α2 adrenergic receptors and histamine receptors is at least a factor of 50. The tracer is displaceable with the 5-HT2 antagonist ketanserin.[46,47]

The IV injected activity ranged from 4.1-7.8 MBq/kg (0.11-0.21 mCi/kg) and the radiopharmaceutical was injected 100 minutes prior to image acquisition. The optimal scanning time, the time when pseudo-equilibral conditions are reached between free and bound radiotracer, was determined in a preliminary study from 90 minutes onwards.

BRAIN PERFUSION TRACER

Perfusion studies were performed with ECD labelled with ^{99m}Technetium. The perfusion tracer ^{99m}Tc-ECD (Neurolite®, Dupont Pharmaceuticals Ltd, Brussels, Belgium) is a lipophilic compound, permitting blood brain barrier passage. It is chemically stable in vitro for several hours after reconstitution. Its fast cerebral retention, rapid blood clearance of metabolites and fast clearance from the extracerebral tissues results in a favourable signal to noise ratio.[48] Care was taken that all procedures provoked minimal excitement of the animal. The catheter was placed intravenously 10 minutes before injection of the tracer to avoid tension accompanying this procedure. The radiopharmaceutical was injected 15-20 minutes prior to the induction of general anaesthesia, in the examination room, free of noise and with dimmed light. The injected activity ranged from 16-32 MBq/kg (0.43-0.86 mCi/kg).

Image acquisition

ANAESTHETIC PROTOCOL

All animals required anaesthesia in both imaging protocols. The anaesthetic protocol was evaluated in prior studies on brain perfusion and receptor binding.[17,19,20] Sedation was obtained with 10-30µg/kg medetomidin hydrochloride IM (Domitor®, Pfizer) and general anaesthesia was induced with 2-3mg/kg iso-propylphenol IV (Rapinovet®, Mallinckrodt) and maintained with halothane (Fluothane®, Zeneca) to effect.

ACQUISITION AND PROCESSING PROTOCOL

All dogs were positioned in ventral recumbence. The head was positioned on a sort of moulage, in order to minimize individual positioning variability. SPECT was performed with a triple head gamma camera (Toshiba GCA-9300A, Dutoit Medical, Antwerp, Belgium), equipped with high resolution fan beam collimators (full width at half maximum (FWHM): 7.8 mm).

For both studies, total acquisition time was 24 minutes, including a transmission (2 x 2 min) prior to the emission (2 x 10 min) acquisition. For each acquisition, 90 projection images were obtained on a 128x128 matrix using a continuous scan mode by rotating each head 120°. The images were reconstructed with filtered back projection after rebinning to parallel data, and applying a Butterworth-filter (cut-off 0.16 cycli/pixel, order 8). Pixel size was 1.72 mm. Uniform Sorensen attenuation correction with a linear attenuation coefficient of 0.12 /cm and triple-energy window scatter correction were applied. The individual image perfusion data were automatically registered to a template, generated from 12 normal dogs, 6 males and 6 females (aged between 12-84 m, mean 49 m; SD: 29), using BRASS software (Brain Registration and Automated SPECT Semiquantification, Nuclear diagnostics). On this template, 11 manually drawn volumes-of-interest (VOI) were defined with inclusion of all grey matter. (Fig 1). The division of the anatomical brain regions was chosen according to the proposal of Redding.[17,49]

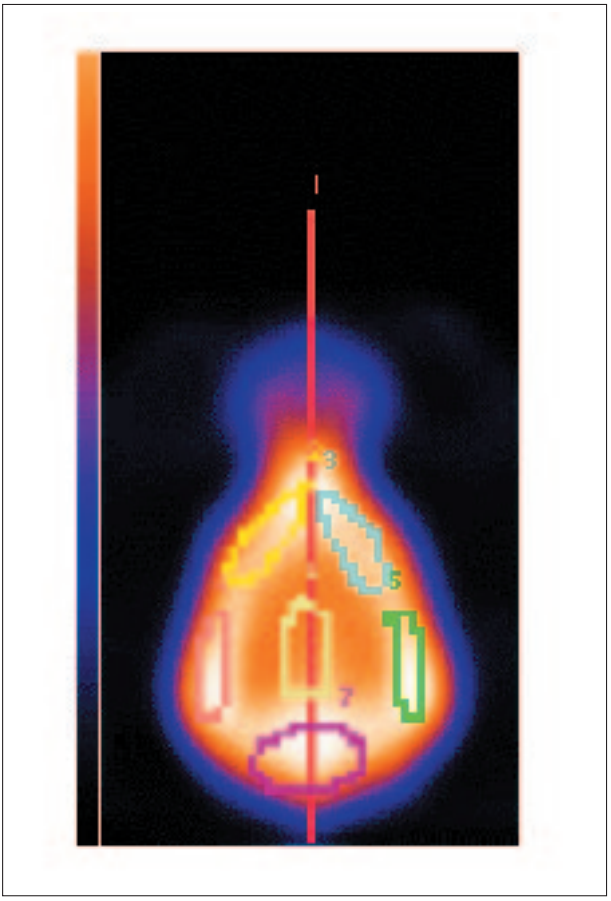

Figure 1: Horizontal slice at the level of the lower mid-brain, showing the manually drawn volumes of interest on a perfusion image. 2. right frontal cortex; 3. left frontal cortex; 4. left temporal cortex; 5. right temporal cortex; 6. subcortical region; 7. cerebellum

The use of this template in the brain perfusion studies eliminates subjectivity due to operator dependent region definition and the automatic registration facilitates the fitting procedure. This template could not directly be used for the serotonin-2A ligand studies since automatic registration is not applicable for the majority of these studies because of the higher intersubject variability and lack of anatomical reference. For the serotonin-2A ligand studies, the emission data were matched with the emission ^{99m}Tc-ECD perfusion data with the aid of their corresponding transmission data, showing sufficient contrast to recognize bone and sinuses. Fitting of these data was performed with the Multimodality software of Nuclear Diagnostics (Hägersten, Stockholm, Sweden). This computer program, displaying images in a dual window setting, allows for manual co-registration by providing tools for scaling, rotation and translation images in all three dimensions. The values of the parameters used to register the transmission computed tomography (TCT) map of the radioligand data to the TCT map of the perfusion data were then applied to the radioligand emission data. Since regions of interest were predefined on the perfusion data, we were able to extent the division of anatomical regions to the serotonin-2A receptor radioligand data.

Data interpretation

The uptake in the cerebellum (a region free from 5-HT2A receptors) was used as a reference for non-specific binding in addition to free ligand. Radioactivity measured in the cortical areas was assumed to represent the total activity (i.e. specific + non specific activity + free ligand).[50] The binding index (BI) was operationally estimated as (counts/pixel in regional cortex) / (counts/pixel in cerebellum). This binding index is directly related to the *in vivo* receptor density and affinity under near equilibral conditions.[51] Concerning the perfusion data a regional perfusion index was obtained by normalising the regional counts per pixel to the registered cerebellar activity per pixel * 100.

Statistical analysis

The equality of age, weight, injected dose per kg body weight of ^{123}I-5I-R91150 and ^{99m}Tc-ECD between the two groups was evaluated according to the Student's t-test. The equality of gender-distributions was evaluated through Pearson's Chi Square. Paired Student's t-statistics were applied to evaluate left-right differences within diagnostic groups. Independent Student's t-statistics were applied to evaluate differences in binding index and perfusion index between diagnostic groups. Pearson correlation analysis was used to examine any relationships between binding index, perfusion index and injected tracer dose per kg body weight. In order to evaluate radioligand binding and perfusion differences across groups, a general linear model procedure was applied and significance levels were adjusted for multiple comparisons through Bonferroni-correction.

Results

Besides ventricular enlargement, no structural abnormalities were present on CT images obtained from all aged dogs.

Description of population

Aged dogs had a mean age of 119 months (SD 18; range 96-144) and reference dogs (young to middle age) had a mean age of 49 months (SD 29; range 12-84), yielding a statistical significant difference (t= -7.0; P< 0.001). Gender was not significantly different (χ^2=0.17; P=0.68) between aged dogs (5 males; 7 females) and the young to middle age group (6 males; 6 females). There was no significant difference in weight (t= -1.20; P=0.24) between aged dogs (mean 33.4 kg; SD 9.8) and reference dogs (mean 29.3 kg; SD 6.6). The dementia index, according to Uchino[8] was below 21 in all aged dogs, indicating absence of cognitive dysfunction.

Regional brain perfusion

Injected dose of the radiopharmaceutical ^{99m}Tc-ECD per kg body weight was not significantly different (t=0.37; P=0.72) between aged dogs (mean 0.72 mCi/kg; SD 0.12) and reference dogs (mean 0.74 mCi/kg; SD 0.09). Regional brain perfusion in none of the studied regions in both populations was correlated with injected dose.

Table 2 depicts regional cerebral perfusion. Regional brain perfusion was significantly lower in aged dogs compared to the reference dogs in the right (t= 2.56; P= 0.02) and left frontocortical region (t= 3.60; P< 0.001), right (t= 4.07; P= 0.001) and left temporocortical region (t=3.18; P=0.01). (Fig 2). There was also a significant lower perfusion (t= 3.67; P= 0.001) in the subcortical region in aged dogs compared to reference dogs. There were no other significant differences between the reference and aged subjects.

Regional cerebral perfusion						
Region	Reference subjects (N=12)		Aged subjects (N=12)		Statistics	
	Mean	SD	Mean	SD	t	P
Right Frontal	91.4	4.0	87.6	1.9	2.56	0.02
Left Frontal	92.5	4.9	87.5	2.1	3.60	< 0.01
Right Temporal	92.9	4.1	86.5	3.6	4.07	0.001
Left Temporal	92.2	4.4	85.8	5.3	3.18	0.01
Right Parietal	97.4	3.7	95.6	2.0	1.51	0.15
Left Parietal	96.9	4.5	94.0	2.6	1.93	0.07
Right Occipital	97.7	4.0	95.0	3.2	1.79	0.09
Left Occipital	96.8	4.0	94.2	2.7	1.90	0.07
Subcortical	95.3	3.5	87.0	7.0	3.67	0.001
Subcortical-cortical ratio	1.01	0.03	0.96	0.07	2.08	< 0.05
Right fronto-occipital ratio	0.94	0.03	0.92	0.03	1.06	0.30
Left fronto-occipital ratio	0.96	0.05	0.93	0.04	1.51	0.14

Table 2: Mean and standard deviation of the regional cerebral brain perfusion in cortical and subcortical regions as assessed with 99mTc-ECD as perfusion tracer, in aged dogs and reference dogs.

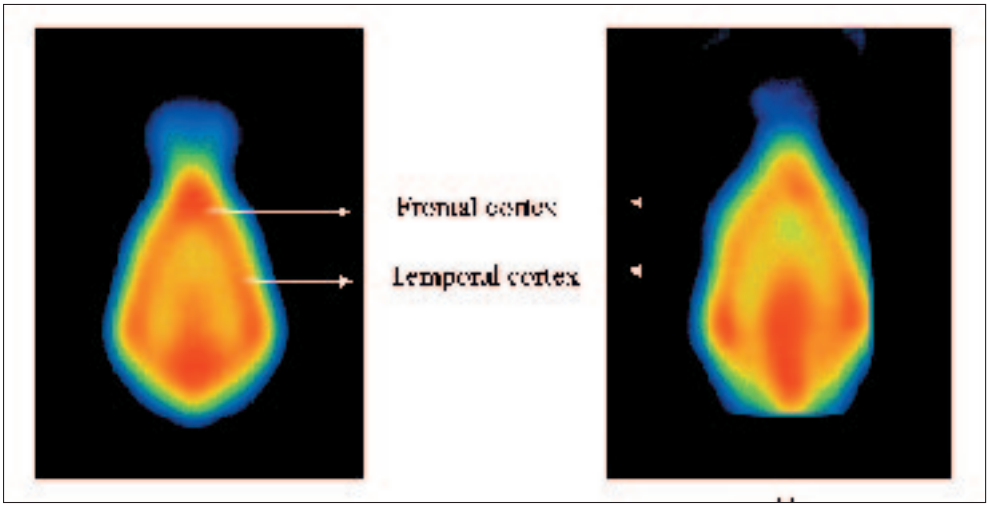

Figure 2: Horizontal slices at the level of the lower mid-brain demonstrate decreased perfusion in the fronto- and temporocortical brain regions in a reference dog (A) compared to an old dog (B)

Comparisons of left-right, cortical-subcortical and anterior-posterior perfusion ratios are presented in Table 3. In the aged dogs population there was a significant difference between right and left perfusion in the parietal cortex ($r= 0.82$; $P< 0.01$). Right and left frontal ($r= 0.58$; $P< 0.05$) and temporal ($r= 0.87$; $P< 0.001$) perfusion were correlated. Cortical and subcortical perfusion were significantly correlated ($r= 0.60$; $P< 0.05$) in the reference but not in the aged group.

Regional cerebral perfusion								
Regional Paired comparisons	Reference subjects (N= 12)				Aged subjects (N= 12)			
	Difference		Paired T statistics	Correlat Statistics	Difference		Paired T statistics	Correlat Statistics
	Mean	SD			Mean	SD		
R-L Frontal cortex	-2.00	2.52	-1.29	0.80**	0.08	1.83	0.16	0.58*
R-L Temporal cortex	0.75	4.07	0.64	0.91***	0.67	2.84	0.81	0.87***
R-L Parietal cortex	0.50	3.55	0.49	0.64*	1.58	2.39	2.29*	0.49
R-L Occipital cortex	0.83	2.95	0.98	0.70**	0.83	3.51	0.82	0.32
Subcortical-Cortical	0.52	3.03	0.60	0.60*	-3.80	6.60	2.00	0.35
Right Fronto-Occipital gradient	-6.25	2.6	-8.33***	0.42	-7.42	3.32	-7.75***	0.25
Left Fronto-Occipital gradient	-4.33	4.52	-3.32**	0.84*	-6.67	3.7	-6.24***	-0.16

*P< 0.05; ** P< 0.01; *** p< 0.001

Table 3: Mean and standard deviation of the left-right and subcortical-cortical regional brain perfusion. as assessed with 99mTc-ECD as perfusion tracer, in aged dogs and reference dogs. Correlation coefficients are calculated.

There was a significant correlation between the mean global cortical perfusion and age in the whole population (r=-0.36; p=0.04), which was absent in both populations when considered as two separate groups. (Fig 3).

Concerning the regional cerebral blood flow, a significant correlation between age and left frontal perfusion (r=-0.71; P< 0.001) and right frontal perfusion (r= -0.52; P< 0.01) was found.

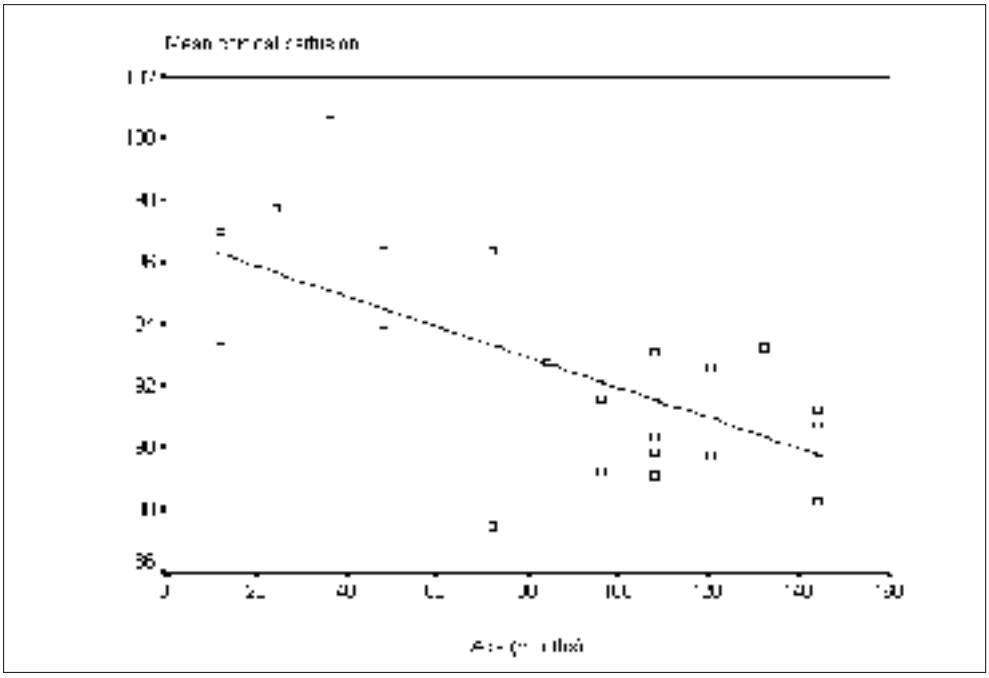

Figure 3: Scatterplot of mean cortical brain perfusion and age in the total population

SEROTONIN-2A RECEPTOR BINDING INDEX

Injected serotonin-2A tracer (^{123}I-5I-R91150) dose per kg body weight was not significantly different (t= -0.03; P=0.98) between aged dogs (mean 0.19 mCi/kg; SD 0.08) and reference dogs (mean 0.19 mCi/kg; SD 0.06). In both the aged dogs and reference dogs group, the serotonin-2A binding index in all studied regions was not correlated to injected dose per kg body weight.

