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Cerebral Hemodynamic Disturbances in Motor Neuron Disease

Matthew J. Murphy

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Cerebral Hemodynamic Disturbances in Motor Neuron Disease

(Spine title: Cerebral Hemodynamics in Motor Neuron Disease)

(Thesis format: Integrated-Article)

by

Matthew J. Murphy

Graduate Program
in
Medical Biophysics

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A thesis submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

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ABSTRACT AND KEYWORDS

An association between motor neuron disease (MND) and dementia was first realized in the late 1800s, yet substantiating research and a description of dementia as part of the clinical syndrome would not appear until the 1990s. In the last two decades, medical imaging has investigated cerebral blood flow changes in the motor and non-motor cortex to correlate with motor dysfunction and clinical dementia, respectively. The aim of this thesis is to describe early cerebral hemodynamic disturbances with the goal to determine a marker for cognitive decline in MND.

Chapter 2 describes the relationship between changes in cerebral hemodynamics and cognition in primary lateral sclerosis (PLS) patients compared to normal controls. Neuropsychological testing revealed subtle frontotemporal changes characterized by executive dysfunction that were associated with global increases in mean transit time (MTT) in grey and white matter, and increased cerebral blood volume (CBV) in the frontotemporal grey matter.

Chapter 3 presents a longitudinal clinical study of early cerebral hemodynamic changes in amyotrophic lateral sclerosis (ALS) patients without evidence of cognitive impairment at study onset. This Chapter characterized the relationship between duration of disease and MTT in the cortical grey matter. MTT was found to be the most sensitive indicator of early cerebral hemodynamic change accompanying disease progression in ALS. Furthermore, these findings corroborate the trend of increased MTT in the absence of cognitive impairment found in PLS patients in Chapter 2, and may further indicate that hemodynamic changes may occur before the onset of cognitive impairment.

The aim of Chapter 4 was to elucidate a biological mechanism for increased MTT described in the previous Chapters 2 and 3. A rabbit model of global hypotension was used to demonstrate that MTT is an indicator of cerebral perfusion pressure (CPP).

A spectrum of cognitive dysfunction has now been described in MND. The use of sensitive neuropsychological testing has enabled us to identify patients with mild changes in cognitive function from those who are cognitively intact. With the help of this stratification, we were able to show that changes in MTT was associated with disease progression and cognitive impairment. The experimental data presented in this thesis suggest that vascular factors may contribute to cognitive dysfunction in MND.

Keywords:

CT Perfusion, cerebral blood flow, cerebral blood volume, mean transit time, motor neuron disease, primary lateral sclerosis, amyotrophic lateral sclerosis, frontotemporal dementia, cognitive impairment

CO-AUTHORSHIP

Chapter 2 has been adapted from the paper entitled "Cerebral Haemodynamic changes accompanying cognitive impairment in primary lateral sclerosis", accepted for publication in *Amyotrophic Lateral Sclerosis* (In Press), by: Matthew J. Murphy, G.M. Grace, M.C. Tartaglia, J.B. Orange, X. Chen, A. Rowe, K. Findlater, R.I. Kozak, M. Freedman, M.J. Strong, and T-Y. Lee. Ting-Yim Lee and Michael Strong were responsible for the design of this study, provided supervision throughout the project, and reviewed the manuscript. Gloria Grace performed all neuropsychological testing and contributed to the preparation of the manuscript for those sections pertaining to her work. Carmela Tartaglia assisted in segmentation analysis of the cerebral images and revising the manuscript. Xiaogang Chen programmed the software used for analysis of data in this paper. The remaining authors were part of the clinical team who helped to recruit patients, collect clinical data, and revised the final manuscript. I was responsible for performing the study with Ting-Yim Lee, collecting and analyzing the data, and writing the manuscript.

The contents appearing in Chapter 3 have been adapted from the paper entitled "Widespread haemodynamic disturbances occur early in amyotrophic lateral sclerosis", submitted to *JNNP – Journal of Neurology, Neurosurgery, and Psychiatry* in August 2008 by: Matthew J. Murphy, G.M. Grace, M.C. Tartaglia, J.B. Orange, X. Chen, A. Rowe, K. Findlater, R.I. Kozak, M. Freedman, T-Y. Lee, and M.J. Strong. Ting-Yim Lee and Michael Strong designed this study, provided supervision throughout the project and reviewed the manuscript. Gloria Grace was responsible for obtaining and analyzing all

neuropsychological data. Xiaogang Chen assisted with the analysis of this paper and designed the software used for analyzing data from this study. The remaining authors were part of the clinical team who helped to recruit patients, collect clinical data, and revised the final manuscript. I was responsible for performing the study with Ting-Yim Lee, collecting and analyzing the data, and writing the manuscript.

Chapter 4 is a manuscript entitled "Evaluation of mean transit time as index of cerebral perfusion pressure in experimentally graded systemic hypotension", by: M.J. Murphy, K.M. Tichauer, D. Ouimet, X. Chen, and T-Y. Lee, to be submitted to *AJNR – American Journal of Neuroradiology*. This study was designed and supervised by T-Y. Lee. Animal care and experimental procedures were supervised by D. Ouimet. Software used for analysis of parametric maps was written by X. Chen. K. Tichauer contributed to preparation and revision of manuscript. I was responsible for contributing to the study design, collection and analysis of data and writing the manuscript.

EPIGRAPH

“Ideas were not divided into good and bad, only those that worked and those that didn't work. And although an idea might not work for several reasons, one part of that idea might have merit. But you couldn't pluck the ripe part if the whole idea went unspoken”

- Gary Kinder, *Ship of Gold: In the Deep Blue Sea*

DEDICATION

For Mom,

whose words of encouragement, love, and support have guided me
throughout my life and education to this day - and beyond.

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The work contained within this thesis has developed over the last five years and is only made possible by the collaborative effort and support of those surrounding me. First and foremost, I would like to thank my supervisor Dr. Ting-Yim Lee for giving me the opportunity to study in his lab, allowing me the freedom to find my own way while at the same time never letting me stray too far and for all the support he has shown me. However, it is his kindness and support towards my endeavors outside of the lab that I have appreciated the most – allowing me to maintain a balance between life and school during the tenure of my PhD. Thank you. Furthermore, I would like to thank Ting's wife Maggie for all the kind conversation we have shared, the support she has shown me, and of course, the wonderful parties she has hosted over the years.

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LIST OF ABBREVIATIONS

5-HT _{1A}	5-hydroxytryptamine
ACh	Acetylcholine
ACZ	Acetazolamide
AD	Alzheimer Disease
ADC	Apparent Diffusion Coefficient
AIF	Arterial Input Function
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised
Ca ²⁺	Calcium
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
Cho	Choline
CMRGlu	Cerebral Metabolic Rate of Glucose
CMRO ₂	Cerebral Metabolic Rate of Oxygen
Cr	Creatine
CST	Corticospinal Tract (or Pyramidal Tract)
CT	Computed Tomography
CTP	Computed Tomography Perfusion
CVR	Cerebrovascular Reserve
DTI	Diffusion Tensor Imaging
EDCF	Endothelial Derived Constricting Factor
EDHF	Endothelial Derived Hyperpolarizing Factor
EMG	Electromyography
FA	Fractional Anisotropy
fALS	familial Amyotrophic Lateral Sclerosis
FDG	¹⁸ Fluro-2-deoxy-D-glucose
fMRI	function Magnetic Resonance Imaging
FMZ	[¹¹ C]-flumazenil
FTD	Frontotemporal Dementia

FTLD	Frontotemporal Lobar Degeneration
FVC	Forced Vital Capacity
GABA	Gamma-Aminobutyric Acid
ICP	Intracranial Pressure
IRF	Impulse Residue Function
LMN	Lower Motor Neuron
MAP	Mean Arterial Pressure
MCI	Mild Cognitive Impairment
MD	Mean diffusivity
MND	Motor Neuron Disease
MRI	Magnetic Resonance Imaging
MRS	Proton (H^1) Magnetic Resonance Spectroscopy
MTT	Mean Transit Time
NAA	<i>N</i> -acetylaspartate
NE	Norepinephrine
NVU	Neurovascular Unit
OEF	Oxygen Extraction Fraction
PET	Positron Emission Tomography
PGE ₁	Prostaglandin E ₁
PLS	Primary Lateral Sclerosis
rCBF	Regional Cerebral Blood Flow
sALS	sporadic Amyotrophic Lateral Sclerosis
SOD1	Superoxide Dismutase 1 gene
SPECT	Single Photon Computed Tomography
SPM	Statistical Parametric Mapping
TDC	Time-Density Curve
UMN	Upper Motor Neuron
Xe/CT	Xenon-enhanced Computed Tomography

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

1.1.1 Motor Neuron Disease

Motor neuron disease (MND) refers to a condition characterized by the progressive degeneration of motor neurons and can include upper and/or lower motor neuron tracts (1). Thus, MND is used variably to describe neurological conditions involving neurodegeneration. For the purpose of this thesis, MND will be used as an umbrella term covering a spectrum of different clinical phenotypes involving neurodegeneration of the motor neurons. More specifically, the research conducted in this thesis will focus on two phenotypes of MND; amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis (PLS).

1.1.2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis, more colloquially known as ALS or Lou Gherig's disease, is the most common variant of MND (2) and was first described by Charcot in 1869. Although it is considered the most common variant, ALS remains a relatively rare disease with a reported population incidence between 1.47 and 2.7 per 100,000 per year (average of 1.89) (3), with the majority of patients succumbing to the disease within 3 to 5 years from the time of onset. The term "amyotrophy" refers to muscle wasting, while "lateral sclerosis" refers to the thickening or hardening of the lateral tracts of the spinal cord following degeneration of cortical spinal tracts (CST) as evidenced only upon

autopsy (1). The classical clinical description of ALS involves signs attributed to the degeneration of both the upper (UMN) and lower motor neurons (LMN). In the former, symptoms include subtle impairments of motor control, changes in vocal tone and clarity, changes in gait and ensuing loss of agility. Involvement of the latter clinically includes atrophy, weakness and fasciculation (4). Symptoms commonly arise as distal weakness of a limb (spinal onset) or dysarthria and dysphagia (bulbar onset) (5). Although the features of the disease may seem well defined, many are subtle in origin or may be non-specific to the disease and are often overlooked as a normal age-related changes, often leading to a diagnostic delay of greater than 1 year from time of onset (4); a considerable delay considering the rapid course of the disease. Diagnosis of the disease is estimated to be correct 95% of the time (1) when it is guided by the El Escorial criteria which include evidence of LMN degeneration by clinical, electrophysiological, or neuropathological examination; evidence of UMN degeneration by clinical examination; and progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination (6).

Although the disease was first characterized over one century ago, the aetiology of the disease remains unknown for the vast majority of cases. The clinical phenomenology describes ALS as either sporadic (sALS) or familial (fALS) in origin, with 90% of patients being described as sporadic, that is having no known etiology. The latter designation, fALS, accounts for 10% of all cases in ALS (7), of which many genes have been proposed to play a causal role. A fifth of these cases are associated with mutations in copper-zinc superoxide dismutase gene (SOD1) (8). SOD1 is the only gene that has been confirmed to cause classical ALS and has over 100 different mutations

described in the literature (9). The primary function of this protein is to act as an antioxidant enzyme, lowering the steady-state concentration of superoxide by the conversion of these toxic superoxide radicals to oxygen hydrogen peroxide, but when mutated, it can also cause disease by free radical toxicity leading to neuronal cell death or apoptosis.

Since its first description, the clinicopathological definition of ALS has progressed to incorporate a complex heterogeneous phenotype. Although motor neuron degeneration still remains as the central component of the disorder, it is now recognized that the phenotypical variability of the disease now includes non-motor manifestations. Contemporary clinical descriptions of ALS have evolved to recognize cognition as part of the disease spectrum (10), which will be covered in more detail in the following sections.

1.1.3 Primary Lateral Sclerosis

Primary lateral sclerosis is the designation used for MND when the degeneration is confined to the UMN, sparing the involvement of LMN, and having an idiopathic, non-familial origin (11). In the hundred years to follow the first description of PLS in 1874 by Charcot, little progress has been made on this rare disease that accounts for 5% of all clinical MND diagnoses (12). Recently, Pringle et al. proposed clinical diagnostic criteria for PLS that specified a minimum duration of 3 years from the time of onset with minimal lower motor neuron involvement and denervation potentials observed on electromyography (EMG) (13). This report was one of the first to attempt to define clinical characteristics and diagnostic criteria needed to delineate this disease from ALS, which in itself is a contentious issue. Whether PLS exists as a discrete entity or along a

continuum that may inevitably converge with ALS is heavily debated (13-20). The distinction between this apparent dichotomous phenomenology may involve the initial diagnosis and how stringent one is when employing diagnostic criteria. One criticism of the criteria proposed by Pringle et al. is the explicit acceptance of "occasional" LMN pathology as demonstrated by EMG recordings and fasciculation, rendering the criteria to be less specific to PLS. Subsequent studies using this criteria demonstrated that patients converted to ALS as late as 25 years from the time of diagnosis (21). More recently, Gordon et al. provided new more stringent criteria for clinically defining PLS that increased the sensitivity of diagnosis (22). By extending the diagnosis window to 4 years in conjunction with the absence of EMG findings, muscular atrophy and fasciculation, they were able to categorize PLS into clinically pure PLS and UMN-dominant ALS. Beyond 4 years the probability of developing LMN signs is about 0.23; had the authors followed the Pringle criteria 31% of their patients would have been misdiagnosed with PLS. Furthermore, those having subtle signs of LMN involvement and fasciculation at diagnosis progress to develop a more severe disability likened to that of ALS as measured by the ALS functional rating score – revised edition (ALSFRRS-R) and forced vital capacity.

As noted, the clinical characteristics of PLS often overlap with that of ALS, making the diagnosis difficult. The central issues for resolving PLS as a distinct disease from ALS are not merely academic, but reside in the fact that during the course of PLS there is the possibility of converting to ALS and with it more dire prognostic ramifications. To date, the defining characteristic unique to PLS is longer duration of survival due to a slowly progressive disease course. Unfortunately, due to the rare nature

of the disease there is a paucity of data concerning pathology and functional changes in PLS. However, with the advent of medical imaging and neuropsychological testing, paradigms that exploit cognitive changes in ALS have recently been applied to PLS. To this end, fundamental descriptions of cognitive and associated cerebral functional changes have been described in PLS and these changes warrant further interrogation.

1.2 COGNITIVE DYSFUNCTION IN MND

As previously described, MND was traditionally considered a disorder of the motor system sparing cognitive functions. However, it is now known that cognitive dysfunction represents a component of the disease that encompasses a striking spectrum of disorders ranging from mild cognitive impairment to frontotemporal dementia (FTD) (5). FTD is one of three subtypes of frontotemporal lobar degeneration (FTLD) characterized by the loss of neurons in the frontal or anterior temporal lobe of the cerebral cortex, and is the most commonly described subtype in ALS (23). FTD is a far less common pathology than Alzheimer disease (AD), and in contrast to AD produces early changes in behaviour, executive function, and language, whilst leaving memory relatively intact.

Cognitive dysfunction in MND has only recently gained the attention of the scientific community despite being described over a century ago and being proposed to be part of the syndrome as early as 1932 (24). The delay in recognizing cognitive decline as part of the syndrome of disorders in ALS is likely rooted in the fact that little was understood about the changes occurring and the relatively insensitive metrics being employed for determining these changes. First, the rapid progression of

neurodegeneration and rapid mortality of the disease often overshadows any subtle changes that arise in cognition. Second, co-morbid depressive symptoms often mimic cognitive decline (5). The prevalence of depression in ALS varies depending on the report, but it is generally acknowledged that patients have higher depression scores than controls, even if clinically relevant levels of depression aren't achieved (25). Thirdly, patients present with dysarthria and decreased motor control, which commonly interfere with testing paradigms employed to determine the extent of cognitive decline. Lastly, mental status changes in ALS have been described as fronto-executive in nature, giving rise to impairment in executive functioning and some language characteristics (5, 24, 26), differing from conceptual knowledge and memory impairment that characterizes other more common variants of dementias (5). Thus, there has been a shift in cognitive testing paradigms to become more sensitive to the type of impairment present in ALS. With raised awareness of these problems, contemporary studies have been designed to alleviate the influence of these co-morbid features and elucidate changes in cognition that may accompany the disease and have provided more accurate information on concomitant changes in cognition.

Evidence of cognitive dysfunction in MND is becoming more recognized, although reports of the incidence and extent of dysfunction still remain to be settled. ALS, the most prominent variant of MND, has enjoyed the bulk of the neuropsychological studies to date while PLS has only recently gained attention in this field.

1.2.1 A Spectrum Disorder: Mild Cognitive Impairment to Frontotemporal Dementia

Cognitive impairment in ALS comprises a continuum of conditions that range from no discernable deficit to FTD. Although there is general agreement that cognitive dysfunction is present in ALS, the incidence has proven to be widely variable between studies. Early studies of cognitive impairment were rudimentary in their approach and did not discern between mild impairment and dementia. Thus, early studies dichotomized patients into impaired or cognitively intact. Results of several studies indicated that a range of 36% to 70% of patients had some degree of cognitive impairment (27-29). Although these studies did not differentiate by degree of impairment, they provided evidence for cognitive changes and stimulated more in-depth examination.

When patients were stratified according to the level of impairment, the incidence of dementia originally was estimated to be only 2% to 5% among ALS patients. However, recent data suggest that the incidence rate may be as high as 23% to 52% (29, 30). Variability in the incidence rate of dementia can be attributed to small sample sizes, selection bias, diverse definitions of cognitive impairment (i.e. number of abnormal tests, individual test threshold for impairment), and differing test batteries (31). Currently, no consensus exists on the definition of cognitive impairment and the metrics employed to measure the level of impairment. From past experience and outcomes, it has become obvious that although tests such as the Mini Mental Status Exam (32) are highly sensitive to other forms of dementia, they are not appropriate for assessing frontotemporal cognitive changes associated with MND. Thus, the necessity for harmonization standards is now widely recognized by the MND community, and guidelines arising from

discussions at the Second International Frontotemporal Dementia in ALS Research Conference (London, Ontario, June 2007) are currently being drafted.

In the largest neuropsychological study to date assessing cognition in ALS, Ringholz et al. (2005) examined 279 consecutive sALS patients attending their clinic and compared them with 129 healthy controls (33). This study found cognitive impairment in 47% of the patients, with 15% of the whole cohort having features consistent with FTD. An important outcome of this study was that the cognitive impairment did not correlate with depression scores or the severity or duration of motor or bulbar symptoms. Furthermore, the site of onset (bulbar versus limb) was not predictive of the level of impairment or the pattern of performance. This work was corroborated by a recent study of 23 ALS subjects by Murphy et al. (2007) that found similar frontotemporal impairment in 11 patients. Of these 11 patients, 5 met the Neary criteria (34) for FTLD (2 with FTD, 2 with semantic aphasia and 1 with progressive non-fluent aphasia) while 6 displayed either mild cognitive or behavioural deficits (35). Thus, these recent studies indicate that about half of ALS patients are subject to some level of cognitive dysfunction, while a subset of these individuals have FTD as defined by the Neary criteria (34).

Primary lateral sclerosis is much rarer than ALS, and for this reason the neuropsychological changes remain largely undefined and excluded from the clinical syndrome until recently. The recent recognition of cognitive changes occurring in ALS has helped to refocus the topic on PLS due to the numerous overlapping features of the two diseases. In their seminal paper describing clinical features and diagnoses criteria, Pringle et al (1991) found that psuedobulbar affect occurred in some patients, but without a detailed assessment of neuropsychological changes all patients were regarded as

cognitively intact. Subsequent studies began to note cognitive dysfunction as “intellectual impairment with preservation and disorientations” without administering any detailed neuropsychological assessment (36).

To date there exist only three studies that systematically assessed the neuropsychological element of PLS. The first was a relatively small study by Caselli et al (1995) that reported cognitive impairment observed by neuropsychological tests (NT) sensitive to frontal lobe function and memory. These results were substantiated in a larger cohort of 20 PLS, of which 16 had significantly more errors in frontal lobe and/or premotor functions when compared with age- and education-matched controls (17). The neuropsychological abnormalities were considered moderate in all cases with no individuals considered for dementia. The latest assessment of cognition in PLS by Piquard and colleagues (2006) has provided the most detailed assessment to date (37), with the pattern and incidence of impairment similar to that of ALS. It was found that 17 of 20 patients had premotor and/or prefrontal deficits, although none was considered demented.

The present studies on cognition in ALS and PLS indicate that there are many commonalities in the cognitive profile of the two diseases. Both have apparent deficits in the frontotemporal region, which are associated with executive dysfunction, albeit much less pronounced or severe in the PLS subtype. Dementia is not reported as part of the clinical syndrome of PLS in the literature; in contrast, the incidence rate of dementia for ALS is ~15%.

1.2.2 Longitudinal Assessment of Cognition

The aforementioned studies have determined the level of cognitive impairment at different time points of MND and established that a spectrum of dysfunction is present. However, longitudinal data is a necessity to determine if cognition, like motor function, in MND is susceptible to rapid decline with disease progression. Understanding this facet of disease will provide a more complete knowledge of the underlying pathology and determine if all patients are predisposed to cognitive dysfunction. Furthermore, biomarkers may be unveiled in patients who present with intact cognition but progress to cognitive impairment at the late stages of disease, to help predict cognitive decline and targeted treatment for such a disorder.

A paucity of longitudinal studies assessing cognitive decline in ALS is attributed to patient recruitment problems and high attrition rates (25, 38). Recent studies have found that rate of cognitive decline in ALS was dramatically different from that of progression of other facets of the disease. A large longitudinal study assessing cognitive function in 52 patients found that cognition was impaired early in the disease but it did not substantially decline with disease duration but maintained a more stable course than motor function (38). Patients were studied at baseline and followed up every 4 months for the next year and if possible at 18 months post baseline. These results were corroborated by the work of Kilani et al (2004) who described no progression of cognitive impairment over a 12 month study period (25). As a caveat, the lack of progression documented in these studies may be due to late recruitment of patients. Early recruitment of patients may detect early cognitive changes that would have been missed if patients were not studied at the time of onset (39). Furthermore, Robinson et al (2006)

found deterioration of cognitive performance measured over a six month time period, with 7 of 19 subjects showing progression of abnormal cognitive performance. Furthermore, the authors of this study speculate that if followed for a longer duration the 12 subjects who remained stable over six months may in fact progress to cognitive impairment and possibly FTD. All measured cognitive deficits at any time point in the above longitudinal studies were frontotemporal in nature and confined to mild changes, with no patient being classified as demented. These studies represent a starting point for further longitudinal investigation and provide important information on recruitment and duration of follow up studies.

1.3 MEDICAL IMAGING IN MND

Raised awareness of cognitive deficits and cortical abnormalities in MND, together with the advent of advanced imaging, have provided the opportunity to develop more specific imaging techniques to study these changes *in vivo*. Imaging studies are important in developing a better understanding of the progression and pathology of MND because not only does it have the ability to detect changes at multiple time points in the same patient, imaging studies also provide a correlate for histopathology.

1.3.1 Magnetic Resonance Imaging

Histopathology of disease is an end-stage measurement and gives no indication of how disease progresses. Magnetic resonance imaging (MRI) is a powerful imaging modality capable of examining structure and function *in vivo* with proven diagnostic value. In the past, MRI was used for indirect diagnosis by eliminating pathologies that

mimic MND. However, recent advances in MRI technology have broadened its use and it is now routinely used in research studies to identify *in vivo* UMN dysfunction. Upper motor neuron markers are important to improve diagnostic certainty, expedite treatment and provide more timely access to clinical trials.

1.3.1.1 Anatomical Imaging

Anatomical studies used to be performed with computed tomography (CT) to assess gross structural changes occurring in the brain. This technique eventually gave way to the use of magnetic resonance imaging (MRI) because of its superior soft tissue contrast allowing precise anatomical localization of atrophy. Another important advancement is the development of 3D image registration methods to align an individual patient's anatomy to standard brain atlases (40, 41).

Whether brain atrophy exists in patients with ALS and PLS and if so to what extent is controversial due to the high variability in neuroimaging results (42). Historical accounts of atrophy in the precentral gyrus in PLS patients was identified indirectly by enlargement of the adjacent central sulcus (13, 43) and were found to be associated with disease duration (44, 45). However, with modern imaging techniques atrophy is now more easily visualized in ALS (46) and is associated with cognitive impairment (35), as is evidenced in Parkinsonism-dementia complex with similar frontotemporal impairments (47).

