A multifaceted retrospective analysis of the association between Zolpidem administration and increased brain perfusion and function in neurologically compromised patients.

A research project submitted in partial fulfilment of the degree:

Magister Scientiae in Human Physiology
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by

Stephanus Petrus Jansen van Vuuren
B.Sc. Hons (Cum Laude) (Pret.), H. Cert, ROHA SAIOH
29059951

Department of Physiology
Faculty of Health Sciences
University of Pretoria
0002
South Africa

Website: http://www.up.ac.za
Zolpidem Intervention - Effect on Brain Perfusion & Function

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**M.Sc. Human Physiology**

through the Department of Physiology, at the Faculty of Health Sciences, University of Pretoria.

**Candidate**

*Name:* Stephanus Petrus Jansen van Vuuren  
*Student number:* u29059951  
*Department:* Human Physiology  
Faculty of Health Sciences  
University of Pretoria  

*Email:* petriejvv@netactive.co.za  
*Contact Number:* 083 294 7573

**Supervisor**

*Name:* Prof Peet du Toit  
*Department:* Human Physiology  
*Position:* Associate Professor  
Faculty of Health Sciences  
University of Pretoria

**Co-supervisor**

*Name:* Dr Nozipho Nyakale  
*Department:* Nuclear Medicine  
*Position:* Specialist  
Pretoria Academic Hospital

**Head of Department**

*Name:* Prof DH van Papendorp  
*Department:* Human Physiology  
Faculty of Health Sciences  
University of Pretoria
The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

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Approval Certificate

New Application

Ethics Reference No.: 46/2014

Title: A multifaceted retrospective analysis of Zolpidem's ability to improve brain perfusion and function in neurologically compromised patients.

Dear Mr Stephanus Petrus Jansen van Vuuren

The New Application as supported by documents specified in your cover letter for your research received on the 30/01/2014, was approved, by the Faculty of Health Sciences Research Ethics Committee on the 08/05/2014.

Please note the following about your ethics approval:

- Ethics Approval is valid from 2 years
- Please remember to use your protocol number (46/2014) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:

- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

_________________________________
Dr R Sommers; MBChB; MMed (Int); MPharMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Tel:012-3541330 ☎ Fax:012-3541367 ☎ Fax2Email: 0866515924 ☎ E-Mail: fhsethics@up.ac.za
Web: //www.healthethics-up.co.za ☎ H W Snyman Bld (South) Level 2-34 ☎ Private Bag x 323, Arcadia, Pta, S.A., 0007

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ABSTRACT

This project represents one of the foundation steps to a collaboration between the Department of Human Physiology, University of Pretoria and the Nuclear Medicine Department at Steve Biko Academic hospital.

Following the initial discovery of the surprising effect zolpidem has on patients in persistent vegetative states in 1999 by Dr H.W. Nel - namely that zolpidem administration results in a significant qualitative increase in brain function, to the extent that patients were able to once again communicate and respond appropriately to their surroundings - much data has been collected by both Dr Nel as well as the Nuclear Medicine Department of Steve Biko Hospital.

Over the course of twelve years SPECT scans have been carried out on patients of various pathologies both before and after a course of zolpidem. To this day, both assessment and follow up of these and new patients is still being done by the Nuclear Medicine Department and Dr Nel. As this vast collection of data grows it has become increasingly daunting for a single research team to consolidate all this information into a usable form and an outside team has been deemed necessary to facilitate this process.

The primary goal of this study was to quantify the neurological perfusion changes following zolpidem administration within responder patients. This was achieved through reprocessing and semi-quantification of the existing SPECT scan records held by the Pretoria Academic Hospital. Within the group of responder patients (n = 29), 22 patients (~76%) presented a significant increase in perfusion within at least one lesion with a range of 4.5 - 46.1% (mean = 11.9%). In opposition to this finding non-responsive lesion perfusion decreased with a significant mean change of -14.5%. For both sets the p-value was determined to be <0.01. Of all lesions measured (n = 85) 32% displayed increased perfusion after zolpidem administration, whereas 30.6% presented with a perfusion decrease.

It was determined that only one lesion is required to respond to zolpidem in a positive manner to facilitate positive functional improvements with a given patient. In a small minority of patients post-zolpidem functional improvements seems to be connected to wide-spread cortical changes as opposed to singular lesional improvements.

This study provides further evidence of zolpidem’s paradoxical action in a subset of brain damaged individuals. Unique quantification of results allows for additional insight and provides further understanding the physiological changes associated with zolpidem administration.

Keywords: Physiology, Nuclear Medicine, Zolpidem, Stilnox, Ambien, SPECT, Semi-quantification, Perfusion, Brain damage, Rehabilitation
ACKNOWLEDGEMENTS

It has been an absolute pleasure to be entrusted with this project and to contribute to the ever growing field of research into the paradoxical effects of the drug zolpidem. It is my utmost privilege to acknowledge the following individuals for their contributions to the completion of this dissertation:

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Thank you.
Dedicated to:

*My mother, Lorraine, an inspiration.*
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<tr>
<td>µg/L</td>
<td>Microgram per Litre</td>
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<tr>
<td>4-HNE</td>
<td>4-Hydroxynonenal</td>
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<tr>
<td>5-HTP</td>
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<td>AIF</td>
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<td>Death-Associated Protein Kinase 1</td>
</tr>
<tr>
<td>DISC</td>
<td>Death Inducting Signalling Complex</td>
</tr>
<tr>
<td>DLPFC</td>
<td>Dorso-Lateral Prefrontal Cortex</td>
</tr>
<tr>
<td>DRP1</td>
<td>Dynamin Related Protein 1</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>ELAM-1</td>
<td>Endothelial Leukocyte Adhesion Molecule</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
</tr>
<tr>
<td>fas receptor</td>
<td>Tumour necrosis factor receptor superfamily member 6</td>
</tr>
<tr>
<td>FC</td>
<td>Fully Conscious</td>
</tr>
<tr>
<td>FC</td>
<td>Febrile Convulsions</td>
</tr>
<tr>
<td>FKBP38</td>
<td>FK506-binding protein 8</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>g/mol</td>
<td>Grams / Mole</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-Aminobutyric Acid</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glial Fibrillary Acidic Protein</td>
</tr>
<tr>
<td>Gpi</td>
<td>Globus Pallidus Interna</td>
</tr>
<tr>
<td>HC</td>
<td>Hydrocephalus</td>
</tr>
<tr>
<td>HCO3⁻</td>
<td>Ionic bicarbonate</td>
</tr>
<tr>
<td>HE</td>
<td>Herpes Encephalitis</td>
</tr>
<tr>
<td>HIF</td>
<td>Hypoxia-inducible factors</td>
</tr>
<tr>
<td>HMPAO</td>
<td>Hexametazine</td>
</tr>
<tr>
<td>IBM</td>
<td>International Business Machines Corporation</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular Adhesion Molecule 1</td>
</tr>
<tr>
<td>ICAM-2</td>
<td>Intercellular Adhesion Molecule 2</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial Pressure</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin 1</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>Int</td>
<td>International</td>
</tr>
<tr>
<td>IP3R</td>
<td>Inositol triphosphate receptors</td>
</tr>
<tr>
<td>IPSC / IPSP</td>
<td>Inhibitory Post Synaptic Currents / Potential</td>
</tr>
<tr>
<td>IU</td>
<td>Intrauterine</td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun N-terminal kinase</td>
</tr>
<tr>
<td>K⁺</td>
<td>Ionic Potassium</td>
</tr>
<tr>
<td>KeV</td>
<td>Kiloelectron Volt</td>
</tr>
<tr>
<td>LD</td>
<td>Lateral Dorsal Nucleus</td>
</tr>
<tr>
<td>LoC</td>
<td>Level of Consciousness</td>
</tr>
<tr>
<td>LP</td>
<td>Lateral Posterior Nucleus</td>
</tr>
<tr>
<td>MBQ</td>
<td>Megabecquerel (derived unit of radioactivity)</td>
</tr>
<tr>
<td>MCS</td>
<td>Minimally Conscious State</td>
</tr>
<tr>
<td>MD</td>
<td>Medial Dorsal Nucleus</td>
</tr>
<tr>
<td>MEG</td>
<td>Magnetoencephalogram</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>ml</td>
<td>myo-Inositol</td>
</tr>
<tr>
<td>MND</td>
<td>Motor Neuron Disease</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>MSN</td>
<td>Medium spiny neurons</td>
</tr>
<tr>
<td>mSv</td>
<td>millisievert (derived unit of ionising radiation dose)</td>
</tr>
<tr>
<td>mV</td>
<td>Millivolt</td>
</tr>
<tr>
<td>MVA</td>
<td>Motor Vehicle Accident</td>
</tr>
<tr>
<td>N</td>
<td>Sample Population</td>
</tr>
<tr>
<td>Na⁺</td>
<td>Ionic sodium</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetyl-aspartate</td>
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</table>
Zolpidem - effect on neurological function & perfusion in brain damage