Table 4 depicts regional serotonin-2A binding indices. Regional serotonin-2A binding index was significantly decreased in aged dogs in the right (t= 3.67; P= 0.001) and left frontal cortex (t= 5.53; P< 0.001) compared to the reference group. (Fig 4). There were no other significant regional differences between the reference and aged subjects.

Serotonin-2a Receptor Binding Index						
	Reference subjects (N=12)		Aged subjects (N=12)		Statistics	
	Mean	SD	Mean	SD	t	P
Right Frontal	186.6	8.2	170.9	12.3	3.67	0.001
Left Frontal	188.1	8.2	165.3	11.8	5.53	<0.001
Right Temporal	163.3	16.3	160.0	21.3	0.42	0.68
Left Temporal	160.4	17.9	156.3	22.5	0.50	0.62
Right Parietal	132.8	32.2	134.3	35.0	-0.11	0.95
Left Parietal	131.5	28.3	139.9	35.1	-0.65	0.55
Right Occipital	147.9	20.3	138.2	33.6	1.74	0.10
Left Occipital	138.9	20.9	125.1	25.9	1.15	0.26
Thalamus	120.9	36.8	97.8	27.0	1.76	0.09
Subcortical-cortical ratio	1.0	0.03	0.96	0.07	2.08	0.05
Right fronto-occipital ratio	1.28	0.17	1.41	0.34	-1.16	0.26
Left fronto-occipital ratio	1.38	0.21	1.42	0.43	-0.3	0.77

Table 4: Mean and standard deviation of the left-right and subcortical-cortical regional brain perfusion. as assessed with 99mTc-ECD as perfusion tracer, in aged dogs and reference dogs. Correlation coefficients are calculated.

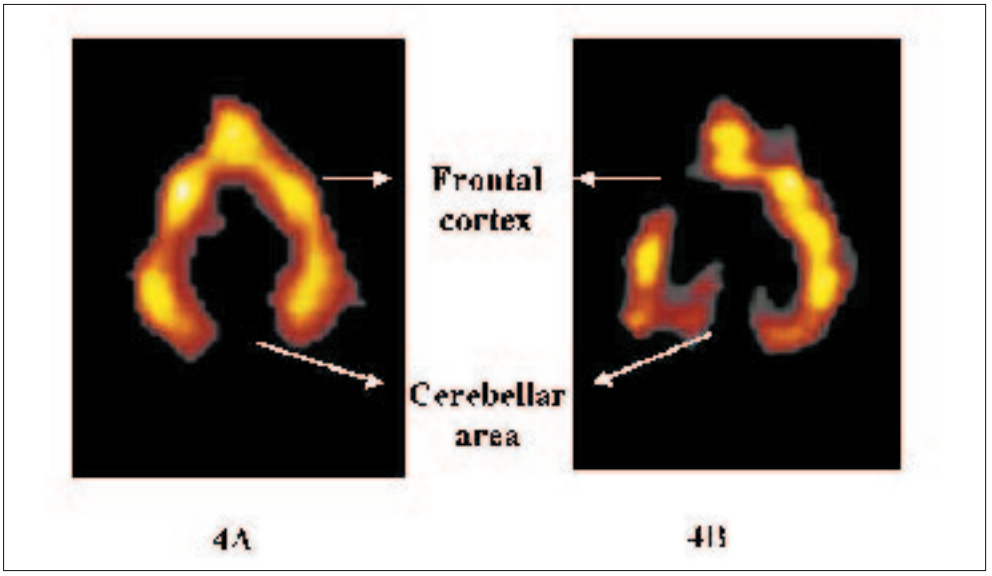

Figure 4: Horizontal slices at the level of the lower mid-brain. Higher radioactivity is registered in the left frontocortical region of the reference dog (A) compared to the old dog (B). Note the lack of radioactivity in the cerebellar area.

Comparison of serotonin-2A binding index ratios concerning left-right, cortical-subcortical and anterior-posterior brain regions are presented in Table 5. A significant difference between the right and left sided binding index in the frontal cortex ($t= 2.7$; $P= 0.02$) was present in the aged group. The serotonin-2A binding index in right and left cerebral cortex was correlated only in the parietal cortex ($r= 0.80$; $P= 0.002$) in the younger population. In the aged group, correlation with age was found in the frontal ($r= 0.82$; $P= 0.001$), the temporal ($r= 0.50$; $P= 0.01$), in the parietal ($r= 0.72$; $P= 0.009$) and in the occipital ($r= 0.75$; $P= 0.005$) cortex. A significant lower binding index was found in the subcortical region both in the reference population ($t= -3.68$; $P= 0.004$) and in the old dogs population, ($r=-5.36$; $P< 0.001$) compared to cortical binding.

Serotonin-2a Receptor Binding Indexn								
Regional Paired comparisons	Reference subjects (N= 12)				Aged subjects (N= 12)			
	Difference		Paired T statistics	Correlat Statistics	Difference		Paired T statistics	Correlat Statistics
	Mean	SD			Mean	SD		
R-L Frontal cortex	- 1.5	7.7	- 0.67	0.57	5.7	7.3	2.67 *	0.82 **
R-L Temporal cortex	2.8	15.8	0.62	0.58	3.8	21.9	0.59	0.50 **
R-L Parietal cortex	1.3	19.6	0.24	0.80**	-5.6	26.4	-0.73	0.71 **
R-L Occipital cortex	9.0	20.6	1.51	0.50	3.1	24.6	0.43	0.75 **
Subcortical-Cortical	-35.3	33.2	-3.68**	0.50	-49.8	32.1	-5.4***	0.03
Right Fronto-Occipital gradient	38.7	19.6	6.85 ***	0.29	42.8	29.4	5.04 ***	0.51
Left Fronto-Occipital gradient	49.2	22.2	7.67 ***	0.026	40.2	29.0	4.80 **	0.70 *

*P< 0.05; ** P< 0.01; ***P< 0.001

Table 5: Mean and standard deviation of the left-right and subcortical-cortical regional serotonin-2a binding index, as assessed with the 123I-5I-R91150 tracer, in aged dogs and reference dogs. Correlation coefficients are calculated.

A significant correlation was present between age and right frontal serotonin-2A binding index (r= -0.60; P= 0.04). This is plotted as Fig 5.

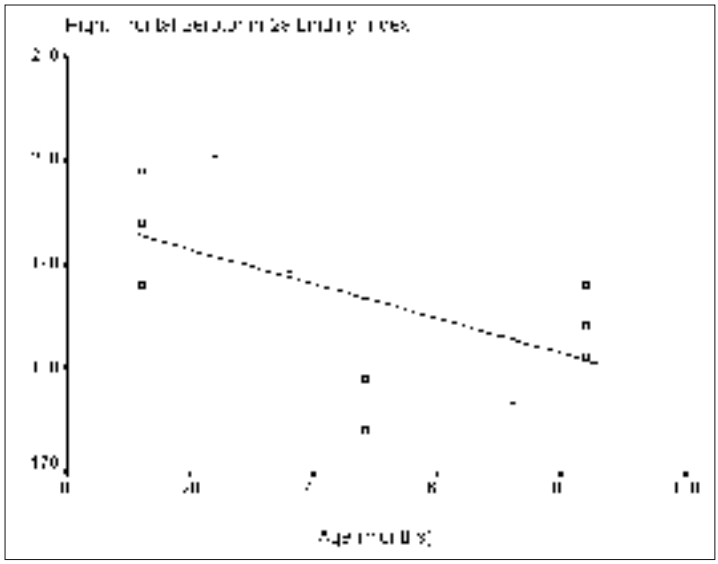

Figure 5: Scatterplot of right frontal serotonin 2a binding index and age in reference dogs population.

REGIONAL BRAIN PERFUSION AND SEROTONIN-2A BINDING INDEX

There were no significant correlations between serotonin binding index and regional brain perfusion in the reference population and in the population of aged dogs.

Discussion

Cerebral perfusion studies

In this study, decreased global cerebral perfusion was present, combined with a significant regional decrease in fronto-, temporocortical and subcortical region perfusion in the aged group compared with the reference group. Regression analysis revealed a significant decrease of perfusion in the frontal cortex with age. Our finding of a global reduction of cortical perfusion is in line with most, but not all, findings in human medicine literature indicating a decrease in global age-related brain perfusion and metabolism.[22,23,27,52] However, in dogs, a dynamic contrast-enhanced MRI study could not reveal global vascular volume changes related to age.[13] With regard to regional changes, a decrease in both left and right sided frontal and temporal regions and the subcortical region was found in this study. This finding is comparable to most human studies showing a decrease in metabolism and perfusion with age, mainly in the fronto-, temporo-, parietocortical and hippocampal, limbic area and basal ganglia[22,23,25-27,53] and is also in line with the findings in a study on regional metabolism in dogs, demonstrating continuous decline of the metabolic rate in some brain areas including the frontal and temporal cortical regions after the age of six years[29]. In human studies, these age related changes in the rCBF are already evident in early to mid-adulthood in the absence of cognitive decline or other functional degenerative processes[14,54] and in dogs a significant decrease in metabolic rate is found between 1 and 6 years in the majority of examined brain regions[29]. We could not demonstrate these early and mid-adulthood changes in rCBF. To our belief, the relatively small number of reference subjects in the adulthood group lacks power to investigate the age of onset of perfusion decline in normal dogs.

The regional reduced uptake of the radiopharmaceutical in the aging brain could result from a decreased blood supply as a result of a tendency to cerebral vasoconstriction caused by decreased distensibility of the vessel wall due to altered vascular innervation and responsiveness[21] and/or amyloid angiopathy[55]. Moreover, β-amyloid accumulation is most consistently found in the frontal cortex at an early stage in the canine aging process[56] which is in line with the perfusion alterations in the frontal cortical area in this study. On the other hand, due to tissue loss or reduction in neuronal synapses, local requirements for energy and oxygen might be reduced, resulting in decreased blood flow. A third reason of decreased blood supply could lie in the fact that enzymatic retention of the radiopharmaceutical itself might be altered with age.[22,25]

A significant anterior-posterior perfusion gradient, to the detriment of the frontal regions, was found in the aged group. This is in accordance with human PET and SPET studies[14,23,25,28] Indeed, the posterior brain (cerebellum, parieto-and occipitocortical region) seems to be less vulnerable to aging effects. This finding could fit the clinical observation that the cognitive, affective and behavioral changes, seen with aging, are more related to altered functioning of the frontal cortex region and that

the posterior regions, involved in receptive sensory functions (occipital cortex) are less involved in the aging process.[25] Only in the parietocortical region, a right/left hemispheric difference was found in the aged subjects. Although there is no clear explanation for this asymmetry, this finding is in line with some human studies, indicating that the left hemisphere appears to be more vulnerable to aging effects.[25,28] However, other studies only found minimal or no hemispheric differences and assumed symmetry between homologous regions.[23,26,53]

No gender influence was noted in this series. Since no functional imaging studies have been performed on dogs, no comparison with former findings is possible. Nevertheless, a post-mortem study in aged dogs on ventricular size revealed no gender differences.[12] In humans, a controversial and probably marginal gender difference with PET and SPET studies is noted for the frontal, temporal, parietal and cerebellar areas.[14,24,25,57]

In human studies, increased standard deviations are found in the aged group in most cerebral regions, presumably due to intersubjective variable cerebral atrophy.[22,26] However, in this study, intersubject variability in rCBF values was lower in the aged group compared with the reference group, most likely due to the smaller age variability in the aged group.

SEROTONIN-2A RECEPTOR BINDING STUDIES

A decreased binding index was found only in the fronto-cortical area in the aged group compared to the reference group. In humans, a more widespread age related reduction is found with ^{18}F-altanserine (a selective 5-HT2A ligand for PET) and with the same ^{123}I-5-I-R91150 ligand for SPECT, involving the whole neocortex.[34,58] In one study with 18F-N-methylspiperone (a PET ligand for dopamine D2 and serotonin 5-HT2A receptors), the decline of 5-HT2 receptor availability with age was greater in the frontocortical area than in the occipitocortical region.[32] In monkeys, reduced binding was observed in the cortical areas but no decrease was found in the subcortical regions.[35]

Tracer uptake did not seem to be influenced by age related regional perfusion changes since no correlation with perfusion was present.

No influence of gender was found in this series, which is in accordance with findings in human studies.[34,58]

METHODOLOGICAL CONSIDERATIONS

Firstly, in contrast to human studies, general anaesthesia is required to perform these examinations. With regard to the perfusion studies, the radiopharmaceutical was injected 15 minutes prior to induction of general anaesthesia, at which stage the tracer is already trapped intracellular.

Concerning the ligand studies, global cerebral blood flow alterations as result of the general anaesthesia, are inevitable and may influence the pseudo-equilibral state of the tracer. However, it is unlikely that the regional variation of activity is due to effects of anaesthetics. Also, all dogs received exactly the same anaesthetic protocol for both studies.

Secondly, in order to obtain semi-quantitative perfusion data, the different regions have to be compared with a reference area, unaffected by the physiological or pathological alterations being investigated. In this study, cerebral regions were normalized to the cerebellum, since this region seems to be least affected by aging changes in humans and in dogs.[26,28,59-61] When using total counts as a normalization entity, perfusion impairment in some regions, influencing the total brain counts, might result in false relative rCBF changes.[26] Another methodological consideration implies crossed cerebellar diaschisis. In this study reductions in cortical perfusion in the old dog population were symmetrical, hence, if existent, one would expect bilateral "crossed" reductions in cerebellar perfusion. Due to the use of the semi-quantification method this phenomena cannot be ruled out. However, it is unlikely that this would affect the semi quantitative results of altered cortical perfusion. Based on the aforementioned positive criteria of using the cerebellum as reference region, possible crossed cerebellar diaschisis effects were neglected.

Thirdly, cortical atrophy may increase the partial volume effects (PVE), confounding the semi-quantitative measurements of both perfusion and ligand data. The answer to this problem is co-registration of functional and structural data, such as obtained with MRI, providing voxel based morphometry and correction for partial volume effects. In human literature, these corrections for PVE have been mainly performed on PET studies on brain metabolism in research on Alzheimer disease, confirming the regions of hypometabolism after correction for atrophy.[62,63] In conclusion, although part of the metabolic alterations seen with PET are the result of structural changes, these changes reflect decrease in neuronal size and shrinkage of synapses and therefore may relate to age induced functional and, coupled, perfusion alterations.[23] Concerning the influence of cortical atrophy on the binding parameters of the 5-HT2A ligand, it was shown in one human study, with the radioligand ^{18}F-altanserin, that the wide spread cortical reduced activity remained after partial volume correction, obtained by MRI derived regional correction factors.[33]

Conclusion

A database is generated for the normal aging canine brain, including perfusion data and binding parameters for the selective 5-HT2A ligand. A significant decreased perfusion is noted in the fronto- and temporocortical regions and the subcortical area. Binding parameters for the ^{123}I-5-I-R91150 selective ligand decrease in the frontal regions, but only the right frontal area is correlated with age. Although partial volume effects cannot be ruled out based on this investigation, studies on human brain find similar age related changes, remaining when corrected for atrophy. When examining the diseased brain it is essential to have normal age matched data. Another important conclusion of this report is that age correction should be considered when interpreting perfusion and serotonin-2A radioligand data in functional imaging studies of the dog's brain.

Future research, focusing on the regional perfusion changes and alterations in binding parameters of the serotonin-2A receptor, in the very old aged dogs and dogs with a declined cognitive status, is mandatory. A comparative investigation between different sized dogs would be interesting since it is shown in dogs that size is inversely correlated with lifespan.[64] Further studies should also include a larger number of dogs in the group younger than 96 months, to evaluate the linearity of the decrease of the serotonin-2A radioligand binding since in humans a more rapid decline is observed in early adult life.[58,65] These studies should also explore the time course of aging effects on regional perfusion indices. Another possible field of future investigation is the impact of pharmacologic interventions on brain perfusion and receptor kinetics in old aged and clinically demented dogs. It would be of considerable interest to extent this methodology to the investigation of aging effects in the canine brain to other neurotransmitter systems. The radioligands currently implemented in research and clinical evaluation of normal and pathological aging are ^{123}I-2 β-carbomethoxy-3 β-(4-iodophenyl) tropane (^{123}I β-CIT), for visualization of the dopamine and serotonin transporters, and ^{123}I-epidepride or 123I iodobenzamide (IBZM), for the D2 receptor. Unfortunately, at this moment not all neurotransmitter systems can be evaluated with SPET, due to the technical difficulties encountered in the production of selective radioligands.