Regional atrophy is inconsistently reported upon routine visual inspection of T2- and proton-weighted MRI images. In the presence of overt FTD, atrophy of the frontotemporal region is a well described hallmark of the disease (48), and is consistently

reported in ALS with FTD (ALS/FTD) (5, 49). However, in ALS without comorbid FTD, there are disparate reports of the presence and extent of atrophy (50, 51). The inconsistency arises in accurately visualizing atrophy in patients with minimal or no cognitive change. Recent advances in MRI, specifically voxel-based morphometry (VBM), have reduced the operator dependence in determining areas of tissue change. VBM is a fully automated, operator-independent whole brain image analysis technique that analyzes grey and white matter densities of individual voxels of MRI scans. This voxel-wise comparison uses standardized parametric statistics to compare differences in patient groups with a known normal data base, thus eliminating any *a priori* hypothesis about the locations of differences (52).

The association of atrophy with cognitive impairment has recently been challenged in an eloquent MRI study by Chang et al (2005). Using a voxel-based MRI method to study morphological patterns of brain atrophy in cognitively normal ALS and ALS/FTD they found that both groups had common patterns of grey matter atrophy when compared with controls (49). The changes were observed to be widespread throughout the grey matter of the frontotemporal region, specifically the motor, premotor, prefrontal and temporal cortices. More pronounced atrophy existed in the ALS/FTD patient group, particularly in the left hemisphere. A corroborative study found similar patterns of grey matter change, but no white matter differences in patients without "overt signs of cognitive impairment" as assessed by subjective observation with no formal NT assessment (42). Furthermore the authors concluded that white matter changes may not have been evident because the patients were only mildly affected by the disease, but the presence of grey matter changes may indicate structural changes occurring prior to

functional changes. In contrast to the Chang et al study showing the sparing of white matter early in disease, a recent study has described changes in white matter in patients without cognitive dysfunction (53). This study tested for mild cognitive impairment to exclude demented patients and select unimpaired (ALSu) and impaired (ALSi) patients. Upon visual inspection there were no differences in atrophy between either of the two groups and controls. When VBM was performed, white matter densities in the two ALS groups were similar in all regions but different from those in controls, with changes in ALSu being less extensive, indicating the presence of a continuum of changes in white matter densities. Most importantly, this study indicated the presence of structural changes in extramotor areas before the onset of cognitive dysfunction, whereas other studies have indicated similar findings in non-demented ALS patients but have not formally tested for subtle cognitive change (54, 55).

The introduction of advanced imaging techniques such as VBM have further characterized frontal lobar atrophy in patients with ALS/FTD and laid the ground work for investigations into non-motor structural changes. Methodological differences had resulted in discrepant structural changes in the motor and non-motor areas in early studies. These differences include first, variable criteria to discern mild cognitive impaired from cognitively-intact ALS patients (28, 56); second, structural changes are not assessed with the more reproducible volumetric quantification techniques (56). More recent studies have used VBM to uncover covert structural changes in patients who are either cognitively intact or mildly cognitively impaired (53). This technique has found similar patterns of changes that were present early in cognitively normal ALS and became exacerbated as the disease progressed to FTD (49, 53). The structural changes in

extramotor areas in the absence of cognitive dysfunction may indicate the existence of a continuum in pathology associated with worsening cognitive impairment before clinical signs become evident.

Heterogeneity in atrophy may also result from the differing genetic background of patients. fALS patients, specifically those with the homozygous D90A mutation of SOD1, had less volume loss in the motor cortex but significantly greater reductions bilaterally in the anteromedial frontal lobes when compared to sALS (57). Another reason for the differing patterns of cortical atrophy is differing neuronal vulnerability, which further complicates the phenotype expressed in ALS. It can be argued that results of neuroimaging studies will be more accurate and reproducible if ALS patient groups are stratified based on familial versus non-familial backgrounds, the presence or degree of cognitive impairment and the severity of disease.

Furthermore, MRI has been proven useful for detecting CST changes implicated in ALS. The CST neurons arise from pyramidal cells in the cerebral cortex and transmit nerve impulses from the motor area to the spinal cord to initiate precise movements of voluntary skeletal muscles. Changes in the CST are visualized by MRI as areas of hyperintense signal on T2-weighted and fluid-attenuated inversion-recovery (FLAIR) images and are reported most often in the posterior limb of the internal capsule (58). However, this form of MRI is qualitative and has been shown to have low sensitivity and specificity for detecting UMN involvement in ALS (51, 59), thus CST changes have been investigated with alternate advanced quantitative MRI techniques as described in the next section.

1.3.1.2 Diffusion Tensor Imaging

The degeneration of UMN tracts in ALS involves the primary motor and premotor cortex, resulting in degeneration of the associated motor fibre bundles and gliosis along the CST. Degeneration of nerve fibre tracts (white matter) can be assessed using an MRI method known as diffusion tensor imaging (DTI). The premise of DTI is that in an intact nerve fibre bundle, diffusion is limited by anatomic structures to be in the same direction as the fibre tract, but when the fibre is damaged, motion of water is less restricted and it may diffuse freely in multiple directions. DTI visualizes the overall orientation of fibre tracts and their integrity by measuring the preferred direction of water diffusion (60). Voxels are assigned values ranging from 0 to 1 and the value represents the ratio of diffusivity along the direction of fibres compared to across them. The ratio is known as fractional anisotropy (FA). An FA value of 1 implies a single anisotropic dominant direction of diffusion or an intact fibre tract whereas a value of 0 implies an isotropic or lack of directional dependence of the diffusion coefficients or a damaged fibre tract. A second diffusion parameter measured with MRI is the apparent diffusion coefficient (ADC). The ADC is a measure of the directionally averaged magnitude of diffusion and relates to the regional integrity of brain tissue, whereas FA reflects the degree of alignment of cellular structures (58). Thus, changes in tissue structure can be assessed with DTI and ADC which examine the changes of diffusion characteristics of water.

Since a primary hallmark of ALS and PLS is CST degeneration, many studies have focused on *in vivo* methods for monitoring these changes. The use of DTI for *in vivo* investigation of nerve fibre tract pathology has shown promising results in the

detection of lesions in ALS. Within the CST, most DTI studies generally focus on pathology incorporating the posterior limb of the internal capsule (PLIC) (61-66), cerebral peduncle (61, 65), corona radiata (64, 67) and the pons and pyramids (64, 65). Measurement of FA consistently shows alteration of diffusion properties in the PLIC in all reported studies. The PLIC contains CST fibres that are very tightly packed and aligned in a single direction, unlike areas of the pons and medulla that have transverse fibres as well as nuclei and roots of cranial nerves associated with them (58). The diffusion anisotropy is very high in the internal capsule due to its inherent structural characteristics, thus when tissue integrity is lost it may be easier to measure the defect against the normal background. A study of 31 patients with ALS measured changes in FA within the CST and the extramotor white matter and documented widespread changes in all regions measured in the CST (68). Furthermore, FA reductions in this study were restricted to the CST, as all extramotor white matter areas had FA within normal limits.

DTI provides the opportunity to assess changes of the CST *in vivo* and possibly provides an indirect marker of disease progression. Measurement of decreased FA in the CST is associated with disease severity and increasing UMN involvement as assessed with UMN tests specific to neurological impairment (61, 62, 68). One study found that FA in the PLIC had a good sensitivity of 95% but a poor specificity of 71% in detecting patients with ALS (63). DTI may be most useful as a quantitative technique for monitoring progression of UMN pathology since it can detect changes in FA in patients without clinical signs of UMN involvement.

1.3.1.3 Proton Magnetic Resonance Spectroscopy

Unlike conventional MR images that essentially represent the spatial distribution of water protons and their relaxation properties after the excitation with a radiofrequency pulse, magnetic resonance spectroscopy (MRS) is based on the behaviour of protons and other nuclei (phosphorous and carbon) in different metabolites within the brain, which exist at much lower concentrations than water (1-10mM vs 70mM) (69). Typically, proton MRS studies in MND focus on three metabolites in the brain; *N*-acetylaspartate (NAA), choline (Cho), and creatine/phosphocreatine (Cr). The function of NAA in the brain has not been fully determined, but is known to be exclusively localized to neurons and their processes in the adult human brain (51). Cho and Cr are present in all brain cells and are functionally important to the phospholipid membrane and energy metabolism, respectively (50). Because NAA is localized specifically to neurons, it is considered to act as an *in vivo* marker for neuronal loss and/or viability, which occurs in MND. Metabolic measurements can be expressed as absolute units reflecting concentration, or as part of a ratio which normalizes the concentration to an internal reference. For MND, NAA is commonly assessed based on a regional NAA/Cr. Cr is chosen as the denominator because its components are in chemical equilibrium, which is expected to be unaffected by neurodegenerative disease processes.

MRS is commonly employed in ALS and PLS to examine degeneration of the motor neurons of the motor cortex, brain stem and spinal cord, with the CST being the main fibre tract commonly examined. The first description of altered NAA/Cr in patients with ALS was published by Pioro et al. (1994). They showed that the NAA/Cr ratio was decreased most prominently in the motor cortex and to a lesser extent in both the

premotor area and primary sensory cortex (70). Decreased NAA was postulated to reflect loss or dysfunction of motor neurons and put forth as a marker for progression of UMN involvement in disease. To this end, longitudinal studies have attempted to determine the relationship between disease progression and metabolite concentrations, but have provided conflicting results. Mitsumoto and colleagues (2007) found significant decreases in NAA concentration of the primary motor cortex in ALS and PLS when compared to controls at baseline measurements, but did not find significant progression with time, possibly due to the delay of baseline measurements from the time of diagnosis (24 months) (71). Conversely, other studies have well described progression of metabolic concentrations throughout the course of disease (72, 73). Unrath et al (2007) recently described significant NAA decline in bilateral motor cortices over a six month period with changes as early as 3 months from baseline (73). These results were corroborated at 3 months from baseline in a similar study, but found that no changes prior to this time point (73). Further examination into white matter and other metabolites (myoinositol, glutamate, and glutamine) provided no significant differences from controls at any time point.

Thus, MRS may be clinically useful in detecting UMN involvement and evaluating its progression. Despite differences in methodology, techniques and patient cohorts, UMN involvement is associated with decreased NAA, both as an absolute and relative measurement. Furthermore, with increasing MRI field strengths becoming clinically available, increased efficacy in monitoring disease progression, pathogenesis and possibly treatment can be expected. Although most studies have been successful in

determining differences in the primary motor cortex, few have focused on changes in extramotor areas including the occipital, frontal and parietal cortex (70, 74, 75).

Given the paucity of literature concerning extramotor involvement, further detailed examination is warranted. Investigation with MRS may help unveil some underlying pathology and perhaps provide an early indicator contributing to the etiology of the disease. In particular, it is of interest to determine if cognitive change in MND is associated with perfusion and neuronal deficits and to determine their temporal relationship.

1.3.2 Nuclear Medicine Imaging

Nuclear medicine imaging makes use of radiopharmaceuticals, which are radioactively labelled pharmaceuticals, to assess cellular and subcellular function in the body. Nuclear medicine, in particular positron emission tomography (PET), has been used extensively to describe the spectrum of pathological and functional change in ALS including metabolic deficits (76, 77), neuronal loss (78-84), microglial activation (85), and perfusion studies both at rest (86-88) and during activation (2, 89-92). Furthermore, single photon emission computed tomography (SPECT) has also been used, predominately for investigation of cerebral perfusion abnormalities in MND (47, 93-102). Thus, nuclear medicine techniques provide a wide range of diagnostic applications to unveil pathological and functional abnormalities in perfusion, metabolism and neuroglial populations.

1.3.2.1 Neuroglial Imaging

Although the pathogenesis of ALS remains unclear, it is now recognized to be a multisystem disorder incorporating both motor and extramotor regions of the brain. The use of radio-labelled analogues of biological molecules permits the study of specific cell populations by targeting their receptors *in vivo*. Since degeneration of pyramidal cells is a hallmark of ALS, the 5-hydroxytryptamine (5-HT_{1A}) receptors on this cell type are specifically targeted with the PET ligand [¹¹C]-WAY100635 to provide a detailed account of this cell population. Reductions in pyramidal cell counts have been described in ALS using statistical parametric mapping (SPM) in conjunction with this technique, with the greatest differences in the frontotemporal regions, cingulate and lateral precentral gyri (82). Interestingly, changes were not limited to this area but were diffusely distributed throughout the cortex in agreement with other imaging studies (85, 86, 95, 103). It was postulated that widespread reductions in 5-HT_{1A} receptor binding reflects damage to the cortical pyramidal neurons, although further investigation would be needed to determine that a functional change in receptor binding was not the cause instead.

Further investigation of pyramidal cell and interneuron loss has been interrogated by targeting the gamma-aminobutyric acid (GABA_A) receptor, which is widely expressed throughout the cortex, with the PET ligand [¹¹C]-flumazenil (FMZ). Decreased FMZ binding occurred globally throughout the cortex, with the most striking difference in areas away from the motor cortex (78). Decreased FMZ binding in the prefrontal cortex where pyramidal cells are sparse may reflect interneuronal dysfunction. In a related study, significantly decreased FMZ binding in the frontotemporal region was

associated with executive dysfunction (84), indicating that neuronal dysfunction is related to cognitive decline.

Another pathological feature implicated in ALS is activation of microglia in the wake of dying or dysfunctional neurons. PET can exploit this pathology using the radioligand PK1195 that binds specifically to the peripheral benzodiazepine receptor expressed in the central nervous system in activated, but not resting, microglia (85). Activated microglia were present throughout the cortical and deep grey matter structures of the brain in the absence of any extramotor symptoms, indicating that pathological change may occur before clinical manifestations of extramotor areas are detectable.

Positron emission tomography has provided a fundamental description of neuronal and glial involvement in ALS and PLS patients *in vivo*. With increasing knowledge of cell biology and pathology implicated in these diseases, along with the introduction of novel radiolabelled tracers, PET imaging will continue to provide important markers of disease progression and perhaps aid in determining drug efficacy in future therapeutic trials.

1.3.2.2 Activation Imaging

The premise behind activation imaging is that neuronal activity can be indirectly assessed by measuring the blood flow increases from baseline during an activation task (104). Activation imaging has been used to determine the relationship between impairment in cognitive or motor tasks and abnormal areas of the cortex in ALS. Using a random movement joystick task, ALS patients impaired on verbal fluency tests displayed, as expected, marked activation of the sensorimotor cortex in the hand and arm

area on the contralateral side. Additionally, unexpected activation in the contralateral face area and adjacent non-motor cortical regions (91, 92) may suggest accessory non-specific sensorimotor area recruitment in response to limb weakness (2). When ALS patients are compared to patients with exclusive LMN involvement, ALS patients exhibit reduced rCBF at rest in addition to abnormal bilateral activation patterns. Since the distinguishing characteristic between these groups was UMN involvement in ALS, reductions in rCBF may indicate a loss of pyramidal cells in the cortex of ALS (2).

Activation imaging with PET has shown an association between cognitive involvement and decreased CBF using activation tasks specific to the areas of impairment. A study of non-demented ALS patients with deficits on the Written Verbal Word Fluency test had reduced activation of PET measured CBF in prefrontal cortex when performing a verbal (letter) fluency test (89). Although not clinically demented, rCBF abnormalities were present in patients impaired on letter fluency measures when compared with unimpaired ALS patients and healthy controls. Thus, the use of activation paradigms sensitive to executive dysfunction, namely verbal fluency, in ALS has described decreased CBF activation in frontal, limbic, and anterior thalamic regions associated with cognitive dysfunction (51, 89, 91).

In conclusion, activation paradigms coupled with PET measurement of CBF have found extramotor abnormalities in demented and non-demented ALS patients with executive dysfunction when compared to controls or unimpaired ALS patients. Recently, advanced functional MRI (fMRI) techniques have corroborated these results with the added benefit of increased resolution for accurate localization of brain structures (103). Together, these data demonstrate abnormal function in the extramotor cortex that is

related to the level of cognitive impairment, and in the motor cortex as evidenced by abnormal CBF response to motor activation tasks.

1.3.2.3 Metabolic Imaging

Positron emission tomography utilizes a [^{18}F] labelled glucose analogue, [^{18}F]-fluro-2-deoxy-D-glucose (FDG), to assess regional cerebral metabolic rate of glucose (rCMRGlu). The [^{18}F] labelled glucose analogue is transported into cells by glucose transporters and phosphorylated by hexokinase into [^{18}F]-2-DG-phosphate. The phosphate version of FDG cannot be metabolized further and accumulates intracellularly. Accumulation of this tracer is detected as it releases positrons in the decay process, which annihilate with electrons to produce two high energy (0.51 MeV) gamma photons which in turn can be detected by a PET scanner.

The original PET FDG study of ALS found with repeat scans over time an association between worsening clinical presentation and decreasing CMRGlu throughout the cortex in 3 of 4 patients (76). Further investigation provided evidence for reduced rCMRGlu in the motor-sensory cortex of ALS associated with UMN degeneration (77). These studies provided the foundation for further metabolic investigation into extramotor involvement and associated cognitive dysfunction. It has been shown that ALS patients with mild cognitive impairment characterized by executive dysfunction have impaired glucose metabolism throughout the cortex and subcortical structures (86). In particular, hypometabolism in the right thalamus and left caudate nucleus were correlated with mild neuropsychological deficits based on word fluency tests. Increased level of impairment in ALS patients with progressive dementia is associated with decreased CMRGlu in all

four cortical regions, but not the cerebellum, when compared with non-demented ALS and controls (87, 88). Reductions in metabolism were noted in non-demented ALS patients as subtle global cortical changes (88), with only the sensorimotor cortex being significantly reduced when compared with controls (87).

Thus, changes in metabolism have been documented in patients with ALS using PET with [¹⁸F]- FDG. Hypometabolism in ALS is associated with motor and cognitive dysfunction and can be an indicator for the severity of the respective dysfunction. More importantly, the aforementioned studies provide confirmation that like the progression of motor disturbances, cognitive decline is a continuum as evidenced by similar and worsening pattern of CMRGlu among unimpaired, mildly impaired and demented ALS patients.

1.3.2.4 Perfusion Imaging

Single photon emission computed tomography (SPECT) has been applied to study cortical perfusion changes associated with motor and cognitive decline in ALS. Changes in cortical perfusion have been associated with UMN involvement and motor dysfunction as rated by the ALSFRS-R or other neurological tests reflective of UMN function. In a recent study of 55 non-demented ALS patients, hypoperfusion in the motor cortex demonstrated a significant correlation with motor dysfunction and was found to be asymmetrically distributed, mainly affecting the right insular cortex and the pre-motor areas (102). However, given the well established coupling between brain function, metabolism and perfusion under steady state conditions (87, 105, 106), the focus of this

thesis will continue to build on the existing literature describing the relationship between cognitive patterns and cerebral perfusion.

The comorbid presentation of FTD and ALS is characterized by a reduction in frontotemporal perfusion (96, 100). However, akin to hypometabolism, hypoperfusion in non-demented individuals with ALS is also evident in this region (47), further arguing for the presence of a continuum towards dementia (99). An eloquent study by Waldemar et al (1992) presented one of the first systematic descriptions of differences in perfusion based on the presence or absence of dementia (94). First, the whole cohort of ALS patients was compared with normal controls and a global reduction in cerebral perfusion was present in the ALS group, with prominent reductions in the frontal cortex, subcortical white matter and hippocampus. Next, assessment of individual SPECT data determined that 8 of 14 patients had abnormal rCBF distribution maps. Of the 8 patients with abnormal rCBF, 3 were demented, yet no patient with a normal rCBF distribution map was demented. Thus, it seems possible to differentiate between demented and non-demented groups based on abnormal patterns of rCBF. The authors note that of the 8 patients with abnormal maps, the 5 non-demented patients also displayed no mild cognitive impairment, although no specific testing for frontal lobe dysfunction was performed. Thus, without the use of testing paradigms sensitive to executive dysfunction, one cannot state with certainty that the 5 remaining patients with abnormal rCBF distribution maps were not mildly cognitively impaired. As discussed above, perfusion deficits in the extramotor area were extensive and have been documented to occur in the absence of dementia (88, 93, 98).

Perfusion imaging with SPECT has provided fundamental evidence for functional changes in ALS with and without dementia. Perfusion deficits are widespread and are associated with changes in motor and cognitive function in their respective areas of the cortex. Future work is warranted to determine the temporal profile of perfusion deficits with respect to onset of disease, as well as the association between severity of perfusion deficits and cognitive impairment.

1.3.3 Advanced Computed Tomography Imaging

Historically, computed tomography (CT) imaging was used for anatomical scans in ALS to determine the extent of atrophy, but quickly lost ground to MRI with its exquisite soft tissue contrast. CT imaging for perfusion has been relatively underutilized in ALS despite its ability to perform rapid, high resolution measurements of CBF, CBV and MTT in cortical and deep subcortical structures without the need for radioactive tracers.

Xenon enhanced CT (Xe/CT) uses stable xenon gas as a diffusible tracer and as an x-ray contrast agent because its linear attenuation coefficient is similar to iodine. The gas is inhaled, dissolves in blood in the lungs, is transported to the brain via blood flow, and diffuses rapidly into the parenchyma. A Xe/CT study involves continuous inhalation of xenon (25-30% balance oxygen) to establish a steady (saturation) state in the brain before switching to room air and the desaturation phase ensues (107). Xenon concentration in a brain region is then estimated by CT scanning, as the concentration is proportional to the attenuation of x-rays passing through that region. The arterial concentration of xenon is estimated from the end-tidal concentration. The brain and

blood (arterial) concentration vs time curves are then used in conjunction with the Kety equation to calculate regional cerebral blood flow (97). Kobari et al (1996) used this method to describe perfusion deficits in the frontal cortex of ALS patients with UMN dysfunction, which are in agreement with previously mentioned studies using PET and SPECT. Moreover, the frontal cortex was assessed on a regional basis and it was found that only the upper part of the posterolateral frontal cortex was reduced in ALS with UMN dysfunction when compared to ALS in the absence of UMN signs (97). Therefore, motor neuron involvement was aptly described by perfusion changes using the Xe/CT technique.

CT Perfusion (CTP) imaging is another method for measuring perfusion with a CT scanner and is the method utilized for the studies conducted in this thesis. Unlike Xe inhalation, the tracer used is ordinary iodinated x-ray contrast agent and is injected into a peripheral vein. The principle of this technique relies on the assumptions that the tracer is uniformly mixed with blood and that enhancement (increase in CT number from baseline, that is, before contrast arrival) in a brain region or blood vessel is proportional to the tracer (contrast) concentration (108). Once the tracer is injected, the scanner operates in cine mode whereby the couch remains stationary and the preselected slice location(s) of the brain is(are) scanned continuously to monitor the passage of the bolus of tracer through the brain. The passage of the tracer is made up of three distinct phases. The first phase is the baseline before the arrival of tracer, which establishes a background level of attenuation of the brain in CT number. The second phase begins at the arrival of the bolus of tracer at the brain and lasts until the start of the washout period. The third and final phase is the completion of the washout of tracer and a return toward baseline

levels of attenuation. For the purpose of this thesis, a four-slice CT scanner was used; thus a series of images covering 4 slices over a 2cm segment of the brain is produced. This image set was then transferred to a workstation for processing to calculate quantitative CBF, CBV, and MTT parametric maps.