NH₃ Ammonia
NMDA N-Methyl-D-aspartic acid
nNOS Neuronal NO Synthase
NO Nitric Oxide
NoC No Control / Reference Region
Noxa Phorbol-12-myristate-13-acetate-induced protein 1
ONOO⁻ Peroxynitrite
P Significance Value
p38 p38 mitogen-activated protein kinases
p53 Tumour suppressor p53
per os Oral Administration
PERP p53 apoptosis Effector Related to PMP-22
PET Positron Emission Tomography
Pf Parafascicular Nucleus
PKA cAMP-Dependent Protein Kinase
PKC Ca²⁺/Phospholipid-Dependent Protein Kinase
PKCθ Protein Kinase C-Theta
PLases Phospholipases
PLSases Phospholipid Scramblases
PMNL Polymorphonuclear Leukocytes
PUMA p53 Upregulated Modulator of Apoptosis
REM Rapid Eye Movement
ROI Region of Interest
ROS Reactive Oxygen Species
RS Reye Syndrome
Rxn Reaction
SAM Synthetic Aperture Magnetometry
SD Standard Deviation
SMC Supplementary Motor Region
SPECT Single Photon Emission Computed Tomography
SPSS Statistical Package for the Social Sciences
TBI Traumatic Brain Injury
TBPS Agent T-Butylbicyclophosphorothionate
TCTP Fortilin
TNF-α Tumour Necrosis Factor Alpha
ToS Time of Scan
UK United Kingdom
US United States
VA Ventral Anterior Nucleus
VCAM-1 Vascular Adhesion Molecule-1
VL Ventral Lateral Nucleus
VPL Ventral Posterolateral Nucleus
VTC Ventral Thalamic Complex

XIV
Zolpidem - effect on neurological function & perfusion in brain damage

<table>
<thead>
<tr>
<th>WB</th>
<th>Whole Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFS</td>
<td>Waterhouse-Friderichsen Syndrome</td>
</tr>
<tr>
<td>xx:yy</td>
<td>Hours:Minutes</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction to zolpidem and pathophysiology of brain damage
1.1 INTRODUCTION

1.1.1 History

To our knowledge, the first recorded instance of zolpidem having a paradoxical restorative effect, on the compromised neurological function of a patient dates to the year 1997.\(^1\) A case is documented in which 10 mg zolpidem, four times daily, was found to improve the akinesia and rigidity in a patient diagnosed with Parkinson’s disease, without inducing drowsiness. Yet in the absence of further evidence this publication was met with some resistance due to concerns about the safety of repeated daily zolpidem administration in the elderly, citing the increased risks of falls in senior patients associated with sedative administration.\(^2\)

Two years later Clauss et al describe the first case of zolpidem’s potential use in the management of disorders of consciousness.\(^3\) They published the case of a 28 year old man who was admitted to hospital following a road traffic accident with a small intracerebral haemorrhage in the left lentiform nucleus (putamen & globus pallidus\(^4\)) and thalamic areas. After 3 years in a vegetative state, two of which were spent completely mute, the patient was roused to consciousness following the chance administration of 10 mg zolpidem and succeeded in uttering the phrase “mammie”. This significant shift in level of consciousness was accompanied by a reduction in hyperaesthesia and spasticity.\(^3\) The drastic change in level of consciousness caught the attention of the media and the imagination of both the public and research groups around the globe.

1.1.2 Efficacy

Studies documenting the remarkable effects of zolpidem have been reported largely in the form of case reports. Zolpidem has been found to be useful in a minority of patients in a myriad of conditions, including but not limited to - catatonia of schizoaffective disorder,\(^5\) post-anoxic minimally conscious states,\(^3, 6-9\) post-stroke Broca’s aphasia,\(^10\) post-traumatic diaschisis,\(^11\) quadriparestes of central pontine myelinolysis,\(^12\) compulsive behaviour while awake,\(^13\) dementia with apraxia,\(^14\) bradykinesia, akinesia, dystonia in Parkinson’s, post-levodopa dyskinesias in Parkinson’s,\(^1, 15-17\) vertical saccadic eye movements and Parkinsonism in progressive supranuclear palsy,\(^18, 19\) restless leg syndrome,\(^20\) blepharospasm,\(^21\) post-anoxic spasticity,\(^22\) spinocerebellar ataxia,\(^11\) psychostimulant induced depression,\(^23\) Anti-N-Methyl-D-Aspartate Receptor Encephalitis.\(^24\)
1.1.3 Epidemiology

Of these pathologies, the damage due to stroke and associated forms of anoxic-type injury represent one of the largest opportunities for zolpidem to become an accepted intervention, due to their high rates of occurrence. According to data published by the World Health Organisation, stroke is the second leading cause of death worldwide, (Figure 1.1) with an estimated annual mortality rate of 6.7 million. The global incidence of stroke is estimated to be between 45 - 75 stroke cases per 1 000 patients per year in the >65 age group (Figure 1.2). Considering that 8% of the world’s population, or 524 million individuals, are believed to be over the age of 65, even a conservative estimate approximates 24 million strokes annually. Although zolpidem might not be able to increase the odds of short term survival, it may offer a glimmer of hope to the millions of people left with long term sequelae after a stroke or similar forms of brain damage.

Following this data on stroke, the second leading cause of event-related acquired brain injury is traumatic brain injury (TBI), with an estimated 10 million people affected annually. The global incidence is approximately 235 cases per 100 000 population. The average mortality rate is 11% of all cases. This review will focus on these types of brain injury, not only due to their epidemiological relevance, but additionally, due to how well the epidemiological data is reflected in the research data. This statement will be expanded on in later chapters.

1.1.4 Conclusion

Despite these shocking statistics there is still very little that can be done to recover function in a permanently damaged brain. Zolpidem offers a unique management approach, which despite the unfavourable response figure of ~6 - 10% (Table 1.1), due to the staggering number of cases and likelihood of disability after brain damage, offers the potential of improved neurological function to millions.
Figure 1.1: Death and Stroke, illustrated statistics. Adapted from the WHO. (1) Leading causes of global mortality, according to 2012 WHO statistics. An estimate of TBI can be garnered from the road injury statistic, the majority of deaths following these injuries stem from brain trauma. (2) Age-standardised incidence of stroke per 1 000 individuals above the age of 65 from seven different countries. Error bars represent 95% confidence intervals.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Design</th>
<th>Total (N)</th>
<th>Category (N)</th>
<th>Responders (N)</th>
<th>Injury</th>
<th>LoC</th>
<th>T</th>
<th>Dose (mg)</th>
<th>Effect</th>
<th>Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Du, B. et al. 2014&lt;sup&gt;29&lt;/sup&gt;</td>
<td>Prospective open-label cohort</td>
<td>165</td>
<td>82</td>
<td>83</td>
<td>Contrecoup / Space-occupying compression Brainstem injury</td>
<td>VS/UWS</td>
<td>Various</td>
<td>10</td>
<td>CSI &amp; burst suppression improved, ↑ perfusion in damaged regions</td>
<td>SPECT</td>
</tr>
<tr>
<td>Whyte, J. et al. 2014&lt;sup&gt;30&lt;/sup&gt;</td>
<td>Placebo-controlled, double-blind, crossover study</td>
<td>83</td>
<td>4</td>
<td>Various (Responders: 3 TBI 1 Hypoxia)</td>
<td>VS/UWS &amp; MCS</td>
<td>&gt;4 m</td>
<td>10</td>
<td>↑ Responsiveness, ↑ social interaction, ↑ environmental interaction, Roused out of MCS (2)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Thonnard, M. et al. 2013&lt;sup&gt;31&lt;/sup&gt;</td>
<td>Prospective open-label cohort</td>
<td>60</td>
<td>2</td>
<td>2</td>
<td>TBI</td>
<td>VS/UWS &amp; MCS</td>
<td>9 y &amp; 5 y</td>
<td>10</td>
<td>Emergence from MCS (N=1), functional use of objects (N=1), intentional Communication</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>Anoxia</td>
<td>MCS</td>
<td>1 y</td>
<td>10</td>
<td>↑ CRS-R, Reproducible command following, object recognition</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>Metabolic</td>
<td>MCS</td>
<td>1 y</td>
<td>10</td>
<td>↑ CRS-R, object localisation</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>56</td>
<td>0</td>
<td>Various</td>
<td>VS/UWS &amp; MCS</td>
<td>&gt;4 w</td>
<td>10</td>
<td>↓ CRS-R</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Nyakale, N.E. et al. 2010&lt;sup&gt;32&lt;/sup&gt;</td>
<td>Physician blinded-randomised observational trial</td>
<td>23</td>
<td>10</td>
<td></td>
<td>Various, majority TBI / CVA</td>
<td>MCS &amp; FC</td>
<td>&gt;6 m</td>
<td>10</td>
<td>↑ perfusion, TFES score fell by mean 19.4% in responders 5.17% in non-responders</td>
<td>SPECT</td>
</tr>
<tr>
<td>Whyte, J. &amp; Myers, R. 2009&lt;sup&gt;33&lt;/sup&gt;</td>
<td>Multicentric, double-blind, randomized study</td>
<td>15</td>
<td>1</td>
<td>Various, majority 8 TBI 5 Anoxia</td>
<td>VS/UWS, MCS</td>
<td>1 m - 23 y</td>
<td>10</td>
<td>VS/UWS changed to MCS, ↑ CRS-R score, visual pursuit, response to command</td>
<td>MRI</td>
<td></td>
</tr>
</tbody>
</table>
1.2 ZOLPIDEM