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CHAPTER 7

Functional brain imaging of serotonin-2A receptors in impulsive dogs: a pilot study

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Summary

Impulsive, disinhibited behaviour in dogs, exposed as aggression, has major impact on public health. Measures might be taken without real understanding of the underlying pathology. It would therefore be both of pathophysiological as well as clinical relevance if a research paradigm could be developed to investigate this aberrant behaviour in dogs. This article first aims at reviewing literature concerning functional-anatomical and biochemical research on animal impulsivity. Based on this research, a hypothesis involving the prefrontal serotonin-2A receptor in the frontal cortex is generated and the feasibility of estimating the 5-HT2A receptor with Single Photon Emission Computed Tomography (SPECT) and the highly selective receptor radioligand ^{123}I-5-I-R91150 is presented in a pilot study. Confirmation of this hypothesis may offer an in vivo research tool to investigate behavioural disorders and a modality to monitor the effects of pharmacologic interventions and behaviour therapy.

Introduction

Definition and classification of aggressive and impulsive behaviour

In literature, there is a striking lack of agreement on definitions and concepts related to aggression and impulsivity.[1] A range of experts with a divergent expertise, from ethologists, zoologists, veterinarians and law enforcement officials are involved in the study of aggressive and violent behaviour, with each his own viewpoint and own operant definition and classification.[2]

Aggression in animal research can be defined as "a manifestation of competition, the active demand by two or more individuals for a common limited source, such as food, reproduction (access to mating), or territory". In this context, predatory aggression, intermale aggression, maternal aggression and territorial aggression are described. These types of behaviours are in-born and instinctive in nature and hence, are not deviant, although they can be beyond control.[2] Domestication of animals included the acceptance of these types of animal aggression by the humans. The aggressive behaviour of the animal is considered as normal and is tolerated as long as it is appropriate in relation to the environmental conditions and stimuli.

Disinhibition and *impulsivity* in human research are defined as "acts related to inadequate self-control or impaired impulse-control".[3] In animal research, impulsivity is related to the "incapacity to wait or to delay response".[4] The "inappropriateness" of impulsive behaviour can be illustrated by the literal definition of the word impulse as " a sudden spontaneous inclination or incitement to some usually unpremediated action".[5] Since this impulsive and disinhibited behaviour is inappropriate and difficult to foresee, compared to more predictable and "appropriate" aggressive behaviour, it is more threatening and must therefore serve as a focus for treatment. Moreover and beyond the academic discussion of the most correct term, this inappropriate "impulsive behaviour", often aggressive in nature, seems to have a different biological basis as compared to the appropriate "aggressive behaviour".[3] Since human biological psychiatric research offers an acceptable research hypothesis on impulsive behaviour, often but not always violent in nature, the research strategy described in this article is directed to the study of "impulsive animal subjects" and not to "highly aggressive" ones that show no impulsivity.

Anatomical brain-behaviour relationships in aggression and impulsivity

As a general conclusion of anatomical studies, both subcortical and cortical brain regions were found to be involved in aggression and impulsivity. Initially based on ablation and stimulation studies in animals, a model of aggressive drive, driven by the subcortical limbic system, and supervisory inhibition, directed by the (pre)frontal cortex was proposed from the 30s to the 60s and further developed and refined up to now.

In this model, on one hand, the *limbic system* can be considered as a primitive system, directed towards survival, where unrefined feelings are generated and pleasant versus unpleasant emotions are discriminated. It constitutes of the hypothalamus, amygdala, the hippocampus, septal nuclei and anterior cingulate gyrus. The *hypothalamus* is the central core from which all emotions derive their motive force but the emotional states that are elicited in the hypothalamus are very primitive (pleasant versus unpleasant), undirected (reflexive) and unrefined (on/off manner)[6] and seem to provide the immediate discharge of tensions in an almost reflexive manner, without concern or understanding of its consequences. For instance, stimulation of the lateral hypothalamus induced extremes in emotionality, including intense attacks of rage accompanied by biting and attack upon any moving object.[7] If the hypothalamus nucleus is destroyed, aggressive and attack behaviour is abolished.[6] In contrast to the primitive hypothalamus, the more recently developed *amygdala* is pre-eminent in the control and mediation of all higher-order emotional and motivational activities, including aggression. Firstly, it is able to modulate and even control rudimentary emotional forces governed by the hypothalamus.[6] Secondly, the amygdala, via environmental surveillance, can discover a potentially threatening stimulus, and then act to excite and drive the hypothalamus to take appropriate action.[6] The role of the amygdala is described as necessary for the animal's selection of aggressive (or submissive) behaviour appropriate for a given social situation[2] and lesions disrupt the ability of the animal to conform its aggressive (or submissive) behaviour to established social norms.

On the other hand, already since the third decade of the twentieth century, it was described that the *prefrontal cortex* is involved in the behavioural inhibition of drives. This finding was based on the observation of an abnormal voracity and aggressiveness in animals with large frontal lesions.[8] These ablation studies had far more than a passing historical significance since the early reports in which "placidity" in primates after frontal lesions was described, led to the common neurosurgical practice of Egas Moniz. He instituted prefrontal lobotomy as a form of treatment for certain emotional disorders, especially aggressive conduct disorders, in human psychiatry. The behavioural consequence of ablation studies largely depended on the anatomical region of the prefrontal cortex that was removed. The prefrontal cortex in mammals can be divided in a medial frontal cortex, comprising the orbital and inferior convexity, and a lateral frontal cortex, comprising the dorsal convexity. Both lesions of the inferomedial frontal cortex and the dorsolateral cortex are associated to a possible increase in aggressive conflicts, albeit based on totally different underlying cognitive mechanisms.

Biochemical substrates in aggression and impulsivity

The influence of the neurotransmitters serotonin, norepinephrine and dopamine, and of hormones, particularly sex hormones, is demonstrated in aggressive behaviour in animals.[9] In biological psychiatry, most research on impulsivity was carried out with probes investigating the

serotonin system. Studying the impact of the serotonin system on behaviour can be done by indirect and direct studies. *Indirect studies* involve the measurement of serotonin and its principal metabolite 5-HIAA in plasma and cerebrospinal fluid (CSF), the manipulation of the serotonergic system by interfering in the synthesis, by quantification of the serotonin receptors on blood platelets and by influencing the serotonergic system with pharmacological interventions.[10-17] *Direct studies* involve brain measurements of serotonin and its metabolites via *in vivo* microdialysis studies, assessment of receptor density and function with postmortem autoradiographic and immunohistochemical studies and with functional brain imaging studies *in vivo*.[18-23] Most studies point at a deficient serotonergic system. However, important research limitations lie in the quick postmortem alterations of brain receptors, in the unproven correlation between peripheral and central nervous measurements and in the imprecise targeting of one type of neurotransmitter or receptor. But besides methodological problems, an important shortcoming of the majority of these techniques is the absence of any topographical localization of the biochemical deficit.

FUNCTIONAL NEUROIMAGING TECHNIQUES

Both single photon emission computed tomography (SPECT) and positron emission tomography (PET) can be used for *in vivo* imaging of the brain using specific radioligands. These techniques offer access to the "living" brain, creating the possibility to investigate pathophysiology of behavioural disorders such as aggression and impulsivity, to investigate the effect of environmental stimuli and to evaluate the effect of pharmacological interventions. Another important advantage is that the interaction of different neurotransmitter systems can be investigated with so-called challenging experiments, where altered binding properties of a specific radioligand to receptors of a particular neurotransmitter system may be explored by manipulation of another neurotransmitter system.[23] In the context of the investigation of impulsivity and aggression, functional neuroimaging studies with SPECT or PET, using specific radiolabelled tracers to assess brain perfusion or metabolism, demonstrated prefrontal hypoperfusion or hypometabolism in the prefrontal and temporal cortex in violent humans.24 (Fig 1).

Recently, the feasibility of the serotonin-2A receptor radioligand ^{123}I-5I-R91150 in the estimation of the 5-HT2A canine brain receptor index was demonstrated.[25,25,26] (Fig 2). In the further described pilot study, this specific radioligand was used to investigate the 5-HT2A receptor binding index in impulsive, aggressive dogs.

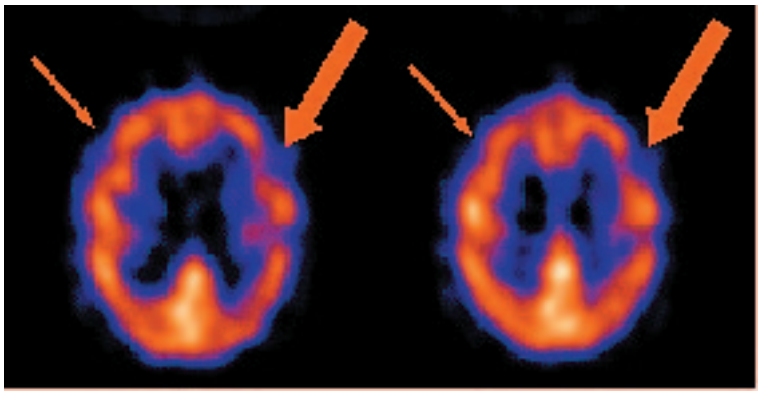

Figure 1: Perfusion defects are seen in the left fronto-cortical brain region (large arrows) compared to the contralateral side (small arrows) in an aggressive human patient. These SPET images (transversal slices) are obtained with the radiopharmaceutical ethyl cysteinate dimer (ECD) labeled with 99m-Technetium.

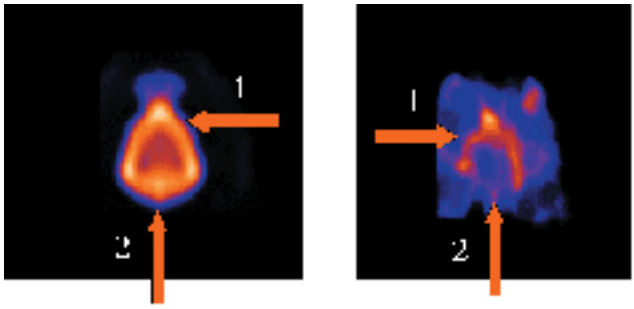

Figure 2: The left image demonstrates the regional brain perfusion in a normal dog. The right image reflects the distribution of the serotonin-2a receptor, visualized with the specific radioligand 123I-5-I-R91150. Both horizontal slices are taken at the same level in the brain, for comparison. Arrow 1 marks the frontocortical area. On the right image this region shows high radioactivity as compared with the region marked with arrow 2. This activity represents binding of the radioligand with the receptor. In the cerebellar area, a region void of 5-HT2A receptors, low radioactivity is seen on both images (arrow 2).

Methodology and results

In a pilot study, including 4 impulsive, aggressive dogs compared to age matched non-impulsive individuals, a significant difference was found in 6 of the 10 brain regions examined (the frontal, temporal, parietal and occipital cortical region, the subcortical area and the cerebellum (used as a reference region)). The demographical and behavioural data of these dogs are depicted in Table 1. These biting incidents were unpredictable, without the classical preceding warning ritual signals and more, the severity of the attacks were out of proportion to the provoking stimuli. All four dogs were referred after thorough examination by clinicians, experienced in the examination and therapy of behavioural disorders in dogs. The imaging examination was performed with the specific radioligand ^{123}I-5-I-R91150. The selectivity of this ligand for 5-HT2A receptors with regard to other neurotransmitter receptors such as other 5-HT receptors, including 5-HT2C and 5-HT1A, dopamine receptors, adrenergic receptors and histamine receptors is at least a factor of 50.[27,28] The examination was performed using a triple head gamma camera, dedicated for brain investigations. The Mann Whitney U test was used to compare differences in regional binding index between the impulsive and the normal dogs. Level of significance was set at $p < 0.05$.

The binding index was significantly increased in the frontal, temporal and occipital region. The binding in the mean cortical regions was also significantly higher. There was no difference present between the two groups in the subcortical regions.

Breed	Age	Sex	Victims	Bites without warning	Severity of bites	Predictability of bites
GR	12m	M	- Owner - Family, friends - Strangers	>5 bite incidents	Superficial wounding	No prodromal warning signs
R	48m	M	- Owner - Family, friends - Strangers	>5 bite incidents	Perforating bites	No prodromal warning signs
LR	18m	M	- Owner - Family - Strangers	>5 bite incidents	Superficial wounding	No prodromal warning signs
JR	20m	M	- Owner - Family, friends - Strangers	>5 bite incidents	Severe wounding	No prodromal warning signs

Table 1: Demographical and behavioural data
Legend: GR= Golden Retriever; JR= Jack Russell ; LR= Labrador Retriever ; R= Rotweiler

Discussion

This pilot study demonstrates a higher serotonin-2A binding index in the frontal, temporal and occipital cortex. The involvement of serotonin dysfunction in impulsivity, found is this pilot study, is in keeping with the literature. As was mentioned before, up to now, in vivo studies demonstrating involvement of the serotonergic system in impulsivity, consisted mainly of indirect studies. First, in research on the feasibility of the 5-HIAA-CSF probe, a significant correlation between levels of 5-HIAA in the cortex and in the cerebrospinal fluid was demonstrated[29] and it was shown that the level of 5-HIAA in CSF is reflecting presynaptic serotonergic activity in brain[30]. Numerous studies applied this paradigm to the study of impulsivity and aggression in humans, mammals and rodents. In research in humans, the reduction of 5-HIAA levels in CSF in violent suicide attempters, in patients with increased lifetime aggressive incidents and in subjects committing impulsive offences is one of the most established and replicated findings in biological psychiatry.[31,32] Measurements of 5-HIAA concentration in the cerebrospinal fluid of non-human primates were inversely correlated with escalated aggression and wounds requiring medical intervention[33] or with excessive mortality due to aggressive interactions and risk taking behaviour (leaving the flock at a young, immature age, performing leaps in trees at dangerous heights and over risky long distances)[34,35,36]. In dogs, a study showed that CSF 5-HIAA was lower in the aggressive group as compared to normal subjects. A very interesting finding in this report was that the levels of 5-HIAA were significantly lower in a subgroup of dogs, exhibiting impulsive aggression compared to dogs that warned before biting.[37] This was an indication that an impaired serotonergic turnover was especially related to impulsivity, and to a lower extent to aggression.

Secondly, and also indirect in nature, experimental challenges that interfered negatively with the synthesis of serotonin were carried out. In human studies, dietary tryptophan depletion lead to behavioural inhibition in aggressive patients[38] or lead to impulsivity in individuals with a genetic vulnerability to alcohol abuse[39]. Muricidal behaviour in rats was increased by a dietary reduction of tryptophan (a serotonin precursor), by drugs eliciting relatively selective axonal degeneration of serotonin-containing neurons, or by blocking tryptophan hydroxylase – the rate-limiting enzymes for serotonin synthesis –with p-chlorophenylalanine (PCPA).[2,40,41]

Tryptophan free food mixtures increased and tryptophan rich mixtures decreased aggression in monkeys.[42] This might implicate that tryptophan-supplemented low protein diets could be used to reduce aggression in dogs.[43]

A third important support for the role of serotonin in impulsive and aggressive behaviour lies in the use of serotonergic enhancing drugs such as the selective serotonin re-uptake inhibitors and the serotonergic tricyclic anti-depressants. In humans, the anti-aggressive effect on impulsive aggressive personality-disordered individuals is demonstrated.[44] In monkeys, fluoxetine-treated subjects had significantly lower scores on a social impulsivity index then did drug-free subjects.[36] In dogs,

fluoxetine was demonstrated to be useful in the management of dominance aggression in dogs.[45] In rats, aggressive behaviour could be reduced with the treatment of citalopram.[46] In animal studies agonists of the serotonin-1A and serotonin-2A receptor reduced impulsivity in rats.[47]

Concerning our finding on the serotonin-2A receptor, in human medicine, a peripheral model on platelets is available to evaluate the brain serotonin-2A receptor status.[48] These platelet studies on impulsive subjects, such as impulsive suicide attempters, demonstrated an increased platelet 5-HT2A binding.[44] It would be of great practical interest to study the possible correlation of brain receptor occupancy with platelet receptor binding in dogs. This would, in theory, allow screening on blood samples, providing a tool that may give access to a large series of normal and impulsive individuals. But, this pathway has to be elucidated further, since a study exists that demonstrate that alterations of 5-HT2A receptors on platelets may not indicate similar changes in central 5-HT2A receptors.[49]

In this pilot study, mostly cortical regions and not subcortical regions seem to be inflicted in impulsive aggressive behaviour in dogs. This finding could be partially artefactual due to the limited number of subjects and due to the methodological problem of adequately scanning subcortical regions, resulting from technical limitations of the SPECT technique. Nevertheless, there is a reasonable hypothesis that can explain the disturbed frontal cortex binding index and its link to frontal cortex related behavioural disturbances in impulsive dogs.