Parametric maps are calculated on a GE workstation (GE Healthcare) using proprietary software (CT Perfusion, GE Healthcare) that uses a deconvolution method for the calculation. There are two sets of data in the raw dataset; the arterial and the tissue time vs enhancement curve (TDC) (Figure 1.1). As discussed before, enhancement is the increase in attenuation of x-rays in a brain region or blood vessel due to the presence of the iodinated contrast agent and is measured in CT numbers. The arterial and tissue time

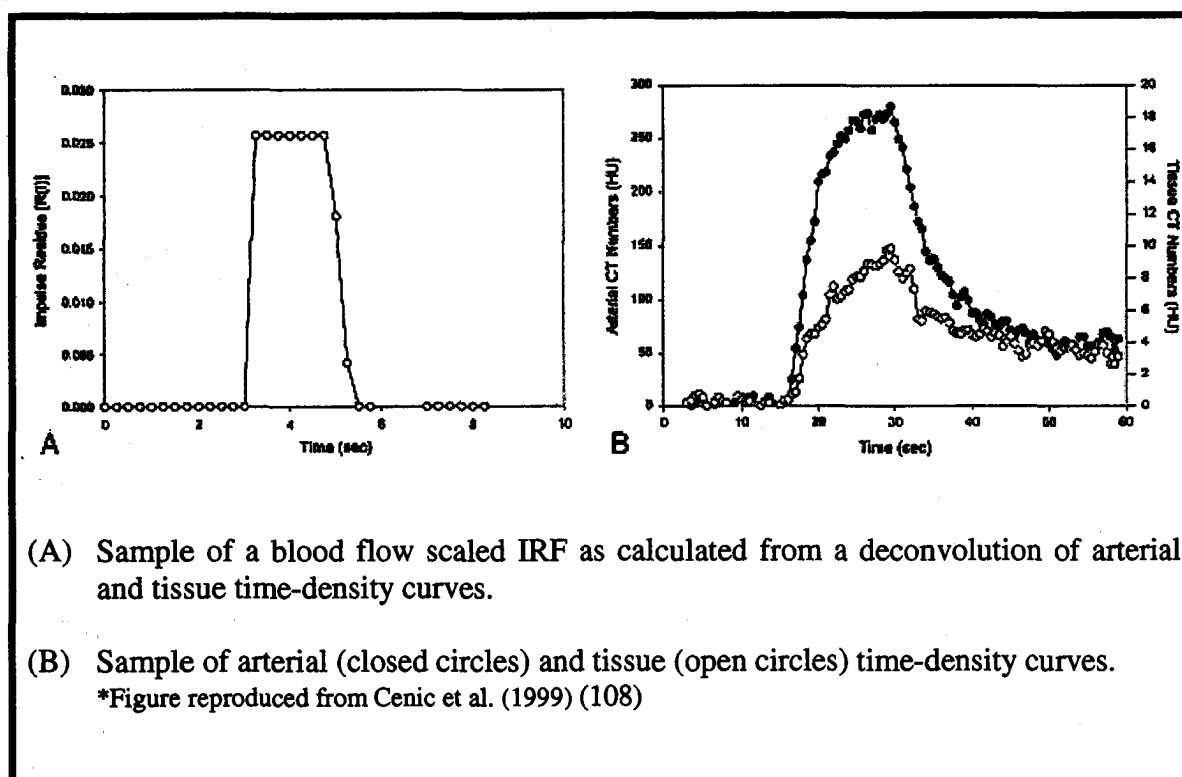


FIGURE 1.1: Sample IRF and Arterial and Tissue Time-Density Curves (TDC).

vs enhancement curves are deconvolved to determine the blood flow scaled impulse residue function (IRF) (109). The IRF models the passage of a tracer (contrast) bolus of unity mass delivered to the brain instantaneously via the arterial input and has the general shape shown in Figure 1.1. The IRF rises instantaneously to a height of unity since the bolus of contrast is assumed to be delivered to the tissue instantaneously. After the initial rise, the IRF maintains a plateau of unity height for a period which is the minimum time for the tracer to traverse the capillary network. The plateau region is followed by a washout phase towards baseline when the tracer (contrast) exits the brain region with blood flow. The height of the blood flow scaled IRF is equal to CBF and the area is CBV. MTT is then determined from the Central Volume Principle which relates the parameters by the equation: $MTT = CBV/CBF$ (110). The above described calculation is repeated for every region of the brain to produce parametric maps for all three parameters.

The cerebral arteries used for measuring the arterial time vs enhancement curve or arterial input function (AIF) are usually smaller than the resolving power of the scanner. Because of averaging of the true arterial enhancement with those of the surrounding pixels – partial volume averaging effect (108), the AIF will be underestimated. Partial volume averaging of the AIF is corrected by multiplying the AIF by the ratio of the area under the time vs enhancement curve for the sagittal sinus to that of the AIF, on the assumption that the sagittal sinus is larger than the resolving power of the CT scanner. The corrected AIF is then deconvolved with the individual TDC from each voxel for the calculation of the CBF, CBV and MTT parametric maps.

1.4 NEUROVASCULAR UNIT

Normal brain functioning requires a supply of oxygen and nutrients in a temporally and spatially correlated manner with respect to brain activity, a process known as neurovascular coupling. Neurovascular coupling is achieved through intercellular communication between neurons, astrocytes and blood vessels, which are functionally coupled and collectively comprise the neurovascular unit (NVU). The NVU forms where the pial arteries penetrate deeply enough into the brain that the Virchow-Robin space disappears, allowing the basement membrane of the arteriole to physically contact the astrocytic endfeet (111). Although CBF may be regulated by the individual elements of the NVU, cerebrovascular tone is most often maintained through the concerted action of the elements comprising the NVU.

1.4.1 The Astrocyte

Historically, astrocytes have been viewed as non-excitable structural cells with menial function. Astrocytes were originally described by Virchow as 'brain glue' that provided the structural scaffold of the brain (112). However, knowledge of this cell has evolved and is now widely accepted as the integral element physically bridging communication between the neuron and blood vessel (113). Astrocytes outnumber neurons in the brain 10:1, occupy up to 50% of the brain volume, and each cell has a distinct spatial domain with its processes contacting other astrocytes, neuronal synapses and blood vessels (114). These specialized cells are also responsible for controlling other non-neuronal brain cells during development and injury (112). This non-neuronal communication includes the release of chemokines that activate receptors on other cells

to signal the attraction and spatial positioning of microglia and lymphocytes during inflammatory reactions and after injury (115, 116). This may be especially pertinent when studying MND, as astrocytes are activated during neurodegeneration. In the context of this thesis, astrocytes will be reviewed with attention to their contribution to perfusion and metabolic coupling to neuronal activity.

1.4.1.1 Astrocytes in Neurovascular Coupling

Because of their anatomical attributes and proximity to contractile elements of blood vessels, astrocytes are well poised to contribute to the regulation of cerebral blood flow. Astrocytes occupy distinct spatial domains with very little overlap and thus are anatomically endowed to transduce signal to multiple other cellular elements (112). The dendritic processes (>100,000) communicate with other astrocytes forming a syncytium for signal relay, as well as with neuronal synapses to sense brain activity (112, 117). Each astrocyte has at least one endfoot process that terminates on a blood vessel, allowing communication of signal and regulation blood flow in the wake of neuronal activity. Significant evidence has mounted over the last decade describing this new regulatory role for astrocytes. The seminal paper by Zonta et al (2003) described calcium (Ca^{2+}) wave oscillations in astrocytic endfeet and postulated a neuronal trigger for this phenomenon. More specifically, experimentation described a response to neuronally released glutamate which bound to metabotropic glutamate receptors on the astrocyte. The coupling of this receptor to a G-protein complex causes a signalling cascade that terminates in an intracellular rise of astrocytic Ca^{2+} . With increasing stimulus strength, these waves propagate away from the soma and into astrocytic endfeet that abut on a

microvessel, in the case of this study causing temporally and spatially related vasodilation (118). Contrary to these findings, Mulligan and MacVicar (2004) documented the opposite vascular response to increased intracellular Ca^{2+} in a more temporally related manner than previously shown (119). They proposed that Ca^{2+} elevations activated the mobilization of an arachidonic acid (AA) pathway that ultimately causes constriction by depolarizing the smooth muscle cells of the vessel wall. Both studies determined that the astrocyte was capable of transmitting signal from the neuronal synapse to the vasculature to influence vascular tone, but had inconsistent results most likely due to differences in experimental methodology. Further experimentation was performed *in vivo* and demonstrated the role of astrocytes in functional hyperemia by photolysis of caged Ca^{2+} (120). Uncaging of Ca^{2+} in the astrocytic endfeet caused a rapid vasodilation which was blocked by cyclooxygenase inhibitors, but again no vasoconstriction was found. Identifying the mechanism for the dichotomous vasomotor response reported in the literature was first described in an eloquent study by Metea et al (2006). It determined that the type of vasomotor response was influenced by the level of nitric oxide, a mechanism that may modulate the production of different arachidonic acid metabolites (121). Other vasogenic factors released from astrocytes that are implicated in the regulation of cerebrovascular tone include potassium (K^+) and prostaglandins (118, 122, 123).

1.4.1.2 Astrocytes in Neurometabolic Coupling

Much akin to neurovascular coupling, astrocytes also play a critical role in neurometabolic coupling to ensure that the delivery of metabolic substrates reflects the

level of neuronal activity. Astrocytes surround the neuronal synapse and actively sequester glutamate, the main excitatory neurotransmitter in the brain, from the synaptic cleft to maintain the synaptic functioning of the neuron. Clearance of glutamate by the astrocytes is accomplished through two main mechanisms. First, glutamate can bind to a metabotropic glutamate receptor which is coupled to a G-protein complex causing increased intracellular Ca^{2+} as described above, which in turn activates the mobilization and metabolism of arachidonic acid into different vasoactive compounds. Secondly, the majority of glutamate is sequestered into the astrocytes by excitatory amino acid transporters (EAAT) through the co-transport of 3 sodium ions (Na^+) (124). The co-transported Na^+ stimulates aerobic glycolysis in astrocytes (125), in turn producing lactate that is shuttled to the activated neuron as the primary energy source (126). Thus, functioning as part of the neurovascular unit, the astrocyte is integral in sensing neuronal activity and integrating both a vascular and metabolic response.

1.4.2 The Blood Vessel

Cerebral blood vessels are unique from most other vessels in the body; endothelial cells lining the lumen form tight junctions to create the blood brain barrier. Intracerebral arterioles consist of endothelial and smooth muscle cells, and a basement membrane, all encapsulated by astrocytic endfeet and innervated by intrinsic neurons arising from the central nervous system. Arterioles become progressively smaller and evolve into capillaries with the smooth muscle cell being replaced by pericytes, the contractile element of capillaries (111). Smooth muscle cells modulate vasogenic tone in response to chemical and mechanical stimuli. Regulatory chemical signals originating

from endothelial cells include the vasodilators NO, endothelial derived hyperpolarizing factor (EDHF), prostacyclin and prostaglandin E₂ and I₁, and the vasoconstrictors endothelin 1, endothelial derived constricting factors, thromboxane A₂ and prostaglandin F_{2α} (111, 127-130). Furthermore, the smooth muscle cells in the blood vessel also respond to vasoactive molecules released from the astrocytes as previously described. Thus, the vessel modulates vasomotor tone by integrating signals from mechanical stimuli caused by local hemodynamics, vasoactive mediators from the endothelium and signals transduced by the astrocyte from neuronal activity. A combination of these complex signals contributes to the resting tone of the vessel and to the vasoactive response to increased neuronal activity under physiologically normal conditions. Pathological conditions affecting any one of these components may lead to compromised neurovascular coupling.

1.4.3 The Perivascular Nerves

Blood vessels located within the brain at the level of the NVU are innervated by intrinsic neurons arising from both subcortical pathways and interneurons (111). Perivascular nerves terminate within one micrometre of the vessel and act directly on the vessel or indirectly through the astrocytic endfoot as part of the NVU (131). Thus, neurotransmitters released from activated perivascular neurons may interact with receptors on either astrocytes or directly with the cells of the vasculature.

The subcortical vasoactive pathways originate from structures deep within the brain in the nucleus basalis, locus coeruleus, or raphe nucleus and contain acetylcholine (ACh), norepinephrine (NE) or serotonin, respectively (131). Electrical or chemical

stimulation of these structures can elicit both an increase and decrease in cortical CBF. Furthermore, anatomical studies have found that projections from these structures terminate perivascularly, with receptors for ACh, NE and serotonin present on both smooth muscle cells and astrocytes. The presence of receptors on both cell types for said neuromodulators implicates that regulation of CBF may involve modulation of the signal by astrocytes. More specifically, projections from the basal forebrain neurons contain ACh and NO that increase cortical flow when stimulated and attenuate flow when pharmacologically inhibited or blocked. The receptors mediating this response have been identified as muscarinic M5 receptors (132, 133) and astrocytes have a multitude of muscarinic ACh receptors, further strengthening the argument that astrocytes are involved in modulating flow via neurochemical pathways.

Studying neural influence on cerebrovascular tone is difficult because many experimental preparations disrupt the neuronal tract of interest. The local interneurons present a unique way to study neurovascular coupling because brain slice preparations can be used without disruption of the neuronal tract. Brain slice preparations allow the study of single cell depolarization and simultaneous visualization of the associated vascular response. A subgroup of GABA interneurons has recently been studied and described to incite both a constrictive and dilative effect on local microvessels (134). Depending on the type of interneuron, they may release constrictive (somatostatin, GABA) or dilatory (vasoactive intestinal peptide, NO, ACh, GABA) factors. In keeping with the concept of neurovascular coupling, it is of interest that astrocytes, like endothelial and smooth muscle cells of the cortical microvessels, also express a multitude of receptors for these vasoactive agents (131, 134). Of further interest, interneurons are

not capable of producing upstream dilation due to the limited interconnectivity. However, since the majority of perivascular nerves terminate on astrocytic endfeet, and since there is a known functional syncytium of glia for transmitting signal upstream (135), interneuronal modulation of blood flow through the NVU is plausible.

1.4.4 Neurovascular Coupling

Recent advances in imaging and knowledge of the cellular biology have provided insight into our understanding of how cerebral perfusion and metabolism are regulated via inputs from the endothelium, neurons, and astrocytes. It is the close association of these elements that makes cerebral blood vessels unique from other vascular beds in the body, with the most distinctive feature being the spatial association between neurons and astrocytes (127). Collectively, these three elements regulate vascular tone as one functional unit through a series of complex interactions and maintain adequate perfusion and delivery of metabolic substrates even under normal conditions. Under disease conditions, neurovascular coupling is often disrupted because the disease may affect the functioning of one of the cell types of the NVU. Diseases that are known to disrupt neurovascular coupling include hypertension, Alzheimer's disease, ischemic stroke and hypercholesterolemia (127, 136).

1.5 CEREBRAL PERFUSION PRESSURE

Cerebral perfusion pressure is the net pressure gradient driving blood delivery to the brain and is estimated as the net difference between MAP and the intracranial pressure (ICP) (137-139). Clinically, when ICP is assumed to be within the normal

physiological range, and since it is only obtained through invasive techniques, CPP is often estimated by the MAP (105). Under normal conditions when CPP is constant, any change in CBF is likely caused by an alteration in the cerebrovascular resistance (CR), which is the result of a change in vessel diameter as reflected by CBV. Thus, CBF is determined by the ratio of regional CPP (CPP) to regional cerebrovascular resistance (CR) (105), as denoted by Equation 1:

$$\text{CBF} = \text{CPP}/\text{CR} \qquad \text{Equation 1.1}$$

Under normal physiological conditions CBF is tightly regulated and remains relatively constant over a range of mean arterial pressures, a phenomenon known as autoregulation (138). Under pathological conditions causing perturbations in CPP, autoregulatory mechanisms are in place to maintain the oxygen and nutrient supply to the brain.

1.5.1 Normal CPP and Hemodynamics

Under normal conditions CPP, CBF and CBV are all tightly coupled with each other within the range of autoregulation (105). Normal values for hemodynamic and metabolic parameters of grey matter as measured by PET in humans are as follows (mean, range): CBF (50, 35-76 ml.100g⁻¹.min⁻¹), CBV (3.2, 2.2 -3.7 ml.100g⁻¹), cerebral metabolic rate of oxygen (CMRO₂) (3.3, 2.7 – 4.2 ml.100g⁻¹.min⁻¹), oxygen extraction fraction (OEF) (0.43, 0.27 – 0.67) (140).

1.5.2 Stages of Hemodynamic Compromise

Hemodynamic mechanisms for ensuring adequate delivery of nutrients are imperative to avert any potential damage to the brain under circumstances of decreased CPP. The brain is intricately designed to protect against ischemia, both morphologically and functionally. The architecture of the cerebral vasculature is such that many areas of the brain are perfused by collateral flow, a primary mechanism maintaining flow in regions compromised by occlusions or stenoses of vessels. The primary source of collateral flow comes from the vessels comprising the circle of Willis (141). Further compensation from secondary collateral flow arises from branches of the external carotid artery including the leptomeningeal and ophthalmic arteries (142). When collateral flow cannot compensate for decreases in perfusion pressure, functional mechanisms ensue, which will now be discussed.

1.5.2.1 Stage I: Autoregulation

In the normal human brain, autoregulation is a well accepted phenomenon. It can be defined as a regulatory mechanism for maintaining CBF at a constant level in the face of decreased CPP. Autoregulation operates over a wide range of MAP from 60 to 150 mmHg and fails outside of these limits (138, 139). The autoregulatory limits of MAP are often modulated by disease pathology, for example, chronic systemic hypertension may cause a shift towards a higher range of values. Furthermore, pathological states that cause local or global intracranial hypertension such as space occupying lesions or traumatic brain injury may also affect autoregulation either locally

or globally. For the scope of this thesis, further review will focus on the lower limit of autoregulation when CPP is decreased below normal values.

Taking equation 1 into consideration, under conditions of decreased CPP, CBF is maintained only by a proportional change in CR facilitated by alterations in the CBV. Thus, when CPP begins to fall, CBV increases to decrease vascular resistance to maintain flow. Although CBF has been proposed to be maintained at a constant level throughout autoregulation (105), experimental data have shown otherwise (140, 143, 144). Within the limits of autoregulation, a 10% decrease in MAP produces slight decreases in CBF (2-7%), for a total decrease of 18% throughout the range (143, 143). Thus, compensation for these small changes in CBF throughout autoregulation are coupled with increases in OEF, albeit small, but adequate for maintaining normal oxygen metabolism (137). Studies of CBV with decreasing CPP have reported variable results which may be an indication of the complexity of the components comprising a measure of CBV. A measurement of CBV takes into account of arterial, capillary and venous compartments, as well as parenchymal and pial components (144). Historically, increases in CBV were well described and accepted within the autoregulatory plateau (105, 145, 146), however recent data suggests that increases in CBV may be much less pronounced or absent within this range (137, 147). It is possible that these discrepant results may be caused by differences in measurement techniques or experimental models. Furthermore, it has been proposed that within autoregulation the response to decreased CPP is inherently variable (144).

Thus, autoregulation most likely involves complex interactions of many physiological parameters to ensure normal oxygen metabolism. Decreases in CPP may

cause small alterations in CBV while maintaining CBF near normal levels. Any residual decreases in CBF may be further compensated by increased OEF.

1.5.2.2 Stage II: Increased Oxygen Extraction Fraction

Beyond the lower limit of autoregulation, CBV may increase slightly (10-20%) (137, 147), stay elevated (145), or continue to increase resulting in a large magnitude of change (up to 150%) (146). Stage II is defined as the point at which changes in CBV can no longer compensate for decreases in CPP to maintain CBF and is passively decreasing with CPP. To maintain a supply of oxygen for metabolism, the brain increases the OEF in the face of decreased blood delivery (137). Thus, Stage II hemodynamic failure is marked by an exaggerated increase in OEF over that witnessed during autoregulation, and marked decreases in CBF, while $CMRO_2$ is maintained.

1.5.2.3 Stage III: $CMRO_2$ Compromise

Regulatory mechanisms ensure that $CMRO_2$ remains constant during Stage I and II of hemodynamic failure. Stage III is defined as the point at which the capacity for compensatory increases in CBV and OEF are exceeded and normal $CMRO_2$ is no longer maintained (105, 140, 144). Further reductions of CPP beyond this point will compromise $CMRO_2$ and metabolic failure will ensue leading to cellular death.

1.6 CEREBROVASCULAR RESERVE

The inherent structure and morphology of the brain is such that it maintains adequate levels of nutrient and oxygen delivery in the face of reduced perfusion pressure

through the previously discussed mechanism of autoregulation. In autoregulation, vessels play a dynamic role in regulating blood delivery by reducing or increasing vascular resistance via dilation or constriction of resistance vessels. The intrinsic ability of the vessels to dilate and augment flow is known as the cerebrovascular reserve (CVR). However, there is a finite limit for reducing the cerebrovascular resistance, at which point CVR becomes exhausted with flow and nutrient delivery becoming compromised. The physiology of cerebrovascular reserve as a function of perfusion pressure is described in Figure 1.2. Assessing the status of the CVR gives an indication of the local CPP and describes areas of the brain at risk of ischemia.

1.6.1 Assessment of CVR

Understanding perfusion dynamics and the risk of hypoperfusion at the lower limit of autoregulation is clinically relevant in many diseases and is most pertinent to the research contained within this thesis. Even though reductions in CPP are characterized by changes in CBF and CBV, taking single measurements of these parameters to determine CPP status may not be the preferred approach due to the inherent measurement variability in CBF and CBV (150). There are two principal approaches for assessing the cerebral functioning along the CPP continuum with respect to cerebral perfusion. The first approach takes quantitative measures of oxygen extraction fraction (OEF), cerebral metabolic rate of oxygen (CMRO₂), and/or CBF with PET using ¹⁵O₂ and C¹⁵O₂ as the tracers. Areas with exhausted CVR will be identified by increased OEF and decreased CBF as compared with normal areas of the brain. The second method measures CVR by measuring the ratio of CBF (or CBV) at basal levels to that after the administration of a

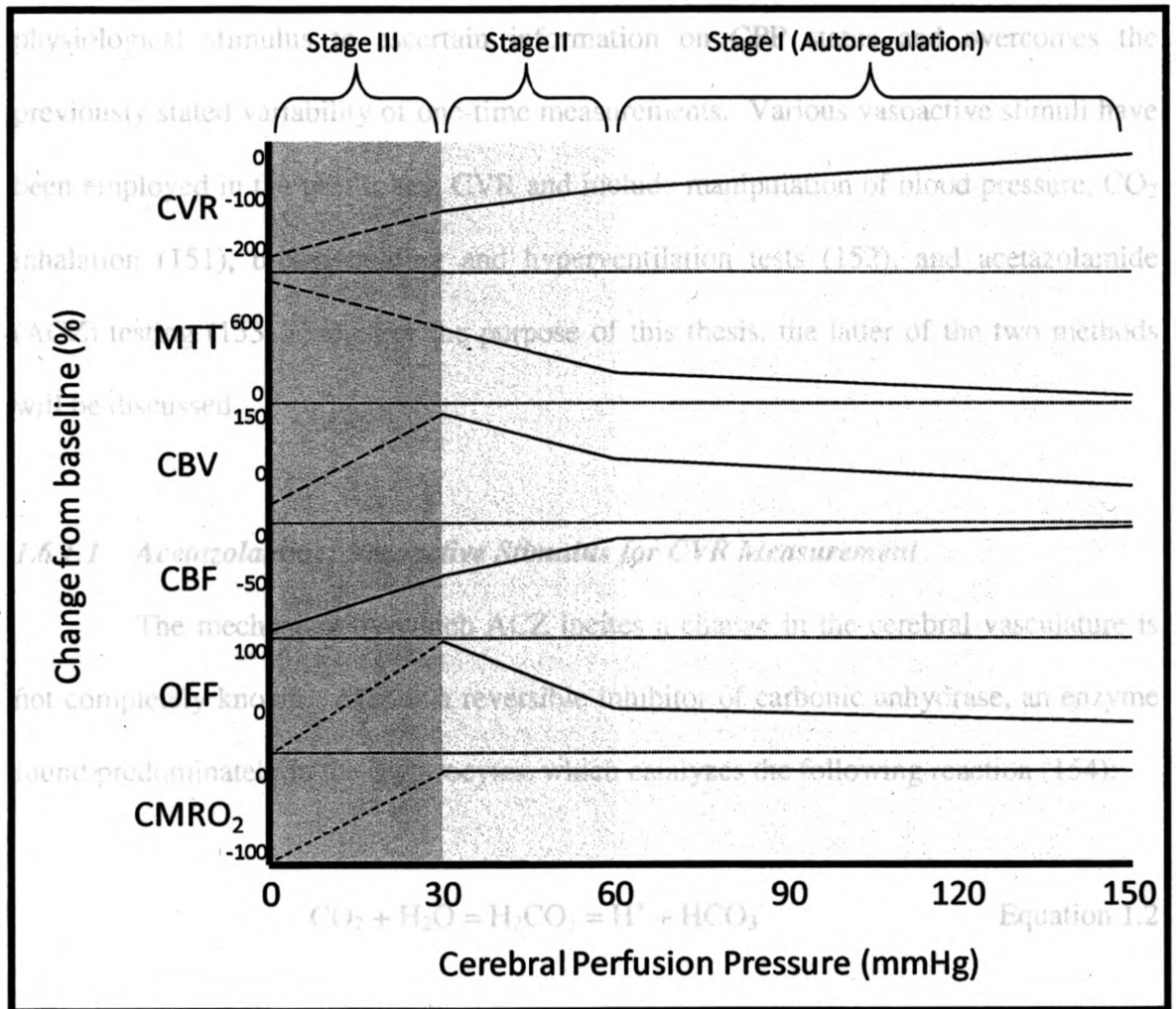


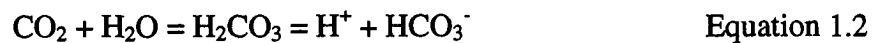
FIGURE 1.2: Hemodynamic Response to Reductions in CPP. Illustration of hemodynamic and metabolic responses to progressive decreases in CPP, categorized as three stages of hemodynamic failure. Most investigations have found that CBV increases throughout the autoregulatory range (Stage I), with slight decreases in CBF causing compensatory increases in OEF to maintain CMRO₂. Furthermore, these small perturbations in CBV and CBF are translated into increases in MTT. Within Stage II (ischemia without infarction), MTT increases more rapidly with further increases in CBV and decreases in CBF. This stage is further marked by dramatic increases in OEF with maintenance of CMRO₂. Stage III, hemodynamic and metabolic failure, signified by decreasing CBV to near basal levels, complete dependence of CBF on CPP, continued exaggerated increases in MTT, and the collapse of OEF and CMRO₂. The behaviour of each parameter in Stage III is represented with dashed lines, as the proposed changes with CPP have yet to be experimentally validated.

