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Functions of the Cholinergic System in the Morbidities Associated with Alzheimer's Disease and the Further Evaluation of Tools for the Molecular Imaging of this System

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Dedication

This thesis is dedicated to my family.

Acknowledgements

If I were to express fully here the thanks and appreciation which each person named below deserves, then this section would assuredly become far, far larger than the actual thesis. Thus, the following is a necessarily abbreviated acknowledgement and description of, and a thank-you to, the people without whom the accomplishment of this thesis would have been impossible, in a vaguely chronological order divided into poorly defined, possibly random, groupings.

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Preface

The material presented in this thesis represents original research performed by the author, with the exception of the radiolabelling components of the work described in Section 4.2. All experiments were conducted in accordance with ethical principles and the prevailing ethical requirements: either the European Community Council Directive 86/609/EEC for the care of laboratory animals or the Australian National Health and Medical Research Council guidelines on the care and use of animals in research in conjunction with the approval of the Animal Care and Ethics Committee of the University of Sydney. The material presented is for submission to the two universities listed in an approved cotutelle agreement and has not been submitted for a higher degree to any other university or institution.

Publications / Presentations

Quinlivan, M., Chalon, S., Vergote, J., Henderson, J., Katsifis, A., Kassiou, M., Guilloteau, D. (2007) Decreased vesicular acetylcholine transporter and α4β2 nicotinic receptor density in the rat brain following 192IgG-saporin immunolesioning. *Neuroscience Letters,* 415:97-101

Quinlivan, M. Henderson, J., Katsifis, A., Guilloteau, D., Kassiou, M, Chalon, S. (2007) Diminished nicotinic receptor density and an impairment in attention but not memory in the 192 IgG-saporin model of AD. *Neurodegenerative Diseases,* 4(S1):247

Quinlivan, M., Chalon, S., Vergote, J., Henderson, J., Katsifis, A., Kassiou, M., Guilloteau, D. (2007) Deficits in reflexive attention but not object-recognition memory n the rat following cortical lesioning with the specific anti-cholinergic immunotoxin 192 IgG-saporin. *In preparation*

Henderson, J., Schleimer, S., Allbutt, H., Dabholkar, V., Abela, J., Jovic, J., **Quinlivan, M.** (2005) Behavioural effects of parafascicular thalamic lesions in an animal model of parkinsonism. *Behavioural Brain Research,* 162:222-232

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Quinlivan, M., Mattner, F., Papazian, V., Zhou, J., Katsifis, A., Emond, P., Chalon, S., Kozikowski, A., Guilloteau, D., Kassiou, M. (2003) Synthesis and evaluation of Iodine-123 labelled tricyclic tropanes as radioligands for the serotonin transporter. *Nuclear Medicine & Biology,* 30:741-746

Abbreviations

6-OHDA	6-Hydroxydopamine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ADHD	Attention deficit hyperactivity disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
ANOVA	Analysis of Variance
APP	Amyloid precursor protein
$A\beta_{(n)}$	B-Amyloid protein (n = # of amino acids)
CBF	Cholinergic basal forebrain
Cbm	Cerebellum
ChAT	Choline acetyltransferase
CPu	Caudate / putamen
CT	Computed tomography
CTD	Cued target detection
Ctx	Cortex
d2	T2 discrimination index ({tN – tF}/{tN + tF})
DAB	Diaminobenzadine
DBB	(Nucleus of the) Diagonal band of Broca
DV	Dorsal – ventral
ECD	Bicisate
^{18}FDDNP	2-(1-(6-[(2-[18F]fluoroethyl)(methyl)amino]-2-

	naphthyl)ethylidene)malononitrile
FDG	2-Fluorodeoxyglucose
FOV	Field-of-view
GABA	γ-amino-butyric acid
Hip	Hippocampus
*I	Radioiodine
IBVM	Iodobenzovesamicol
icv	Intra-cerebroventricular(ly)
IHC	Immunohistochemistry
IR	Immunoreactive
ITI	Inter-trial interval
iv	Intravenously
keV	Kilo electron-volts
mAChR	Muscarinic acetylcholine receptor
MAO	Monoamine oxidase
MCI	Mild cognitive impairment
MCSRT	Multiple choice serial reaction time task
MFB	Medial forebrain bundle
MMSE	Mini mental-state exam
MRI	Magnetic resonance imaging
MS	(Nucleus of the) Medial septum
N, F	Novel object, Familiar object

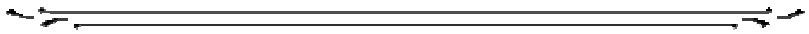
NAC	Nucleus accumbens
nAChR	Nicotinic acetylcholine receptor
NBM	Nucleus basalis magnocellularis
NFT	Neurofibrillary tangle
NMDA	*N*-methyl-*D*-aspartate
NORT	Novel object recognition task
$p75^{NGFr}$	p75 (low affinity) nerve growth factor receptor
PBS	Phosphate-buffered saline
PD	Parkinson's disease
PET	Positron emission tomography
PHF	Paired helical fragment
PIB	Pittsburgh compound B
PLSD	Protected least squares difference
PMT	Photomultiplier tube
PRh	Perirhinal cortex
rCBF	Regional cerebral blood flow
RN	Radionuclide
ROD	Relative optical density
ROI	Region of interest
RP	Radiopharmaceutical
RT	Reaction time
SAP	192 IgG-saporin