Based on theories with respect to the role of frontal cortex in behaviour[50], two major parts of the mammal brain, the orbito-frontal and the dorsolateral prefrontal cortex may play a role in the regulation of behaviour. First, regarding the orbitofrontal and medial cortex, there is evidence that at least a part of the canine's medial prefrontal cortex – the pregenual area – is, like the orbital cortex in the monkey, essential for the inhibition of inappropriate behavioural responses.[8] For example, lesions in monkeys and humans and medial frontal lesions in dogs and cats were related to an increased number of aggressive conflicts because these subjects were found to be more easily distractable, and hence overreacting to stimuli. The subjects suffer from the basic inability to inhibit behavioural reactions when they need to be inhibited.[51] Secondly, lesions of the dorsolateral cortex can also add to desinhibitive behaviour. For example, large lobectomies that spare the posterior orbital area tend to increase the aggressiveness of the monkey.[52] The effect is peculiar because it occurs in conjunction with a general diminution of the communicative expressions that normally accompany aggression. There is a decrement of threats and of all those symbolic gestures and moves with which the normal monkey precedes aggression or asserts dominance and position in the hierarchy. The dorsolateral monkey attacks others, often without apparent motive or warning, not guided by the customary interchange of signals.[8] The precise neuropsychological deficits in dorsolateral animals, like dorsolateral humans, are executive in nature.[53] Executive functioning can be defined as the capacity to organize cognitive-specific resources to allow development of contextually sensitive plans and flexible responses.[54] Hence, the dorsolateral cortex can be interpreted as the siege of execution capacity of most other cognitive functions and motor behaviour, based on sensory input. Dysexecutive subjects fail to

interact with the environment because of unplanned and unorganized responses, often unpredicted and hence impulsive in nature. Research in monkeys and large animals showed that dorsolateral animals had a marked delayed-response deficit[8], incapacity to use previously patterns of avoidance[55] and failed integration and the recognition of communicative signals[56]. Dorsolateral deficits not only provoke behaviour deficits but also reduce learning abilities because of reduced working memory capacities.[54] This has an impact on behavioural therapy strategies in human psychiatry but presumably also in veterinary medicine.

In general, further exploration of this technique makes sense, not only regarding research on the pathophysiology of this aberrant behaviour, but also from a clinical point of view. At least, this investigation can add to the public debate on canine aggressive behaviour. Indeed, canine aggression towards man, often in an impulsive way, has major impact on the victims, varying from superficial wounds to permanent disability and even lethal physical damage, especially in children.[57] Besides physical injury, it has also important consequences on the psychological function of the attacked person, with post-traumatic stress disorder and avoiding behaviour as possible consequences.[58] Since dog bites are recognized as a public health problem[59], authorities are "forced" to take measures, varying from elimination of dogs that provoked incidents to restrained breeding. In the absence of objective tests, providing measurable data on evaluating and predicting canine impulsive aggression, evidence-based preventive action, such as breeding restriction, and therapeutic interventions are not available, and hence, elimination of subjects-at-risk is the most logical solution in practice in the majority of cases.

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CHAPTER 8

Evaluation of the brain 5-HT2A receptor binding index and regional brain perfusion in the impulsive aggressive dog measured with SPET

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"Violence has persistently resisted policies intended to control it. It is possible that these policies have been failing because they address the consequences rather than the origins of violence"

J.Volavka, Neurobiology of violence, 1995

Summary

Impulsive aggression in dogs has major impact on human Public Health. A better insight in the pathophysiology of this phenomenon can lead to a more adequate diagnosis and treatment. Indirect *in vivo* research on peripheral body fluids and *post mortem* studies in animals and humans indicate a deficient serotonergic system in general and disturbances in the serotonin-2A receptor in particular.

To include dogs in this study, owners were asked to describe the overall behaviour of the dogs and their conduct during aggressive assaults. Finally, 19 dogs were retained for this study, showing, according to different behavioural specialists, impulsive aggressive behaviour. Functional imaging studies were performed on all of these dogs. Single photon emission tomography (SPET) was used to measure regional brain perfusion using ^{99m}Tc-ethyl cysteinate dimer (ECD). The 5-HT2A receptor binding properties were investigated using the selective radioligand ^{123}I-5-I-R91150.

Whereas no alterations were found in the regional cerebral perfusion, a significant increased uptake of the serotonin-2A radioligand was noted in all cortical areas.

Introduction

Impulsive, aggressive behaviour is experienced as distressing for the victim and for the perpetrator. Moreover, unpredictable aggressive behaviour towards people living in close relation with dogs has a major impact, varying from superficial wounds to permanent disability and even lethality.[1] Apart from physical injury, these insults have also important psychological consequences on the victim.[2]

Research on impulsivity and its functional neuroanatomical substrate in the living subject has a long tradition. Already in the nineteenth century case studies reported on the involvement of the (pre)frontal cortex in impulsive behaviour. Famous case reports, such as Phineas Gage, indicated that anatomical lesions to the prefrontal cortex could alter social adequate behaviour into impulsive unadapted behaviour.[3] Research animal studies demonstrated that ablation of the frontal cortex or prefrontal cooling experiments could provoke disinhibited behaviour and resulted in unplanned, unmediated reactions.[4,5]

From a neurobiochemical viewpoint, the involvement of a deficient serotonergic system in impulsive behaviour is one of the most established findings in biological psychiatry in human medicine.[6-16] Studies in other species, such as primates, dogs and rats also demonstrate a prominent role of serotonin in social adequate and inadequate behaviour.[17-25] The influence of the serotonergic system on behaviour in animals and man has been investigated with direct and indirect studies. Indirect studies consist of measuring biological markers on peripheral body fluids. In research on impulsivity and aggression most often the measurement of 5-hydroxy indol acetic acid (5-HIAA), the major serotonin metabolite, has been carried out in cerebrospinal fluid. Studies in rats and silver foxes indicated that socialization and domestication over generations is paralleled by the acquirement of higher 5-HIAA concentrations, and hence, higher brain serotonin turnover.[26-28] On the other hand, in primates, involved in escalating aggressive incidents leading to severe wounds, and in dogs, attacking in an unpredictable way, an inverse correlation was found between impulsive aggressive behaviour and CSF 5-HIAA.[19,22,29-31] Likewise, human studies demonstrated a reduction of 5-HIAA in violent suicide attempters[8,14,32,33] and individuals showing impulsive outward-directed aggressive behaviour[34,35].

The involvement of serotonin in behavioural disorders can also indirectly be deducted from the fact that dietary reduction of tryptophan (the precursor of serotonin) leads to increased impulsivity and disinhibited behaviour in aggressive patients[36,37] and muricidal behaviour in rats[38]. Moreover, tryptophan rich mixtures lead to decreased aggression in monkeys.[39] The serotonergic turnover can also be enhanced by pharmacological interventions, using serotonergic agonistic drugs, resulting in reduced aggression and impulsivity in humans, monkeys, dogs and rats.[12,17,25,40,41]

Besides evidence of the aforementioned involvement of the serotonin system in impulsivity, some indirect studies focus more specifically on the postsynaptic receptor systems. One model measures the serotonin-2A receptor density on blood platelets to indirectly estimate serotonin-2A

density. This has been extensively studied in humans, revealing a significant increased receptor serotonin-2A density in suicidal patients and impulsive aggressive individuals.[42-47]

Possible confounders of these indirect measurements of the function of the serotonergic system include that they may not truly reflect events in the brain[48] and that they lack spatial information and give no specifications on post-synaptic binding and receptor specificity.

The development of suitable radiopharmaceuticals for functional imaging modalities enable investigations on brain perfusion and –metabolism and can, therefore, help in the evaluation of the functional status of the frontal cortex and the subcortical structures in vivo. Moreover, this technique allows visualization and evaluation of several neurotransmitter systems and their receptors *in vivo* with highly selective radioligands.

With PET imaging modalities, perfusion and metabolism can both be evaluated quantitatively. SPET radiopharmaceuticals image perfusion and regarding the ^{99m}Tc-labelled tracer N,N''-1,2-ethylene-diylbis-L-cysteine diethyl ester dihydrochloride (ECD), also to some extent metabolism. The advantage of SPET is that it is a more accessible technique and economically more advantageous compared to PET. In humans, disturbed perfusion was demonstrated in murderers and aggressive individuals with SPET[49,50] and altered metabolism was found with PET[51]. Previous SPET studies in dogs, using the radiopharmaceutical ^{99m}Tc-ECD, demonstrated the regional distribution pattern of brain perfusion in a healthy reference and in aging dogs.[52,53]

Studies on the serotonin-2A receptor have been performed in humans and animals both with PET and SPET. A selective serotonin-2A receptor radioligand, ^{123}I-5-I-R91150, was synthetised for SPET imaging.[54-57] This radioligand has been used to investigate the serotonin-2A receptor status in normal volunteers[57], human patients with recent suicide attempts[58] and in patients with eating disorders[59]. In dogs, the feasibility and regional distribution pattern of this tracer was first demonstrated[60,61,61], followed by the investigation of the binding properties of this receptor radioligand in a healthy reference group and in a group of aged dogs[53].

In this study, the results of regional cerebral perfusion and the 5-HT2A receptor binding index, visualized using SPET, in the impulsive, aggressive dog are presented.

Materials and methods

Demographical data

Nineteen dogs (15 males, 4 females, mean age 31.5 months), showing impulsive aggression, defined as aggressive assaults without warning and, therefore, unpredictable in nature (table 1), were compared to a reference group (6 males, 6 females, mean age 49 months) of dogs which never showed this behaviour. None of the included dogs had a history of treatment with psychotropics or (tryptophane-rich) diet. None of the dogs in both groups had a history of neurological disorders. In the reference group one male dog was neutered, in the aggressive group four male dogs were neutered. Breeds varied substantially in the aggressive group (table 1). In the reference group all dogs included were shepherd type dogs. All dogs from the aggressive group were referred by behavioural consultants.

Behavioural consult

In the consult, general behaviour of the dog, showing unprovoked aggression towards man or other dogs, was discussed. In addition to the detailed questioning on the dog's general behaviour, the circumstances and the physical manifestation of the aggressive insults, and especially their predictability, were evaluated. The questionnaire was a compilation of tests as proposed in the literature[62-64] and was adapted towards recognising especially impulsive behaviour, including repeated questions in order to control the objectivity of the answers of the owners (Ödberg, personal communication)

ID N°	Breed	Age	Gender	Victims	Bites without warning	Severity of bites
1	BB	24m	M	- Family, friends - Strangers	R	0 → 4
2	R	48m	MC	- Family, friends - Strangers - Dogs	R	0 → 4
4	R	12m	M	- Owner - Dogs	3	0 → 1
5	DD	20m	M	- Family, friends - Strangers	R	0 → 3
6	BS	54m	M	- Family - Strangers	R	1
7	JR	20m	MC	- Family, friends - Strangers	R	0 → 4
8	GR	24m	M	- Owner, family, friends - Strangers	R	3
9	R	18m	M	- Family	R	0 → 1
10	KS	18m	FC	- Family, friends - Strangers	R	1
11	D	24m	M	- Family , friends - Strangers	R	0 → 3
12	BM	42m	F	- Family, friends - Strangers	4	0 → 1
13	BS	60m	F	- Owner, family - Strangers	R	0 → 1
14	BB	12m	F	- Owner, friends - Strangers	R	0 → 1
15	BS	76m	MC	- Friends - Strangers - Dogs	R	0 → 4
16	PB	18m	M	- Family - Strangers - Dogs	R	0 → 2
17	LR	18m	MC	- Owner, family - Strangers	R	0 → 1
18	EB	72m	M	- Family - Strangers - Dogs	R	0 → 2
14	GR	12m	M	- Owner, family, friends - Strangers	R	2 → 3
3	DD	24m	M	- Family, friends	R	0 → 1

Table 1: Breed age, gender and impulsive behaviour characteristics in the aggressive dogs
Legend: BB= Berger de Beauce; BS= Belgian shepherd; PB= Pitbull; LR= Labrador Retriever; EB = English Buldog; R= Rotweiller; DD= Deutsche Dogge; KS= Kaukasian Shepherd; JR= Jack Russel; GR= Golden Retriever; D= Dobberman; BM= Bull mastiff;
M= male; F= female; MC= male castrated; FC= female castrated
Severity of bites: 0 = no wounds; 1= blue mark; 2 = superficial wound; 3 = laceration; 4 = medical treatment; R = > 5 bite accidents;

Imaging procedures

The examination procedures were performed according to good animal practice and were approved by the local ethical committee. Computed tomographic brain imaging was performed on separate days due to safety precautions concerning general anaesthesia, to rule out anatomical brain pathology (scanner Pace Plus, GE Medical Systems, Wisconsin, USA).

The two functional imaging examinations, brain perfusion SPECT and brain 5-HT2A receptor binding SPECT, were performed on separate occasions to account for the half-life of the radionuclides. The head was positioned on a sort of moulage, in order to minimize intra-individual positioning variability.

RADIOPHARMACEUTICALS

Brain perfusion tracer

Perfusion studies were performed with the ^{99m}Tc-labelled tracer N,N''-1,2-ethylene-diylbis-L-cysteine diethyl ester dihydrochloride (ECD; bicisate; Neurolite™). This tracer, which preponderantly indicates cerebral perfusion, is a lipophilic compound, permitting blood brain barrier passage. ECD was used in this study because it is Technetium labelled and is chemically stable *in vitro* for several hours after reconstitution, making it more convenient to handle than ^{99m}Tc-hexamethylpropylene amine oxime (HMPAO). Moreover, the fast cerebral retention, rapid blood clearance of metabolites and fast clearance from the extracerebral tissues, result in a favourable signal to noise ratio, providing superior imaging qualities and higher lesion detection sensitivity compared to HMPAO.[65-68]

The IV injected activity ranged from a mean of 0.70 mCi/kg (SD: 0.17) in the aggressive group to a mean of 0.74 mCi/kg (SD: 0.09) in the reference group. Care was taken that all proceedings provoked minimal excitement of the animal. The catheter was placed intravenously 10 minutes before injection of the tracer to avoid tension accompanying this procedure The radiopharmaceutical was injected 20-25 minutes prior to induction of the general anaesthesia, in the examination room, free of noise and with dimmed light.

Serotonin-2A receptor binding tracer

The receptor binding studies were performed with ^{123}I-5-I-R91150. The tracer is synthesized by electrophilic substitution on the 5-position of the methoxybenzamide group of R91150, followed by purification with high-performance liquid chromatography. The product has a radiochemical purity of more than 99 % and is sterile and pyrogen free. A specific activity of 10 Ci/μmol is obtained.

The tracer is a 5-HT2A antagonist with high affinity (K_d = 0.11 nM) and selectivity for 5-HT2A receptors. The selectivity of the ligand for 5-HT2A receptors with regard to other

neurotransmitter receptors such as other 5-HT receptors, including 5-HT2C and 5-HT1A, dopamine receptors (D1 and D2), adrenergic receptors (α1 and α2) and histamine receptors is at least a factor of 50. The tracer is displaceable with the 5-HT2 antagonist ketanserin[54-56,60]. The IV injected activity ranged from a mean of 0.19 mCi/kg (SD: 0.07) in the reference group to 0.21 mCi/kg (SD: 0.09) in the group of aggressive dogs. The radiopharmaceutical was injected 90-100 minutes prior to image acquisition. The optimal scanning time, the time when pseudo-equilibral conditions are reached between free and bound radiotracer, necessary for semi-quantification of the regional binding index, was determined from 90 minutes onwards in a preliminary study.[60]

Anaesthetic protocol

All animals inevitable required anaesthesia in both imaging protocols. The anaesthetic protocol was evaluated in prior studies on brain perfusion and receptor binding.[52,60] Sedation was obtained with 10-30mg/kg medetomidin hydrochloride IM (Domitor®, Pfizer) 30 minutes prior to the examination and general anaesthesia was induced with 2-3mg/kg iso-propylphenol IV (Rapinovet®, Mallinckrodt) and maintained with halothane (Fluothane,®, Zeneca) to effect.

Acquisition and processing protocol

All dogs were positioned in ventral recumbence, with the head placed on a moulage to ensure similar intra-individual positioning. SPECT was performed with a triple headed gamma camera (Toshiba GCA-9300A, Dutoit Medical, Antwerp, Belgium), equipped with high resolution fan beam collimators (FWHM 7.8 mm) and ^{153}gadolinium rods for transmission scanning purposes.

For both studies, total acquisition time was 24 minutes, including a transmission (TCT) (2 x 2 min) prior to the emission (2 x 10 min) acquisition. For each acquisition, 90 projection images were obtained on a 128 x 128 matrix using a continuous scan mode by rotating each head 120°. The images were reconstructed with filtered back projection after rebinning to parallel data, and application of a Butterworth filter (cut-off 0.16 cycles/ pixel, order 8). Pixel size was 1.72 mm. Uniform Sorensen attenuation correction with a linear attenuation coefficient of 0.12 /cm and triple-energy window scatter correction were applied according to standard clinical settings, as described previously.[69] The individual image perfusion datasets were automatically registered to a template, generated from 12 normal dogs, 6 males and 6 females (aged between 12-84 m, mean 49 m; SD: 29), using BRASS software (Brain Registration and Automated SPECT Semiquantification, Nuclear diagnostics). The use of this template in the brain perfusion studies eliminates subjectivity due to operator dependent region definition and the automatic registration facilitates the fitting procedure, necessary to

compensate for intraindividual differences in anatomical brain size and shape, and in head positioning. On this template, 11 manually drawn volumes-of-interest (VOI) were defined with inclusion of all grey matter. (Fig 1).