Over the past 5 years there has been a distinct increase in the number of large scale studies conducted on zolpidem’s action in brain damage. The results of these are summarised in Table 1.1. Using data from the only four studies which each had a randomised sample group, while simultaneously publishing data on both the number of zolpidem responders and non-responders, the likelihood of a steady-state brain damage patient responding to zolpidem in a positive manner can be estimated at ~10%, for a collective sample of 181 patients. It’s important to note, that in the described studies, virtually all responder patients were derived from three sources of brain damage, anoxia, TBI & stroke.

This response figure can arguably be amended to 6% based on how the reader wishes to interpret the outlying data in the form of the large number of responder patients in the Nyakale et al study. The nature of this spike cannot be determined in the absence of additional evidence. Therefore, approximate likelihood of a given acquired brain injury patient, responding to zolpidem in a positive manner, can be stated as ~6 - 10%.

This estimate is in no way meant to serve as attempt at establishing a firm response statistic for the use of zolpidem in brain damage. It is merely meant to allow for the discussion of zolpidem’s efficacy in light of existing data and to give the reader a vague sense of the efficacy of the drug. A true patient-response statistic would require very large sample sizes under similar conditions, and would ideally be limited to specific diagnostic groups, all studied under the same conditions.

1.2.1 Pharmacology

Zolpidem (C₁₉H₂₁N₃O, molecular mass: 307.395 g/mol) is an imidazopyridine hypnotic with rapid onset and short duration of action after oral administration. From examination of the structure presented in Figure 1.2, it is evident that the overall molecule is generally non-polar. The electron lone pairs on the oxygen atom do not have a sufficient influence on the overall polarity of the compound to impede transport across the blood brain barrier in any significant manner.
Zolpidem - effect on neurological function & perfusion in brain damage

The drug is rapidly absorbed from the gastrointestinal tract with a mean maximum plasma concentration of 121 µg/L reached within 1.6 hours of oral administration of a 10 mg dose. Within the dosage range of 5 - 20 mg per day zolpidem has been found to obey linear kinetics without any accumulation of the drug. Metabolism is carried out by a collection of cytochrome P450 isoenzymes. Hepatic CYP3A4 carries out approximately 60% of drug metabolism. The final metabolic products are 3 inactive compounds which are eliminated primarily through renal excretion. In healthy volunteers, zolpidem’s elimination half-life following a 10 mg dose is 2.5 hours.

Zolpidem is a benzodiazepine receptor agonist with the unique property of having high binding affinity for the GABA_\textsubscript{A} (gamma-Aminobutyric acid) receptor’s \(\alpha_1\)-\(\gamma_2\) subunit junction as illustrated in Figure 1.3. Most GABA_\textsubscript{A} receptor agonists bind to the GABA_\textsubscript{A} receptor at sites that are distinct from the primary GABA binding site, causing an allosteric modification which increases the likelihood of GABA binding or potentiates its action once bound. These mechanisms promote receptor action and increase the chloride influx for a given concentration of GABA. Classical barbiturates increase the duration the ion channel remains open, benzodiazepines and benzodiazepine-like drugs such as zolpidem, cause their target receptor’s ion channels to open with greater frequency.
Zolpidem effect on neurological function & perfusion in brain damage

Figure 1.3: Extracellular portion of α₁ and γ₂ GABAₐ receptor. Adapted from Richter L, et al. GABA binds to the junctions between α and β subunits whereas zolpidem only binds to the junction between α₁ and γ₂. General benzodiazepines display a significantly wider range of compatible subunit conformations. Gamma-Aminobutyric acid (GABA), Benzodiazepine (BZ).

Traditionally indicated for short-term insomnia at a dosage ranging from 5 - 10 mg per day, zolpidem’s pharmacological profile differs from that of the conventional benzodiazepine hypnotics in that it produces sedative and/or hypnotic effects at much lower doses than those active against convulsions and motor function. Additionally it is virtually devoid of myorelaxant activity.

1.2.2 Side-Effects

The most commonly occurring side effect of excessive dosages up to 140 mg, is uncontrolled drowsiness. A small number of cases presenting with respiratory depression have been reported for dosages in the 140 to 400 mg range. Overdosages up to 1400 mg have been successfully treated in the clinical setting. Management of overdose includes gastric evacuation as well as administration of flumazenil, usually completely reversing symptoms associated with acute overdose. Figure 1.4 examines the main side effect spectrum associated with zolpidem administration.
Zolpidem - effect on neurological function & perfusion in brain damage

1.2.3 Binding in brain damage

It has been confirmed that zolpidem’s unexpected action in brain damaged patients is via the GABA$_A$ receptor and not an unforeseen binding site.$^{46}$ In healthy individuals flumazenil blocks benzodiazepine action via competitive inhibition of the benzodiazepine binding site. In animal studies, brain damaged zolpidem responder baboons fail to present with any positive neurological perfusion changes if administered flumazenil following zolpidem administration.$^{46}$ The conclusion is drawn that due to this very specific inhibition, and flumazenil not having any known alternate binding sites, zolpidem mediated cognitive improvements are due to action at GABA$_A$ receptors.$^{46}$

Additional studies have determined that it’s not the GABA$_A$ receptor in general, but rather zolpidem’s high receptor specificity, binding primary to the GABA$_A$ $\alpha_1\gamma_2$ subunit junction, which is vital for its restorative action. Diazepam and zopiclone, a pair of non-specific GABA$_A$ agonists were administered to zolpidem responder patients after a sufficient zolpidem clearance period.$^{47, 48}$ Diazepam and zopiclone
retained their sedative action and no improvement of any kind was noted. A similar
trend of eliciting no positive response was met by placebo as well. Both diazepam
and zolpidrone have a much wider range of target GABA_A isoforms than zolpidem.
The conclusion is drawn that zolpidem’s highly selective GABA_A binding is vital to its
restorative action.\textsuperscript{47, 48}

This is also supported by the dissociation constant values for the interactions
between zolpidem and GABA_A receptor isoforms. The $\alpha_1$ isoforms favour ligand-
receptor complex formation relative to other isoforms: $K_D \alpha_1\beta_2\gamma_2 = 2.1 \times 10^{-7}$ M (at
physiological temperature), $\alpha_2^- \& \alpha_3^- \beta_3\gamma_2 \sim 1.5 \times 10^{-6}$ M. $\alpha_5$ conformation isoforms
have next to no appreciable zolpidem binding. The remaining $\alpha_x\gamma_2$ isoforms do not
interact with zolpidem.\textsuperscript{49, 50}

Having established that zolpidem’s action is due to GABA_A receptors containing the
$\alpha_1\gamma_2$ isoform, it would be pertinent to examine this receptor in depth to gain an
understanding of how it acts on a cellular level; to contextualise the action zolpidem
administration has on the brain.
1.3 GABA<sub>A</sub> RECEPTORS

GABA is synthesized through the decarboxylation of glutamate. It is the primary inhibitory neurotransmitter in the mammalian central nervous system where it typically plays the role of reducing neuronal firing through the promotion of a hyperpolarised state. GABA<sub>A</sub> receptors are cys-loop, ligand gated, ion channels (Figure 1.5). GABA<sub>A</sub> receptors exist as pentameric protein complexes, assembled from a variable combination of five subunits from a pool of at least 16 potential subunits, drawn from seven distinct gene families (α<sub>1</sub>-6, β<sub>1</sub>-3, γ<sub>1</sub>-3, δ, ε, θ, and π), not counting the recently reclassified ρ subunit family, formerly thought to form GABA<sub>C</sub> receptors. The ρ subunit does not co-assemble with classical GABA<sub>A</sub> subunits and is therefore beyond the scope of this dissertation.

![GABA<sub>A</sub> receptor structure](image)

**Figure 1.5.1 & 5.2: GABA<sub>A</sub> receptor structure** (1) Side-on view of the full structure of a pair of GABA<sub>A</sub> receptors, illustrating the extracellular, membrane spanning and intracellular domains. Different colours represent different subunits. The basal blue structure is gephyrin, which has been implicated in GABA<sub>A</sub> receptor synaptic clustering. (2) Top-down view of a pair of GABA<sub>A</sub> receptors embedded in the cellular membrane, clearly illustrates the pentameric nature of the receptor. Custom molecular model was generated for illustrative purposes utilising MODELLER © and Swiss-PdbViewer.