*Adapted from Nemoto et al. (148) and Derdeyn (149)

physiological stimulus to ascertain information on CPP status and overcomes the previously stated variability of one-time measurements. Various vasoactive stimuli have been employed in the past to test CVR and include manipulation of blood pressure, CO₂ inhalation (151), breath holding and hyperventilation tests (152), and acetazolamide (ACZ) testing (153, 154). For the purpose of this thesis, the latter of the two methods will be discussed.

1.6.1.1 Acetazolamide: Vasoactive Stimulus for CVR Measurement

The mechanism by which ACZ incites a change in the cerebral vasculature is not completely known. ACZ is a reversible inhibitor of carbonic anhydrase, an enzyme found predominately in the erythrocytes, which catalyzes the following reaction (154):



It is commonly accepted that ACZ creates a metabolic disturbance through shifting the above chemical equilibrium to the left, causing an increase in CBF via increases in extracellular partial pressure of carbon dioxide (pCO₂) and decreases in extracellular pH and end-tidal pCO₂ (155-157). Vostrup et al (1989) proposed that these phenomena are explained by the fact that CO₂ and water produced by glycolysis are transformed into H⁺ and HCO₃⁻ by carbonic anhydrase located in the erythrocytes (158). Thus, if this enzyme is blocked by ACZ, the dissociation of carbonic acid into H⁺ and HCO₃⁻ is also blocked and carbonic acidosis results. Carbonic acid then easily crosses the erythrocyte membrane resulting in an increased extracellular level of carbonic acid,

thus altering the extracellular pH and pCO₂. Furthermore, carbonic anhydrase in the lungs transforms H⁺ and HCO₃⁻ back into water and CO₂, thus explaining decreases in end-tidal pCO₂ after the administration of ACZ. Lastly, there is evidence suggesting that carbonic anhydrase is located directly in the walls of the small cerebral arteries and arterioles (159), thus it may be possible for ACZ to alter cerebral tone by acting directly on the vasculature, however there is currently no scientific study supporting this theory (154).

Cerebrovascular tone at the level of the small resistance arterioles is regulated in part by pH and pCO₂ as previously discussed. West et al (1992) has recently described a pH-dependent mechanism regulating calcium channels in the smooth muscle cells lining small cerebral arterioles, which may be important in explaining the vasodilatory effects of ACZ (160). Thus, ACZ may have both direct and indirect (metabolic) effects on the vasculature.

1.6.1.2 Dose Dependency and Kinetics of Acetazolamide

A current review describes the dose dependency and kinetics of ACZ on CBF or cerebral blood velocity (154). Both oral and intravenous methods have been used to deliver different doses of ACZ to determine the optimal dose and time course for measuring the hemodynamic response. Dosages range from 250 – 1200 mg and are associated with increases in CBF and cerebral blood velocity changes ranging from 1.45 – 90% increases over basal levels. The current clinical protocol is to intravenously inject 1000 mg of ACZ and measure a maximal CBF response at 10-30 minutes post-ACZ administration (161-165).

1.6.2 Implications of CVR Measurements

CVR reflects the dilatory capacity of the cerebral arterioles to a vasoactive stimulus and is used to assess adequate regulatory function of CBF. A normal CVR maintains the supply of blood and nutrients to the brain under variations of MAP (CPP) within the autoregulatory plateau, while an impaired CVR is associated with increased risk of stroke or ischemia, particularly, at the lower reaches of the autoregulation (166). Evaluating CVR plays an important role in determining the pathophysiology behind cerebral hemodynamic compromise and thus provides information on possible subsequent cerebral events. Predominantly, CVR testing is used to identify hemodynamic compromise in symptomatic patients with a predisposition to increased risk of stroke due to cerebrovascular disease (166-168). A study by Yonas et al. (1993) demonstrated that patients whose blood flow fell by more than 5% after administration of ACZ had a 12.6 times greater chance of stroke in the subsequent two year period than those with CBF that increased or fell by less than 5% (166). It is this paradoxical blood flow response, known as the "steal phenomenon", that is the hallmark for increased risk of stroke as it indicates the inability of the vessels and their collaterals to adequately maintain CBF.

CVR testing has been shown to be an accurate indicator of end stage cerebrovascular compromise (161, 169, 170). The chief focus of assessing CVR is to determine the cerebral regions subject to chronic borderline hypoperfusion, usually due to occlusion or stenosis of large cerebral arteries (150). However, within the limits of autoregulation when cerebrovascular reserve is still intact, another surrogate for CPP may be needed as CVR is not reliable when the hemodynamic compromise is mild (171).

1.7 MEAN TRANSIT TIME AS AN INDICATOR OF CPP

Mean transit time, which is mathematically equivalent to CBV/CBF (172, 173), is a measure of the amount of time it takes for a blood borne agent to traverse the vascular bed. MTT is particularly advantageous as an indicator for changes in CPP because the ratio depends on the discontinuity of the coupled relationship between CBF and CBV. Within the autoregulatory range, the response to decreased CPP involves reciprocal increases in CBV and constant or decreased CBF. Thus, because MTT takes both into account, it may provide a more sensitive indicator of CPP within autoregulation. As the lower limit of autoregulation is reached MTT becomes exaggerated, as further increases or consistency in CBV cannot attenuate the fall of CBF with decreasing CPP.

MTT, or its inverse, have been widely used as a clinical marker for assessing regional CPP deficits in cerebrovascular disease and assessing regions of the brain chronically at risk of hypoperfusion (173, 174). Furthermore, it has been experimentally determined to be a sensitive marker for CPP over a wide range of perfusion pressures (137, 146). However, it is yet to be determined how measurements of MTT compare with the clinical gold standard (CVR) both within and outside of autoregulatory limits.

1.8 RESEARCH OBJECTIVES

The objective of this thesis is to describe early cerebral hemodynamic disturbances with the goal to develop a marker for cognitive decline in MND. The research was divided into three scientific papers with the following individual objectives:

1. Identify a relationship between cerebral hemodynamic measurements using CT Perfusion imaging and cognitive status determined by neuropsychological testing in cohort of patients with primary lateral sclerosis (Chapter 2).
2. Characterize the relationship between duration of disease, progression of cognitive impairment and hemodynamic disturbances in a group of cognitively intact ALS patients (Chapter 3).
3. Investigate biological mechanisms in an experimental model to account for increased cerebral MTT (Chapter 4).

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CHAPTER 2

CEREBRAL HEMODYNAMIC CHANGES ACCOMPANYING COGNITIVE IMPAIRMENT IN PRIMARY LATERAL SCLEROSIS

2.1 INTRODUCTION

Primary lateral sclerosis (PLS) is a rare neurodegenerative disease, comprising about 5% of all MND cases presenting at neuromuscular clinics (1). Since its first formal description, the issue of whether PLS is a discrete nosologic entity or a disorder that inevitably converges into ALS has remained a topic of controversy (2-8). Because of the rarity of PLS, there are few reported studies that describe its full clinicopathological spectrum and particularly the extent to which nonmotor manifestations may occur. Brain perfusion studies are perhaps the least explored facet of this disease, even though in a related MND such as ALS, significant changes in cerebral perfusion and metabolism have been documented (9-16).

Typically, the cardinal features of PLS consist of insidiously progressive spasticity with pseudobulbar affect and spastic dysarthria. The failure to develop signs of lower motor neuron dysfunction, either clinically or electrophysiologically, within 3 years of symptom onset differentiates this entity from the more common disorder amyotrophic lateral sclerosis. Although PLS is classically described as sparing intellect (2), there is evidence that cognition may be affected during the disease course. In a study of 9 patients, Caselli and colleagues observed mild cognitive deficits most reflective of frontal lobe dysfunction in all (17). In a study of 20 PLS patients, Le Forestier and colleagues found that 16 had moderate deficits in frontal and/or premotor functions (7). More recently, these results were corroborated in an extensive neuropsychological study

examining 20 patients with PLS (18). This study described qualitatively similar dysfunction to ALS with all but three patients having signs of premotor and/or prefrontal cortical deficits.

Many authors consider PLS and ALS to be closely related disorders, if not two diseases within a continuum of age-dependant motor neuron degenerations. Although classical descriptions of ALS focused on degeneration of motor neurons, it is now recognized as a multisystems disorder in which cognitive impairment may be part of the pathological spectrum of the disease process (19, 20). The neuropsychological deficits in ALS range from mild cognitive or behavioural impairment to a more progressive form of frontotemporal dementia (FTD) in which the Neary criteria are fulfilled (20-25). Although cognitive impairment in ALS was once thought to be rare, the current literature indicates that when tested using paradigms sensitive to frontal lobe dysfunction, upwards of 50% of ALS patients exhibit some degree of impairment with up to 20-30% of the patients fulfilling the criteria for FTD (21, 26-28).

Cognitive changes in ALS have been associated with alterations in cerebral perfusion and metabolism as demonstrated using functional neuroimaging techniques, including PET (9, 11, 13-15), and [^{99m}Tc]-d,l-HMPAO (16) or N-Isopropyl-p- ^{123}I -iodoamphetamine (^{123}I -IMP) (10, 12, 29) SPECT. Given the large pool of literature concerning cerebral perfusion and related cognitive impairment in ALS, there is comparatively little known regarding these changes in PLS. In order to examine the association between cognitive dysfunction and cerebral perfusion in PLS, we have carried out a study of eighteen PLS patients and seven non-PLS controls to assess the degree of cognitive impairment and associated hemodynamic changes.

2.2 METHODS

2.2.1 Participants

Eighteen PLS patients (9 male, 9 female) meeting the Pringle criteria (2) and seven controls subjects (4 male, 3 female) were recruited from the Motor Diseases Clinic at the University of Western Ontario from 2003-2005. All patients signed a consent form for research approved by the University of Western Ontario Human Research Ethics Board. Disease severity was measured using the ALSFRS-R (30). All patients and controls underwent the same battery of neuropsychological tests (NT) followed by a CT perfusion (CTP) head scan. NT were used to stratify PLS patients into two groups based on the number of abnormal test scores; Cognitively-intact PLS patients (PLS) - those having zero or one abnormal score (n=14) and PLS patients with cognitive impairment (PLSci) - those having 2 or more abnormal test scores (n=4).

2.2.2 Neuropsychological Evaluation

Neuropsychological functioning was assessed in all subjects using 9 neuropsychological tests with 13 variables to address four major domains of cognitive functioning, focusing particularly on frontal-executive skills (Table 2.1). Tests were chosen to minimize the requirements for speech production and upper limb motor skills.

2.2.3 Imaging and Analysis

A 4-slice CT scanner (GE LightSpeed Plus; GE Healthcare, Waukesha, Wis) was used for the CTP scan, during which time blood pressure (BP), end tidal CO₂ (EtCO₂), oxygen saturation (O₂sat), breathing frequency (BF) and heart rate (HR) were monitored.

Table 2.1: Summary of Neuropsychological Tests and Variables. Testing battery consisting of 9 neuropsychological tests with 13 variables to address four major domains of cognitive functioning in all PLS and control subjects.

Domain	Test	Measured Variable
Executive Skills	Controlled Oral Word Fluency (49)	Total number of words
	Thurstone Written Word Fluency (50)	Total number of words
	Delayed Alteration Test (51)	Number of errors
	Wisconsin Card Sorting Test (52)	Perseverative responses
		Total errors
Attention and Concentration	Consonant Trigrams Test (53)	Mean score - 3 conditions
Visual-Perception/ Visual-Construction	Block Design (WAIS-III) (54)	Age scaled score
	Motor Free Visual Perception Test - Revised (55)	Number correct
Memory	Recognition Memory Test (56)	Number of words correct
		Number of faces correct
		Number of words: Trials 1-5
		Delayed recognition
		Delayed recall - number of words

Blood gases were taken in the clinic before and after the study to control for oxygen, carbon dioxide, bicarbonate, and pH levels. Four 5mm slices were localized to the level of the basal ganglia using a non-enhanced CT head scan. Once the appropriate slices had been determined patients were scanned continuously at this table location for 49 seconds at 80 kVp and 190 mA and with an intravenous injection of 50mL of iodinated contrast agent (Omnipaque 300, GE Healthcare) at a rate of 2-3.5 ml·s⁻¹ using a methodology previously described (31). The software CT Perfusion (GE Healthcare) was used to

calculate cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT) from dynamic images acquired with the CTP scan(32). Hemodynamic parametric maps were regionally analyzed based on the segmentation of constituent lobes of the cortex (frontal, temporal, parietal, and occipital), deep grey matter structures (basal ganglia and thalamus), and deep white matter using a custom program written in IDL (Version 5.6, Research Systems Inc., 2002).

2.2.4 Statistical Analysis

Differences in neuropsychological test scores were determined by converting test scores into T-scores (mean of 50, SD of 10) based on normative samples stratified for age and when available for education. A T-score less than 30 (i.e., more than 2 SD below the mean) on a specific measure was considered an abnormal score. Results for physiological and hemodynamic measurements are given as the mean \pm SD values. Since assumptions of normality were violated in the PLSci group non-parametric statistical methods were employed. A Kruskal-Wallis analysis was used as the omnibus test with post hoc Wilcoxon tests where appropriate, with $p < 0.05$ considered significant. A Pearson correlation was performed to determine an association between hemodynamic parameters with disease duration and education, with $p < 0.05$ considered significant.

2.3 RESULTS

2.3.1 Clinical Characteristics

The clinical characteristics of patients are presented in Table 2.2. PLS patients ranged in age from 44 to 72 years (mean, 59.1 ± 8.7 years), whereas the control subjects

age ranged from 34 to 63 years (mean, 52.8 ± 9.9 years). No significant difference in age was reported between groups.

Table 2.2: Demographics and Clinical Characteristics of PLS Patients.

Patient	Sex	Age at Symptom Onset	Age at Diagnosis	Site of Onset	Symptom at Onset	Current Bulbar Symptom	Overt Diabetes	Cardio-vascular Disease	Smoker
1	M	35.3	53.3	B	D	Yes	No	Yes	No
2	M	47.8	57.9	r/L	W	Yes	Yes	Yes	No
3	F	56.6	67.7	lL	W	No	No	Yes	No
4	F	55.8	60.7	lL	S	No	No	No	Yes
5	M	29.1	43.2	B	D	Yes	No	No	Yes
6	F	51.6	63.4	r/L	W	No	No	No	No
7	M	41.3	48.6	lL	W	No	No	No	Yes
8	F	47.8	55.4	r/L	S	No	Yes	Yes	No
9	M	34.3	41.6	r/L	S	Yes	No	No	No
10	M	56.7	62.9	lL	S	Yes	No	No	Yes
11	F	36.0	64.9	r/L	S	Yes	No	No	Yes
12	F	58.1	68.3	B	D	Yes	No	No	Yes
13	M	29.8	45.8	r/L	S	Yes	No	Yes	No
14	F	58.4	67.8	rL	W	Yes	No	Yes	No
15	M	32.4	40.4	rL	W	No	No	No	No
16	F	51.4	58.3	lL	W	Yes	No	No	No
17	M	50.3	56.1	rL	S	Yes	No	Yes	No
18	F	44.5	53.8	r/L	W	Yes	Yes	No	No

B = bulbar; r = right; l = left; L = Leg(s); D = dysarthria; W = weakness; S = spasticity

2.3.2 Neuropsychological Results

The neuropsychological studies were performed on all 18 PLS patients. As a group, PLS patients scored broadly within normal limits (mean T-scores greater than 40) on all cognitive measures. However, there was considerable heterogeneity observed in scores when the data were examined on a case by case basis. Neuropsychological data were analyzed using a stringent threshold of 2 SD below the mean for impairment criteria, resulting in 4 PLS patients (or 22 %) being rated as impaired. In the PLSci group, abnormal scores were found on tests measuring executive functions including, in order of frequency, oral word fluency, source memory (recognition memory trial of the

Rey Auditory Verbal Learning Test), problem-solving ability (Wisconsin Card Sorting Test – Total errors), nonverbal working memory (Delayed Alternation Test), and verbal learning efficiency (Rey Auditory Verbal Learning Test – Number of Words Trials 1-5). The cognitive test scores for each of the four PLSci patients is presented in Table 2.3. All other PLS patients had zero or only one abnormal score. All control subjects in this study were cognitively normal, with no abnormal neuropsychological scores.

2.3.3 Imaging Results

No hemispheric differences existed within groups for all hemodynamic parameters measured. Thus, the data presented are cerebral regions averaged over both hemispheres. PLS patients and control subjects were found to be statistically similar for all hemodynamic parameters in all regions analyzed. The majority of the differences of this study were found between the PLSci and control groups. Eight of the PLS patients had concomitant cerebrovascular disease or diabetes as described in the patient's medical

Table 2.3: Cognitive Results of PLSci Patients. Cognitive results of 4 PLSci patients showing that verbal word fluency was the most commonly observed test with a deficit. Bolded T-scores signify scores that are greater than 2 SD below the mean, indicating an abnormal test score.

Test	Measured Variable	T-Scores of PLSci Patients (N = 4)			
		# 1	# 2	# 3	# 4
Controlled Oral Word Fluency	Total number of words	27	27	27	50
Thurstone Written Word Fluency	Total number of words	35	---	31	49
Delayed Alteration Test	Number of errors	16	---	---	57
Wisconsin Card Sorting Test	Perseverative responses	58	51	32	39
	Total errors	55	51	28	43
Consonant Trigrams Test	Mean score - 3 conditions	---	31	33	48
Block Design (WAIS-III)	Age scaled score	53	---	43	53
Motor Free Visual Perception Test - Revised	Number correct	60	47	47	63
Recognition Memory Test	Number of words correct	---	43	50	50
	Number of faces correct	---	57	57	40
Rey Auditory Verbal Learning Test	Number of words: Trials 1-5	44	31	35	26
	Delayed recall - number of words	41	37	41	37
	Delayed recognition	47	10	40	23

vascular cognitive impairment. However, when the data was analyzed both as a whole cohort and with the removal of those with concomitant cerebrovascular risk factors, there was no significant impact of either cerebrovascular disease or diabetes on the cerebral perfusion study results of the whole cohort. Hence, the whole cohort of 18 PLS patients was used for analysis. Implications of this approach in our analysis will be examined in the discussion.

2.3.3.1 Grey Matter Analysis

Hemodynamic parameters for each group are presented in Table 2.4A. Global trends of decreased CBF and increased CBV and MTT were evident in the PLSci group when compared with the other two groups. In the frontotemporal region CBV was significantly increased in PLSci compared with controls. However, MTT proved to be the most sensitive in detecting differences based on cognitive status as shown by significantly elevated values in all regions of the PLSci group when compared to controls with the exception of the temporal lobe ($p=0.055$). Figure 2.1 displays the hemodynamic trends as mentioned with MTT being the most pronounced.

2.3.3.2 White Matter Analysis

White matter hemodynamic changes between groups closely resembled those in the grey matter and are presented in Table 2.4B. MTT was again found to be the most sensitive indicator of changes between groups with significant increases reported in the frontal and occipital regions of the PLSci group as compared with controls. The temporal and parietal region did not reach significance, but displayed the same trend.

Furthermore, a Pearson correlation analysis was performed to determine if the difference between groups for hemodynamic parameters could be explained by group differences in disease duration or education. Neither age nor the number of years of education provided a significant relationship with any hemodynamic parameter measured in any region of interest. Lastly, since the groups were stratified based on neuropsychological performance, it was of interest to determine if an interaction existed between disease duration or education with group membership. There existed no

Table 2.4A: Comparison of Cerebral Hemodynamic Results for Grey Matter According to Group.

Parameter	Region	Control		PLS		PLSci		Kruskal-Wallis	Wilcoxon	
		Mean	SD	Mean	SD	Mean	SD		Control vs PLSci	PLS vs PLSci
CBF	Frontal	38.7	3.5	38.6	4.3	36.2	5.5	-	-	-
	Temporal	39.5	4.3	39.8	3.9	36.4	6.2	-	-	-
	Parietal	44.2	4.8	41.8	4.4	36.5	5.5	-	-	-
	Occipital	39.6	4.2	37.3	4.9	32.1	4.3	-	-	-
	Basal Ganglia	40.5	5.9	40.6	3.6	36.4	5.2	-	-	-
	Thalamus	37.6	6.0	37.2	5.2	31.6	5.2	-	-	-
CBV	Frontal	1.52	0.17	1.68	0.18	1.82	0.12	*	*	*
	Temporal	1.61	0.20	1.83	0.20	1.89	0.16	*	*	*
	Parietal	1.82	0.31	1.83	0.26	1.97	0.15	-	-	-
	Occipital	1.83	0.24	1.97	0.23	2.04	0.18	-	-	-
	Basal Ganglia	1.48	0.18	1.67	0.17	1.73	0.10	*	-	*
	Thalamus	1.55	0.21	1.75	0.22	1.87	0.09	*	*	*
MTT	Frontal	2.37	0.29	2.63	0.34	3.04	0.27	*	*	-
	Temporal	2.47	0.41	2.78	0.41	3.17	0.44	-	-	-
	Parietal	2.47	0.34	2.65	0.44	3.27	0.26	*	*	-
	Occipital	2.82	0.60	3.22	0.59	3.84	0.42	*	*	-
	Basal Ganglia	2.22	0.28	2.48	0.32	2.88	0.25	*	*	-
	Thalamus	2.50	0.45	2.88	0.56	3.60	0.39	*	*	-

CBF (ml/100g/min); CBV (ml/100g); MTT (seconds); * = significant (P <0.05); - = not significant

Table 2.4B: Comparison of Cerebral Hemodynamic Results for White Matter According to Group.