The template is generated from the addition of all images from the reference group. On this mean image the volumes-of-interest are manually drawn, creating a regionmap. This template with its regionmap are then used for automatic registration of patient data.

The division of the anatomical brain regions was chosen according to the proposal of Redding.[52,70] Regional radioactivity was normalized to the activity registered in the global cerebellum (both hemispheres). A similar template for the receptor radioligand studies could not be used since automatic registration is not applicable for the majority of these studies due to higher intersubject variability and lack of anatomical reference. For the serotonin-2A ligand studies, the emission data were matched with the emission ^{99m}Tc-ECD perfusion data with the aid of their corresponding transmission data, which show sufficient contrast to recognize bone and sinuses. Fitting of these transmission data was performed with the Multimodality software of Nuclear Diagnostics (Hägersten, Stockholm, Sweden). This software, displaying images in a dual window setting, allows for manual co-registration by providing tools for scaling, rotation and translation images in all three dimensions. The values of the parameters used to register the transmission computed tomography (TCT) map of the radioligand data to the TCT map of the perfusion data were then applied to the radioligand emission data. Since regions of interest were predefined on the perfusion data, we were able to extend the division of anatomical regions to the serotonin-2A receptor radioligand data.

The uptake in the global cerebellum (a region poor of 5-HT2A receptors)[71-73] was used as a reference for non-specific binding in addition to free ligand. Radioactivity measured in the cortical areas was assumed to represent the total activity (i.e. specific + non specific activity + free ligand). The binding index (BI) was operationally estimated as (counts/pixel in regional cortex) / (counts/pixel in cerebellum). This binding index is proportional to the concentration of available receptors under pseudo- equilibral conditions.[74]

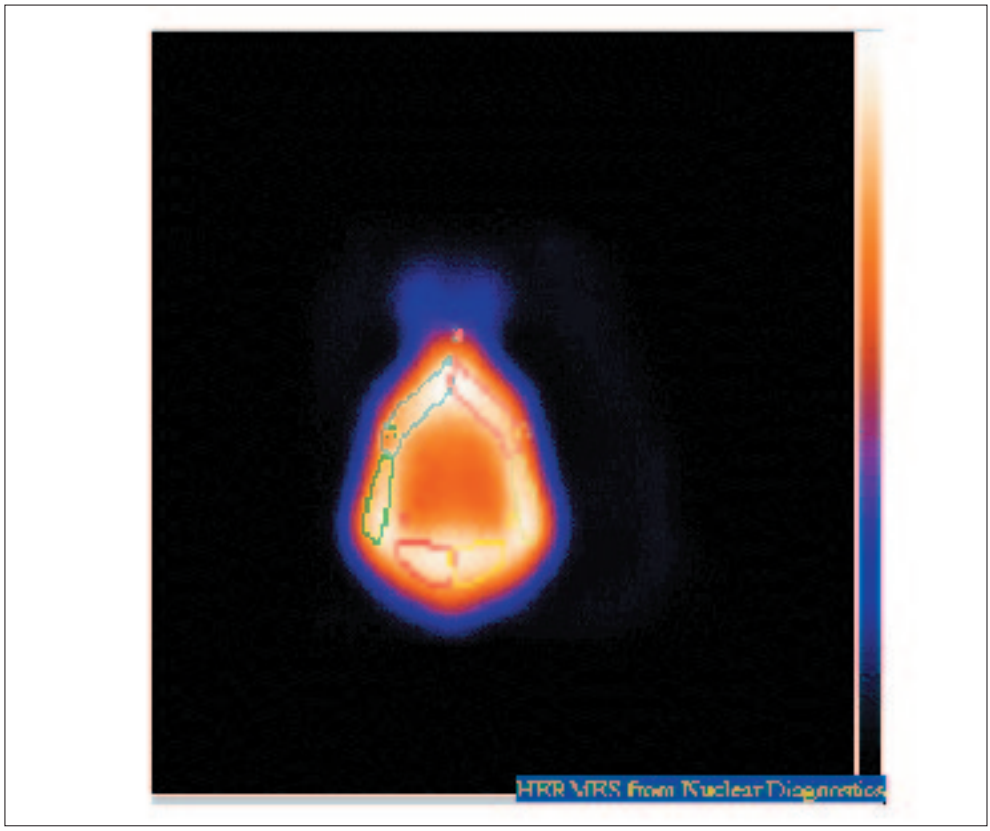

Figure 1: The template is generated from the addition of all images from the reference group. On this mean image the volumes-of-interest are manually drawn, creating a regionmap. This template with its regionmap are then used for automatic registration of patient data.

Statistics

The equality of age, weight, injected dose per kg body weight of ^{123}I-5I-R91150 and ^{99m}Tc-ECD between the two groups was evaluated according to the independent samples Student's t-test. Since age was different, although not statistically significant (P= 0.08), age was taken into account as confounding variable, since interaction between age and perfusion and binding index of the serotonin radioligand are reported in literature.[53,75-81]

The equality of gender and gonadal status were evaluated with the Fischer Exact's test. Both in the reference group and in the impulsive aggressive group, there were no differences between the male and female subjects concerning the perfusion index and the ^{123}I-5-I-R91150 binding index. A selective interaction between gonad status and perfusion and serotonin receptor radioligand binding index in impulsive dogs was inspected with scatter plots, comparing frontal perfusion and receptor radioligand binding index in neutered versus intact dogs. Since no differences between the intact and neutered subjects concerning regional serotonin-2A binding index and concerning regional cerebral blood perfusion were found, in the impulsive aggressive group, gender and gonad status were not taken into account as a covariate in further analyses. (Fig 2, 3).

There was no significant difference in weight between impulsive dogs and reference dogs. Since injected dose of tracers was adjusted to weight, weight as such was not taken into account as a covariate.

Paired Student's t-statistics were applied to evaluate left-right differences within diagnostic groups. In order to evaluate binding and perfusion differences across groups, a general linear model procedure was applied. Pearson correlation analyses were used to examine any relationships between binding index, perfusion index and injected tracer dose per kg body weight.

	Demographical and experimental data			
	Impulsive subjects (N=19)	Reference subjects (N=12)	Statistics	
Demographical data	Mean (SD)	Mean (SD)	t / χ^2	P
Age	31.5 (24.1)	49.0 (29.6)	t = 1.81	0.08
Weight	35.5 (15.6)	29.3 (6.6)	t = -1.29	0.21
Gender	15 M / 4F	6M / 6F	χ^2= 2.82	0.13 (FE)
Gonad status	15 I / 4 N	11 I / 1 N	χ^2= 1.52	0.36 (FE)
Experimental data	Mean (SD)	Mean (SD)	t	P
Inj dose/kg ^{123}I-R91150	0.22 (0.09)	0.19 (0.07)	t = -0.98	0.34
Inj dose/kg ^{99m}Tc-ECD	0.71 (0.17)	0.74 (0.09)	t = 0.64	0.53

FE = Fisher Exact's Test

Table 2: Demographic and experimental data from impulsive and reference subjects. Gender: Male (M) versus Female (F) subjects. Gonad status: Intact (I) versus Neutered (N) subjects. In the experimental data, the injected dose per kg body weight of the respective tracers is given.

Results

Demographical data

Although statistically not significant (χ^2=2.82; P=0.13), gender was different between the group of impulsive dogs (15 males; 4 females) and the group of reference dogs (6 males; 6 females). A selective interaction between gender and perfusion and between gender and binding index of the serotonin-2A ligands in impulsive dogs was inspected with scatter plots, comparing frontal index in males and females, and demonstrated that both perfusion and radioligand binding index of the female subjects fell in the range of these of the male individuals. (Fig 2, 3). Gonad status, intact versus neutered, was not significantly different between impulsive dogs and reference dogs. Neutered subjects fell in the range of the intact subjects in the impulsive group. (Fig 2).

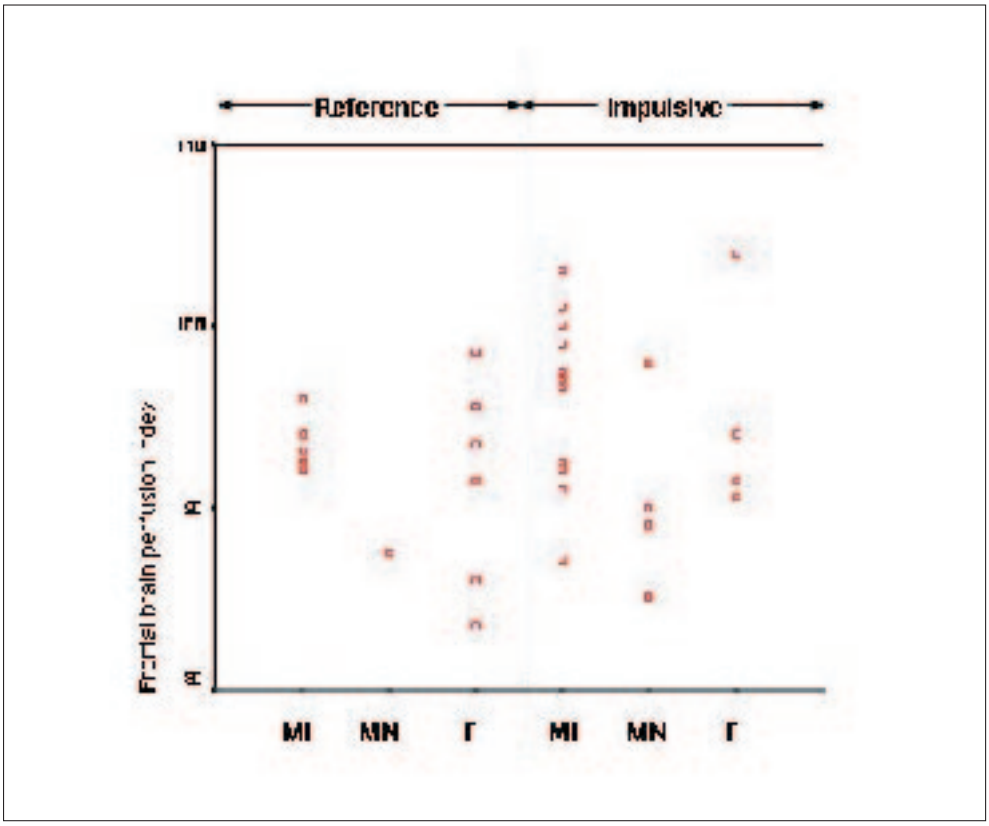

Figure 2: Scatter plot of the frontal perfusion index of all subjects (reference and impulsive dogs). Reference subjects and impulsive subjects are separated visually by the black bold line.
Legend: M= male; F= female; MI= male with intact gonads; MN= male neutered; FI= female with intact gonads; FN= female neutered

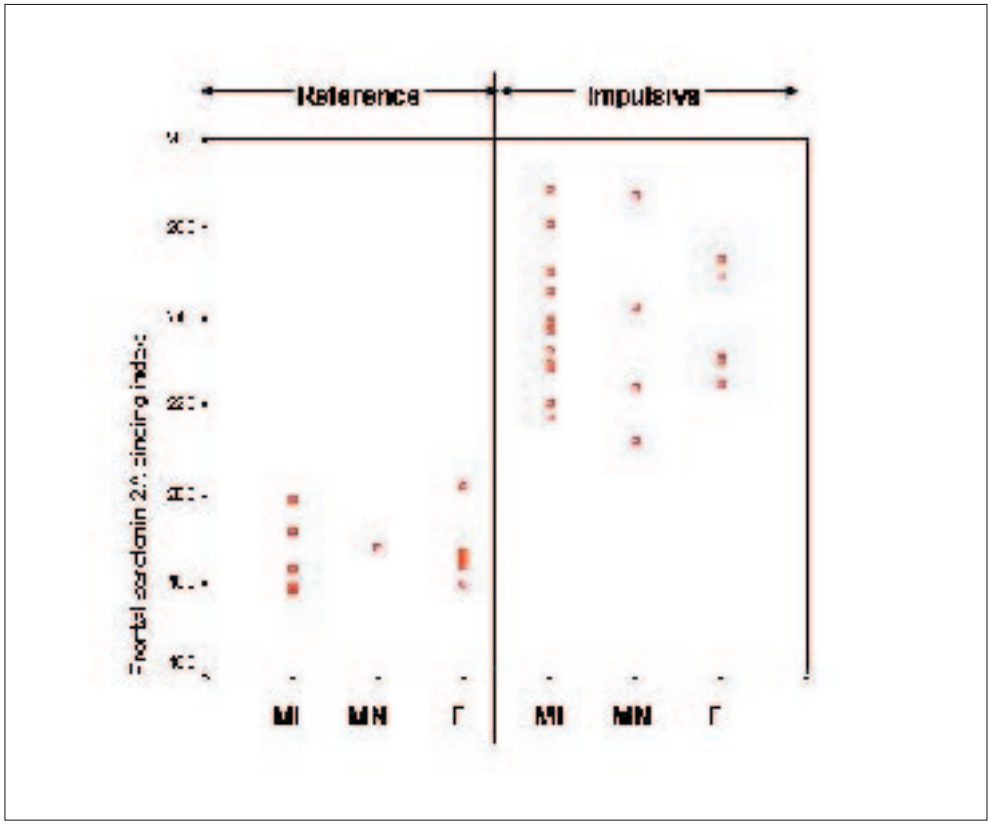

Figure 3: Scatter plot of the frontal serotonin-2A binding index of all subjects (reference and impulsive dogs). Reference and impulsive subjects are separated visually by the black bold line.
Legend: M= male; F= female; MI= male with intact gonads; MN= male neutered; FI= female with intact gonads; FN= female neutered.

Behavioural consults

The number of assaults and the severity of bites are summarized in Table 1. The main complaint in all cases was that the bite incidents were unpredictable and that the provoking stimulus was not in proportion to the intensity of the attack and associated with positive signals (for example, petting….) as well as with negative (for example, punishment….) interactions. Usually multiple bites were registered during the incident, not always leading to medical intervention. It is marked that owner and family members are most often the assaulted persons. Concerning eating habits, most animals in the aggressive group, were labeled greedy. Most owners reported decreased learning abilities of their dogs in the sense that it seemed difficult to learn them to do or not to do things. Submissive behaviour, nervousness, hyperkinetic behaviour was frequently observed and tail chasing, self mutilation and excessive barking was occasionally reported.

Regional brain perfusion

Injected dose per kg body weight of ^{99m}Tc-ECD was not significantly different between the two groups (Table 2) nor correlated with the regional brain perfusion. Both in the group of the impulsive dogs and the reference dogs, there were no significant left-right differences in regional brain perfusion index in none of the regions (Table 3).

Left-Right comparison of cortical brain perfusion index								
	Impulsive subjects (N=19)				Reference subjects (N=12)			
	Right Mean (SD)	Left Mean (SD)	t	P	Right Mean (SD)	Left Mean (SD)	t	P
Frontal	93.9 (5.6)	94.4 (5.4)	-0.54	0.60	91.4 (1.4)	92.5 (1.3)	-1.29	0.22
Temporal	91.5 (9.9)	92.6 (9.3)	-1.26	0.22	92.9 (1.2)	92.2 (1.3)	1.40	0.19
Parietal	100.2 (7.0)	98.9 (7.4)	1.43	0.17	97.4 (1.1)	96.9 (1.3)	0.49	0.64
Occipital	99.8 (7.4)	99.0 (6.2)	0.74	0.47	97.7 (1.2)	96.8 (1.2)	0.92	0.38

Table 3: Left-Right comparison of cortical brain perfusion index from impulsive and reference subjects. Means and Standard Deviation (SD) data are given. All data are age corrected to the mean age of the group (37.9 months) applying linear correction.

Mean regional brain perfusion index (mean of left and right regional perfusion) was not significantly different between impulsive and reference dogs in all cortical regions and in the cortex as a whole (mean cortical) (Table 4). There was also no significant difference in regional brain perfusion index between the two groups in the subcortical region. Neither the cortical-subcortical gradient, calculated as the mean cortical to subcortical perfusion index ratio, nor the anterior-posterior ratio, calculated as the frontal cortex to occipital cortex perfusion index ratio, were different between the impulsive and reference dogs (Table 4).