1.3.1 Receptor-Ligand Interaction

Receptor - GABA interaction results in the opening of a central ion channel which is permeable to chloride (Cl<sup>-</sup>), and to much lesser extent, bicarbonate (HCO<sub>3</sub><sup>-</sup>). The influx of negative ions establishes a hyperpolarised state. Subsequent activation of sodium (Na<sup>+</sup>) - potassium (K<sup>+</sup>) antiporters (Na<sup>+</sup>/K<sup>+</sup>-ATPase) result in the efflux of two potassium ions and influx of three sodium ions in an attempt to restore normal resting potential. Under normal conditions this membrane channel’s baseline activity can be responsible for up to two thirds of a neuron’s energy expenditure.
The net result of these processes is a diminished chance of a successful action potential occurring in the post-synaptic neuron. This inhibitory process is typically referred to as an inhibitory postsynaptic potential and can be electrically measured as a dip in the membrane potential. Short term inhibition is mediated through synaptic GABA_A receptors, whereas long term tonic inhibition is the result of low ambient levels of GABA binding to extra-synaptic receptors.

Synaptic GABA_A receptors are responsible for modulating benzodiazepine sensitivity and typically contain α_{1(2, 3, 5)} β_{2(3)} subunits in various combinations with themselves and other subunits as well as a single γ_{2} subunit. The remaining confirmations are associated with extrasynaptic GABA_A receptors. The final assembled structure contains a variety of allosteric binding sites which may directly or indirectly influence the eventual binding of GABA to the receptor. Through purification of benzodiazepine receptors from cortical tissue and immunoprecipitation with specific antibodies it has been determined that virtually all GABA_A receptors contain β family subunits, 75% contain γ_{2} subunits (synaptic) and 20 - 30% contain δ family subunits (extrasynaptic). The regional selectivity of the γ_{2} subunit allows the conclusion to be drawn that zolpidem acts primarily, if not entirely, through synaptic GABA_A receptors. The regional distribution of the primary GABA_A isoforms is elaborated on in Figure 1.6.

GABA_A receptor sensitivity to benzodiazepines is mediated through the alpha (α) family of subunits. Benzodiazepines bind to synaptic GABA_A receptors containing α_{1}, α_{2}, α_{3}, or α_{5} subunits with comparable affinity. GABA_A receptors expressing the α_{1} subunit correspond to the benzodiazepine omega one (ω_{1}) receptor. GABA_A receptors containing α_{2}, α_{3}, or α_{5} subunits correspond to ω_{2} benzodiazepine receptors. The benzodiazepine receptor nomenclature does not allow for detailed specificity, as such this discussion will refer to GABA_A receptor subunits by using specific individual designations.

Due to the specificity of zolpidem binding, the α_{1} subunit will be the primary focus of this analysis. This subunit mediates the sedation, ataxia, amnesia and partially, the anticonvulsant effects associated with zolpidem administration. Myorelaxation, anxiolysis and further anticonvulsant activity is primarily associated with the α_{2} and/or α_{3} subunits which zolpidem shows a drastically significantly reduced affinity for with no appreciable binding to any of the other subunits.
Figure 1.6: GABA_A Receptor Distribution in Rat and Man. Adapted from Amunts L et al. & Rudolph U & Knoflach F. Pie chart represents approximate distribution of GABA_A receptor subtypes that are known to exist in vivo within the murine brain. Within rats receptor subunit α_1 is expressed in cortical regions, thalamus, globus pallidus and hippocampus. α_2 is expressed in hippocampus, cortical regions, striatum and nucleus acumbens. α_3 expression is identified in the cortex, and reticular thalamic nucleus. α_5 expression is confined to deep cortical layers and the hippocampus. Collective GABA_A staining in the human brain is shown to illustrate the resemblance to murine staining. Figure heavily modified from references: 59, 60
1.3.4 Tolerance

GABA<sub>A</sub> receptor ligands affect sensitivity, synthesis and degradation of GABA<sub>A</sub> receptors. Post-binding there is an uncoupling of the allostERIC linkage between GABA and benzodiazepine sites. Receptor inactivation would be expected to be particularly strong for drugs with a high receptor affinity, which zolpidem displays for α<sub>1</sub> subtype receptors. Following zolpidem-receptor interaction, case studies have found a rapid disappearance of either sedative effects, in neurologically normal patients, or neuro-activating effects in brain damaged patients, despite plasma concentration only beginning to fall after the effects have already begun to diminish. This evidence suggests an acute tolerance model. Theoretically acute tolerance could be caused by the formation of a local antagonistic metabolite or due to acute receptor site adaptation. Since there are no known active metabolites which have been identified for zolpidem, down-regulation of GABA<sub>A</sub> receptors is speculated to play a role in zolpidem pharmacology.

In the traditional benzodiazepine model, acute tolerance development is considered a plausible first step in the development of chronic tolerance. Yet, chronic tolerance has not yet been reported in zolpidem responders. Many responders remain responsive for the remainder of their treatment with the drug, often spanning many years and multiple daily doses. This observation is supported by evidence from formal studies, finding that despite zolpidem’s rapid onset, short duration of action and pronounced short term tolerance, in both animals and humans, there are few indications of long-term adaptive adverse events.

A conceivable mechanism by which tolerance could potentially develop, occurs during prolonged exposure, at high doses and without a washout period. Under these conditions alterations in mRNA synthesis may cause changes in expressed GABA<sub>A</sub> receptor subunits, thereby reducing the sensitivity of the GABA receptor to GABA or its agonists. These long exposure phenomena would not be expected during intermittent short-lasting exposure. It is possible that it is precisely due to its short half-life that zolpidem is not typically associated with development of tolerance when used in a dosage controlled manner.

Tolerance to zolpidem does not develop in mice, in contrast with the effects of classical benzodiazepines. Cases in man have been reported where very high doses in the order of 70 - 400 mg daily, taken over prolonged periods of time, do result in tolerance development. Yet these dosages far exceed the average dosages encountered in literature studying the use of zolpidem in brain damage. As such chronic tolerance is not believed to be a therapeutic risk for zolpidem’s use in brain damage.
1.3.5 Conclusion

Having reviewed the basic pharmacology of zolpidem, it would be pertinent to establish exactly what environment it is exposed to in the damaged brain, this in an effort to lay the foundations for the following chapter, in which the paradoxical awakening effects of zolpidem will be discussed.
1.4 STROKE & ISCHAEMIA

Irrespective of the individual aetiology of acute strokes, compromised vascular supply to the brain or parts thereof is the primary event in the majority (85 – 90%) of acute strokes. Poor respiratory reserve and a near complete reliance on aerobic metabolism results in brain tissue being particularly vulnerable to the deleterious effects of ischaemia.71

Cerebral ischaemia leads to profound cellular responses. Initially these are limited to the site of the infarct, but with time, remote regions are altered as well.72 These changes include activation and proliferation of local glia along with the attraction of blood derived leukocytes to the site of ischaemic brain damage. Intracellular calcium concentration is rapidly raised following the infarct facilitating the release of excitatory acids, promoting acidosis and the production of oxidative free radicals. Each of these factors is likely to play a key role in neuronal damage. The inflammatory response which follows cellular destruction and immune activation may contribute to secondary deterioration or delay recovery.72 These processes will be examined in depth, to gain a better understanding of the environment during and after brain damage.