Parameter	Region	Control		PLS		PLSci		Kruskal-Wallis	Wilcoxon	
		Mean	SD	Mean	SD	Mean	SD		Control vs PLSci	PLS vs PLSci
CBF	Frontal	22.4	3.9	22.8	3.1	20.7	4.3	-	-	-
	Temporal	22.9	4.9	22.8	3.5	20.4	3.9	-	-	-
	Parietal	23.5	3.9	23.7	3.2	17.8	3.0	*	-	-
	Occipital	24.2	3.8	22.3	3.3	18.4	3.8	-	-	-
CBV	Frontal	0.92	0.11	1.04	0.10	1.10	0.12	-	-	-
	Temporal	1.02	0.14	1.14	0.14	1.15	0.07	-	-	-
	Parietal	1.08	0.17	1.11	0.13	1.14	0.07	-	-	-
	Occipital	1.06	0.12	1.13	0.14	1.13	0.13	-	-	-
MTT	Frontal	2.50	0.32	2.77	0.44	3.24	0.32	*	*	-
	Temporal	2.78	0.60	3.07	0.61	3.49	0.59	-	-	-
	Parietal	2.81	0.57	3.84	0.51	3.89	0.47	*	-	-
	Occipital	2.69	0.49	3.10	0.59	3.76	0.45	*	*	-

CBF (ml/100g/min); CBV (ml/100g); MTT (seconds); * = significant (P <0.05); - = not significant

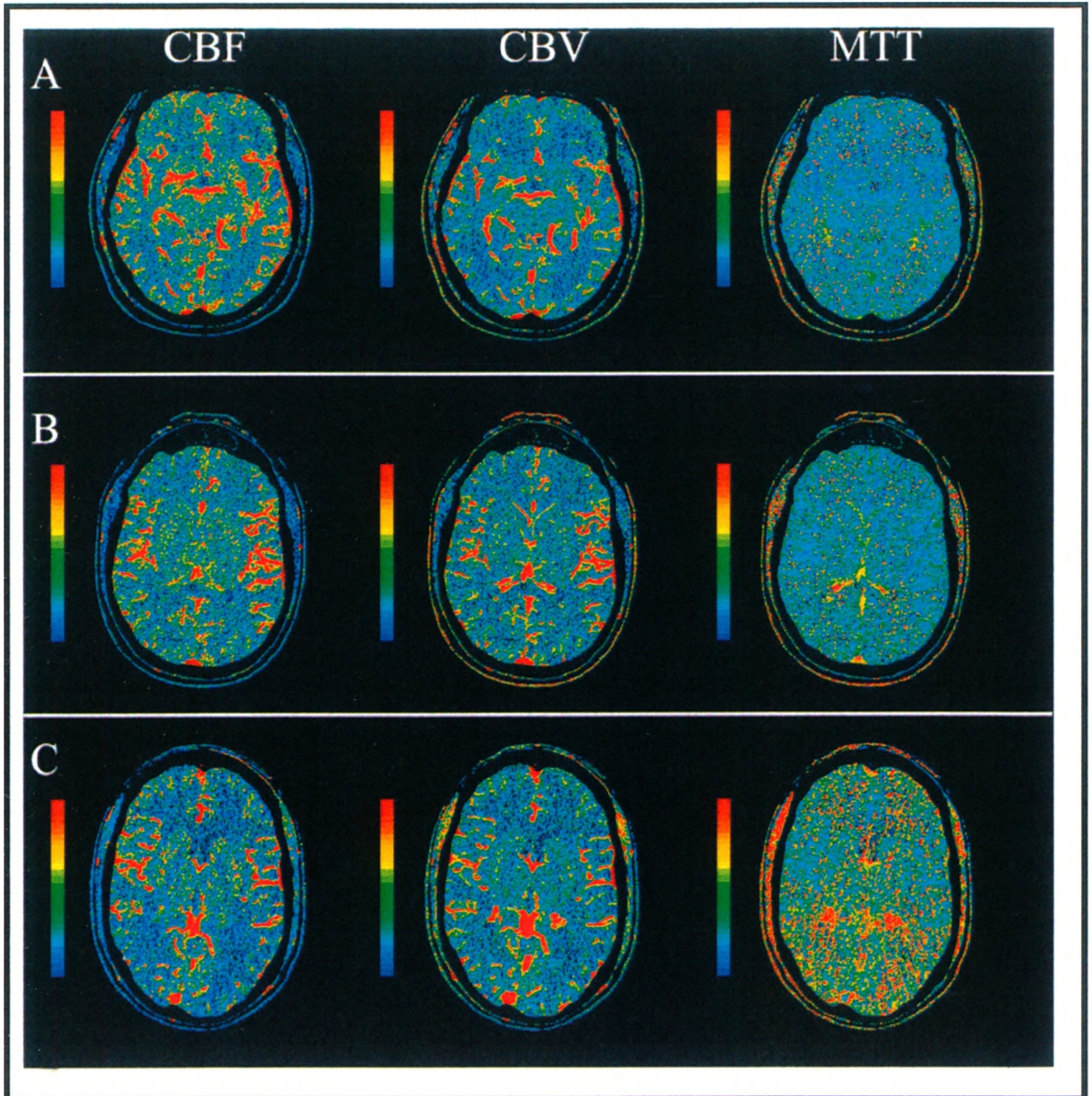


Figure 2.1: Hemodynamic Parametric Maps by Group. Hemodynamic parametric maps of patients from the Control (A), PLS (B), and PLSci (C) groups demonstrating a decreasing trend of CBF and an increasing trend of MTT. Changes in trends of CBV are much less pronounced. CBF, cerebral blood flow (range: 0-120 ml/100g/min); CBV, cerebral blood volume (range: 0-6ml/100g); MTT, mean transit time (range: 0-12 secs).

statistical difference between the two PLS groups with respect to disease duration or between the controls and the two PLS groups for education. Blood gases were analyzed in a similar manner and there was no statistical difference between either PLS group. Physiological parameters measured during the CT scan are presented in Table 2.5. PLSci had significantly elevated mean arterial pressure when compared with the controls, but otherwise was similar to both groups in all other parameters.

Table 2.5: Physiological Data During CT Scan.

Parameter	Control		PLS		PLSci		Kruskal-Wallis	Wilcoxon	
	Mean	SD	Mean	SD	Mean	SD		Control vs PLSci	PLS vs PLSci
EtCO ₂	39.6	2.5	38.4	3.7	42.4	1.7	-	-	-
BF	15.8	1.5	18.6	2.4	12.8	1.2	*	-	-
BP	91.8	9.3	106.1	10.4	108.7	9.9	*	*	-
HR	63.0	9.5	79.0	7.7	72.7	21.0	*	-	-
O ₂ sat	96.8	1.9	95.5	2.0	97.3	1.3	-	-	-

EtCO₂, end tidal CO₂ (mmHg); BF = breathing frequency (breaths/min); BP = blood pressure (mmHg); HR = heart rate (beats/min); O₂sat = tissue oxygen saturation (% oxygenated hemoglobin); * = significant (P < 0.05); - = not significant

2.4 DISCUSSION

We have investigated the relationship between cognitive function and cerebral hemodynamics in 18 PLS patients. We found that cognitive impairment was associated with hemodynamic compromise, including significant increases in MTT and trends of increasing CBV and decreasing CBF. Overall, 22% of our patients were classified as cognitively impaired based on our relatively stringent criteria. However, none of these patients were considered to demonstrate a frontotemporal dementia. Consistent with

previous studies in ALS(33), the cognitive impairment was generally absent or subtle for the majority of PLS patients. When impairment was present, it was characterized by executive dysfunction, with oral word fluency being the most frequently impaired measure.

The neuropsychological assessment utilized in this study minimized the impact of speech and motor dysfunction in testing outcomes, with many of the tests requiring no speech production and minimal motor requirements (Motor Free Visual Perception Test – Revised, Delayed Alternation Test, and Recognition Memory Test). Other tests requiring motor ability were adapted if necessary to reduce motor requirements (e.g., Wisconsin Card Sorting Test cards were placed by the examiner). In addition, some tasks allowed for either written or oral responses depending on the patient's capabilities (e.g., Rey Auditory Verbal Learning Test, Consonant Trigrams Test, Thurstone Written Word Fluency). The only task that required significant manual control, Block Design, did not result in abnormal scores in either the patient or control subjects. Although oral-motor dysfunction may affect a patient's performance on the timed oral word fluency task (Controlled Oral Word Fluency), it has been previously shown for ALS patients that the vast majority of words (90 percent) are generated in the first half or 30 seconds of the oral word fluency task (21). This suggests that the limiting factor in this task is associated with thought production of the words rather than oral-motor speed in speaking the words. Previous studies are also consistent with this finding, noting a lack of effect of bulbar dysfunction (34) or specifically dysarthria (35) on oral word fluency results.

Similar to ALS (9, 11-15), PLS patients in this study exhibit cognitive dysfunction associated with disturbances of cerebral hemodynamics that are

proportionate to the level of cognitive impairment. When 8 PLS patients with concomitant cerebrovascular disease or diabetes were removed from the analysis this remained true. In the aforementioned 8 PLS patients, group membership was split between the PLS (n=7) and the PLS_{ci} (n=1). Since the majority of these patients were from the cognitively intact group, it provides evidence that their concomitant disease pathology may be mild in nature since both are known risk factors for vascular cognitive impairment (VCI) (36), thus helping to explain the similar hemodynamic outcome between the analyses.

The observed tendency for lower CBF in PLS_{ci} versus PLS is consistent with literature regarding CBF in ALS (9, 16), but perhaps did not reach significance due to the small sample size. Of note, Tanaka *et al.* (13) observed that regional CBF (rCBF) and cerebral metabolic rate of oxygen (CMRO₂) were significantly lower in ALS patients with dementia in all four cortices of the brain when compared to healthy controls and ALS patients without dementia. These authors suggested that in the absence of distinct neurological differences between ALS patients with cognitive impairment and those without, abnormalities in CBF and CMRO₂ may be reflective of intellectual deterioration. Although we do not have direct evidence to support this statement, our findings of hemodynamic compromise in PLS_{ci} are consistent with this.

Since differences in perfusion parameters are reported to be associated with both age (37) and gender (38) the current study aimed to minimize the influence of both. There were no significant differences for age between groups, thus not influencing perfusion measurements in this study. Gender-matching between groups was more difficult with fewer controls than patients participating in the study, although similar

proportions of males to females were maintained between groups. Slight differences in group gender composition should have little influence on perfusion measurements as described by a recent study that found 13% greater whole brain and grey matter perfusion in a group of women when compared with a group of age-matched men.(37) Thus, given similar group gender composition in this study and reportedly marginal and controversial gender differences in perfusion (39), perfusion measurements made herein are believed to be minimally influenced by these slight group differences. Recently, Le Forestier *et al.* (6) examined 9 PLS patients with PET using the early uptake of [¹¹C]-Flumazenil to estimate the distribution of rCBF as compared with healthy volunteers. The patient group had foci of abnormally low blood flow bilaterally in the fronto-opercular region, predominantly in the precentral gyrus, but not limited to this area. The distribution of the PET tracer described in this study was further corroborated in a small study of 4 PLS patients (40) describing a qualitatively similar pattern to that seen in sporadic ALS (41). Similarly, using HMPAO-SPECT, Caselli *et al.* (17) observed hypoperfusion bilaterally in the posterior frontal cortex in all 6 patients studied, with temporal involvement in 2 patients. Pringle *et al.* (2) observed diminished FDG uptake in the pericentral cortex in two patients with PLS in association with frontal lobe dysfunction.

We have observed that hemodynamic changes in PLSci are not confined to a single anatomic or functional region, but in fact are seen to extend globally throughout all four cortices and the underlying white matter, as well as into the deep grey matter structures of the brain. The frontotemporal region had the most prominent perfusion deficit with cortical decreases in CBV, with associated frontal lobe changes in MTT in

both cortical and subcortical regions when compared with controls. These results are in agreement with similar studies examining regional differences in perfusion and metabolism in cognitive impairment in ALS (9, 11, 13) and PLS (2, 17).

Although CBV is rarely addressed in neuroimaging studies of MND, it is of interest as it provides insight into the autoregulatory state. CBV is altered by dilation of resistance vessels to reduce cerebrovascular resistance, thereby counteracting reductions in cerebral perfusion pressure (CPP) in a phenomenon known as autoregulation (42). Cerebral perfusion pressure is the net difference between the arterial pressure driving blood into the cerebral circulation and the microvascular resistance opposing it (43). We observed an increasing trend of CBV in the PLSci patients when compared to the PLS and control group. Interestingly, with respect to the cortex only the frontotemporal region in the PLSci group had significantly increased blood volume when compared with the control group. This is of particular interest since many studies indicate that this area is predominantly affected in executive dysfunction and can be visualized with CBF or $CMRO_2$ measurements, albeit many of these studies describe patients with more pronounced cognitive deficits. Thus, in cases of mild cognitive impairment as presented in this study, changes in CBF and $CMRO_2$ may not be readily apparent as this mechanism of preferential cerebral dilation allows CBF to stay within the normal range, although slight changes in CBF are documented to occur during autoregulation (44, 45). The autoregulatory mechanism just described was observed in our PLSci group, and has been elucidated in experimentation of decreased CPP using primates (46) and suggested in humans (47). Thus, it is plausible to consider that the change in CBV observed in our patients may be indicative of changes in CPP.

We have also observed increases in MTT in PLSci patients. Defined as the ratio of CBV:CBF, MTT is measured in time and is an indicator of flow velocity (42, 43) and quantifies the average amount of time that it takes the contrast agent in blood to move from the arterial input, through the capillary network, and exit via a venous output. A change in MTT is inversely related to CPP and is a sensitive measure to hemodynamic change as the resultant MTT is amplified by small reductions in CBF and increases in CBV (48). Furthermore, in an unpublished data set using CTP imaging, our group has demonstrated that MTT is an accurate surrogate marker for CPP and may be indicative of early endothelial dysfunction.

CTP scanning was used in this study as a novel technique to describe cerebral perfusion in PLS. The findings of this study are similar to other imaging modalities used describing decreased perfusion in ALS patients with cognitive impairment when compared to cognitively intact ALS and control groups. One advantage of CTP is that it not only provides high resolution anatomical images of cortical and subcortical structures, but is capable of producing parametric measurements of CBF, CBV and MTT with one rapid scanning procedure (49-sec) without the need of any radioisotopes. (31) Furthermore, a short scan time may be more comfortable for patients with dysphagia while facilitating less movement during the scan. A possible drawback of our technique may be the limited coverage as compared with other imaging modalities such as PET and MRI, which are capable of whole brain imaging. Thus, our 2-cm slab of cerebral tissue is carefully selected to ensure a scan plane with representation from all cortical lobes and deep grey matter structures. In the present study, MTT was significantly higher in the PLSci group in all grey matter regions when compared with the controls, save the

temporal lobe, as was the trend when compared with the PLS group. The white matter analysis supported this trend with significantly higher MTT in the frontal and temporal subcortical regions when compared to the control group. To our knowledge this is the first study assessing MTT differences in PLS or cognitive impairment. Given our findings showing the increased sensitivity of MTT over CBF, which is predominantly employed in hemodynamic studies, we believe that MTT may be a valuable tool for assessing mild changes in cognitive impairment that may otherwise be overlooked with CBF and CBV.

The current study has demonstrated that PLS patients are subject to cognitive decline in association with changes in cerebrovascular hemodynamic parameters, and most specifically alterations in MTT. Although many of the clinical characteristics of cognition and changes in hemodynamic status in PLS are similar to those documented in ALS, this study does not necessarily suggest that these two diseases lie on the same continuum, nor can it suggest that they are distinct entities. These data demonstrate only that many of the clinical characteristics found in ALS are also present in PLS, although to a lesser degree. Of note however, we have observed that the analysis of MTT provided a sensitive indicator correlated with cognitive change in PLS, suggesting that studies of MTT may provide clinicians and researchers with a valuable surrogate marker for the presence of cognitive impairment in PLS, and by extension, ALS.

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CHAPTER 3

WIDESPREAD CEREBRAL HEMODYNAMICS DISTURBANCES OCCUR EARLY IN AMYOTROPHIC LATERAL SCLEROSIS

3.1 INTRODUCTION

Historically, amyotrophic lateral sclerosis (ALS) has been viewed as a progressive neurodegenerative disease confined to the voluntary motor system giving rise to diffuse muscle wasting, weakness and spasticity. A growing body of evidence now describes widespread manifestations of the disease outside of the motor system. Moreover, studies in neuroimaging (1, 2), pathology (3) and neuropsychology (4, 5) have provided a fundamental description of changes in extramotor regions, both cortical and subcortical in nature.

Contemporary views of ALS now assert that cognitive change is a prominent extramotor manifestation and part of the clinical characterization of the disease. To this end, studies have stratified patients based on the presence or absence of overt frontotemporal dementia (FTD) in an attempt to elucidate possible mechanistic differences in pathology between the two groups. As a first approximation, it was successful and provided a basis for current knowledge and gave a first description of differences between the two clinical phenotypes. However, it is now recognized that impairment levels are variable and can range from mild cognitive or behavioural impairment to FTD, and may in fact represent a continuum of dysfunction (6, 7). When cognitive change is prospectively tested with paradigms sensitive to frontal lobe

dysfunction, upwards of 50% of ALS patients exhibit some degree of impairment short of dementia, while up to 20-30% fulfil the criteria for FTD (7-9).

Perfusion and neuroimaging studies have provided evidence for the link between neuropsychology and pathology in cognitive impairment. When ALS is accompanied by cognitive impairment or dementia, as demonstrated by specific neuropsychological testing, concomitant changes in cerebral perfusion and metabolism have been noted. These results have been suggested using various imaging methodologies including positron emission tomography (PET) (10-14), [^{99m}Tc]-d,l-HMPAO (15) or N-Isopropyl-p- ^{123}I -iodoamphetamine (^{123}I -IMP) (16-18) single photon emission computed tomography (SPECT). A preponderance of the dysfunction described in the literature focuses on the prefrontal cortex when examining extramotor involvement in ALS (5, 10, 13, 14, 19). Much less is known about early perfusion changes in the extramotor area away from the frontal lobe, although studies have demonstrated widespread cortical and subcortical involvement in the disease through pathology (3) and advanced MRI techniques (1, 2).

Furthermore, imaging and cognitive studies in ALS have predominantly employed cross sectional methods at discrete time points to resolve changes in their respective areas with few focusing on the temporal course of these changes. Even less is known about the association between cognitive decline and cerebral hemodynamics in ALS with respect to time, even though longitudinal studies have been highlighted (20) as a necessary step to help define and understand disease progression and its manifestations. We thus assessed regional cerebral perfusion as a function of cognitive dysfunction in

ALS. We used CT perfusion (GE Healthcare) (21) to examine hemodynamic changes in a prospective longitudinal study that systematically assessed cognitive function in ALS.

3.2 METHODS

3.2.1 Participants

Fourteen ALS patients (9 men, 5 women), age 34 to 69 years (mean, 52.4 ± 9.4 years) and eleven spousal controls (6 men, 5 woman), age 34 to 63 years (mean, 53.9 ± 8.2 years) were recruited from the Motor Diseases Clinic at the University of Western Ontario from 2003-2007 to study the possible relationship between cognitive and hemodynamic changes that occur during disease progression. All subjects gave informed consent for the study which was approved by the University of Western Ontario Health Sciences Research Ethics Board. Disease severity was measured using the revised ALS functional rating scale (ALSFRS-R) (22) and diagnosed as clinically definite for having the disease based on the El Escorial criteria (23). Upon admission to the study, both groups completed an initial assessment consisting of a cognitive test battery and a CT Perfusion (CTP) head scan. Patients and controls completed follow-up visits involving the same tests, although some withdrew early from the study due to medical or personal reasons. Time periods for analyses were stratified as initial assessment and periods post initial assessment: T0; initial assessment ($n_{ALS} = 14$, $n_C = 9$), T1; 0-12 months ($n_{ALS} = 8$, $n_C = 5$), T2; ≥ 13 months ($n_{ALS} = 6$, $n_C = 4$).

3.2.2 Neuropsychological Evaluation

Nine neuropsychological tests consisting of 13 variables were employed to assess four major domains of cognitive functioning, with a particular emphasis on frontal-executive skills as described in Table 3.1.

Tests were specifically chosen to minimize the requirements of speech production and upper limb motor skills. All patients and controls completed neuropsychological testing on the morning of CTP scanning at all time points. Neuropsychological data were analyzed using a threshold of 2 SD below the mean to determine impairment for a specific test score, with two abnormal test scores being the criteria for CI (24).

3.2.3 Imaging and Analysis

A 4-slice CT scanner (GE LightSpeed Plus; GE Healthcare, Waukesha, Wisconsin) was used for the CTP scan, during which time blood pressure (BP), end tidal CO₂ (EtCO₂), oxygen saturation (O₂sat), breathing frequency (BF) and heart rate (HR) were monitored. Blood gases were taken before and after the study to control for oxygen, carbon dioxide, bicarbonate, and pH levels. Four 5mm slices were localized to the level of the basal ganglia using a non-enhanced CT head scan. Once the appropriate slices had been determined patients were scanned continuously at this location for 49 seconds at 80 kVp and 190 mA and with an intravenous injection of 50mL of iodinated contrast agent (Omnipaque 300, GE Healthcare) at a rate of 2-3.5 ml·s⁻¹ using a methodology previously described (21). The software CT Perfusion (version 3, GE Healthcare) was

Table 3.1: Summary of Neuropsychological Tests and Variables.

Domain	Test	Measured Variable
Executive Skills	Controlled Oral Word Fluency	Total number of words
	Thurstone Written Word Fluency	Total number of words
	Delayed Alteration Test	Number of errors
	Wisconsin Card Sorting Test	Perseverative responses
		Total errors
Attention and Concentration	Consonant Trigrams Test	Mean score – 3 conditions
Visual-perception/ Visual-construction	Block Design (WAIS-III)	Age scaled score
	Motor Free Visual Perception Test - Revised	Number correct
Memory	Recognition Memory Test	Number of words correct
		Number of faces correct
	Rey Auditory Verbal Learning Test	Number of words: Trials 1-5
		Delayed recognition
	Delayed recall - number of words	

used to calculate parametric maps of hemodynamics: cerebral blood flow (CBF), cerebral blood volume (CBV), and mean transit time (MTT) from dynamic images acquired with the CTP scan (25). MTT, defined as the ratio of CBV:CBF, is an indicator of flow velocity and quantifies the average time period for blood borne contrast agent to move from the arterial input, through the capillary network, to a venous output.

Cortical lobes (frontal, temporal, parietal, and occipital), deep grey matter structures (basal ganglia and thalamus), and subcortical white matter were segmented manually according anatomical landmarks. Average values in the manually drawn regions of interests (ROIs) were determined with a custom program written in IDL

(Interactive Data Language, Version 5.6, Research Systems Inc., 2002) from the hemodynamic parametric maps.

3.2.4 Statistical Analysis

Neuropsychological test scores were converted to T-scores (mean of 50, SD of 10) and the difference of the T-scores was calculated based on normative samples stratified for age and when available for education. A T-score less than 30 (i.e., more than 2 SD below the mean) on a specific measure was considered to an abnormal score. Results for physiological and hemodynamic measurements are given as the mean \pm SD values. Since assumptions for normality were violated within groups, a nonparametric analysis was employed. Comparison of ALS and control groups were made with a Wilcoxon test with $p < 0.05$ considered significant.

3.3 RESULTS

3.3.1 Clinical Characteristics

Fourteen ALS patients and 11 controls were studied at T0, all completing neuropsychology and cerebral perfusion assessments. T0 was assessed on average 11.8 months (range, SD; 1-62, 19.5 months) post diagnosis, with T1 and T2 being assessed at 6.1 (4-8, 1.4) and 17.0 (13-25, 4.3) months post T0, respectively.

There was no significant difference in age at the time of study between the patient and control groups. The mean age of onset at the first symptom for ALS patients was 49.8 ± 9.7 years, with the nature of the first symptom being bulbar in 5 patients and limb-onset in 9 patients. At the time of testing, all patients had bulbar symptoms.

3.3.2 Neuropsychological Results

The results of the neuropsychological tests indicated that all 14 ALS patients were cognitively normal at T0. In the control group at T0, neuropsychological testing identified two controls as cognitively impaired and were subsequently excluded from the study; leaving nine cognitively normal controls included. For T1 and T2, all patients and controls remained cognitively normal with the exception of two ALS patients. These two patients achieved the minimum threshold for consideration of CI by scoring abnormally on two tests, at which point they were excluded from the ALS group analysis. Unfortunately, a grouping of 2 patients is insufficient to perform statistical procedures on as a separate group and thus is further excluded from any formal statistical analysis.

3.3.3 Imaging Results

No hemispheric differences were found in each of the hemodynamic parameters. Thus data is presented as the average of both hemispheres for the cerebral region of interest. Values of physiological parameters measured during the CTP scan are displayed in Table 3.2. No significant differences existed between groups with the exception of mean arterial pressure at T0.

For time period T0, no significant differences or trends were apparent in any hemodynamic parameter measured for any region between the ALS and control groups. The follow up T1 measurements were assessed on average 6.1 and 6.2 months after T0 for ALS and control groups, respectively. The temporal region had significantly increased MTT in the ALS group compared with controls; otherwise no significant

Table 3.2: Physiological Data During CT Scan.