	Brain perfusion index				
	Impulsive subjects (N=19)	Reference subjects (N=12)			
	Mean (SD)	Mean (SD)	Diff. (SED)	t	P
Frontal	94.1 (5.1)	92.0 (4.4)	2.1 (1.8)	-1.21	0.24
Temporal	92.1 (9.43)	92.5 (4.17)	0.4 (2.9)	0.16	0.87
Parietal	99.6 (7.0)	97.2 (3.7)	2.4 (2.2)	-1.09	0.28
Occipital	99.4 (6.3)	97.3 (3.7)	2.1 (2.0)	-1.06	0.30
Mean cortical	96.4 (4.9)	94.7 (3.3)	1.7 (1.6)	-1.05	0.30
Mean Subcortical	94.9 (8.4)	95.3 (3.5)	0.4 (2.6)	0.14	0.89
Costro-caudal gradient	0.96 (0.08)	0.95 (0.03)	0.01 (0.02)	-0.44	0.66
Cortical-Subcortical gradient	1.02 (0.09)	1.00 (0.03)	0.02 (0.03)	-1.03	0.31

Table 4: Comparison of brain perfusion index between impulsive and reference subjects in cortical and subcortical brain regions. An anterior-posterior gradient and a cortical-subcortical gradient is calculated. All data are age corrected to the mean age of the group (37.9 months) applying linear correction. Post-hoc correction for multiple comparisons was done through Bonferroni correction. Group means and Standard Deviations (SD) are given and differences (Diff) and Standard Error of the Difference (SED) are calculated.

5-HT2A RECEPTOR BINDING INDEX

The injected dose per kg body weight of 123I-5I-R91150 was not significantly different between the two groups (Table 2) and did not correlate with the regional binding index of the ligands.

No significant left/right binding index differences were found in the cortical areas neither in the aggressive impulsive group, nor in the reference group (Table 5).

Left-Right comparison of cortical serotonin-2A binding index

	Impulsive subjects (N=19)				Reference subjects (N=12)			
	Right Mean (SD)	Left Mean (SD)	t	P	Right Mean (SD)	Left Mean (SD)	t	P
Frontal	241.0 (19.8)	236.3 (15.5)	1.56	0.14	186.6 (8.2)	188.0 (8.2)	-0.67	0.52
Temporal	218.7 (23.8)	219.0 (35.0)	-0.04	0.97	163.3 (16.3)	160.4 (17.9)	0.62	0.55
Parietal	157.2 (32.4)	160.6 (36)	-0.53	0.60	132.8 (32.2)	131.5 (28.3)	0.24	0.82
Occipital	177.2 (24.1)	176.7 (35.3)	0.10	0.92	147.9 (20.3)	138.9 (20.98)	1.51	0.16

Table 5: Left-Right comparison of cortical serotonin-2A binding index from impulsive and reference subjects. Means and Standard Error data are given. All data are age corrected to the mean age of the group (37.9 months) applying linear correction. Post-hoc correction for multiple comparisons was done through Bonferroni correction.

Mean regional serotonin-2A binding index (mean of left and right regional binding index) was significantly different between impulsive and reference dogs in all cortical regions and in the global cortex (mean cortical) (Table 6) (Fig 4). There was no significant difference in serotonin-2A receptor binding index in the subcortical regions. Neither the cortical-subcortical gradient, calculated as the mean cortical to subcortical binding index ratio, nor the anterior-posterior ratio, calculated as the frontal cortex to occipital cortex binding index ratio, were different between the impulsive and reference dogs (Table 6).

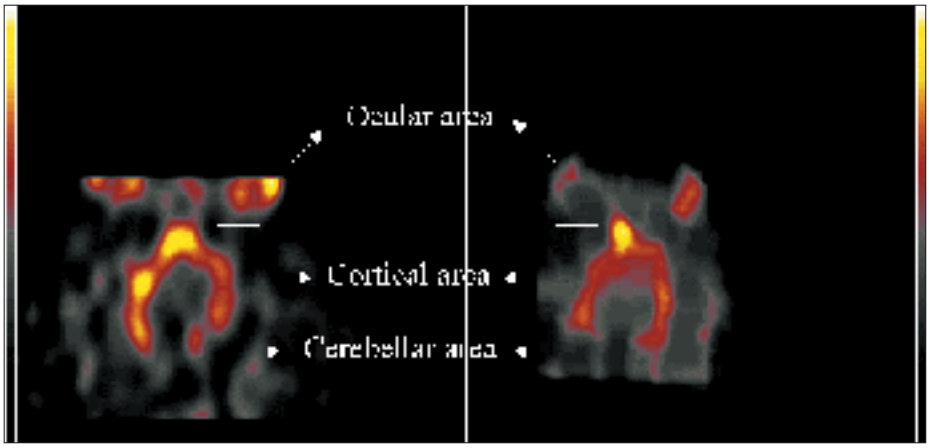

Fig 4: Serotonin-2A radioligand horizontal images from (A) a normal reference dog and (B) an aggressive dog. Increased activity is most visible in the left cortical areas of image B.

	Serotonin-2A binding index				
	Impulsive subjects (N=19) Mean (SD)	Reference subjects (N=12) Mean (SD)	Diff. (SED)	t	P
Frontal	238.7 (16.5)	187.3 (7.2)	51.3 (5.1)	10.1	<0.001
Temporal	218.9 (25.9)	161.8 (15.2)	57.1 (8.2)	6.9	<0.001
Parietal	158.9 (31.22)	132.2 (28.7)	26.7 (11.2)	-2.4	0.02
Occipital	177.0 (28.1)	143.4 (17.8)	33.6 (9.1)	3.7	0.001
Mean cortical	198.4 (17.4)	156.2 (11.2)	42.2 (5.7)	7.4	<0.001
Mean Subcortical	126.2 (40.1)	120.9 (36.8)	5.3 (14.3)	0.37	0.71
Costro-caudal grad	1.39 (0.30)	1.32 (0.17)	0.07 (0.10)	0.69	0.49
Cortical-Subcortical grad.	1.88 (1.16)	1.69 (1.58)	0.19 (0.49)	0.38	0.71

Table 6: Comparison of serotonin-2A binding index between impulsive subjects and reference subjects in cortical and subcortical brain regions. An anterior-posterior gradient and a cortical-subcortical gradient is calculated. All data are age corrected to the mean age of the group (37.9 months) applying linear correction. Post-hoc correction for multiple comparisons was done through Bonferroni correction. Group means and Standard Deviations (SD) are given and differences (Diff) and Standard Error of the Difference (SED) are calculated.

Regional brain perfusion and serotonin-2A binding index

There were no significant correlations between serotonin binding index and regional brain perfusion in the reference population and in the impulsive dogs population (reference group: all regions > temporal cortex: r=-0.31; P= 0.32 and the impulsive group: all regions > occipital cortex: r= 0.13; P= 0.57).

Discussion

The major findings of this report are a statistically higher mean serotonin-2A binding index in all cortical regions, but not in subcortical regions, in the impulsive subjects as compared to the reference subjects but no mean regional perfusion differences between the two groups. The absence of gender differences for serotonin-2A binding and regional brain perfusion is in line with the study of 5-HIAA in aggressive and reference dogs[22], human impulsivity studies on platelets[82], studies on primates[29]. In terms of the gonad status, a larger proportion of dogs in the impulsive aggressive group was neutered. As neutering is a common treatment in problems with aggression in animals, as was in this group, it can certainly affect the study results. A correlation between plasma testosterone and aggressive acts in male offenders was found in humans[83], and an interaction between the sex-hormone system and the serotonergic system was suggested in a study on aggressive behaviour in rats[84]. However, in this study, the frontal binding index measurements of the neutered subjects fell in the range of the intact subjects. The reported decreased learning abilities in the impulsive aggressive group might be related to frontocortical dysfunction. This is shown with frontal ablation or cooling experiments resulting in rats and primates, resulting in impairment of learning and task performances.[4,85] The absence of perfusion deficits in this study is in disagreement with human studies, where a decreased frontal and subcortical perfusion and metabolism is found in murderers and aggressive individuals.[49-51,86,87]

The statistically higher serotonin-2A binding index in all cortical regions found in this study in the impulsive subjects, confirm the involvement of the serotonergic system in impulsive aggressive behaviour. In so far, as methodological differences do not preclude the following comparisons, our results are in line with the higher levels of 5-HT2A receptor binding in post-mortem brains of suicide victims[42,88,89], but not with a report on reduced prefrontal 5-HT2A binding index, measured with the same serotonin-2A receptor ligand as used in this study, *in vivo* in patients that very recently attempted suicide[58].

Confounders, such as the impact of possible post-mortem brain alterations *in vitro*, the impact of the acute stress-relief of stress episode in the immediacy of a very recent suicide attempt, the distinction between outward-directed (assault) and inward-directed (suicide attempt) aggression *in vivo* and the absence of impulsivity ratings in the *in vitro* and *in vivo* aforementioned studies, make a reasonable comparison difficult.

It is tempting to make a hypothetical link between the results of the indirect studies and the results of our study that showed a significant higher cortical serotonin-2A binding index. Following the classical theory on receptor alterations, theoretically, a mechanism of up-regulation of post synaptic 5-HT2A receptors as a compensatory change for the reduced presynaptic serotonergic activity could be put forward. However, typical up- and down-regulation of receptors from over- and underexposure to 5-HT does not appear to apply to these type of receptors. Lesions to the serotonergic cell bodies,

leading to a dramatic reduction in presynaptic serotonin levels leave the post-synaptic serotonin-2A receptors unchanged.[90] In addition, serotonin-2 receptors not only will down regulate in the presence of agonists but also, paradoxically, in the presence of antagonists.[91,92] The exact mechanism of this reduction is not clear but may be related to the receptor itself by downregulation or internalization or to changes in gene transcription and/or translation.[91] On the other hand, serotonin-selective uptake inhibitors (SSRI's), increasing the serotonin level in the synaptic cleft, induce variable effects on the receptor.[91] Hence, it is unlikely that a classical receptor up-regulation, accompanying a decrease of serotonin pre-synaptically, will explain the findings in this study.

Another putative mechanism for up-regulation of 5-HT2A receptors in the brain may be related to modulation through other neurotransmitter systems or through neuro-endocrinological pathways, such as the hypothalamic-pituitary-adrenal axis (HPA axis). In chronic stress situations, the $\alpha 2$ adrenergic receptors show altered functioning[93,94], possibly contributing to disturbances in related receptor systems such as the serotonergic system. It has been demonstrated in rats that administration of dexamethasone or exposition to stress (forced swimming) causes an up-regulation of 5-HT2A receptors without any modification of 5-HT or 5-HIAA concentrations in the brain.[95,96]

Methodological considerations

We must take into consideration that the serotonin-2A binding index might be artefactually increased.

First, an increase in cerebral blood perfusion could deliver more tracer to the brain. But this is unlikely in this study since no significant differences in regional brain perfusion were found between impulsive and reference subjects. The trend (p=0.08) towards a higher perfusion in the cortical regions in the impulsive subjects is unlikely to account for the large significant differences in serotonin-2A binding index.

Second, external manipulations such as medication or diets could act as confounding factors. This is unlikely since all dog owners and all treating veterinarians or behavioural therapists were interviewed and all pharmaceutical or dietary interventions were ruled out. In conclusion, all dogs were psychopharmacological medication-naïve and were not on a diet.

Third, a confounding factor may include breed variability, since the reference group consisted predominantly of shepherd-type dogs while the aggressive impulsive group was composed of a larger diversity of breeds. Westergaard et al. (1999) demonstrated that small between-species variations were accompanied by large differences in CSF 5-HIAA in primates but concluded that this finding also accounted for interspecies temperament difference (high intensity, escalating aggressive acts and wounding accompanying lower CSF 5-HIAA.[29] Moreover, none of the species-specific subgroups of the dogs in this study were out of the range of the other species subgroup members and also, shepherd type dogs were also included in the impulsive group. However, we cannot rule out that the heterogeneity of the impulsive aggressive population is an influencing factor for the observed difference. Finally, this study could not demonstrate whether this finding was state-dependent or trait-related. Indeed, owners came to search for advise often immediately after a serious assault by the dog. Nevertheless, due to methodological reasons, all dogs, both the reference and the impulsive subjects, had to wait at least two weeks for their first scan. All dogs were kept and treated in comparable conditions. By consequence, the findings tend to be rather trait-related than state-dependent.

There are also methodological considerations due to the animal research paradigm, more specifically, in contrast to human studies, the need of anaesthesia in animals and the smaller cortical area and brain volume compared to human brain.

First, in contrast to human studies, general anaesthesia is required to perform these examinations. The anaesthetics used in this study have a predominantly global effect on the brain (cortical, subcortical and cerebellar region) without hemispheric preponderances, as opposed to the more regional effects of some anaesthetics such as ketamine hydrochloride. Given the relative quantitation with reference to the cerebellum, a potential effect of anaesthetics would be ruled out. Also, with regard to the perfusion studies, the radiopharmaceutical was injected 20-25 minutes prior to induction of general anaesthesia, at which stage the tracer is already trapped intracellularly.

Concerning the serotonin-2A radioligand studies, global cerebral blood flow alterations as result of the general anaesthesia, are inevitable and may influence the pseudo- equilibral state of the tracer. But, it is unlikely that the regional variations, found in this study, are due to the effects of anaesthetics, since all dogs from both groups received exactly the same anaesthetic protocol for both studies, precluding individual effects of different products.

Second, in order to obtain semi-quantitative perfusion data, the different regions have to be compared with a reference area, unaffected by the physiological or pathological alterations being investigated. In the perfusion study, cerebral regions were normalized to the cerebellum, since this region seems to be least affected by aging changes.[97-100] When using total counts as a normalization entity, false relative rCBF changes might result from impairment in some regions, influencing the total brain counts.[97] In the radioligand study, the cerebellum was also used as a reference region since this structure contains few 5-HT2 receptors. This procedure has the disadvantage that it is dependent on a very secure estimation of cerebellar activity and that a small artifact herein can have large impact on other regional brain activity estimations. On the other hand, it has the advantage that small differences in activity in brain regions have a greater chance to be discovered since they are not included in the reference region. Moreover, to our knowledge, there are no reports that the cerebellum is involved in the pathophysiology of impulsive aggressive behaviour.

Future research directions

Future studies should at first replicate these findings in larger populations. It would be of interest if peripheral measurements of serotonin metabolites and platelet serotonin-2A receptor binding index, preferentially with the same radioligand, could be assessed simultaneously. This would offer the opportunity to have access to a larger number of animals. In addition, further research should also include neuro-endocrinological measurements, such as HPA-axis hormones and sex-hormones.

Furthermore, future research should aim at follow-up studies in both populations. Since repeated examinations from a radio protective point of view are more acceptable for dogs, compared to humans, due to their limited life-span, and since repeated, state-of-the-art performed, anaesthesia is well-tolerated, this offers the opportunity to examine individuals on several occasions, evaluating the "state" or "trait" nature of pathological alterations and monitor perfusion and serotonin-2A binding index after therapeutic interventions. In this time interval, different intervention-strategies, ranging from pharmaceutical to behavioural therapy, could be evaluated and compared.

Conclusion: The imaging techniques used in this study can help to improve insight information on the pathophysiology of impulsive behaviour and the diagnosis of subjects or groups at risk. This may lead to more adequate preventive breeding programs in stead of the "blind" elimination of breeds. In addition, animal research on impulsivity can potentially serve as a representative model for the study of impulsive behaviour in humans.

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GENERAL DISCUSSION

General discussion

Gradually, more and more research is focussing on clinical behavioural problems encountered in small animals, and this from a veterinary clinical point of view as well as concerning the possible use of larger animal models in research on human brain disorders and related deviant behaviour.[1]

In the discussion we will address two aspects of this work. In the first part we will raise the methodological issues and in the second we will discuss the clinical applications.

METHODOLOGICAL ISSUES

Functional versus structural imaging

Structural neuroimaging studies with CT or MRI offer insight in brain-behaviour relationships that involved anatomical lesions of the brain. But very often, behavioural disturbances are not accompanied with structural lesions. Therefore, structural imaging is only helpful to rule out organic pathology. Functional imaging modalities, including functional magnetic resonance imaging (fMRI), magnetic resonance spectroscopy (MRS), PET and SPET offer the opportunity to examine the functioning brain *in vivo*. Moreover, with the introduction of functional imaging, using SPET and PET, in the research of brain-behaviour relationship, the possibility is created to quantify perfusion, metabolism and the neuroreceptor status *in vivo*.

SPET as a research tool

We used the SPET technique in this studies, despite the better resolution and more accurate quantification, provided by PET. Nevertheless SPET remains a more economical and applicable modality for general use, since it does not require a cyclotron in the immediate vicinity of the camera. It also remains a valid modality to evaluate regional binding kinetics and to provide semiquantitative indices of regional perfusion and receptor binding. Some technical aspects have been discussed in the preceding chapters.

Partial volume effects and attenuation

We did not subdivide the fronto-cortical and subcortical region in smaller structural and functional regions. In these studies, partial volume effects would increase inter-and intra-individual variability to an undesirable extent. Also, the lack of detail on the CT images, precluded adequate subdivision of the fronto-cortical and subcortical regions, as is done in human studies. Nevertheless, even with this division, partial volume effects cannot be excluded and can result in an underestimation of regional radioactivity values. A second technical factor is the attenuation applied to the acquired data. The broad beam (uncollimated source) attenuation coefficient used in this study was 0.12/cm

which is slightly lower than the narrow beam (collimated source) attenuation coefficient used for correction for attenuation of photons with an energy of 140 keV in water (0.15/cm).[2] The usefulness of a non uniform attenuation coefficient (NUAC) is beyond any doubt in studies of heterogeneous structures such as the thoraco-abdominal region but the clinical relevance for brain SPET is under debate.[2] Brain is a rather homogenous structure, although the thickness of the skull at certain parts can influence attenuation, mainly in the superficial cortical structures and the cerebellum. When applying uniform attenuation, the superficial cortical activity will be underestimated, compared to the deeper structures (subcortical regions), resulting from increased attenuation of the overlying skull (paradoxical skull effect).[2,3] Since we used group comparisons, the effects are expected to be minimal.