1.4.1 Topography

Cerebral tissue undergoing stroke induced ischaemia consists of three concentric regions:

(i) The inner core, undergoing severe ischaemia. The centre of the ischaemic core is perfused at 10 - 12 ml of blood per 100 g of tissue per minute or less (10 – 25% of the norm), presenting with necrosis of both neuronal and supporting glial elements. Tissues in this region typically sustain permanent damage within minutes.71, 73, 74

(ii) The area immediately around the ischaemic core, between it and the penumbra (outermost layer) is perfused at less than 18 – 20 ml per 100 g per minute, the result is a combination of necrotic and apoptotic tissue, the extent of which depending on the degree of hypoperfusion. Neurological tissues in this category are at risk of dying within hours.71, 73, 74

(iii) A surrounding outer layer of moderate ischaemia (penumbra). Blood supply by collateral vessels slows down the degenerative processes and the resultant perfusion of the region is 60 ml per 100 g per min. Despite functional disorder, these neurons are still metabolically intact. The penumbra contains cells which are the most viable targets for revival through timely therapeutic intervention. 71, 73, 74
It’s important to note that the penumbra is not stable. Due to the raised concentrations of extracellular glutamate and aspartate in the lesion core, spreading to adjacent tissues, repetitive peri-infarct depolarisations are triggered in the surrounding tissue. The extent of arterial blockage is proportional to the number of peri-infarct depolarisations. These depolarisations impose significant metabolic strain on penumbra cells to that extent that reserves of ATP are depleted and apoptosis follows. The growth of the ischaemic core into the penumbra can be prevented by pharmacologic blocking of peri-infarct depolarisations.\textsuperscript{72}

In terms of survival following an ischaemic event, white matter has shown to have a distinct advantage.\textsuperscript{75} It is more resistant to initial ischaemia due to a lower metabolic rate, with an ischaemic threshold of $\sim$20.8 ml per 100 g per minute compared to grey matter which has an ischaemic threshold of $\sim$34.6 ml per 100 g per minute.\textsuperscript{76, 77} Expressed virtually no NMDA receptors and only a sparse population of AMPA receptors, white matter is also more resistant to the damaging cascades once ischaemia sets in. The implications of this will become clear during the discussion to follow.\textsuperscript{78} More importantly, white matter has been shown to have a calcium regulation autoprotective feedback loop mediated by adenosine, furthering its resistance to excitotoxic events.\textsuperscript{79}

As far as grey matter is concerned, not only is it more vulnerable to ischaemia but also displays differential vulnerability between regions. Utilising MRI, it has been shown that following global hypoxia the superficial and deep grey matter are the most vulnerable to ischaemic damage. These are typically the regions with the greatest metabolic demand.\textsuperscript{80} The hippocampus appears to have the greatest vulnerability of ischaemic damage, followed by the basal ganglia then the frontal lobe.\textsuperscript{81} The brainstem and cerebellum appear to have some resistance when compared to the aforementioned regions.\textsuperscript{80}

\subsection*{1.4.2 Initial Processes}

The initial occlusion causes oxygen levels to rapidly decline, with the result that aerobic ATP synthesis grinds to a halt. Lack of ATP results in the failure of ion pumps to maintain the correct resting potential. This leads to neurons gradually depolarising, associated with cessation of spontaneous activity followed by a 5 to 10 mV hyperpolarisation.\textsuperscript{72}

This process of depolarisation is not solely due to failure of ionic pumps, but also hyperexcitability following initial ischaemia. Cortical ischaemia causes persistent functional alterations in the affected area. Brief hypoxic-hypoglycaemic episodes have been shown to increase expression of two N-methyl-D-aspartate (NMDA) components, including the NR2C subunit of the NMDA receptor, which is not expressed under normal conditions. Studies have also shown an increase in NMDA-
R1 messenger RNA. These changes to NMDA receptors induce hyperexcitability especially within the prevailing peripherally raised calcium levels.\textsuperscript{72}

\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are not spared modification, ischaemia is accompanied by a reduction in expression of the GluR2 subunit of the AMPA receptor. The modified AMPA receptors lacking GluR2 are more permeable to ionic calcium, contributing to the prevalent ionic fluxes.\textsuperscript{72}

The effects and extent of this hypersensitisation have been studied - investigations were performed on regions around the ischaemic core: field potentials were recorded from cortical layer II neurons with a stimulating electrode placed in layer VI, below the recording electrode. A double-pulse stimulation protocol was used to study paired-pulse inhibition. The resultant recordings revealed that post-stroke, inhibition was decreased in the ipsilateral brain hemisphere down to the rhinal fissure. This alteration was of sufficient magnitude to measure clearly from 1 day after stroke. Alterations in general excitability were found in the contralateral cortex as well. These were not confined to the mirrored region of brain but also present in surrounding cortical regions. During this period multiple epileptiform discharges may be noted in cortical recordings.\textsuperscript{72}

The broad implications of the receptor level changes discussed here can be surmised thusly - Four hours after ischaemic induction a hypometabolic lesion core has formed surrounded by a halo of tissue with considerably increased metabolism. Distal brain areas ipsilateral to the lesion present with increased metabolism. This hypermetabolism can be seen for over four hours post stroke over the majority of the cortex and shifts into pronounced hypometabolism 24 hours after the thrombotic event.\textsuperscript{72}

1.4.3 Ionic Dysregulation

If vascular occlusion / ischaemia persist for more than a few minutes ischaemic depolarisation begins in earnest. Sodium, chloride and calcium ions, along with water, flow into cells. Intracellular calcium rises from 10 to 100 mmol/L, although this is an intrinsic defence aimed to activate stress survival pathways, the extent of calcium flux ends up being one of the primary causes of cell death.\textsuperscript{71, 72, 82} Chloride levels shift from 10 to 40 mmol/L and the intracellular sodium level increases from approximately 25 to 50 mmol/L. During this flux potassium is expelled from cells rapidly elevating the extracellular levels to 80 mmol/L. These dramatic alterations in resting ion concentrations cause swelling of neurons and glial cells, potentially causing cytotoxic oedema. These events are not unique to stroke, any severe anoxic or hypoxic event will trigger the same cascade.\textsuperscript{71, 72}
Calcium influx causes disordered activation of wide range of enzyme systems, namely proteases, lipases and nucleases. These enzymes and their metabolic products, such as oxygen free radicals damage cell membranes, genetic material, and structural proteins in neurons, ultimately leading to cell death via the intrinsic apoptotic pathway.\textsuperscript{72} Figure 1.7 summarises a selection of the various major calcium induced cellular death pathways.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.7.png}
\caption{Outline of the apoptotic pathways surrounding ischaemic calcium influx. Adapted from Balzer F. During ischaemia p53 is upregulated through the genetic damage caused by free radicals, as well as via the upregulation of c-Jun N-terminal kinases (JNK), Hypoxia-inducible factors (HIF) and p38 directly by ischaemia. p53 is also upregulated through the activation of calpains by calcium influx. One of the primary actions of p53 activation is the assembly of the death inducting signalling complex (DISC) which arises through the trans-membrane fas receptor. The DISC directly induces apoptotic events. p53 triggers the activation of a wide variety of pro-apoptotic target genes including Bcl-2-associated X protein (BAX), p53 upregulated modulator of apoptosis (PUMA) and the pro-apoptotic protein NOXA. These mediate the disruption of the mitochondrial membrane, releasing further apoptotic factors in the form of cytochrome C (Cytc) and apoptosis inducing factor (AIF) which leads to apoptosis. p53 activation also upregulates production of SIVA protein which upregulates the apoptotic hub protein - apoptotic protease activating factor 1 (APAF-1). APAF-1, in conjunction with Cytc and Caspase-9 forms a complex directly involved in apoptosis. p53 apoptosis effector related to PMP-22 (PERP) is another pro-apoptotic gene upregulated by p53. In short, extensive calcium influx activates numerous apoptotic cascades.\textsuperscript{83}}
\end{figure}
Beyond the effect on internal enzyme systems, the dysregulated entrance of calcium into cells due to the ischaemic cascade triggers the release of excitatory neurotransmitters, namely glutamate and aspartate. Typically glutamate is vital for neuronal excitation and plasticity; however an uncontrolled release in ischaemic brain mediates excitotoxic events through activation of pre-sensitised NMDA, AMPA or kainate receptors. The targets cells for this release of glutamate are either adjacent to primary ischaemic neurons or synaptically downstream to affected tissue. Glutamate binding triggers sodium and calcium influx in neurons that may previously have been able to maintain their metabolic balance. The influx promotes changes similar to those seen in primary ischaemic neurons, namely; over-utilisation of resources in resource deprived conditions and calcium driven apoptosis.

Ischaemia not only facilitates excitotoxicity through sensitisation of glutamate receptors but also inhibits the expression of GABA receptors. Prolonged ischaemia decreases GABA receptors in and around the ischaemic lesion. This reduction is most pronounced in the first week, measurements in animal models reveal a decrease in GABA receptor density to about 60% of normal. Over the course of nine weeks or more, GABA receptor density steadily recovers. Degeneration of GABAergic interneurons in a 0.5 - 1 mm radius around the infarct accounts for some of this GABA receptor loss.

1.4.4 Oedema

As ions move into cells, extracellular water is also drawn in along with it, cytotoxic oedema follows. Excessive internal pressure compromises the structural integrity of both the membrane and cell organelles. Cytotoxic oedema evolves within minutes after injury due to a failure of ATP dependent ion channels as well as the release of oxygen derived free radicals.