Parameter	T0		T1		T2	
	ALS	Control	ALS	Control	ALS	Control
EtCO₂	39 ± 4	39 ± 3	39 ± 2	40 ± 3	37 ± 1	40 ± 3
BF	19 ± 4	16 ± 4	21 ± 8	15 ± 4	21 ± 7	16 ± 4
MAP	106 ± 7*	93 ± 7	97 ± 12	93 ± 3	98 ± 7	95 ± 5
HR	79 ± 13	69 ± 11	83 ± 17	70 ± 13	83 ± 18	69 ± 4
O₂sat	95 ± 3	97 ± 2	95 ± 3	93 ± 3	94 ± 2	95 ± 2

EtCO₂ = end tidal CO₂ (mmHg); BF = breathing frequency (breaths/min); MAP = mean arterial pressure (mmHg); HR = heart rate (beats/min); O₂sat = tissue oxygen saturation (% oxygenated haemoglobin); mean ± SD; * = significant (P < 0.05).

differences existed between the two groups at T1. However, it is noteworthy that ALS patients had trends of reduced CBF and increased MTT in all regions without exception when compared with the control subjects. Lastly, T2 was assessed on average 17.0 and 15.5 months post T1 for ALS and controls, respectively. At T2, in all regions measured for both grey and white matter, the ALS group had trends of decreased CBF and increased CBV. The MTT was significantly higher in all cortical regions and the thalamus of the ALS group when compared with the controls, although significant differences were not found for white matter regions during this time period. Trends of differences in hemodynamic parameters between ALS and controls at T1 and T2 were most pronounced in the cortex.

Figure 3.1 compares the four cortical regions in both groups for MTT.

3.4 DISCUSSION

In this study we measured cerebral hemodynamic parameters in both cortical and subcortical regions in early stage ALS and compared them to normal age matched controls using CT Perfusion imaging over three time periods. Furthermore, an inclusion

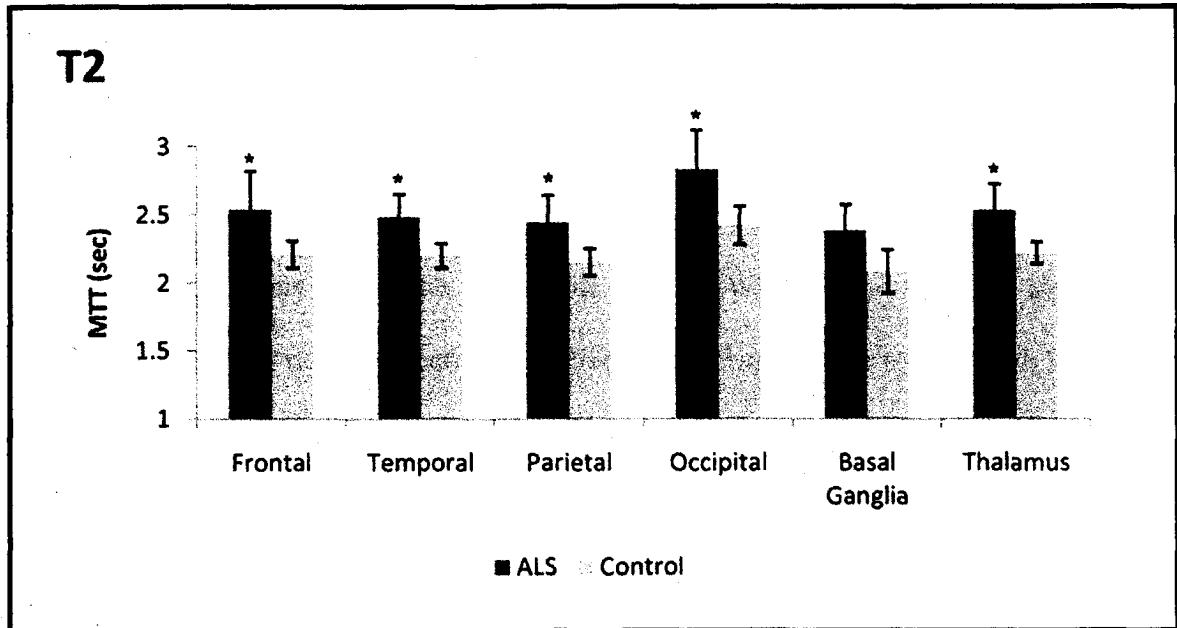


Figure 3.1: Comparison of MTT Between Groups by Region at T2. Mean MTT values (\pm SD) for the 6 grey matter regions measured between ALS patients and controls at T2. MTT was significantly increased in all regions of the ALS group except basal ganglia when compared with controls (* $p < 0.05$).

criterion at the onset of the study was that patients, when tested with a battery sensitive to frontal executive function, had normal neuropsychological profiles. This criterion has merit in that it allows a prospective account of the incidence of cognitive impairment. As well, it ensures early changes in cerebral perfusion are not confounded by impairment level, thus allowing comparison of cognitively intact ALS patients with controls with certainty.

Once thought to be rare, cognitive impairment is now considered as a component of the clinical pathology of ALS. Of the 14 patients studied, two (14%) patients progressed to mild cognitive impairment without developing overt dementia. The incidence of cognitive dysfunction in this study is lower than expected and may be

related to the prospective study design in which patients had no cognitive deficits at baseline. This eliminated patients in whom cognitive dysfunction developed before symptoms of ALS. Secondly, it is possible that the 6 patients that remained cognitively intact to the last time period represent a more stable disease course (24) and may not show cognitive signs within the given time period. With this noted, other longitudinal studies have shown development of cognitive dysfunction similar to ours with little or no progression of cognitive decline over a similar time interval (26, 27).

Pathological studies of cerebral tissue in ALS are predominantly limited to the premotor and motor cortex. However, a limited number of studies have described extramotor involvement that is in agreement with the findings of the current study. Smith et al. (1960) noted degeneration of fibres extending into the frontal (prefrontal cortex) and parietal (precentral gyrus) regions away from the motor cortex. Furthermore, degeneration was noted in the temporal lobe, corpus callosum, and anterior cingulate gyrus (28). Both the thalamus and basal ganglia have also been implicated in ALS in a related study (29). Although histopathology is an excellent technique for accurately quantifying tissue changes, it is an end stage metric that only provides a description of the cumulative changes that occur throughout the disease. The advent of advanced imaging techniques allows visualization of morphological and functional changes *in vivo* throughout disease progression (30).

Our study measured changes in cerebral perfusion parameters (CBF, CBV and MTT) of cortical grey matter, and subcortical grey and white matter at the level of the basal ganglia. We observed no significant differences in any of these parameters in the white matter throughout the duration of the study. Similarly, we found no differences in

the grey matter at T0 and T1 between ALS and control groups. In contrast, at T2 the trend of increased CBV and decreased CBF in all regions in the cortical grey matter leads to a significant increase in the MTT in ALS patients when compared to controls. Our findings are in agreement with those of Tanaka et al. (1993) who described mild reductions in regional CBF (rCBF) and cerebral metabolic rate of oxygen (CMRO₂) in non-demented patients with ALS, and significant reductions of both parameters in the majority of cortical regions measured in ALS with dementia when compared with healthy controls (12). Nakano et al. (2007) described significant decreases in rCBF in ALS patients with associated dementia, but only subtle changes in the non-demented ALS group when compared with controls using SPECT (17). These studies also described subtle changes in cerebral perfusion in ALS patients without dementia in which extramotor areas including the anterior portion of the cingulate gyrus and posterior part of the corpus callosum were involved.

Previous studies have focused largely on CBF and less frequently on CBV. We have shown that while separately these two parameters are not sensitive markers of altered cerebral perfusion in ALS, when used in conjunction with each other and expressed as MTT, which is quantitatively equivalent to CBV/CBF, significant differences between the groups become apparent. This is most evident at T2 and is present throughout the cortex. These findings are also consistent with automated voxel based morphometry (VBM) MRI studies in ALS patients with or without dementia. Abrahams et al. (2005) observed decreased white matter volume in non-demented ALS patients affecting both motor and extramotor regions (1). When the non-demented group from this latter study was further stratified into cognitively impaired and intact ALS

individuals, based on verbal fluency tests, both groups had similar patterns of white matter loss as compared to controls, although more pronounced in impaired ALS patients. The authors concluded that a loss of white matter volume was present early in the disease course of ALS, and that this may precede cognitive impairment. Although VBM studies have documented subtle grey matter structural changes extending beyond the motor cortex that are not obvious on routine visual inspection of T2- and proton-weighted MRI images (2), there are inconsistencies in this technique (31). These inconsistencies may arise from differences due to the study of small patient cohorts or methodological differences. Our study is however in agreement with the majority of studies(1-5, 10, 13, 14, 17, 29, 32) in describing hemodynamic changes outside of the motor cortex, and suggest hemodynamic change, like structural abnormalities, occur early in the disease process and may precede cognitive decline.

Elucidating the cause of these hemodynamic changes and their relationship to cognitive impairment in ALS presents a complex challenge. Our findings suggest that increases in MTT may indicate that vascular compromise is present in ALS. If alterations in vascular perfusion contribute to cognitive decline in ALS, then parallels between perfusion changes in ALS with mild cognitive impairment and vascular cognitive impairment or dementia might be expected.

It is pertinent to this study then that Neary et al. (2002) have described altered vascular perfusion with both MRI and SPECT in the three most common causes of young onset dementia including Alzheimer's disease (AD), FTD, and vascular dementia (VaD) (33). The overlap of atrophy and altered perfusion patterns was similar in many regions between FTD and VaD. FTD had more pronounced changes confined to the

frontotemporal lobe, while VaD displayed more subtle changes in this region and extended diffusely to other regions. More specifically, FTD had severe frontotemporal lobe atrophy, whereas only mild atrophy was observed in VaD in frontal, temporal and parietal regions. In keeping with this finding, in ALS the presence of overt atrophy is only documented in ALS/FTD (31) whereas ALS without dementia have only subtle cortical changes (2, 31, 34) similar to those described for VaD. Furthermore, in a separate study using VBM to compare ALS/FTD and ALS, a similar pattern of grey matter atrophy was found between the two groups, with the ALS/FTD group having significantly increased atrophy of the frontal region (35). When Neary et al. (2002) compared rCBF of FTD and VaD, a similar pattern was present in both dementias, again with more marked reductions in frontotemporal regions of FTD, distinguishing it from AD but to a lesser extent from VaD. VaD had mild changes in rCBF in all cortical regions measured with the most pronounced decreases in the frontal and temporal regions. These findings are similar to the previously described patterns of rCBF in ALS and ALS/FTD. While FTD is undoubtedly part of the pathological spectrum for a subset of patients with ALS (18, 36, 37), the description for VaD provided by Neary et al. (2002) closely resembles accounts of mild cognitive impairment in ALS.

A possible mechanism relating hypoperfusion to cognitive dysfunction may involve astrocytic degeneration and gliosis. Astrocytes lie in close proximity to neurons, are in direct apposition with vessel walls and are now considered important mediators of neurovascular coupling (38). In response to injury, as in neuronal degeneration, astrocytes become reactive and disruption of astrocyte-neural connections may have significant consequences on neuronal integrity and cerebral perfusion (39). Cotman and

colleagues (2001) described global trends for increased astrogliosis in patients with FTD with the most prominent changes in the frontal and temporal region (39). They noted that while the total number of astrocytes was similar in FTD and control brains, the number of degenerating astrocytes was elevated in FTD, although not reaching significance due to the variability within FTD cases. The number of degenerating astrocytes had a significant inverse relationship with cerebral perfusion measured with SPECT. In a related study, astrocytic cell degeneration and death was correlated with disease stage and neuronal loss in the progression of FTD (40). Thus, by extension, if a continuum of cognitive impairment exists that terminates in FTD, the perfusion deficits would also represent a continuum, with mild changes present in early ALS as documented in the current study.

In summary, we have observed early widespread cerebral hemodynamic changes in a group of cognitively intact ALS patients. Differences in MTT between groups became progressively apparent with increased time from baseline measurements in the absence of cognitive impairment. Although these findings cannot implicate vascular compromise as having a causal role in ALS, they do raise the possibility that vascular compromise may contribute to cognitive impairment in ALS. This study also demonstrated that MTT is a more sensitive indicator of perfusion deficits compared with CBF which is commonly used in neuroimaging and perfusion studies. Further research is needed to longitudinally assess patients who progress to cognitive impairment and FTD to help determine a possible relationship between vascular compromise and cognitive dysfunction in ALS.

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CHAPTER 4

EVALUATION OF MEAN TRANSIT TIME AS INDEX OF CEREBRAL PERFUSION PRESSURE IN EXPERIMENTALLY GRADED SYSTEMIC HYPOTENSION

4.1 INTRODUCTION

A reliable and clinically applicable surrogate of regional cerebral perfusion pressure (CPP) would be of substantial benefit to the diagnosis and treatment of cerebrovascular diseases since a predominant hallmark of cerebrovascular disease is a regional decrease in CPP downstream from the site of pathological origin. As the direct measurement of CPP can only be obtained through invasive techniques, it is not clinically practical. Thus, indirect measurements that are sensitive to hemodynamic compensatory mechanisms brought about by the presence of decreased CPP must be used to distinguish areas of the brain at risk. With decreasing CPP, there are two mechanisms that can be recruited to ensure the adequate delivery of substrates to the brain is not compromised. First, if the extent of reduced CPP is within the autoregulatory range (Stage I), vasodilation can be employed to reduce cerebrovascular resistance and maintain flows near the normal range (1, 2). Second, in the case where the reduction in CPP is more severe (Stage II), the fraction of oxygen extracted from the blood can be increased in an attempt to maintain normal levels of cerebral metabolism (1, 3-5). A third stage, Stage III, is defined as the point of failure of all compensatory mechanisms with ensuing metabolic failure.

It follows that the degree of hemodynamic compromise may be used as an indicator of CPP impairment (3). Cerebral hemodynamic compromise caused by a

decrease in CPP is commonly inferred from measurements of cerebral blood flow (CBF), cerebral blood volume (CBV), oxygen extraction fraction (OEF), and/or the cerebral metabolic rate of oxygen (CMRO₂). Several strategies have been developed to assess regional cerebral hemodynamic status *in vivo* for diagnosis of cerebrovascular disease; however, particularly with respect to Stage I cerebrovascular disease when autoregulation remains effective, blood flow effects can be subtle and difficult to distinguish. Within the range of autoregulation, CBV may be more sensitive as it responds inversely to alterations in CPP and thus may provide an indication of hemodynamic status. However, both CBF and CBV values may be reduced under circumstances of reportedly normal CPP when metabolic demands of the tissue are low (3).

Measurement of cerebrovascular reserve (CVR) was proposed as a more accurate method of interrogating cerebral hemodynamics and is currently widely accepted as a clinical method for diagnosing hemodynamic compromise (reduction in CPP) in cerebrovascular disease (6, 7). CVR reflects the dilatory capacity of cerebral arterioles and is determined as the ratio of CBF or CBV at baseline to that after administration of a vasodilatory stimulus (i.e., hypercapnia or acetazolamide). Theoretically, a blunted, non-existent or paradoxical CVR, that is a CVR value less than normal, equal to or less than unity respectively, would suggest the presence of chronic vasodilation prior to the dilatory stimulus, presumably associated with reduced CPP.

Despite the clinical acceptance of CVR as a marker of CPP in cerebrovascular disease, a major drawback of the technique is its reliance on stressing the vasodilatory capacity of the brain, a procedure that may precipitate a stroke in patients with compromised hemodynamics. In response, the measure of the basal mean transit time

(MTT) of blood for a given volume of brain tissue has been proposed as a preferential surrogate of CPP. Under conditions of normal CPP, CBF and CBV are tightly coupled (8); however, this strict coupling is disrupted under conditions of reduced CPP (9). By the Central Volume Principle, MTT is the ratio of CBV to CBF (10). It is particularly sensitive to any disturbances in the coupling between CBF and CBV and has been shown to be sensitive to disturbances in CPP (9, 11). The purpose of the present study was to compare CT Perfusion measurements of both MTT and CVR to a range of CPP in male New Zealand White rabbits.

4.2 METHODS

4.2.1 Animals and Anaesthesia

Ethics approval for all experimental procedures was obtained from the Animal Use Subcommittee of the Canadian Council on Animal Care at the University of Western Ontario. All studies were conducted in male New Zealand White (NZW) rabbits weighing 2.5-3.5 kg. Rabbits were housed throughout the duration of the experiment in separate cages in the animal care facility and provided standard rabbit chow and water *ad libitum*. Prior to all scanning protocols, anesthesia was induced with 3-5% isoflurane. Ketamine (3.0 mg/kg) and diazepam (0.3 mg/kg) boluses were given during intubation to maintain anaesthesia. During scanning, anesthesia was maintained with isoflurane (2.75%), and vecuronium bromide (0.15 mg/kg) was used during acetazolamide (ACZ) challenge to suppress spontaneous respiration caused by increased arterial CO₂ tensions. During each scan, physiological parameters were measured and maintained within normal ranges when possible.

4.2.2 Experimental Design

4.2.2.1 Acetazolamide Time Course

The blood flow and CVR response to ACZ were investigated in a group of normotensive (normal mean arterial pressure) rabbits ($n = 15$). Basal hemodynamic parameters (CBF, CBV, and MTT) were determined by averaging results of two CT perfusion (CTP) scans separated by 10 minutes just prior to *i.v.* infusion of 20 mg/kg ACZ (Sigma). Subsequent scans were performed at 5, 15, 25, 40, and 60 minutes post-ACZ infusion to assess the temporal course of the hemodynamic response. Cerebrovascular reserve was calculated as the percentage-increase in CBF at 25 minutes after the administration of ACZ over basal levels.

4.2.2.2 Acetazolamide and CTP Reproducibility

The previous procedure was repeated in ten rabbits one week later to assess the reproducibility of CTP measurements and the hemodynamic response to ACZ (scanning protocol was truncated to 25 minutes post-ACZ infusion). Two basal measurements were made on each testing day to determine intra- and inter-day variability of CTP measurement. Interday variability of the blood flow response to ACZ was also assessed between testing days.

4.2.2.3 Intracranial Pressure with Varying MAP

Variations in intracranial pressure (ICP) with arterial pressure were investigated. ICP was measured directly via an intraventricular cannula (IVC) in a separate group of rabbits ($n = 8$) that underwent both normotensive and hypotensive

conditions on separate testing days. For chronic implantation of the IVC, anesthesia was induced with 3-5% isoflurane and the scalp was prepared for surgery with a subcutaneous injection of 0.25% bupivacaine (Sensocaine). The central ear vein was then cannulated for constant infusion of a mixture of ketamine (20mg/kg/hr) and diazepam (2.0mg/kg/hr), while isoflurane was turned off for the remainder of the procedure. The scalp over the superior aspect of the skull was excised to expose an area between the coronal sutures, bregma and lambda. A guide cannula (C313G/FS, Plastics One, Roanoke, Virginia) was stereotaxically (Stoelting Co., Wood Dale, Illinois) implanted into the right lateral ventricle of the brain and secured with 3 nylon screws and cyanoacrylate adhesive. For continuous monitoring of ICP an internal cannula (CS313/FS), coupled to a pressure transducer, was fitted to the guide cannula.

4.2.2.4 Hemodynamic Measurements with Varying MAP

In a separate study, rabbits were divided into three groups: normotensive (n = 14), mild hypotensive (n = 6), and moderate hypotensive (n = 9) to investigate the relationship between the hemodynamic parameters: CBF, CBV and MTT and CPP over a range of mean arterial pressures (MAP). Mild and moderate hypotension was induced by an intravenous infusion of prostaglandin E₁ (PGE₁, Alprostadiol, 500 µg/ml) at 1.5 µg/kg/min and 3.0 µg/kg/min, respectively. All groups of rabbits received two basal CTP scans. Both groups of hypotensive rabbits then received their respective hypotensive treatment, followed by a basal hypotensive CTP scan once a steady state MAP was reached. ACZ treatment was given to normotensive and hypotensive animals 5 minutes post basal and basal hypotensive CTP scans, respectively. All groups were then scanned

at 5, 15, 25, 40, and 60 minutes post ACZ. The temporal scanning protocol is given in Figure 4.1.

4.2.3 CT Imaging

All imaging was conducted on a LightSpeed Plus 4-slice CT scanner (GE Healthcare, Waukesha, Wisconsin). Rabbits were placed in the prone position on a custom-made jig and a routine scout was used to localize a 2-cm slab that maximized cortical grey matter volume. The selected slab was used for all subsequent CTP scans in the experiment. The CTP scanning protocol consisted of a continuous 32-second scan using 80 kVp, 80 mA and 1-sec rotation speed while the couch remained stationary. One hundred twenty-five 512 x 512 images were reconstructed with the detail algorithm (reconstruction filter) and a 10 cm field-of-view at 0.25 s intervals for each of the four 5 mm thick slice locations. The limiting resolution of the detail algorithm is ~10 lp/mm or 0.5 mm. Upon commencement of the scan, a bolus injection of 5 ml of contrast agent (iohexol [Ominpaque], 300 mgI/mL; GE Healthcare, Piscataway, NJ) was given at a rate of 0.5 ml/s into the femoral vein with an automatic injector (Medrad, Indianola, Pa).

CT Perfusion calculations of parametric maps are theoretically based on the central volume principle relating CBF to the ratio of CBV to MTT (10). Within a given network of brain capillaries, CBF is the rate of flowing blood per unit time and mass of tissue, CBV is the volume of (flowing) blood in that capillary network per unit mass of tissue and MTT is the average time for the blood to traverse the differential vascular path lengths of the given network. To calculate these parameters from data acquired by CT scanning, blood is rendered detectable by the CT scanner with the injection of

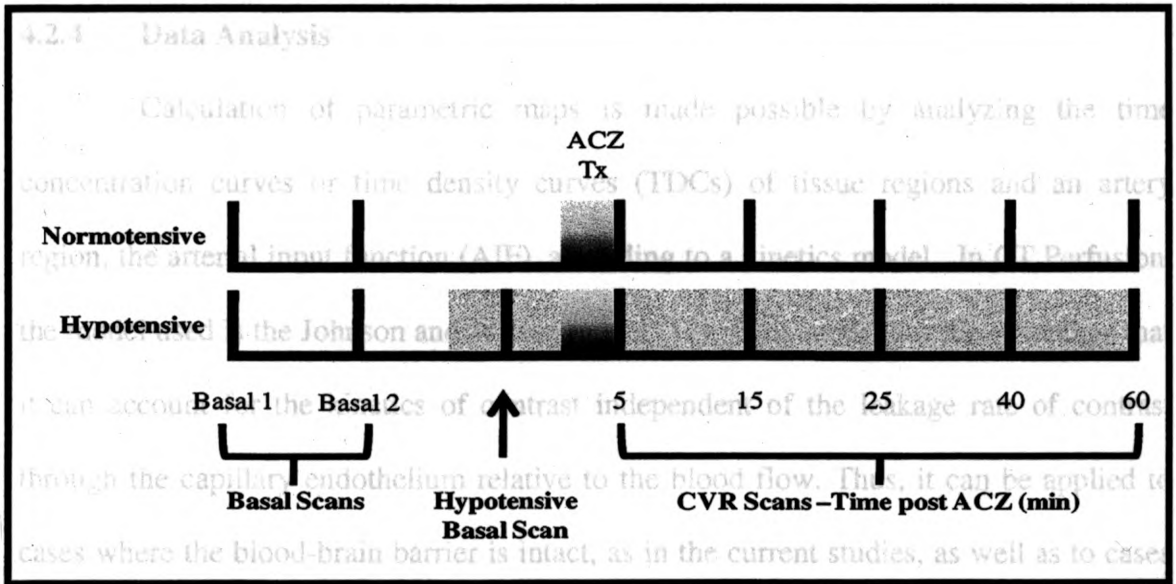


Figure 4.1: CT Perfusion Scanning Protocol. All groups of animals received 2 basal scans to measure normal cerebral hemodynamics. After basal scanning was completed, normotensive animals continued with ACZ administration (ACZ Tx) and CVR scans while hypotensive animals received hypotensive treatment (□) and a basal hypotensive scan before ACZ Tx and subsequent CVR scans. Hypotensive treatment lasted the duration of CVR testing for these animals.

contrast agent as previously discussed. Furthermore, the calculation relies on the assumptions that the contrast concentration in the blood is proportional to the increase in attenuation of x-rays by blood and that the contrast has the same hemodynamic properties of blood. To calculate perfusion, the deposition (concentration) of the contrast within individual regions of the brain, is measured as increases in CT number (also known as Hounsfield Units - HU) by the CT scanner. The kinetics of the tracer (contrast), that is, the time course of the concentration of contrast in a region, is captured by the CT scanner image. An average map was also constructed by averaging all the CT images of a slice together. Parametric maps were analyzed using a region of interest analysis program previously described in Chapter 1 (Section 1.3.3). The raw data set is transferred to a GE workstation (GE Healthcare) for calculation of parametric maps using proprietary software (CT Perfusion, GE Healthcare).