Feasibility of perfusion and 5-HT2A receptor imaging

We demonstrated in these studies that SPET is a valid modality to image and measure both perfusion and the serotonin-2A receptor occupancy in physiological and pathophysiological states of the canine brain. However, to our belief, the technique of functional neuroimaging cannot stand alone. It has to be carried out after careful behavioural examination of the animal and has to be accompanied by anatomical imaging techniques to exclude organic lesions. Ideally, in its research phase, it needs post-mortem validation with autoradiography or immunohistochemical techniques and should be accompanied by investigation of other biochemical markers, to rule out metabolic, hormonal and other neurotransmitter influences and to explore possible peripheral markers, reflecting central alterations.

Applicability in daily veterinary practice

We must also stress the questionable applicability for common daily use in veterinary practice. An important methodological problem concerns the requirement of a brain dedicated gamma camera to optimise resolution and shorten acquisition procedures. Furthermore, receptor imaging requires specific receptor ligands and up to now, radioligands for SPET are scarce. As is the case for most receptor ligands, the tracer used in this study is labelled with ^{123}I (in the order of 500 euros per dog) which is too expensive to apply on routinely basis. It is therefore logical to conclude that this technique offers definite advantages as a research model for behavioural problems in dogs and as a model for certain human brain disease and behavioural abnormalities, but that practical application in veterinary medicine is still limited. Tracer labelling with ^{99m}Technetium might offer a more economical solution.

CLINICAL APPLICATION

The study of the feasibility of this SPET technique, both in the assessment of perfusion as in the evaluation of serotonin-2a binding index in the normal canine brain, was the first goal of our research. Its clinical application was evaluated in the study of the brain of the aging dog and the impulsive dog.

Aging dogs

First, concerning the perfusion alterations found in aged dogs, the increased anterior-posterior gradient observed is in agreement with findings in human aging brain. A possible explanation for this observation could be that regions, serving cognitive and affective behaviour, are more affected than the more sensory areas[4,5], which in turn could account for the cognitive and mood disorders encountered in aging individuals.

Second, the most important methodological confounder concerns the cortical atrophy, accompanying aging alterations. Nevertheless, regional hypometabolism in patients with Alzheimer disease, measured with PET, were confirmed after atrophy corrections based on MRI derived volume correction factors in some human studies.[6,7]

Third, the global decrease found in this study is in line with some but not all human studies on brain perfusion.[8-11] On the other hand, the regional decreases found in this study are comparable with human studies.[5,8-10,12,13] In humans, age related changes in rCBF are already noted in early to mid adulthood without accompanying cognitive decline or other degenerative processes. Concerning the altered 5-HT2A receptor radioligand binding mainly affecting the frontocortical cortex in our study, this is in partial agreement with human and primate studies In most studies a more general cortical decrease is found in normal aging brain[14,15], although some studies report a region dependent decrease and an anterior-posterior gradient in normal aging and in Alzheimer patients.[16,17] The absence of decreased binding in the other cortical regions in our study could also be artefactually induced by the larger standard deviation found in the temporo-, parieto and occipitocortical regions, accounting for a less sensitive detection of marginal decreases in binding index which could therefore mask more general decreases. In addition, it is also noteworthy to mention that a few human studies report a non-linear decline of serotonin-2A radioligand binding with the largest decline before the age of 40.[14,17,18] It could therefore be possible that the observed tendency to decreased binding in other regions, except for the parieto-cortical area, does not reach levels of significance in the age groups we examined. These observations emphasize the need to include a larger population reference dogs in order to validate the time of onset and the evolution of altered binding of the 5-HT2A receptor radioligand in smaller age groups.

Fourth, from previous research on rats and primates it is known that the activity of the serotonergic system also alters during brain maturation. It would therefore be of considerable interest to monitor the development of the serotonin-2A receptor in animals under different rearing conditions. In view of this, the SPET receptor imaging procedure could offer a feasible research paradigm to follow receptor status during brain development.

Impulsive, aggressive dogs

No perfusion alterations were found in this study. In human studies concerning aggressive and antisocial behaviour, focal regional perfusion and metabolism alterations are consistently reported in the prefrontal cortical regions but alterations in other regions differ between studies.[19-24] Since the brain structures of dogs are small, dividing the fronto-cortical and the subcortical structures into their structural and functional subdivisions (dorso-lateral and orbital fronto-cortical region, thalamus, amygdala..) would increase the partial volume effects to an impermissible degree precluding accurate quantification. Therefore we could only evaluate global alterations in regional perfusion, which could allow intra-regional compensation of focal increases and decreases. As an example, in the study of Raine et al (1997) decreased metabolism was present in some parts of the subcortical areas while in other parts increases were found. Another explanation could lie in the possible smaller contribution of the frontal cortex in the regulation of canine behaviour.

Concerning the serotonin-2A radioligand binding, a general increase was found in the impulsive, aggressive group, indicating that the serotonergic system is involved in this behavioural disorder in dogs. This finding is in line with post-mortem evaluation of serotonin-2A receptor binding with autoradiography in suicide victims.[25] We must take care of oversimplification by addressing only one receptor or even one neurotransmitter system. It has to be kept in mind that possible effects of downstream events following receptor activation and interaction of different neurotransmitter systems may contribute to the pathophysiology of the investigated disorders. As an example, we did not investigate the involvement of the adrenergic system. Soubrie and Bizot (1990) found in their experiments with rats that pharmacological enhancement of both serotonin and noradrenergic transmission decreased impulsivity (defined as capacity to wait or tolerance to delay of reward).[26] Furthermore, it is known that the $\alpha 2$ adrenergic receptor is involved in the modulation of the serotonergic system.[27] It has been demonstrated in rats that chronic stress not only results in altered frontocortical densities of the 5-HT2 receptor but also of the adrenergic receptors.[28-32] Also in humans, altered functioning of the adrenergic system has been found in combination with an altered serotonergic system in depression.[33]

The investigation of the effect of the hormonal status on the serotonergic system would make an interesting research subject, since it is known from literature that estrogens have a modulating role.[27,34-36] Not only from a veterinary pathophysiological, but also from a human medicine neuropsychiatric viewpoint, this research could shed a light on the influence of hormones on the

behavioural, affective and cognitive status in humans and could lead to a better understanding of behavioural disorders such as the Pre Menstrual tension Syndrome (PMS), which is now empirically treated with serotonergic drugs.[34]

The possibility to perform longitudinal studies in man is restricted due to radioprotective measures. Therefore studies are limited to cross sectional investigations, or at most one follow-up study, and by consequence, hamper differentiation between state or trait alterations of neurotransmitter systems.[37] It would be of considerable interest to evaluate the impulsive aggressive dogs after 6 months and longer (without any form of treatment) to evaluate the consistency of our findings. Moreover, future studies will also focus on pharmacologic manipulation of the serotonergic system in impulsive, aggressive dogs, to monitor the serotonergic system with both clinical evaluation and functional imaging. This imaging modality provides also the possibility to monitor the effects of diet interventions, e.g. tryptophan augmentation, suggested to influence aggressive behaviour.[34,38,39]

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SUMMARY

Summary

In the Introduction of this thesis, the evolution and technique of imaging involving radionuclides is described. A brief review is given on the history of the evolution of radioactivity for medical purposes. With the introduction of more efficient imaging modalities such as PET and SPET, brain imaging with radionuclides have gained an important place in investigation of normal physiology and of the pathophysiology of neuropsychiatric disorders in human medicine. The technical aspects of functional imaging are further explained in the introduction. In veterinary medicine no comparable imaging studies have been performed.

In the introduction, we postulated some questions regarding the applicability of functional brain imaging in dogs. These questions formed the core of this research project. The results will be summarized per chapter.

1. **Can we measure brain perfusion with functional brain imaging, using the SPET modality, in the canine brain and what is the normal distribution pattern in dogs without neurological or behavioural disorders?**

In the first chapter regional brain perfusion was investigated in 10 normal dogs. In this study ^{99m}Tc-ECD was used to measure perfusion with SPET. Since perfusion is coupled under normal conditions with metabolism and since its trapping mechanism relies on its metabolism from a lipophilic to a hydrophilic compound, it provides also an indirect measure of neuronal metabolism. Neither age nor gender influences were noted but the number of included animals (N=10, age between 1 and 9 years) was too small to draw definite conclusions. An important anterior-posterior gradient was demonstrated. No left to right differences were registered. Methodological confounders were discussed concerning partial volume effect and the effect of the anaesthesia. Since the examined structures are small, partial volume effects may play a significant role. The application of anaesthetics, which is an inevitable procedure, is a second confounder, although the effect is minimalized since the tracer is injected before induction of anaesthesia and is trapped within minutes in the brain, reaching stable values for at least 60 minutes. In the second chapter the application of a standardised, automated method was investigated to semi-quantify regional perfusion in a more objective way with less interindividual variance, offering the opportunity to compare data more accurately. The validity of this procedure was demonstrated in this chapter.

In conclusion, the feasibility of perfusion SPET imaging and the normal regional perfusion indices in normal canine brain are demonstrated in this chapter. An automatic registration procedure, using dedicated software can be used to semiquantify regional activities in order to make inter- and intra-individual comparison more accurate.

2. What are the technical issues to consider when performing receptor radioligand studies?

To outline the technicalities of receptor radioligand studies *in vivo*, we first reviewed the literature in the third chapter, with emphasis on animal studies. The advantage of neuroreceptor imaging is that physiology and physiopathology of neurotransmitter systems can be examined *in vivo*, pharmacodynamics-and kinetics of newly created drugs can be evaluated and the effect of treatment can be monitored. Moreover, the behaviour of the receptor and its transmitters can be followed in their natural surroundings including intra- and extracellular regulation and influences from other neurotransmitter systems. On the other hand, these modulating endogenous influences make interpretation of the results not always straight forward. Also, major difficulties are encountered in the production of radioligands. First, the creation of new ligands for SPET is hampered by the fact that the radionuclide may not alter the binding properties of the used receptor ligand. Technetium is the preferred radionuclide since it is easy to obtain from a generator "on site", but it is also large and can therefore interfere with the stereotactic properties of the ligand, thereby changing its binding characteristics. The other commonly used radionuclide for SPET is ^{123}I, which has the disadvantage that it is expensive, enhances lipophilicity and can also interfere with the binding properties of the ligand. Second, the lipophilicity, a characteristic necessary for blood brain barrier (BBB) passage, increases non specific binding to non target molecules. Third, although the tracer should have high affinity for the receptor, an equilibrial state has to be installed for the quantification procedure, which is precluded when the tracer is trapped too avid when affinity for the receptor is too high. Fourth, the specificity of the radioligand is not always 100% for one receptor and more, since not all subtypes of the different receptor systems are known, specific binding to a receptor subtype might not be so specific. Fifth, (semi)quantification in SPET examinations relies on the ratio method, precluding differentiation between receptor density and receptor affinity. Several neurotransmitter systems have already been imaged in different animal species, all studies in function of research on human disease.

In conclusion, this chapter points at the methodological issues that have to be considered in SPET functional receptor imaging with radioligands. All animal studies performed up to now are performed for human medicine use.

3. Is it possible to use the radioligand, ^{123}I-5-I-R91150 to image and quantify the 5-HT2A receptor in canine brain?

In the fourth chapter the applicability of the specific radioliogand ^{123}I-5-I-R91150 was evaluated in the canine brain. First, in order to investigate the selectivity of the tracer, displacement and blocking studies were performed with the cold (unlabelled) 5-HT2A antagonist ketanserine, displacing the radioligand or making the receptor unavailable for the tracer. Binding was displaceable, proving selectivity and reversibility of binding. Second, in order to semiquantify regional data using the ratio method, a steady state has to be achieved. This implies a stable state between free, non-specific and receptor bound ligand. In order to achieve this situation, no redistribution should occur from other organs, which was demonstrated in this study with time activity curves for most organs. Third, the optimal scanning time, the time that a steady state is achieved for at least the time of the acquisition, was determined from 90 minutes post-injection onwards, lasting throughout the whole biodistribution study (180 minutes).

In conclusion, this chapter describes the biodistribution characteristics of 123I-5I-R91150 and proves the feasibility of this tracer for serotonin-2A receptor imaging in the canine brain.

4. If so, what is the normal distribution pattern of this receptor in the brain of dogs without neurological or behavioural disorders?

In the fifth chapter the regional distribution of the serotonin-2A receptor radioliogand was measured in the normal canine brain. Ten dogs were included, aged between 1 and 9 years and gender was equally distributed. Highest regional radioactivity was found in the fronto-cortical region and lowest was registered in the cerebellum. A significant rostro-caudal gradient was noted in this series, which is not reported in human studies, although not all of these studies report regional activity of all cerebral areas. A possible explanation for the higher frontal radioactivity in this study could be due to species dependent regional distribution of this receptor. Larger studies and post-mortem information are needed to verify this gradient in dogs. Neither age nor gender influences were noted in this study, but, as was mentioned in the perfusion studies, the number of included subjects is too small to draw definite conclusions. Methodological confounders in this study were similar to those described for the perfusion studies, including the inevitable use of general anaesthesia. To minimize effects on the binding parameters of the radioligands, care was taken that anaesthetics used had no direct effects on the 5-HT2A receptor and had furthermore global, rather than regional effects on the brain circulation.

In conclusion, the serotonin-2A receptor tracer ^{123}I-5I-R91150, is suitable for assessing the regional brain 5-HT2A receptor binding index in dogs. Creation of this normal database forms the base for studies on abnormal canine behaviour.

5. What is the influence of age on brain perfusion and binding characteristics of the specific serotonin-2A radioligand ^{123}I-5-I-R91150 in normal aging brain?

In the sixth chapter perfusion and serotonin-2A binding index were evaluated in the normal aging canine brain. Twelve dogs (age >8 years) were compared with a group of young to middle age dogs (N=12, age <8 years). None of the aged dogs suffered from dementia as was determined with a questionnaire. A significant global decrease of perfusion and a regional decrease was found in the fronto- and temporocortical area, with no left-right differences. A linear decline was found between age and cortical perfusion. The 5-HT2A receptor binding index was decreased in the frontal region, again without left to right differences. No correlation was shown between perfusion and binding index of the receptor radioligand, indicating that the decreased binding index of ^{123}I-5-I R91150 in this study was not caused by perfusion alterations. Besides its importance as a database of normal aging dogs for comparison with pathological aging individuals, this study illustrates the necessity of age correction when examining perfusion and serotonin-2A radioligand binding index.

In conclusion, this chapter describes the perfusion and serotonin-2A binding index alterations in the brain of normal aged dogs, imaged and quantified with SPET.

6. Can we include impulsive aggressive behaviour in dogs as a clinical behavioural disorder to investigate with this imaging modality?

In the seventh chapter a research paradigm was expounded concerning the relation of perfusion and the serotonergic system in impulsive aggression, based on a literature study. In this review emphasis is laid on the pathophysiological base of abnormal coping in animals, rendering them unsuitable to socialize and in extremes, to survive. Evidence from literature is given that a dysfunctioning frontal cortex plays a major role in this kind of behavioural disorders since this structure has a major regulating influence on the basic drives of the limbic system. From a neurobiochemical point of view, it is shown that the serotonergic system has an important role in signal transducing in the limbic system as well in the cortical regions and as such, is primordial in the well functioning of these structures. In a small pilot study changes of binding of the serotonin-2A radioligand, ^{123}I-5-I-R91150, were present in dogs showing impulsive, aggressivity.

In conclusion, this literature review and the pilot study confirms the validity of the inclusion of impulsive, aggressive dogs in imaging studies on brain perfusion and serotonin-2A receptor evaluation.

7. Is it possible to demonstrate differences in brain perfusion and/or serotonin-2A radioligand binding using SPET between normal dogs and dogs showing impulsive, aggressive behaviour?

In the eight chapter the status of the 5-HT2A receptor and the cerebral perfusion was investigated in a group of impulsive, aggressive dogs (N=19), compared with a group of normal behaving dogs (N=12). Selection of the impulsive, aggressive animals was based on a questionnaire. None of the dogs suffered from neurological disease. No significant differences were found in brain perfusion but a significant increase in binding index of the serotonin-2A receptor radioligand was found in all cortical regions.

In conclusion, this chapter demonstrates the alterated binding properties of the serotonin-2A receptor radioligand in impulsive, aggressive behaviour in dogs, imaged and quantified with SPET.