An additional form of oedema, vasogenic oedema, results from an increased permeability of brain capillary endothelial cells to macromolecular serum proteins. This increase in permeability is the result of a loss of vascular integrity following the release of proteases such as matrix metalloproteases by compromised cells. Loss of vascular integrity causes the breakdown of the blood-brain barrier. As these enter the extra-cellular space they increase extra-cellular fluid volume which raises intracranial pressure (ICP). This displaces brain tissue, potentially crushing whole structures.
Sustained increase in ICP causes further ischaemia as vascular elements are compressed. Initially acute ischaemia triggers cytotoxic oedema, if prolonged, this gives way to vasogenic oedema. This distinct sequence of events suggests that time is needed for defects to develop in endothelial cells. Studies show that vascular endothelin receptors are upregulated during a stroke, possibly due to the action of protein kinase C as well as mitogen activating protein kinase. 71, 82, 85, 86

1.4.5 Free Radicals

In normal physiological conditions neurons are exposed to a baseline level of oxidative stress from various sources, particularly in the form of free radicals. Free radicals are highly reactive molecules with one or more unpaired electrons which if left unchecked can react with cellular components and products such as DNA, proteins and lipids, causing varying degrees of damage and dysfunction. Research both clinical and experimental has shown that ischaemia significantly increases free radical formation. 87-89

Free radicals formed during stroke induced anoxia include the superoxide radical, hydroxyl radical and nitric oxide. There is some regulation by antioxidant enzymes and free radical scavengers, but these systems are exhaustible, particular in oxygen deprived conditions. 82

During ischaemia, mitochondria become the primary site of free radical generation, producing superoxide anion radicals during a compromised electron transport process. After re-perfusion, additional oxygen free radicals are generated by activated microglia and infiltrating peripheral leukocytes via NADPH oxidase, which causes further damage. 82

NMDA receptor activation and subsequent calcium influx, stimulates nitric oxide (NO) production through the calcium/calmodulin dependant neuronal NO synthase (nNOS). The products of which have been connected to excitotoxicity. Endothelial NOS produced NO acts as a vasodilator, but even externally produced NO can still diffuse across membranes into neurons. 82, 90, 91 Regardless of origin, once within neurons or glia NO reacts with the superoxide radical producing peroxynitrite (ONOO-), another highly reactive oxygen species. Both oxygen-derived and nitrogen-reactive free radicals activate several pathways within the cell, promoting apoptosis and inflammation. 82, 90, 91

The role of free radicals in ischaemia would be incomplete without discussing the effect of reactive oxygen species on lipids. Lipid peroxidation appears to play a major role in ischaemic pathogenesis, generating 4-hydroxynonenal (4-HNE), an aldehyde which covalently modifies membrane transporter proteins, including the Na+/K+ ATPase and the glucose-glutamate transporter. This covalent modification significantly impairs their function. 82, 92
1.4.6 Astrocytes & Calcium

Due to the ionic disturbances found accompanying the initial hyperexcitation in ischaemia, accompanied by massive calcium fluxes, calcium-dependent exocytosis of glutamate from astrocytes is triggered. 74, 93-95

The eventual release of glutamate from astrocytes can occur through a variety of potential mechanisms: (i) reversal of uptake by plasma membrane glutamate transporters,96 (ii) cellular swelling triggering the opening of anion channels,97, 98 (iii) calcium dependent exocytosis,97 (iv) glutamate exchange via the cystine–glutamate antiporter,99 (v) release through ionotropic purinergic receptors100 and (vi) unpaired connexons, communicating with the extracellular space.101 Although the mechanism of action of each of these methods of astrocytic glutamate release have been clarified in normal physiological models, it is still unclear exactly which of these are involved in the altered environment of ischaemia and/or uncontrolled calcium fluxes.82, 102

Cortical astrocytes strongly upregulate glial fibrillary acidic protein (GFAP) expression in the boundary zone of infarcts which GFAP staining methods can detect as early as one day after infarct.72 Within 3 days the entire ipsilateral cortex stains positive for GFAP. The same cannot be said for the contra lateral hemisphere, which despite undergoing metabolic shifts of its own, does not appear to display the same astrocytic activation, suggesting it may not be subject to the same oedematous stressors. GFAP expression is vital to maintain astrocytic mechanical strength in the face of oedematous swelling.72, 103 The primary function of GFAP in the face of neurological trauma is to facilitate the formation of a glial scar of reactive GFAP-positive astrocytes. The ultimate function of the scar is to re-establish the physical and chemical integrity of the damaged region. It forms a physical barrier across the injured site re-sealing any gaps in the blood-brain-barrier. Glial scars promote revascularisation of the damaged site.72, 104

Problematically, despite the physical benefits of the glial scar, they also secrete neuron-specific growth-inhibitory substances, inhibiting axonal regrowth throughout the ischaemic core. Glial scars typically persist for approximately ten weeks post trauma.104
1.4.7 Astrocytes & Potassium

Release of potassium as cells attempt to regulate ionic fluxes serves as one of the primary pathological mechanisms through which induced alterations are propagated via spreading depression. Under normal conditions extracellular potassium released through excitatory processes is absorbed by glial networks (astrocytes). This occurs through passive diffusion, the Gibbs-Donnan effect (membrane impermeable charged ions altering eventual ion distribution) and active transport. Glial cell coupling via gap junctions allows site specific uptake and diffuse distal release of potassium reducing the potassium / charge load on any one specific site. This system can effectively disperse potassium concentrations up to 10-12 mmol/L without excessive ion build-up in peripheral regions. With potassium fluxes as high as 80 mmol/L following stroke, astrocytic dispersion simply can’t act fast enough to prevent compromised ionic gradients. Under these conditions the astrocytic dispersion of potassium does not cease, but instead becomes a mechanism by which uncontrolled ion fluxes spread through the brain.

1.4.8 Spreading Depression

The phenomenon of spreading depression is caused by a combination of raised levels of potassium in any region exceeding this astrocytic buffering capacity and uncontrolled glutamate release. Spreading depression requires a functional glial network to travel through the brain. The eventual ischaemic spreading depression is the result of exhaustion of intracellular potassium and is dependent on NMDA mediated excitement as has been shown in animal models. Post stroke administration of MK801 (Dizocilpine), a non-competitive NMDA antagonist, abolishes remote ipsilateral GFAP immunoreactivity, expression of early genes and remote hypermetabolism followed by hypometabolism. This effect is not extended to the initial lesion.

Spreading depression may be one of the few regulatory methods by which the ischaemic or physically damaged brain can truly conserve resources, despite the initial cost to initiate the reaction, once potassium travels through the brain it establishes a modified resting potential, promoting hyperpolarisation relative to the new extracellular potential, decreasing neuronal firing and facilitating resource conservation.
1.4.9 Calcium Influx and the Endoplasmic Reticulum

Relevant to any type of injury involving large amounts of calcium influx into the cell, endoplasmic reticulum (ER) disturbance is often overlooked in lieu of mitochondrial events. However, events related to the ER may both impair mitochondrial function, and promote apoptosis of the whole cell. BCL-2 proteins, although largely active in the mitochondria, are also found in ER membranes. Several members of the BCL-2 family of proteins modulate ER calcium homeostasis, these include tunicamycin (inhibits N-linked glycosylation) and brefeldin (inhibits ER-golgi transport). Anti-apoptotic proteins including BCI-2 and BCL-XL reduce calcium concentrations within the ER. Pro-apoptotic proteins such as BAX increase ER calcium concentration. There is growing evidence to suggest that BCL-2 family proteins regulate ER calcium concentrations through IP3 receptors.

BCL-2 targeting the ER selectively suppresses induced cell death. BCL-XL targeting of the ER suppresses autophagy. BI1 (BAX inhibitor 1) is a transmembrane protein which interacts with BCL-2 family members. This protein blocks cell death related to oxidative stress or over expression of pro-apoptotic BAX. BI1 also regulates ER calcium concentration, where over expression of the protein reduces ER calcium levels by increasing leakage. The BCL-2 family appear to require BI1 to regulate calcium concentration and fail to reduce ER calcium concentration in the absence of BI1.