4.2.4 Data Analysis

Calculation of parametric maps is made possible by analyzing the time concentration curves or time density curves (TDCs) of tissue regions and an artery region, the arterial input function (AIF), according to a kinetics model. In CT Perfusion, the model used is the Johnson and Wilson model (12). This model has the advantage that it can account for the kinetics of contrast independent of the leakage rate of contrast through the capillary endothelium relative to the blood flow. Thus, it can be applied to cases where the blood-brain barrier is intact, as in the current studies, as well as to cases where the BBB is disrupted, as in brain tumors. In each of the four slices scanned by the CT scanner, a 2 X 2 pixel region was defined in the internal carotid artery to obtain the corresponding arterial TDC. Among the four arterial TDCs generated, the one with the earliest arrival of contrast was used as the arterial input function (AIF). The AIF was then deconvolved with all tissue TDC according to the Johnson and Wilson model to determine the blood flow-scaled impulse residue function (IRF) for individual tissue regions in the brain. It follows that, as discussed in Chapter 1 Section 3.3, the height of the IRF is equal to the CBF, while the area under the IRF is the CBV. Additionally, given the relationship of $MTT = CBV/CBF$ from the central volume principle, MTT can be calculated. Parametric maps of CBF, CBV and MTT were constructed by assembling the corresponding values of each parameter for all tissue regions in a brain slice into an image. An average map was also constructed by averaging all the CT images of a slice together. Parametric maps were analyzed using a region of interest analysis program developed in our lab using IDL (Research Systems, Boulder, Colorado). Large regions of interest (ROI) were hand drawn over the entire cortical grey matter of all four slices with

the help of the corresponding average map and were automatically propagated onto CBF and CBV parametric maps. To reduce the influence of large blood vessels on the average value of CBF and CBV in the defined grey matter ROIs, any vascular pixel with $CBV > 8.0 \text{ mL}/100 \text{ g}$ or $CBF > 250 \text{ mL}/100 \text{ g}/\text{min}$ within a ROI was eliminated from the calculation of the mean (13, 14). Average mean transit time of a ROI was calculated as the ratio of the corresponding ROI average of CBV to CBF.

4.2.5 Statistical Analysis

All statistical analyses were performed using SPSS for Windows (Version 15.0, SPSS, Chicago, Illinois). Determination of significant changes in CBF for the ACZ timecourse was calculated using paired T-tests (Bonferroni-corrected) for each time point compared with the basal CBF measurement. A repeated-measures analysis of variance design was employed to determine the reproducibility of CT Perfusion measurements as well as CVR and was assessed using the coefficient of variation (CV). The relationships between CBF, CBV, MTT and CVR with MAP were assessed using a non-parametric Spearman's rank correlation, as it does not depend on assumptions of linearity. Significant differences for all analyses were defined by $p < 0.05$.

4.3 RESULTS

4.3.1 Acetazolamide Time Course and CTP Reproducibility

The time course of the CBF-response to ACZ is given in Figure 4.2. A significant increase in CBF was observed immediately following injection of ACZ and was sustained until 60 min post-injection. There was no significant difference between

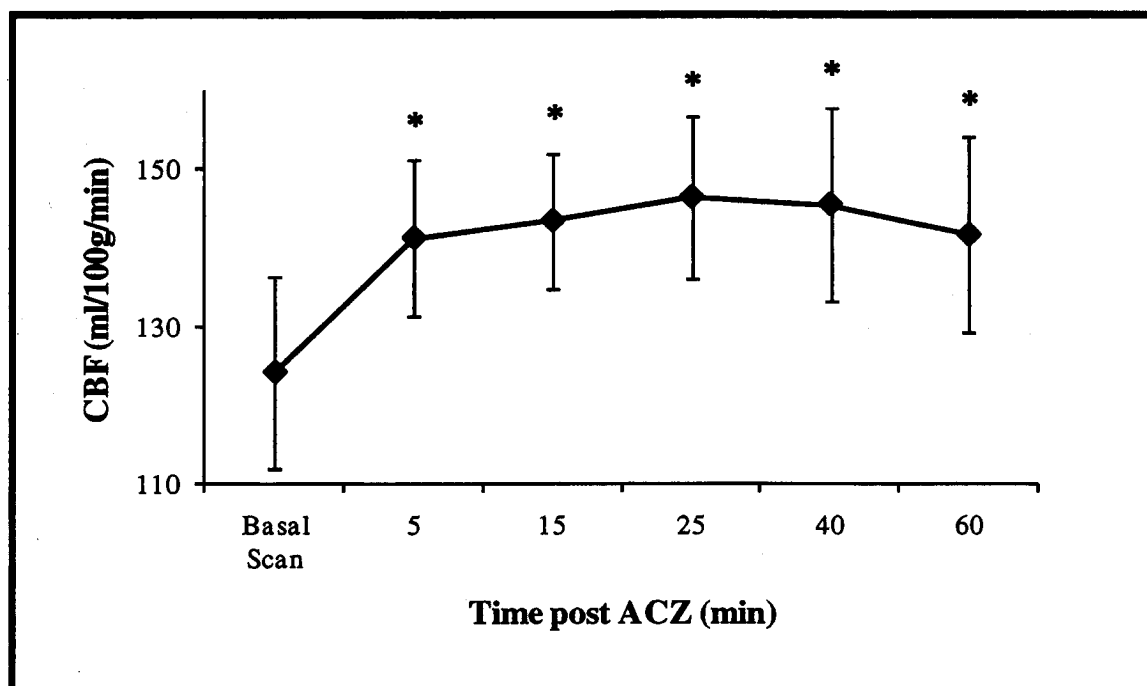


Figure 4.2: Graph of CBF Response to ACZ in Control Animals. Graph of the CBF (mean \pm SD) response to ACZ (20 μ g/kg) for a group of animals ($n = 15$) under normotensive conditions. CBF at all time points post ACZ are statistically similar to each other and are significantly greater than basal levels (* $P < 0.05$)

any time point between 5 and 60 minutes post ACZ. The 25-min time point had the greatest increase from the basal CBF (21.3%) and thus, was used for all calculations of CVR. Within the same testing day (intra-day), the two basal CBF, CBV, and MTT measurements acquired prior to ACZ injection were not significantly different from each other, with a CV of 9.0%, 5.6%, and 7.6%, respectively. The effect of scan day (inter-day) was also found to be non-significant for basal CBF, CBV, MTT and ACZ response measurements with a CV of 16.6%, 10.6%, 17.4% and 8.9%, respectively. Measurements of basal CBF and its response to ACZ for inter- and intra-testing days are shown in Figure 4.3.

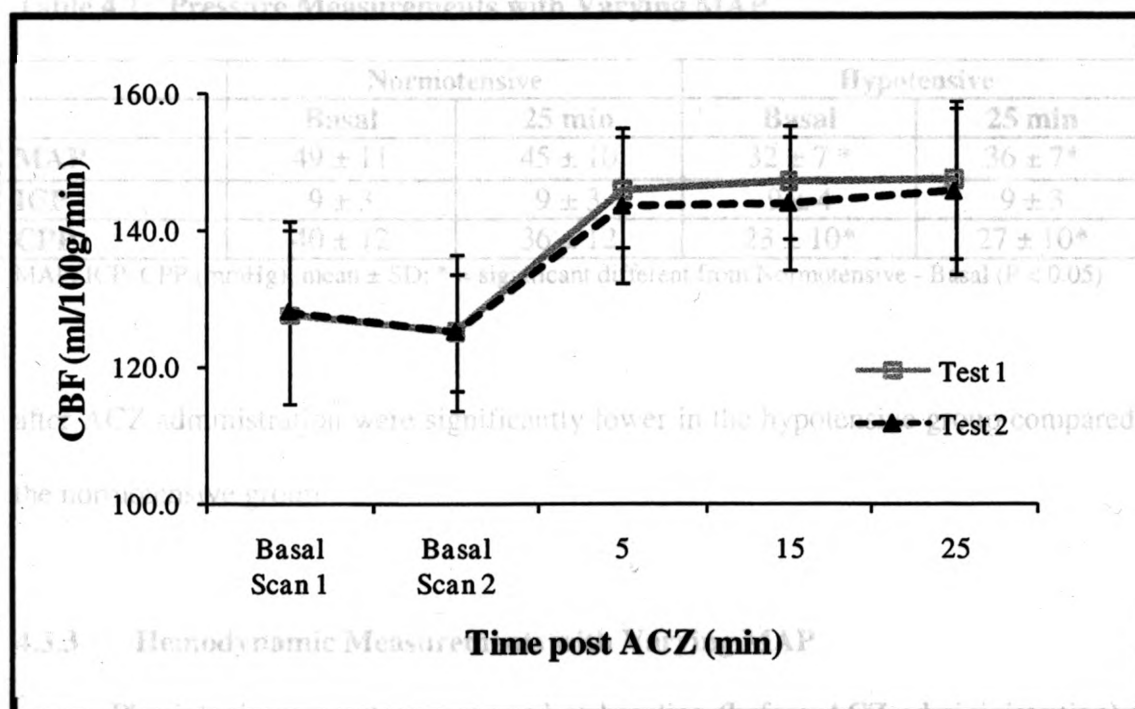


Figure 4.3: Reproducibility Graph of Intra- and Inter-Test Day Variability in CBF and CBF Response to ACZ. Reproducibility analysis of intra- and inter-test CTP measurements of CBF (basal scans) and inter-test CBF response to ACZ (5, 15, 25-mins following ACZ administration). Test 1 and 2 were separated by 7 days. Intra-Test basal CBF measurements were not significantly different and had a CV of 9.0%. Inter-Test measurements were also non-significant for Basal and post ACZ time points with a CV of 16.6% and 8.9%, respectively.

4.3.2 Intracranial Pressure with Varying MAP

Intracranial pressure (ICP), MAP and CPP measurements made at baseline and 25-min post-ACZ for both MAP states are displayed in Table 4.1. ICP remained steady at 9 ± 3 mmHg (mean \pm SD) both before and after ACZ administration and there were no significant differences between normotensive and hypotensive states. Since MAP did not influence ICP, as evidenced by no ICP changes between normotensive and hypotensive states, MAP can be used as a surrogate for global CPP. MAP and CPP both before and

Table 4.1: Pressure Measurements with Varying MAP.

	Normotensive		Hypotensive	
	Basal	25 min	Basal	25 min
MAP	49 ± 11	45 ± 10	32 ± 7 *	36 ± 7*
ICP	9 ± 3	9 ± 3	9 ± 4	9 ± 3
CPP	40 ± 12	36 ± 12	23 ± 10*	27 ± 10*

MAP, ICP, CPP (mmHg); mean ± SD; * = significant different from Normotensive - Basal (P < 0.05)

after ACZ administration were significantly lower in the hypotensive group compared to the normotensive group.

4.3.3 Hemodynamic Measurements with Varying MAP

Physiologic parameters measured at baseline (before ACZ administration) for normo and hypotensive groups are summarized in Table 4.2. There were no significant differences between the parameters of each group. Significant differences after ACZ injection between groups were only reported for end-tidal CO₂, with a reduction in the moderate (35 ± 1 mmHg) group when compared with the normotensive group (36 ± 1 mmHg) (data not shown). The hemodynamic measurements from CTP scans are presented in Table 4.3 for normo and hypotensive groups. At baseline, CBV was not significantly different between groups. Basal CBF and MTT were statistically similar between both mild and moderate hypotensive groups but CBF was significantly reduced (~15%) while MTT was significantly elevated (~16%) in comparison to the normotensive group. Administration of ACZ did not significantly alter the MAP of any group with respect to their respective basal measurement. The difference between CVR of each group was not statistically significant (range; 16.3-22.0%). Similarly, there were no significant differences in the percentage or absolute increase of CBV in response to ACZ

Table 4.2: Basal Physiological Measurements with Varying MAP.

	Normotension	Hypotension	
		Mild	Moderate
MAP (mmHg)	53 ± 12	35 ± 3*	28 ± 6*
HR (min ⁻¹)	273 ± 33	259 ± 34	222 ± 31
Temp (°C)	39 ± 1	39 ± 1	39 ± 1
Ht (%)	34 ± 4	32 ± 2	31 ± 2
EtCO ₂ (mmHg)	40 ± 1	40 ± 2	39 ± 1
p _a O ₂ (mmHg)	314.9 ± 31.5	299.2 ± 55.3	288.9 ± 31.2
p _a CO ₂ (mmHg)	41.8 ± 3.4	41.3 ± 3.3	43.2 ± 3.7
HCO ₃ (mmol/L)	25.7 ± 1.8	25.8 ± 3.6	26.0 ± 2.4
pH	7.42 ± 0.03	7.42 ± 0.05	7.40 ± 0.04
Glu (mmol/L)	6.3 ± 1.5	6.4 ± 1.8	6.9 ± 2.7

MAP = mean arterial pressure; HR = heart rate; Temp = temperature; Ht = hematocrit; EtCO₂ = End tidal CO₂; p_aO₂ = arterial oxygen; p_aCO₂ = arterial carbon dioxide; HCO₃ = bicarbonate; Glu = glucose; mean ± SD; * = P < 0.05 compared with Normotension.

Table 4.3: Hemodynamic Parametric Measurements with Varying MAP.

	Normotension		Hypotension			
			Mild		Moderate	
	Basal	25 min	Basal	25 min	Basal	25 min
MAP	53 ± 12	49 ± 13	35 ± 3 **	38 ± 3*	28 ± 6 **	33 ± 7**
CBF	126 ± 11	149 ± 10**	107 ± 7**	125 ± 5	108 ± 7 **	131 ± 9
CBV	2.17 ± 0.21	2.47 ± 0.19*	2.17 ± 0.16	2.47 ± 0.16*	2.17 ± 0.21	2.44 ± 0.18*
MTT	1.04 ± 0.08	1.02 ± 0.08	1.21 ± 0.03 **	1.19 ± 0.09*	1.21 ± 0.15 **	1.12 ± 0.11
CVR		16.3 ± 9.0		16.7 ± 8.6		22.0 ± 14.4

MAP = mean arterial pressure (mmHg); CBF = cerebral blood flow (ml/100g/min); CBV = cerebral blood volume (ml/100g); MTT = mean transit time (sec); CVR = cerebrovascular reserve (% increase above own group basal measurement), * = P < 0.05, ** = P < 0.01 compared with normotensive basal levels

between groups (range; 12.4-13.8%). Figure 4.4 displays the correlations between all hemodynamic data and MAP. Both MTT and CBF demonstrated statistically significant (P < 0.005) Spearman's rank correlation coefficients of $\rho = -0.642$ and 0.575 , respectively. Correlations of CBV and CVR with MAP were not significant over the range of pressures tested.

4.4 DISCUSSION

Over the range of MAP tested in this study, it was shown that MTT provided a significant correlate for MAP, the main physiological component comprising CPP. Furthermore, changes in MAP did not significantly alter ICP. By extension, since ICP was constant, MTT is also significantly correlated to changes in CPP. These results indicate that basal levels of MTT may be useful for determining regions of compromised CPP.

Our study employed global changes in CPP by manipulating MAP pharmacologically. Two dosages of PGE₁ were used to create varying levels of CPP in groups defined as mild and moderate hypotension. Both hypotensive groups had significantly lower MAP from the normotensive group but were not significantly different from each other, potentially due to the heterogeneous response of individual animals to the two dosages of PGE₁. PGE₁ and its dosage were carefully selected to ensure that they did not confound the results with unexpected changes in cerebrovascular tone, thereby modifying basal levels and the relationship between CBV and CBF. Dosages of ≤ 3.0 $\mu\text{g}/\text{kg}/\text{min}$ of PGE₁ in rabbits are suitable for creating hypotension without significant changes in the diameter of cerebral arterioles and venules (15, 16).

Furthermore, PGE₁ has been confirmed in both rabbits (15, 16) and in humans (17, 18) to maintain local CBF at rest and not to impair CVR to hypercapnia. Thus, PGE₁ is a suitable hypotensive drug for lowering MAP and testing CVR without affecting cerebral hemodynamic parameters measured in this study.

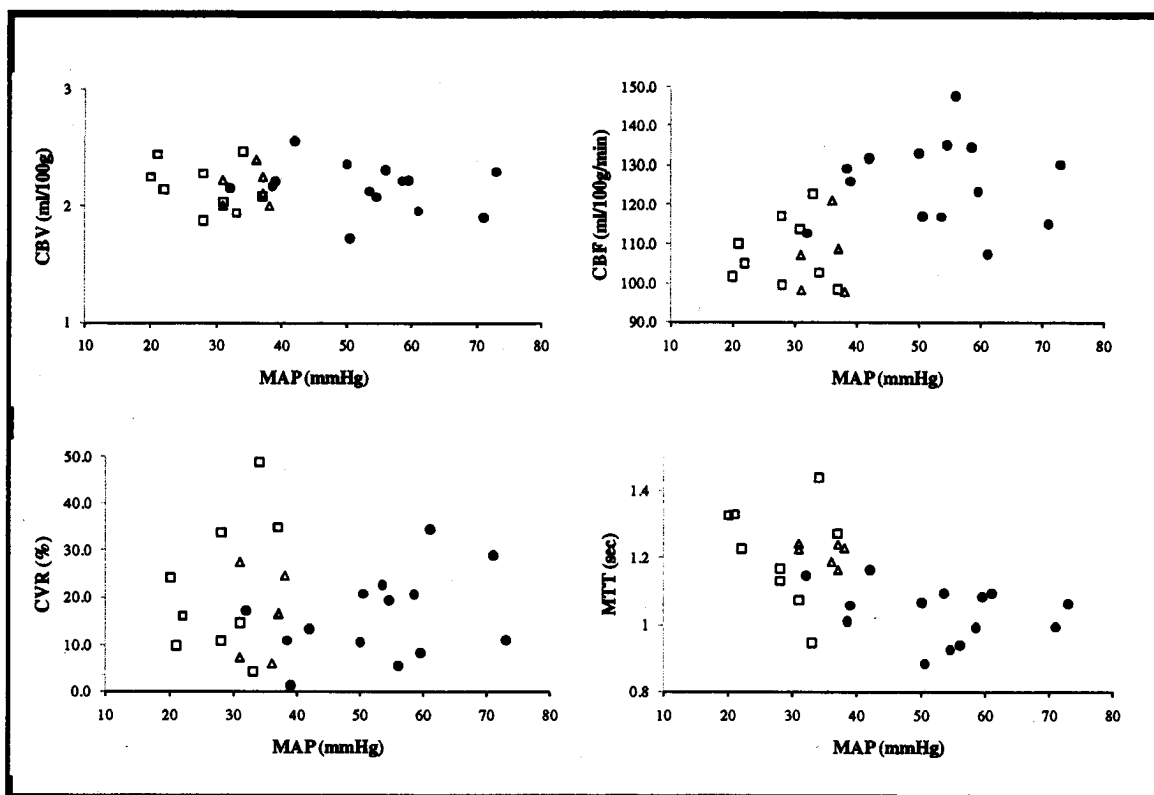


Figure 4.4: Scatter Plots of CBV, CBF, MTT and CVR Against MAP. MTT and CBF produce significant ($P < 0.005$) correlations ($\rho = -0.642$ and 0.575 , respectively) with MAP when normotensive (closed circles), mild (open triangles) and moderate (open squares) hypotensive groups were analyzed together to provide a spectrum of MAPs.

At the first CTP measurement 5-min after administration of ACZ, CBF was significantly increased above the corresponding baseline value and remained significantly elevated until 60-min post in agreement with other experimental data (6). We standardized the measurement of CVR at 25-min post ACZ because that time yielded the greatest increase in CBF (Figure 4.2) and is in close agreement with that used in clinical studies (19). The mean CVR at 25-min post ACZ was 21.3% in a group of control animals; this was less than those measured with a dose that incites a maximal dilation in other experimental designs (6, 20). Furthermore, this study assessed the biological reproducibility of the response to ACZ, which is important in the evaluation of the

efficacy of bypass surgery or endovascular treatment in the clinical setting, or when using a repeated measures experimental design. The coefficient of variation of CBF response to ACZ for individual rabbits was 9.8%. Although our CVR response was lower than expected, the variability in the response is less than the magnitude of response (21 %) and is deemed adequate for repeated studies. ACZ was chosen to test CVR as it is commonly used in the clinical setting and is well characterized in the literature. Alternatively, breathing an increased content of carbon dioxide (CO₂) in air is another method that is used to test CVR (21). A recent study compared the cerebrovascular response of both methods in an animal model and found that both CO₂ and ACZ produced similar results for CVR in all regions of the brain. Moreover, they were significantly correlated ($r=0.93$) with each other, with a regression line not significantly different from the line of identity (20). This data is corroborated by two other studies indicating good correlation between the two methods and thus validate the usage of ACZ in our studies (22, 23).

Global measurement of CPP is defined as the difference between the MAP and the ICP (24), assuming that the venous backpressure is negligible (1). In a previous study, global CPP was estimated to be a function of MAP, as negligible changes in CBV during profound hypotension should not influence changes in ICP (24). To validate this assumption, we measured ICP in a group of normotensive animals followed one week later by hypotensive treatment to ensure that changes in MAP did not alter ICP over the range of MAP to be tested. ICP's, steady in the range of expected values, were not different between both levels of MAP as previously reported in the literature (15, 25). As such, we deemed that further investigation of ICP was not warranted and that all associations between hemodynamic parameters and MAP also apply to CPP.

Measurements of cerebral hemodynamic parameters are used as indicators of cerebrovascular status to determine areas of the brain with altered CPP. During autoregulation, CBF and OEF are maintained within a fairly constant. Constancy of CBF and OEF during decreasing CPP throughout the autoregulatory plateau is largely attributed to the decrease in cerebrovascular resistance facilitated by the dilation of resistance vessels (2). Recently, the relationship between these parameters and CPP has been revised to acknowledge gradual changes occurring throughout the range of autoregulation (26). The authors found that small increases in OEF were present during autoregulation and occurred in the absence of measurable changes in CBV. Furthermore, in the revised autoregulatory model, the authors acknowledged slight reductions in CBF with decreasing CPP as demonstrated in previous work (27). Additionally, the authors pointed out that the autoregulatory mechanism for bringing about these changes, CBV, has recently been a point of controversy. Measurement of compensatory changes in CBV due to decreased CPP within the autoregulatory range have had mixed results; classical views have described an increase in CBV during decreasing CPP (8, 28), while more recently CBV has been demonstrated to stay relatively constant with any changes being insignificant (24, 29). Schumann et al (1998) described significant increases outside the lower limit of autoregulation, with subtle but insignificant differences within the autoregulatory plateau in a PET study of experimental hypotension in baboons (24). The authors found that decreases in MAP during profound hypotension (53%) caused a significant increase in CBV, while moderate reductions in hypotension (43%) produced insignificant CBV changes. However, it is noteworthy to mention that average CBV increase in both groups had a similar magnitude, but only the profound group reached

significance, which may signify variability of the CBV response within the autoregulatory range. Furthermore, measurement of CBV is inherently difficult due to the morphological composition of components comprising this parameter. CBV is a complex summation of arterial, capillary, and venule compartments in addition to parenchymal and pial components while current methods only measure total CBV rather than its individual compartments/components. In addition to the inaccessibility of the individual compartments/components of CBV, the vasodilatory response to decreased perfusion pressure is variable within these different compartments (29). Our study described similar reductions in MAP in minor and moderate hypotension groups, 34% and 47%, respectively, with no significant changes in CBV when compared to the normotension group. Interestingly, similar to Schumann et al. (1998), we reported significant decreases in CBF accompanying the minor and moderate hypotension groups in the absence of any changes in CBV. Discrepancy of documented change in CBV may be a result of experimental design. Insignificant changes in CBV may be due to the variability of CBV response, difficulty in accurately measuring CBV, or an indication of a pre-dilated state, the latter of which would be associated with a reduction or abolishment of a response to a vasodilatory stimulus.