SAMENVATTING

Samenvatting

In de inleiding van dit werk wordt de evolutie toegelicht van ioniserende straling voor medische toepassingen naast een korte uiteenzetting over de techniek van de beeldvorming met radionucliden. Met de introductie van beeldvormende modaliteiten zoals PET en SPET, krijgt deze techniek een belangrijke plaats in het onderzoek naar de normale physiologie en de pathophysiologie van neuropsychiatrische afwijkingen bij de mens. In diergeneeskunde zijn er tot op heden geen vergelijkbare studies uitgevoerd.
We brachten 7 vragen naar voor in de inleiding, betreffende de bruikbaarheid van functionele beeldvorming bij het onderzoek van de hersenfunctie bij de hond, dewelke onderzocht werden in deze thesis. De antwoorden hierop worden per hoofdstuk gegeven.

1. **Is het mogelijk hersenperfusie te meten met behulp van SPET beeldvorming, bij honden? Wat is de normale regionale perfusie bij honden zonder neurologische of gedragsafwijkingen?**

In het eerste hoofdstuk onderzochten we de regionale hersenperfusie bij 10 normale honden. We maakten gebruik van de zoekstof ^{99m}Tc-ECD. Deze methode geeft tevens een indirecte evaluatie van het hersenmetabolisme, gezien onder normale omstandigheden perfusie en metabolisme gekoppeld zijn en gezien de opname van de zoekstof afhankelijk is van metabole conversie van een lipofiel naar een hydrofiel product. Er werden geen leeftijds- noch geslachtsverschillen opgemerkt in deze studie maar het aantal honden was te klein om tot een definitieve conclusie te komen (N=10, leeftijd tussen 1 en 9 jaar). Verder vonden we een belangrijke rosto-caudale gradiënt en was er geen links-rechts verschil. Methodologische problemen werden aangekaart in dit hoofdstuk. Een eerste probleem is het partieel volume effect, dat niet onderschat mag worden gezien de kleine structuren die in beeld gebracht worden. Het niet te vermijden gebruik van algemene anaesthesie is een tweede belangrijke factor, maar het effect is waarschijnlijk niet groot, daar de zoekstof zeer snel opgenomen wordt in de cel na IV inspuiting, die gebeurt vòòr inductie van de anesthesie. In het tweede hoofdstuk wordt een geautomatiseerde registratie en semiquantificatie methode getest voor de perfusie data. Het voordeel van deze procedure is dat de perfusie data automatisch op mekaar worden gepast, waardoor de interindividuele variatie afneemt en de vergelijking van inter- en intra-individuele data accurater maakt.

Het gebruik van functionele beeldvorming voor hersendoorbloeding onderzoek kan toegepast worden bij de hond. Met behulp van de automatische registratie en semiquantificatie procedure daalt de interindividuele variantie gevoelig.

2. Waarmee moeten we rekening houden bij het uitvoeren van receptor radioligand studies?

In het derde hoofdstuk wordt een overzicht gegeven van de literatuur met betrekking tot de technische factoren van receptoronderzoek en quantificatie. Tevens worden ook de studies met betrekking tot dieren bekeken. Het voordeel van neuroreceptor-beeldvorming met PET en SPET is dat de fysiologie en pathofysiologie van neurotransmitter systemen *in vivo* kunnen geëvalueerd worden. Tevens kan men hiermee ook de farmacodynamische en -kinetische eigenschappen van nieuwe medicamenten uittesten. Een bijkomend voordeel, in vergelijking met *in vitro* of *post-mortem* studies, is dat het gedrag van de receptor en de neurotransmitters gevolgd kan worden in hun natuurlijke omgeving met intra- en extracellulaire regelende mechanismen en beïnvloeding van andere neurotransmitter systemen. Deze modulerende endogene invloeden maken echter interpretatie van de resultaten niet altijd éénvoudig. In dit overzicht worden ook de problemen bekeken die rijzen bij het aanmaken van nieuwe radioliganden. Een eerste vereiste voor een bruikbare ligand is dat het isotoop de bindingskarakteristieken van het ligand niet mag veranderen. ^{99m}Technetium is het meest gebruikte isotoop in nucleaire beeldvorming gezien het gemakkelijk en relatief goedkoop te genereren is uit een generator die in de buurt van de camera staat. Het nadeel is dat dit element groot is en al snel interfereert met de stereotactische eigenschappen van de receptor ligand, waarbij deze de mogelijkheid tot binding met de receptor verliest. Het andere veel gebruikte isotoop is ^{123}I. Dit heeft dan weer het nadeel dat het erg duur is, het de lipofiele eigenschap van het complex versterkt en het eveneens bindingseigenschappen van de ligand kan veranderen. De lipofiele eigenschap van de radioligand is noodzakelijk om door de bloed-hersen-barrière te geraken, maar deze eigenschap geeft eveneens aanleiding tot een verhoogde, niet specifieke binding met andere moleculen. Een geschikte receptor radioligand moet over de nodige affiniteit en specificiteit beschikken. Ondanks het feit dat de ligand een hoge affiniteit moet hebben voor de receptor in kwestie, mag deze affiniteit niet te sterk zijn om de semiquantificatie procedure, gebaseerd op een evenwichtstoestand tussen specifiek, niet specifiek gebonden en vrij ligand, niet te belemmeren. Een bijkomend probleem van receptor beeldvorming is dat de meeste gebruikte radioliganden niet 100% specifiek zijn voor de te onderzoeken receptor en dat daarenboven waarschijnlijk nog niet alle subtypes van bepaalde neurotransmitter-systemen gekend zijn, waardoor de veronderstelde specificiteit van binding mogelijk niet zo specifiek is. Als laatste punt dient vermeld te worden dat de semikwantificatie methode geen onderscheid mogelijk maakt tussen verandering in aantal of verandering in affiniteit van de onderzochte receptor.
Verschillende receptoren zijn reeds in beeld gebracht bij dieren voornamelijk in functie van onderzoek naar de pathofysiologie van humane afwijkingen. Onderzoeken in functie van specifieke diergeneeskundige problemen zijn tot op heden nog niet gebeurd.

Dit hoofdstuk bespreekt de methodologische factoren die in acht genomen moeten worden wanneer men receptor onderzoek uitvoert in vivo, met functionele beeldvorming gebruik makend van PET en SPET.

3. Is het mogelijk om de specifieke serotonine-2A receptor radioligand, ^{123}I-5-I-R91150, te gebruiken voor beeldvorming en kwantificatie van deze receptor in hondenhersenen?

In het vierde hoofdstuk werd de toepasbaarheid van de ^{123}I-5-I-R91150 als specifieke radioligand voor de 5-HT2A receptor bekeken. In eerste instantie werd de selectiviteit en de affiniteit van de ligand bekeken met behulp van verdringing en blokkeren van de receptor met de niet radioactief gemerkte concurrerende ligand ketanserine. De radioligand kon verdrongen worden waarbij reversibiliteit en specificiteit werd aangetoond. Bovendien kon de koude ligand, toegediend vóór de radioligand, binding verhinderen. Een tweede belangrijk punt in deze studie was het onderzoek naar het optreden van een evenwichtstoestand tussen niet specifieke en specifieke gebonden en vrije radioligand, een toestand die essentieel is voor semikwantificatie van de regionale activiteit. Deze situatie kan maar optreden als er geen redistributie gebeurt vanuit andere organen. Dit werd aangetoond in deze studie aan de hand van tijd/activiteitscurven voor verschillende organen. Het optimaal scanmoment, het moment dat er een evenwichtstoestand ontstaat gedurende minstens de tijd nodig voor de acquisitie, werd eveneens bepaald. Dit evenwicht werd bereikt vanaf 90 minuten na inspuiting van het radioligand en bleef gedurende de hele biodistributiestudie bestaan (180 minuten).

Als besluit kan gesteld worden dat de specifieke serotonin-2A receptor radioligand, 123I-5-I-R91150, gebruikt kan worden voor het in beeld brengen en kwantificeren van de serotonine-2A receptor in hondenhersenen.

4. Wat is de regionale distributie van de specifieke serotonine-2A radioligand, 123I-5-I-R91150, in normale honden zonder neurologische of gedragsafwijkingen?

In het vijfde hoofdstuk werd de regionale distributie van de serotonine-2A radioligand onderzocht bij 10 normale honden tussen 1 en 9 jaren. De hoogste radioactiviteit werd geregistreerd in de frontocorticale regio's en de laagste, zoals verwacht, in het cerebellum. Er werden geen geslachts- noch leeftijdsverschillen aangetoond maar zoals al vermeld bij de perfusie studie is het aantal honden te beperkt om definitieve besluiten te trekken. De rostrocaudale gradiënt die vastgesteld werd in deze studie is niet terug te vinden in humane studies, waar de corticale activiteit globaal het hoogste is in vergelijking met de subcorticale en cerebellaire activiteit. Nochtans dient opgemerkt dat in een aantal

studies de activiteit van niet alle corticale structuren wordt gerapporteerd. Een mogelijke verklaring voor de hogere frontale geregistreerde radioactiviteit kan de diersoort afhankelijke regionale distributie van 5-HT2A receptoren zijn. In ieder geval dient deze gradient geverifieerd te worden aan de hand van uitgebreidere klinische studies en post-mortale studies. Er werden geen geslachts- noch leeftijdsverschillen aangetoond. Maar, zoals al vermeld voor de perfusie studies, is de groep te klein voor definitieve conclusies. Methodologische problemen waren gelijkaardig zoals beschreven voor de perfusie studies. Om effecten van de anesthesie zo laag mogelijk te houden werden producten gebruikt die geen rechtstreekse invloed hebben op de 5-HT2A receptoren en die eerder een globaal dan een regionaal effect hebben.

Als besluit kan gesteld worden dat deze methode bruikbaar is voor het bepalen van de regionale distributie van de 5-HT2A receptor in hondenhersenen. De registratie van regionale waarden bij normale honden kan als basis dienen voor studies op dieren met afwijkend gedrag.

5. Wat is de invloed van leeftijd op hersenperfusie en binding van de serotonine-2A radioligand in normale verouderende honden?

In het zesde hoofdstuk werd doorbloeding en binding van de 5-HT_2A radioligand bekeken in 12 oude honden (>8 jaren) in vergelijking met een referentie groep (N=12; leeftijd < 8 jaren). Bij geen enkele oude hond werd er dementie vastgesteld (gebaseerd op een vragenlijst). Een significante globale daling van de perfusie en een regionale daling werd vastgesteld in fronto- en temporocorticale regio's, zonder links-rechts verschillen. De binding van de serotonine-2A radioligand was verminderd in de frontale regio, zonder links-rechts verschillen. Er werd geen correlatie gevonden tussen doorbloeding en 5-HT2A radioligand binding, wat erop wijst dat perfusieveranderingen niet aan de basis liggen van deze veranderde receptorbinding. Naast het belang als databank van normale verouderende honden, duidt deze studie ook op de noodzakelijkheid van leeftijdscorrectie bij het uitvoeren van perfusie en serotonine-2A receptor radioliganden studies.

Deze studie beschrijft leeftijdsafhankelijke veranderingen in regionale hersenperfusie en in bindingskarakteristieken van de serotonine-2A radioligand in beeld gebracht met SPET.

6. Zijn de neurobiologische kenmerken van impulsieve agressie van die aard dat we dit afwijkend gedrag kunnen invoegen in deze studie, betreffende perfusie en serotonine-2A receptor beeldvorming?

In het zevende hoofdstuk werd een onderzoeksparadigma ontwikkeld betreffende de relatie tussen hersenperfusie en het serotonerge systeem bij impulsieve agressie bij dieren, gebaseerd op een literatuurstudie. In dit overzichtsartikel wordt vooral aandacht besteed aan de pathofysiologie van abnormaal agressief en impulsief gedrag hetwelk het dier onmachtig maakt normaal te functioneren in groep, waarbij het ook zelfdestructief gedrag kan vertonen en het in het algemeen verlaagde overlevingskansen heeft. Abnormaal agressief gedrag is niet gemakkelijk te omschrijven bij dieren omwille van het feit dat een zekere vorm van agressie essentieel is als natuurlijk middel om te overleven, voedsel te vergaren en zich voort te planten. In de vrije natuur is het voor het in groep levende individu uitermate belangrijk voldoende sociale vaardigheden te bezitten in de omgang met groepsleden, die bepalend zijn voor de rang in de hiërarchie en overlevingskansen binnen deze groep. Als normale vormen van agressie worden maternale, territoriale, prooi gerelateerde en dominante agressie aanvaard. Een slecht functionerende frontale cortex speelt een belangrijke rol in abnormaal gedrag omdat deze structuur een regulerende rol heeft over het limbische systeem. Vanuit biochemisch oogpunt vindt men dat het serotonerge systeem een belangrijke rol speelt in het vlot doorgeven van signalen zowel in de cortex als in het limbische systeem. In een beperkte pilootstudie werd tevens aangetoond, dat veranderingen in binding van de serotonine-2A radioliogand aanwezig waren in de 4 geïnclineerde agressieve individuen.

Op basis van de literatuur studie en de pilootstudie kan besloten worden dat het zin heeft om perfusie en serotonine-2A binding te onderzoeken in agressieve, impulsieve honden.

7. Zijn er aantoonbare verschillen tussen normale honden en impulsieve, agressieve honden wat betreft hersendoorbloeding en bindingskarakteristieken van de serotonine-2A receptor radioligand, gebruik makend van SPET?

In het achtste hoofdstuk worden perfusie parameters en serotonine-2A receptor radioligand bindingen vergeleken tussen een referentie groep honden (N=12) en een groep impulsieve, agressieve honden. De agressief, impulsieve honden werden geselecteerd op basis van een vragenlijst. Geen enkele hond leed aan enige vorm van neurologische afwijking. Perfusie was niet significant verschillend tussen beide groepen, maar er werd wel een significant hogere corticale binding van de serotonine-2A receptor radioligand aangetroffen bij de agressieve groep in vergelijking met de referentie groep.

In dit hoofdstuk wordt aangetoond dat er verandering is in bindingskarakteristieken van de serotonine-2A receptor radioligand in agressieve, impulsieve dieren, in beeld gebracht met SPET.

DANKWOORD

Dankwoord

In mijn dankwoord richt ik mij in eerste instantie tot mijn promotor Prof. Francis Verschooten, waar ik tevens dit werk aan opdraag. Sinds jaren heeft hij voor de nodige ruggesteun gezorgd bij mijn opleiding zowel als radioloog als nadien als “nuclearist”. Zijn nimmer aflatende zorg mij te overtuigen dat “meten” “weten” is en zijn interesse voor het brein en vooral het afwijkende brein, naast het paardenbeen, heeft ertoe bijgedragen dat dit werk is ontstaan. Los van het feit dat hij van in het begin mijn mentor was op gebied van beroepsactiviteiten, is hij ook mijn beste, meest betrouwbare kameraad! Francis, dank je voor wat je al die jaren hebt betekend en nog zal betekenen. Ik hoop dat je, ondanks het einde nadert van jouw zéér productieve carrière, nog met raad en daad meehelpt aan het vervolg van dit verhaal…..

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CURRICULUM VITAE

Curriculum vitae

Kathelijne Peremans studeerde in 1983 af aan de faculteit diergeneeskunde, RUG, met onderscheiding. Gedurende twee jaren was ze werkzaam aan de universiteit van Liverpool, Engeland, waar ze een opleiding genoot in radiologie en orthopedische chirurgie van het paard onder toezicht van Dr. Wynn-Jones. Tijdens die periode volgde ze opleidingen in fractuurbehandeling en arthroscopie bij het paard, in Ohio en in Colorado State University, USA. In 1985 behaalde ze het "Certificate of Veterinary Radiology". Van 1985 tot 1991 werkte ze als assistente op de dienst chirurgie van de huisdieren, faculteit diergeneeskunde, RUG, waar ze zich verder bekwaamde in radiologie, echografie en arthroscopie van het paard onder leiding van prof Verschooten. Als bijkomende opdrachten verrichtte ze anaesthesie van het paard en chirurgie van het rund. Vanaf 1991 werkte ze als tweedelijns consultante in dierenkliniek Noorderwijk-Voortkapel en in opdracht van verschillende dierenartsen, voornamelijk voor radiologie, orthopedische diagnostiek en behandeling van paarden. In 1994 verkreeg ze de titel "Diplomate of the European College of Veterinary Diagnostic Imaging". Vanaf 1996 tot 2001 verplaatste haar werkterrein zich naar een eigen opgebouwde kliniek: "Aan de Watergang", waar ze voornamelijk tweedelijns mankheidsonderzoeken, keuringen en chirurgische ingrepen bij het paard deed. In 1997 ging ze in opleiding voor nucleaire geneeskunde aan het UZ, Gent, onder leiding van prof. Dierckx. In 1999 werd de aanzet gegeven tot hersenbeeldvorming bij de hond met behulp van radionucliden en SPET, dewelke leidde tot deze thesis. Vanaf oktober 2001 is ze fulltime werkzaam op de dienst medische beeldvorming van de huisdieren onder leiding van prof. Verschooten en prof. van Bree. Ze is auteur en co-auteur van verschillende nationale en internationale artikels en was spreker op internationale congressen.