SI	Substantia inominata
SPECT	Single photon emission computed tomography
T1, T2	Trial 1, Trial 2
Thl	Thalamus
TOHR	Tomographe Haute Résolution (High-resolution tomograph)
VAChT	Vesicular acetylcholine transporter
VBP	Vesamicol-binding protein

Resumé / Abstract

Les objectifs de cet projet étaient de participer à la caractérisation de la fonction du système cholinergique dans les processus d'attention et de mémoire, deux domaines cognitifs très diminués dans la maladie Alzheimer (MA), et d'évaluer et de développer des outils pour l'imagerie fonctionnelle moléculaire de cet système afin d'améliorer la connaissance de la MA et autres désordres neurologiques. Afin d'atteindre le premier objectif, la toxine spécifique anti-cholinergique 192 IgG-saporin (SAP) a été administrée aux rates (femelles) « Sprague-Dawley » soit par une voie intra-cerebroventriculaire (icv) soit par voie intra-corticale. Ensuite deux tests, un test du temps de réaction à l'excitation vibrissale et un test de reconnaissance d'objet, ont été effectués aux animaux SAP et témoins pour évaluer leur fonction attentionelle et mnémonique. Le deuxième objectif a été approché de deux manières. D'abord, la densité neuronale relative chez les animaux avec lésions icv a été étudié par autoradiographie *ex vivo* et *in vitro* chez le même animal, à l'aide de deux radiopharmaceutiques :[^{123}I]iodobenzovesamicol (^{123}IBVM) (*ex vivo*, ligand pour le transporteur acétylcholine vésiculaire) et ^{125}I-A-85380 (*in vitro,* ligand pour le récepteur acétylcholine nicotinique). De plus, de nombreuse études *in vivo* et *in vitro* (fantômes physiques) ont été effectués avec un système d'imagerie moléculaire neuve et unique (TOHR), afin de mesurer et d'améliorer ses performances et étudier son utilité pour l'imagerie moléculaire des petits animaux. Les études comportementales supportent l'idée d'une fonction cruciale du système cholinergique dans la processus d'attention normal. De plus, en accord avec la

littérature, aucune déficience mnémonique n'a été observé. Cependant, on peut conclure que les résultats observés chez les animaux avec lésion intracorticale soutiennent l'hypothèse déjà publiée que les nerfs afférents au « *perirhinal cortex* » sont indispensables à la mémoire de reconnaissance d'objet. Dans les études autoradiographiques au ^{123}IBVM, le signal mesuré chez les animaux SAP est inférieur à celui des témoins dans nombreuses régions. La densité de récepteurs nicotiniques est aussi diminuée chez ces animaux, un résultat qui montre très bien les caractéristiques superbes du I-A-85380 radioiodiné. Les études des performances de TOHR démontrent son utilité de principe pour l'imagerie moléculaire des petits animaux.



The aims of this project were to contribute to the elucidation of the role of the cholinergic system in attention and memory, two cognitive processes severely compromised in Alzheimer's disease (AD), and to evaluate and develop tools for the functional molecular imaging of this system with a view to improving knowledge of AD and other neurological disorders. Towards the first aim, the specific anti-cholinergic toxin 192 IgG-saporin (SAP) was administered to female Sprague-Dawley rats via either an intracerebroventricular (icv) or an intracortical route and animals were tested with a vibrissal-stimulation reaction-time task and an object recognition task to evaluate their attentional and mnemonic function, respectively. The second aim was approached in two ways. Firstly, relative

neuronal densities from animals with icv lesions were assessed with both *ex vivo* and *in vitro* autoradiography with the specific cholinergic radiopharmaceuticals [^{123}I]iodobenzovesamicol (^{123}IBVM) and ^{125}I-A-85380, ligands for the vesicular acetylcholine transporter and the nicotinic acetylcholine receptor, respectively. Secondly, a number of *in vivo* and *in vitro* studies were performed on a novel and unique molecular imaging system (TOHR), with which it had been hoped initially to image eventually SAP-lesioned animals, with a view to measuring and ameliorating its performance characteristics and assessing its in-principle suitability for small-animal molecular imaging. The behavioural studies support a critical role for the cholinergic system in normal attentional function. Additionally, in accord with literature evidence, no significant impairment was observed in mnemonic function. It is postulated however that the results observed in the intracortically-lesioned animals support the published hypothesis that cholinergic projections to the *perirhinal cortex* are critical for object-recognition memory. In autoradiographic studies, SAP-lesioned animals demonstrated reduced uptake of ^{123}IBVM in multiple regions. A reduction of nicotinic receptors was also seen in SAP-lesioned animals, a novel finding supportive of the excellent characteristics of radioiodinated I-A-85380. Examination of the performance characteristics of the TOHR support in principle its utility for targeted small-animal molecular imaging studies.