The reactivity of blood vessels to a vasodilatory stimulus indicates the cerebrovascular status and is reflected by changes in CBV and CBF. Our study found no differences among the three groups (normotensive and both hypotensive groups) with respect to basal CBV and increases in CBV measured at 25-mins post ACZ, which resulted in statistically similar increases in CBF. The response to ACZ was much less than expected and was equivalent in all groups. The constancy in basal CBV among the

three groups, as well as similar magnitude of change in response to the administration of ACZ, may well reflect a predilatory state, as differences would be expected between the normotensive and hypotensive states. One limitation of the current study was the use of isoflurane to maintain anaesthesia for the duration of the study. Animals were maintained at 2.75% as required by the Canadian Council on Animal Care and the Animal Use Subcommittee at the University of Western Ontario to ensure an adequate plane of anaesthesia. Isoflurane is a peripheral and cerebral vasodilator associated with decreases in MAP and small increases in ICP (18) and thus may account for lower basal levels of MAP than expected in the rabbit (16). Furthermore, isoflurane has a dose dependent degree of dilation of pial arteries and arterioles (30). At low levels of isoflurane (1.4%) cerebrovascular reserve to changes in arterial CO₂ are maintained, whereas high levels of isoflurane (2.8%), similar to that of the current study, have been shown to attenuate such responses in a canine model (31). Thus, the similarity of CBF response to ACZ in all three groups may indicate existing vasodilation from isoflurane, thus masking differences caused by hypotensive treatment, thereby explaining the similar values of basal levels in all groups.

Autoregulation, by definition, is dependent on MAP. MAP measurement, compared to previous studies in rabbits investigating the lower limit of autoregulation at MAP of 40 mmHg or lower (32, 33), indicated that the normotensive group was approaching the lower limits of autoregulation and the hypotensive groups were below the lower limit of autoregulation. In addition to the low MAP, constant CBV and CVR across all pressures tested were not expected. It would follow that isoflurane may have

caused a dilation that was near the physiological maximum, such that further lowering of MAP was unable to cause additional dilation.

The current study has provided a model for global reductions in CPP causing vasodilation near the maximum physiological dilation of vessels. The inability of reduced perfusion pressure to cause further vasodilation indicates that these areas may be within Stage II of hemodynamic failure. These areas experience "misery perfusion" and are at the highest risk of ischemic incidents as compensatory mechanisms near exhaustion. Clinically, these are considered the border zones of chronic hypoperfusion and may be caused by large artery occlusions, often identified by testing the CVR. The results of this study indicate that MTT may be a more sensitive marker to changes in CPP under these conditions. In the current study we found that CVR had no significant association with CPP, as evidenced by a correlation coefficient (ρ) of only 0.024. Testing was performed in anaesthetized animals with near maximal cerebral vasodilation in all animal groups, as a result CVR was unable to differentiate between different MAP groups. Thus under the experimental conditions used in our studies, CVR was not related to CPP, and could not reveal hemodynamic compromise. Even though isoflurane may not be ideal due to its cerebrovascular effects, it has allowed us to examine global or large regional increases in CBV with concomitant decreases in CPP, as may be seen in large hemispheric occlusions or cerebral edema. MTT was capable of describing a relationship with CPP ($\rho = -0.642$) based on its inherent characteristics that account for changes in both CBF and CBV. Thus, MTT is capable of acting as a surrogate for cerebral hemodynamic compromise in response to decreased CPP. Further study is

needed to investigate the relationship between CVR and MTT with CPP under conditions that more closely mimic the awake state without anaesthetic induced vasodilation.

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CHAPTER 5

CONCLUSIONS AND FUTURE WORK

5.1 SUMMARY

The introduction of this thesis provided an overview of the clinical characteristics of motor neuron disease, in particular ALS and PLS. Furthermore, a detailed account of past imaging studies provided a background on perfusion and metabolic deficits related to the disease, with a particular emphasis on changes accompanying cognitive decline. Few mechanisms have been advanced in the literature to facilitate a mechanistic understanding of perfusion deficits occurring in ALS. Chapter 1 reviewed the regulation of cerebral perfusion at the structural and cellular level and also included compensatory mechanisms responsible for maintaining adequate perfusion. In doing so, it provided a framework to discuss the implications of our findings in the context of the known pathology present in the disease. Collectively, the objective of this thesis was to prospectively measure cerebral perfusion parameters to investigate an association with cognitive decline and unveil a possible biomarker for monitoring disease progression.

The conclusion of this thesis will review the outcome of the three research papers and incorporate their findings and implications into the current clinical view of the disease. Furthermore, this Chapter will discuss the collective findings of this thesis and highlight future areas of research. Although largely successful, the possible limitations of the research contained within this thesis will also be discussed.

5.2 PERFUSION CHANGES WITH COGNITIVE IMPAIRMENT

Chapter 2 prospectively studied cognition and cerebral perfusion in PLS. Past studies of cognition in MND have been hampered by insensitive neuropsychological testing paradigms that prevented accurate stratification of patient cohorts based on cognitive profile, with resultant groups coarsely stratified by the presence or absence of overt dementia. Chapter 2 also describes a more accurate method of patient stratification based on neuropsychological tests specific to frontotemporal dysfunction characteristic of MND. Once stratified into PLS and PLSci, analysis of hemodynamic parameters in the latter grouping showed significant global increases in MTT, with the exception of the temporal lobe. Additionally, the frontotemporal region, an area shown to be most affected by cognitive testing, also exhibited significantly increased CBV. The outcome of this study represented the first time that a prospective analysis produced an association between hemodynamic measures and cognitive decline in PLS.

The use of a single neuropsychological test for cognitive dysfunction that correlates with cerebral perfusion deficits would be convenient for monitoring disease progression, and possibly eliminate the need to employ a full neuropsychological test battery. Previous MND studies have indicated that Verbal Word Fluency is the most sensitive indicator of executive dysfunction in dementia and is significantly correlated with cerebral perfusion deficits (1). Thus, we sought to determine if a similar relationship existed for less advanced cognitive dysfunction present in our study. Consistent with previous studies of dementia in MND, we found that Verbal Word Fluency was the most frequently impaired test in our patient cohort, although no correlation between it and any cerebral hemodynamic parameters was present. Even though no significant correlations

were revealed, we do not feel that this result distracts from the reliability of the relationship between cognitive impairment and hemodynamics found in this study based on the following rationale. First, cognitive changes in this study were subtle with only the minimum criterion being achieved in the PLSci patients. Second, impairment criteria in this study are based on the combination of at least two abnormal test scores. Thus, determining a correlation with only one neuropsychological test in mild cognitive impairment may prove difficult, whereas a correlation with the total number of abnormal tests may be more reliable. To defend this rationale and our findings, we performed a correlation analysis between the total number of abnormal neuropsychological test scores and MTT for all regions. We found a significant correlation between the degree of cognitive impairment and MTT in all regions tested. Thus, in contrast to dementia in ALS, for less extensive cognitive dysfunction found in our study it was not possible to determine a single neuropsychological test that was associated with perfusion deficits.

5.3 WIDESPREAD PERFUSION CHANGES OCCUR EARLY IN DISEASE

In addition to the findings associated with cognitive impairment, Chapter 2 also described our finding of global trends of increased MTT in the cognitively intact patient group. Uncertain whether these changes were indicative of future impairment or the duration of disease at the time of study (36.5 ± 47.4 months), we longitudinally examined a group of cognitively intact ALS patients from disease onset (11.8 ± 19.5 months). Results of this study (Chapter 3) described similar hemodynamic patterns between both normal controls and ALS patients at study onset and T1 (6 months post T0). At the last time point in the study, T2 (17 months post T0), trends of perfusion measurements

observed at earlier time points in the ALS patient group had become significant, in particular, MTT increased in all cortical and thalamic regions compared to controls. These findings demonstrated that perfusion deficits occur early in disease in the absence of cognitive impairment. Only two patients became cognitively impaired during the study period, thus, this study lacked statistical power necessary to determine if any perfusion parameters can be an early indicator of progression to cognitive impairment. To our knowledge this was the first time that a prospective, longitudinal study of ALS incorporated neuropsychological testing and perfusion measurements on the same day. Furthermore, this study found widespread grey and white matter changes in perfusion, involving both motor and non-motor areas, in the absence of any cognitive deficits.

5.4 IMPLICATIONS OF INCREASED MTT

Findings from both Chapters 2 and 3 indicate that cerebral MTT is affected globally in PLS and ALS, both in the presence and absence of cognitive impairment. This is a novel finding for MND, and to our knowledge has not been associated with cognitive impairment in other neurodegenerative diseases. Using an animal model, Chapter 4 explored the relationship between cerebral MTT and cerebral perfusion pressure (CPP).

We created a model of graded hypotension to test the hypothesis that global changes in MTT of the cerebral circulation were related to changes in CPP. While, MTT has been investigated as a marker of CPP in both clinical (2, 3) and experimental (4, 5) studies, it has not been compared against cerebral vascular reserve (CVR), the clinical gold standard for assessing changes in cerebral hemodynamics induced by perturbations

in CPP. This study validated the assumption that global CPP could be approximated by the MAP, as evidenced by constant ICP in the face of moderate decreases in systemic pressure. Furthermore, this study found that MTT ($\rho = -0.642$) and CBF ($\rho = 0.575$) were significantly correlated ($P < 0.005$) with CPP over the range of pressures studied, while CVR did not provide a significant correlation with CPP ($\rho = 0.024$). Thus, MTT provides a non-invasive index of CPP, and may assist in defining areas of the brain at risk of hypoperfusion due to decreased CPP.

Our findings concerning CVR are not consistent with literature and thus deserve some explanation. A limitation of this study was the choice of isoflurane as the anesthetic, as it is a known cerebrovascular dilator (6) that blunts the cerebrovascular response to a vasodilatory stimulus (7). The use of isoflurane suppressed the MAP before the study onset, causing a shift towards the lower limit of autoregulation, reducing the range for study. However, in doing so it has provided a model of near maximal dilation to study the hemodynamic effects of further decreasing CPP.

5.5 EXPERIMENTAL & CLINICAL RELEVANCE

While past studies of ALS and PLS have focused primarily on motor disability and the associated cerebral areas, this thesis has contributed to the mounting evidence of involvement of non-motor areas in the brain. Findings from the PLS study described cognitive change associated with widespread perfusion deficits, most notably in the frontotemporal region, also the area implicated by the cognitive abnormality. The pattern of executive dysfunction and associated perfusion change are characteristic of those described in ALS but less pronounced, lending support to the argument that the two

diseases may share a common pathogenetic pathway. Understanding whether these two diseases are part of the same spectrum of disease processes is paramount, as the prognosis for ALS is much more morbid with a more rapidly progressing disease course than PLS.

Biomarkers to monitor disease progression of PLS and ALS are necessary to determine efficacy of treatment in future clinical trials. The study presented in Chapter 3 focused on recruiting cognitively intact patients near the time of diagnosis to determine if baseline perfusion measurements differed from normal controls, then longitudinally followed these groups to assess cognition and perfusion changes. This study was unique in that it attempted to determine the temporal association between cognition and cerebral perfusion. However, fewer patients became cognitively impaired than expected, with only two reaching the minimum criteria for this designation. These findings, while keeping in mind the exclusion criteria of cognitive impairment at study onset, indicate that cognitive decline may precede clinical presentation of ALS. Furthermore, this study provided evidence for evolution of perfusion deficits that were not present at onset over the time course of the study in the absence of cognitive impairment. Measurements of MTT provide a more sensitive indicator than measurements of CBF or CBV alone, and may provide a means to monitor disease progression.

Lastly, the results of this thesis may indicate that changes in MTT present in our clinical studies are associated with endothelial dysfunction. This postulation was elucidated using an animal model of graded hypotension where MTT was significantly associated with changes in CPP. Furthermore, this study described that near the lower limit of autoregulation, where risk of hypoperfusion is a primary concern, that CVR did not provide an accurate indicator of CPP. Identifying areas of the brain at risk of chronic hypoperfusion is important if intervention is required to prevent ischemia. Furthermore,

CVR was highly variable across the range of pressures tested and not different across all groups. Since CVR is an individualized approach to assess risk of stroke and is the clinical gold standard, it should be predictive across the full range of autoregulation, thus, more work should be done to validate the insensitivity of CVR to CPP drops at the lower limit of autoregulation. Most importantly, this study determined that MTT provides an index of CPP from basal measurements without necessitating two perfusion measurements and a pharmacological dilatory stimulus.

5.6 FUTURE WORK

The work contained within this thesis has provided a better understanding of the clinical, physiological and functional phenotype of MND. It has provided a novel marker of disturbed cerebral hemodynamics in disease progression, which is associated with subtle cognitive change. However, many questions remain. This last section will discuss possible research directions that may help resolve some of the unanswered queries.

5.6.1 Multimodality imaging

The study of cerebral perfusion/hemodynamics in this thesis was limited by the ability to distinguish a pathophysiological mechanism for the documented changes. The use of multiple imaging techniques, such as PET, SPECT, CTP and MRI, would be beneficial to help co-localize regions of functional, metabolic, neurochemical and perfusion change. Recently, the PET radioligand [^{11}C]-flumazenil was used to detect reductions in interneuron population in ALS (8). Based on the literature reviewed in Chapter 1, interneurons abut against astrocytic endfeet and vessel walls to influence

cerebrovascular tone. Thus, using an imaging technique capable of defining areas of the brain with reduced interneuron populations in conjunction with perfusion imaging would help elucidate the possible mechanism behind changes in cerebral perfusion, as noted in this thesis.

Furthermore, loss of astrocytic glutamate transport protein EAAT2 is one of the hallmarks of ALS pathology (9), with up to 95% loss of expression and activity in affected areas of the central nervous system (10). This protein is responsible for removing glutamate from the synaptic cleft and preventing chronic glutamate neurotoxicity, imperative for neuronal survival (11, 12). As previously discussed in Chapter 1, glutamate is co-transported into the cell with 3 Na⁺ ions, a process that has been shown to stimulate glycolysis in astrocytes to produce lactate to meet the energy requirement of activated neurons. Currently, a PET ligand is being developed to target EAAT2 transporters *in vivo* to determine areas of the brain with decreased expression of this protein (personal communication with Dr. Jeffery Rothstein). Using this novel ligand, if available, in conjunction with a metabolic imaging technique, would be of tremendous benefit to our understanding of EAAT2 and metabolic coupling in ALS. Furthermore, it would provide concrete evidence of the protective effect EAAT2 proteins have on motor neuron survival, as documented in a recent mouse model over-expressing this protein (13).

5.6.2 Cerebrovascular Reserve Testing in MND

We have described encouraging results with respect to MTT as a possible marker for cognitive impairment in MND and have suggested that there may be a vascular component involved. If in fact there is a vascular component that contributes to cognitive

impairment or is active in the disease progression, it may be unveiled by testing the cerebrovascular reserve. The CTP technique can sensitively reveal cerebral hemodynamic impairments in patients with stenocclusive disease, which is associated with increases in MTT, by testing the CVR. Thus, it follows that regions with increased MTT in MND may also display a blunted CVR. Testing of CVR in MND would provide a corroborative measure of hemodynamic compromise documented in our study, further implicating endothelial dysfunction as having a causative role in cognitive impairment in MND. The clinical significance of such a finding would highlight the contribution of vascular factors to the disease, and possibly create a therapeutic target for clinical trials.

5.6.3 Mean Transit Time as an Indicator of Cerebral Perfusion Pressure

Lastly, this thesis has presented work that describes MTT as an indicator of CPP in a model of graded hypotension. However, we realize that the choice of anesthetic may not have been ideal for assessing a full range of vascular responses due to the vasodilatory effect of isoflurane. Future work should involve the development of a new experimental anesthetic protocol that is well tolerated by rabbits with minimal cerebrovascular or systemic effects. Once developed, repetition of the study presented in Chapter 4, would permit MTT to be tested over a wider range of CPP, with greater vasodilatory capacity within the range of autoregulation. To date, no study has provided an association of hemodynamic measurements with CPP over a large experimental range and compared it to the gold standard of CVR.

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Appendix A – Human Primary Lateral Sclerosis Ethics Approval



Office of Research Ethics

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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. M. Strong

Review Number: 09671

Revision Number: 2

Protocol Title: Perfusion and Metabolic Imaging in Primary Lateral Sclerosis (PLS)

Department and Institution: Clinical Neurological Sciences, London Health Sciences Centre - University Campus

Sponsor:

Approval Date: 02-Oct-03

End Date: 01-Apr-04

Documents Reviewed and Approved: revised study end date

Documents Received for Information:

This is to notify you that the University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has received and granted full board approval to the above named research study on the date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

This approval shall remain valid until end date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

- a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- b) all adverse and unexpected experiences or events that are both serious and unexpected;
- c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Paul Harding

Lenora Perry, BHSc., Monitoring Officer

Filed: ON
 Date: Oct 2/03
 m

This is an official document. Please retain the original in your files.

UWO HSREB Ethics Approval

09671

Page 1 of 1

Appendix B – Human Amyotrophic Lateral Sclerosis Ethics Approval



Office of Research Ethics

The University of Western Ontario
 Room 00046 Dental Sciences Building, London, ON, Canada N6A 5C1
 Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
 Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. M.J. Strong

Review Number: 09541

Review Date: August 07, 2007

Revision Number: 3

Review Level: Expedited

Protocol Title: A prospective study of rapid CT cerebral blood flow imaging in the detection of cognitive impairment in sporadic amyotrophic lateral sclerosis

Department and Institution: Clinical Neurological Sciences, London Health Sciences Centre

Sponsor: THE AMYOTROPHIC LATERAL SCLEROSIS ASSOCIATION

Ethics Approval Date: August 07, 2007

Expiry Date: December 31, 2007

Documents Reviewed and Approved: End Data Revision

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

- changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- all adverse and unexpected experiences or events that are both serious and unexpected;
- new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. John W. McDonald
 Deputy Chair: Susan Hoddinott

Ethics Officer to Contact for Further Information

Jennifer McEwen (jmcewen4@uwo.ca) Denise Grafton (dgrafton@uwo.ca) Ethics Officer (ethics@uwo.ca)

This is an official document. Please retain the original in your files.

cc: ORE File
 LHRJ

Appendix C – Animal Ethics Approval

01/07/2005 14:58 FAX 519 661 2028

ACVS-UWO

002



October 25, 2004

"This is the Original Approval of this protocol"
 "A Full Protocol Submission will be required in 2008"

Dear Dr. Lee:

Your "Application to Use Animals for Research or Teaching" entitled:

"The Influence of Mean Transit Time as an Index of Cerebral Perfusion Pressure"
 Funding Agency - IntraMural

has been approved by the University Council on Animal Care. This approval is valid from October 25, 2004 to October 31, 2005. The number for this project is **04-089-16**.

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
 If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED

PAIN LEVEL - C

Rabbits	-	NZW	SPF	3mo/2kg	M/F	-	16
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STANDARD OPERATING PROCEDURES

Procedures in this protocol should be carried out according to the following SOPs. Please contact the Animal Use Subcommittee office (661-2111 ext. 86770) in case of difficulties or if you require copies. SOP's are also available at <http://www.uwo.ca/animal/acvs>

- # 310 Holding Period Post Admission
- # 320 Euthanasia
- # 322 Criteria for Early Euthanasia/Mammals/Non-Rodent
- # 333 Post-operative/Post-Anaesthetic Care - Level Three
- # 360 Blood Collection/Volumes/Multiple Species

REQUIREMENTS/COMMENTS

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

c.c. Approved Protocol - T. Lee, J. Hadway, D. Forder
 Approval Letter - J. Hadway, D. Forder

Conference Presentations and Abstracts

Invited Oral:

1. TY Lee, B Murphy, and MJ Murphy. *CT Perfusion in stroke and cognitive impairment.* The 4th International Symposium on CT and MR Brain Perfusion. Chicago, Illinois. (09/2005, non-presenter)
2. MJ Murphy. *Cerebral hemodynamic compromise associated with cognitive impairment in primary lateral sclerosis.* First International Research Workshop on Frontotemporal Dementia in ALS, London, Canada. (05/2005)

Oral Presentations:

1. MJ Murphy, X Chen, T-Y Lee. *Evaluation of mean transit time as an indicator of cerebral perfusion pressure in rabbits with graded systemic hypotension.* Medical Biophysics Seminar Series, University of Western Ontario (09/2007)
2. MJ Murphy, GM Grace, MJ Strong, MC Tartaglia, JB Orange, RI Kozak, X Chen, A Rowe, K Findlater, and T-Y Lee. *A comparison of cerebral hemodynamic parameters in patients with ALS and PLS accompanied by changes in cognitive impairment.* 17th International Symposium on ALS/MND, Yokohama, Japan (12/2006)
3. MJ Murphy, GM Grace, MJ Strong, MC Tartaglia, JB Orange, RI Kozak, X Chen, A Rowe, K Findlater, and T-Y Lee. *A comparison of cerebral hemodynamic parameters in patients with ALS and PLS accompanied by changes in cognitive impairment.* Medical Biophysics Seminar Series, University of Western Ontario (12/2006)
4. MJ Murphy, X Chen, T-Y Lee. *Investigation of cerebral perfusion pressure under varying conditions of MAP.* Medical Biophysics Seminar series, University of Western Ontario (01/2006)
5. Grace GM, Orange JB, Murphy MJ, Lee T-Y, Rowe A, Findlater K and Strong MJ. *Cognitive Impairment in Primary Lateral Sclerosis.* 16th International Symposium on ALS/MND, Dublin, Ireland (12/2005)
6. MJ Murphy, GM Grace, MJ Strong, MC Tartaglia, JB Orange, RI Kozak, X Chen, A Rowe, K Findlater, and T-Y Lee. *Cerebral Hemodynamic Changes Accompanying Cognitive Impairment in Primary Lateral Sclerosis.* Radiology Research Day, Department of Radiology, University of Western Ontario (06/2005)
7. MJ Murphy, X Chen, T-Y Lee. *Evaluation of Cerebral Perfusion Pressure.* Medical Biophysics Seminar Series, University of Western Ontario (03/2005)
8. MJ Murphy, GM Grace, MJ Strong, MC Tartaglia, Kozak, X Chen, and T-Y Lee. *Perfusion Imaging in Motor Neuron Disease.* Medical Biophysics Seminar Series, University of Western Ontario (05/2004)

Poster Presentations:

1. MJ Murphy, GM Grace, MJ Strong, MC Tartaglia, JB Orange, RI Kozak, X Chen, A Rowe, K Findlater, and T-Y Lee. *Cerebral perfusion associated with cognitive function in patients with ALS: A longitudinal study.* 17th International Symposium on ALS/MND. Toronto, Canada (12/2007)
2. MJ Murphy, GM Grace, MJ Strong, MC Tartaglia, JB Orange, RI Kozak, X Chen, A Rowe, K Findlater, and T-Y Lee. *Longitudinal assessment of cognitive function and concomitant changes in cerebral perfusion in patients with ALS.* Second International Research Workshop on Frontotemporal Dementia in ALS, London, Canada (06/2007)

3. **MJ Murphy**, GM Grace, MJ Strong, MC Tartaglia, JB Orange, RI Kozak, X Chen, A Rowe, K Findlater, and T-Y Lee. *Evidence of endothelial dysfunction in Primary Lateral Sclerosis (PLS) with cognitive impairment as measured by CT Perfusion*. The 23rd International Symposium on Cerebral Blood Flow and Metabolism, Osaka, Japan. (05/2007)
4. **MJ Murphy**, X Chen, T-Y Lee. *Evaluation of mean transit time as an index of cerebral perfusion pressure in rabbits with graded systemic hypotension*. The 23rd International Symposium on Cerebral Blood Flow and Metabolism, Osaka, Japan (05/2007)
5. **MJ Murphy**, X Chen, T-Y Lee. *Reproducibility of acetazolamide reactivity and CT Perfusion measurement of cerebral blood flow in rabbits*. 5th Imaging Symposium of the Imaging Network Ontario, Toronto, Canada. (04/2007)
6. **MJ Murphy**, GM Grace, MJ Strong, MC Tartaglia, JB Orange, RI Kozak, X Chen, A Rowe, K Findlater, and T-Y Lee. *Cerebral Hemodynamic Changes Accompanying Cognitive Impairment in Primary Lateral Sclerosis*, 16th International Symposium on ALS/MND, Dublin, Ireland (12/2005)
7. MC Tartaglia **MJ Murphy**, A Rowe, K Findlater, MJ Strong, JB Orange, GM Grace, Kozak, X Chen, and T-Y Lee. *Deficits Observed in Patients with Primary Lateral Sclerosis are Associated with Changes in Cerebral Hemodynamics*, 16th International Symposium on ALS/MND, Dublin, Ireland (12/2005)
8. **MJ Murphy**, GM Grace, MJ Strong, MC Tartaglia, JB Orange, RI Kozak, X Chen, A Rowe, K Findlater, and T-Y Lee. *Mean Transit Time Changes in Primary Lateral Sclerosis Patients with Cognitive Impairment*, First International Research Workshop on Frontotemporal Dementia in ALS, London, Canada (05/2005)