It is proposed that this slow controlled leakage of calcium from the ER reduces the amount of free calcium released upon catastrophic ER stress, if a sudden burst of calcium is released into the cytosol, critical levels will be reached triggering disastrous downstream effectors (figure 1.3). Examples of these include the calcium mediated activation of protein kinase C-theta (PKCθ) and calmodulin-dependent kinase-beta (CaMKKβ) which have been reported to induce autophagy. Figure 1.8 summarises and expands on the events discussed here.
Figure 1.8: Effect of cellular calcium on the endoplasmic reticulum (ER): Adapted from Kim I et al. (1) Inositol triphosphate receptors (IP$_3$R) are believed to be the most likely mechanism by which calcium is able to enter the ER. IP$_3$Rs are inhibited by B-cell lymphoma 2 (BCL-2) and BCL-X$_L$, which require BAX inhibitor 1 (BI1) for their action. BI1 is proposed to inhibit IP$_3$R but this connection is yet to be conclusively proven. BCL-2 associated protein (BAX) as well as BCL-2 antagonist (BAL) counteract BCL-2 and BCL-X$_L$. BCL-2 and BCL-X$_L$ interact with proteins resident in the ER such as B-cell receptor-associated protein 31 (BAP31) and bifunctional apoptosis regulator (BAR), these latter two proteins also interact with each other. (2) Cytosolic calcium stimulates multiple pathways which are involved in triggering cell death. The activation of calcium-sensitive mitochondrial fission protein, known as dynamin related protein 1 (DRP1) has been implicated in BAX-induced release of cytochrome c from mitochondria. (3) Phopholipases (PLases), which are calcium dependant and phospholipid scramblases (PLSases) have been suggested to transfer cardiolipin from the inner to outer membrane of mitochondria. This is the trigger for the insertion of pro-apoptotic BCL-2 proteins & BAX into mitochondrial membranes. (4) NO Synthase activation generates reactive nitrogen species and adds to pre-existing oxidative load. (5) Calpain family cysteine proteases are calcium-dependent and involved in cell death and the inhibition of BCL-2 and BCL-X$_L$. (6) Calcium induces an increase in permeability of mitochondrial membranes, releasing apoptogenic proteins as well as reactive oxygen species (ROS). (7) Protein phophatase B is calcium sensitive, its activation regulates the pro-apoptotic activity
of BCL-2 associated agonist of cell death (BAD) (8) FKBP38 (FK506-binding protein 8) is a peptidyl prolyl isomerase which binds BCL-2 and induces apoptosis. It is activated through the action of the calcium/calmodulin complex. (9) TCTP (fortilin), a calcium binding protein is a suspected modulator of anti-apoptotic BCL-2/BAX family proteins. (10) Calcium influx activates death-associated protein kinase 1 (DAPK1) as well as the related protein DRP1. Both of these contain calmodulin binding domains. DAPK1 can induce either apoptosis or autophagy. (11) Illustrates pathways which trigger autophagy involving the activation of autophagy inducing agents protein kinase C-theta (PKCθ) and calmodulin-dependent kinase-beta (CaMKKβ).

1.4.10 Ischaemia - Immune Response

Several cell populations (endothelial cells, astrocytes, microglia & neurons) within the brain are able to secrete pro-inflammatory mediators following ischaemia. Calcium influx induces various neuro-protective transcription factors, however many of these also induce inflammatory cytokines (IL-1, IL-6 & TNF-α) as well as chemokines (ICAM-1, ICAM-2 & selectins) and generally pro-inflammatory genes (interferon-inducible protein-10). 72, 74, 82

Migration of leukocytes from the bloodstream into tissue and subsequent persistence is regulated by cell adhesion molecules. Surface ligands such as CD11/18 complex and very late activation molecules (lymphocytes) facilitate this process. These interact with inducible receptors found on vascular endothelial cells. Examples of these include intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) and endothelial leukocyte adhesion molecule (ELAM-1). The induction of these receptors will also determine the site of eventual inflammation. Upregulation of ICAM-1 has been found in the cerebral vessels of baboons as early as 4 hours after an ischaemic event, persisting for up to 2 days thereafter. 72 Persistent regional leukocytes were found to be ICAM-1 positive. Human autopsy also identified endothelial ICAM-1 following cerebral infarct. 72

Polymorphonuclear leukocytes (PMNL), primarily neutrophils, are the first hematogenous cells to arrive in an ischaemic lesion. Intravascular PMNLs are present thirty minutes after cerebral artery occlusion and peak at around 12 hours. Inter-neuronal/glial granulocytes are most numerous twenty-four hours after ischaemia and rapidly decline within the next twenty-four hours. 72

There is evidence to suggest that leukocytes are involved in re-perfusion injury as well as microvascular dysfunction. Leukocytes accumulate in post-ischaemic tissue prior to onset of injury. Neutropenic animals exhibit a superior response to ischaemic stroke. Prevention of leukocyte-endothelial cell adhesion protects against injury. Therefore PMNLs are viewed as mediators of re-perfusion injury. 82
The initial phagocytic response is due to local microglia while haematogenous macrophages are recruited with a delay of 5 days to aid removal of debris. Recruited macrophages remain through to resolution.\textsuperscript{72}

Animal studies have shown that a day or two after thrombosis, granulocytes and T-cells adhere to sub-pial and cortical vasculature and infiltrate the extent of the ischaemic lesion. By day three T cells are concentrated in the ischaemic penumbra and ED1+ phagocytes have appeared in the region. The extent of ED1+ phagocytic infiltration dramatically increases between days three to seven forming a ring around the ischaemic lesion. The ischaemic core is only infiltrated by ED1+ phagocytes two weeks after the initial events and the number of accompanying T-cells was decreased.\textsuperscript{72}

1.4.11 Stroke & Ischaemia - Conclusion

The eventual degree of neurological compromise can be summarised as a complex interplay between various factors, including the post-ischaemic inflammatory response exacerbating cell damage, delayed cell death, ischaemic tolerance of the brain and remote effects such as diaschisis.\textsuperscript{82} Figure 1.9 illustrates a concise summary of the events laid out above.
Zolpidem - effect on neurological function & perfusion in brain damage

**Pathways of Ischemic Cell Death**

*Induction of Long-Term Functional Damage*

**Initiators and Activators**

1. ATP
   - Na/K ATPase
   - Membrane Depol
   - Glutamate
   - Ca
2. ATP
   - Na/K ATPase
   - Membrane Depol
   - Glutamate

**Perpetrators**

3. Protease Activity (Calpain)
   - Proteolysis

**Critical Functional & Structural Changes**

4. Membrane Permeability
   - Edematous Cell Change

5. Na/K ATPase
   - Ischemic Cell Change

**Cell Death End Stages**

6. Mitochondrial Dysfunction
   - Homogenizing Cell Change

7. Protein Synthesis
   - Apoptotic Cell Change

8. Proteins & Phospholipids & DNA
   - Autophagic-lytic Cell Change

9. Cytoskeletal Damage
   - -

10. Prolonged Changes in Kinases or Phosphatases
    - -

11. Phospholipase Activity
    - -

12. Free Radicals
    - -

13. Phospholipid Changes
    - -

14. Depolarisation (Depol)
    - -

15. Intracellular (i) Free fatty acids (FFA)
    - -

16. Platelet activating factor (PAF)
    - -

17. Electron transport (e)
    - -

**Figure 1.9: Summary of cellular events following ischaemia / stroke.** Adapted from Lupton P. (1) & (2) The first two columns list the cellular events following ischaemic damage which trigger the metabolic cascades leading to long term alterations. (3) Column three lists the actions that result in the long-term functional alterations listed in column four. (4) Column four lists the changes in cell membrane permeability or function which are known to be connected to ischaemia. No single listed entity links solely to any of the elements contained in column 5. (5) Column five lists the primary cellular morphological forms cell assume after ischaemic insult. Depolarisation (Depol), Intracellular (i) Free fatty acids (FFA), Platelet activating factor (PAF), Electron transport (e).106
1.5 TRAUMATIC BRAIN INJURY

Traumatic brain injury (TBI) is in many ways similar to ischaemia in that ionic fluxes and ischaemia also play a pivotal role. Aside from the initial damage arising from trauma, the fundamental mechanisms behind stroke and TBI are shared. As such these will not be discussed in nearly as much detail as they have already been covered in depth. This review will focus on unique aspects of this specific form of acquired brain injury.107

1.5.1 Initial Trauma

TBI spans a massively wide breadth of clinical entities, everything from the knock a sportsman may receive during his activity of choice, through to severe damage associated with the large forces of a motor vehicle accident. The principal mechanisms of injury are classified as (1) focal neurological damage arising from contact type injuries, causing contusions and laceration of brain tissue, as well as possible intracranial haemorrhage, or (2) diffuse brain damage as a result from acceleration/deceleration and rotational forces. These result in diffuse axonal injury and rapid deafferentation.107, 108 The specific results of the latter processes include widespread severing of synaptic connections, the extent of which is correlated to the severity of the injury.

Neuronal vulnerability to TBI follows a similar pattern to ischaemia, aside from the region directly receiving the mechanical insult, the most metabolically active regions are likely to suffer the greatest damage after trauma. Due to its large concentration of NMDA receptors, the hippocampus is particularly vulnerable to insult, even if completely remote to the initial impact.109

The first stages of TBI, immediately after the direct damage from a traumatic event are associated with impaired regulation of cerebral blood flow (CBF) and metabolism. The resulting metabolic cascades resemble ischaemia and see an accumulation of lactic acid due to anaerobic metabolism taking over as ATP reserves dwindle.107, 109

Accompanying this, as in stroke, is massive release of excitatory amino acids and a shift in normal cerebral ion balance, see Figure 1.10 for details as to the extent of these fluxes after trauma. Excitation & dysregulation of membrane ion channels leads to calcium floods into cells activating lipid peroxidases, proteases and phospholipases. These lead to the formation of free radicals and the activation of caspases, translocases and endonucleases which drives cells towards apoptosis.107 The combination necrosis brought about by the initial trauma and the ionic fluxes comprise not only the integrity of individual neurons, but also the functional integrity of entire neuronal circuits, e.g. the reticular activating system and thalamocortical loops (which mediate consciousness).110
Figure 1.10: Concentration variations in neurological factors present in cerebrospinal fluid after induced fluid percussion brain injury in rats. Adapted from Giza CC. Ionic calcium (Ca$^{2+}$), Cerebral Blood flow (CBF), Cerebral Metabolic Rate (CMR), Ionic Potassium (K$^+$). Of particular relevance is the dramatic rise in glutamate and potassium as well as the decrease in perfusion shortly after injury.$^{110}$

1.5.2 TBI & Cerebral Perfusion

The mechanisms through which ischaemia arises following TBI include, morphological injury resulting from mechanical displacement hypotension in the face of autoregulatory failure, shortage of endothelial NO (causing vasoconstriction) or a lack of cholinergic neurotransmitters (linked to substrate deficiency).$^{111}$

Many investigations have revealed that focal or global ischaemia are frequent components of TBI.$^{107, 112, 113}$ The critical perfusion threshold for development of irreversible damage in TBI is less forgiving than in stroke, TBI: 15 ml per 100 g per min vs stroke: 5 - 8.5 ml per 100 g per min. This can be explained by considering the mechanical initiating component of TBI. While both ischaemia and TBI lead to metabolic stress and ionic perturbations, TBI has the additional component of physical trauma to brain cells, cerebral microvasculature and endothelial cell damage, the effects of which can manifest throughout the brain immediately after injury.$^{107, 112, 113}$
In the early stages of TBI, hyper-perfusion and accompanying hyperaemia may also develop. This may be equally detrimental as ischaemia due to the fact that increases in cerebral blood flow (CBF), not matching specific metabolic demand, increases cerebral blood volume and intracranial pressure. The combination of increased vascular pressure, with post-injury tissue inflammation contributes to cytotoxic events. Increased CBF without an increase in metabolism, as well as a decreased CBF without an associated decrease in metabolism are indicative of a defect in autoregulation of the coupling between CBF and metabolism.\textsuperscript{107, 114}

The primary pair of vascular autoregulatory mechanisms serving to regulate this reactivity are CBF autoregulation and carbon dioxide (CO\textsubscript{2})-reactivity. In a healthy brain an increase in cerebral perfusion pressure (CPP) should elicit vasoconstriction and vice versa in an effort to maintain constant ICP. This reflex is compromised with varying time course after trauma. Vasoconstriction seems to be somewhat more resistant to damage than vasodilation, indicating that patients are more sensitive to damage arising from a decrease in CPP than an increase.\textsuperscript{107, 115}

Cerebrovascular CO\textsubscript{2}-reactivity, the constriction/dilation in response to hypo/hypercapnia is a significantly more robust phenomenon than either of the previous reflexes. CO\textsubscript{2}-reactivity is typically only impaired in patients with very severe brain damage and poor outcomes.\textsuperscript{107, 116}

Cerebral perfusion is not only affected by a failure of regulatory events. Post-traumatic vasospasm is an important secondary insult taking place days after the initial trauma. The chronic depolarisation of vascular smooth muscle arises from compromised potassium channel activity, a reduction of endothelin, vasoconstriction mediated by prostaglandins and reduced availability of NO. Excessive vasoconstriction becomes a cyclical phenomenon with an increase in the production of free radicals and potentiating chemicals released from damaged tissue.\textsuperscript{107, 115, 117}

\subsection*{1.5.3 Metabolic Events}

Beyond the unique vascular events accompanying TBI once ischaemia has developed and damaged neurons release their cellular contents into surrounding tissues, the events that follow on a cellular scale become largely indistinguishable from ischaemic stroke.\textsuperscript{107, 118}

Metabolic failure is related to a failure of ATP generation arising from mitochondrial dysfunction in a hypoxic environment. This results in a reduced nicotinic co-enzyme pool and cellular calcium overload. Excitotoxicity and insufficient blood flow both contribute to the alternate metabolic event of glucose hypermetabolism secondary to ischaemia.\textsuperscript{107, 118}
Massive release of glutamate potentiates these events and contributes to the propagation of metabolic dysfunction. Excitotoxicity and exhaustion of endogenous antioxidants induces the peroxidation of cellular and vascular structures. Damage to mitochondria and DNA facilitate apoptosis.\textsuperscript{107, 118}

TBI induces a complex array of tissue responses causing inflammation in a manner similar to that encountered in re-perfusion injuries of stroke. The release of cellular mediators such as inflammatory cytokines, prostaglandins, ROS and complement cascade induce chemokines and adhesion molecules. These mobilise immune and glial cells which infiltrate and adhere to the regions of significant trauma.\textsuperscript{107, 118}

The removal of tissues destroyed by the initial injury or by the cellular processes thereafter commences hours after injury, lasting for weeks or months until astrocytes eventually produce microfilaments and GFAP, forming the glial scar.\textsuperscript{107, 118}

1.5.4 Traumatic Brain Injury - Conclusion

Traumatic brain injury is a combined assault of mechanical stress and metabolic dysregulation on the brain. The only major difference between stroke and severe TBI is the method of inducing initial distress. There is good evidence that the major metabolic pathways are nearly identical.
1.6 ACQUIRED BRAIN INJURY - GABAERGIC CHANGES

Following the previous discussion elucidating the similarities between the pathological processes surrounding ischaemic events after the leading forms of acquired brain injury, it is pertinent to narrow down the specifics relevant to this discussion, particularly of how these events relate to the GABA_A receptor family and the environment zolpidem may interact with after brain injury.

1.6.1 GABA Receptor Alterations

The excitatory cascades both triggered by and causing the release of glutamate are in part facilitated by the influx of calcium, triggering the fusion of cystolic vesicles with the synaptic portion of the cell membrane leading to neurotransmitter release. This calcium mediated release is by no means limited to glutamate, GABA release from GABAergic neurons is also stimulated by this process but often ignored in reviews on the topic. Measurements taken from fluid in the extracellular space reveal drastically increased levels of GABA hours after ischaemia returning to normal levels after re-perfusion.\(^{119}\)

In addition to calcium dependant processes, the formation of oxidative radicals following mitochondrial dysfunction indirectly promotes release of intracellular stores of GABA. The raised levels of extracellular GABA are maintained by a reversal of action of membrane spanning GABA transporters caused by influxes of sodium. This increased sodium concentration is the result of glutamate induced activation of AMPA receptors.\(^{119}\)

It would not be an excessive logical leap to expect this large GABA efflux to, at least to some extent, reign-in the excitotoxic events previously described, however this is not the case, at least not within the ischaemic or damaged core and penumbra. Although inhibitory neurotransmitters may protect surrounding tissues from dysregulated ionic flux, within directly damaged regions, calcium dependant activation of phospholipase A_2 promotes the formation of arachidonic acid and its metabolites such as prostaglandins and thromboxanes. Each of these compounds directly inhibits the action of the GABA_A receptor, promoting the run-away changes described previously.\(^{119}\) The large GABA release also drives receptor internalisation which requires time after re-perfusion to normalise.\(^{119-121}\)
Multiple animal studies\textsuperscript{120, 121} have shown that following cerebral ischaemia there is a decrease in binding by radioactive labelled GABA\textsubscript{A} chloride blocking agent 1-butylbicyclophosphorothionate (TBPS) within the dendritic fields of the CA1 area of hippocampal neurons as well as striatal neurons for up to 27 days, potentially longer in humans. This is thought to be, in part, the result of neuronal degradation, but drastic receptor down-regulation is not improbable and is known to occur in other neurological regions due to raised concentrations of extracellular GABA.\textsuperscript{119-121}

Murine photothrombosis induces a distinct pattern of change in GABAergic receptor distribution. There is significant down regulation of nearly all GABA receptor isoforms in cortical, hippocampal and thalamic neurons throughout both hemispheres after cortical thrombosis induction. Subunits \(\alpha_1, 2 \ & 5\) along with \(\gamma_2\) are down-regulated to various degrees. There is an upregulation of subunit \(\alpha_3\), relative to other subunits in the contralateral cortex homotopic to the original lesion, implying unique GABAergic modulation of the cortical site contralateral to the lesion. These changes can be blocked through administration of NMDA antagonist MK-801. Connecting these changes to glutamate and calcium induced excitation.\textsuperscript{122}

After an ischaemic event extracellular GABA concentrations are raised up to 600x above the baseline concentration.\textsuperscript{122} Due to the uncontrolled GABA release associated with aforementioned calcium fluxes this GABA diffuses freely through the extracellular environment. This causes both acute receptor alterations with initial synaptic release as well as slow changes if ischaemia persists beyond a few minutes. Figure 1.11 details the nature of these two categories of changes.\textsuperscript{123}
GABA receptor down regulation is not the only factor impeding GABA’s ability to prevent run-away ionic shifts, paradoxically the down regulation itself may contribute to neural damage-control. As large calcium, glutamate and chloride cascades commence, drastically affected neurons being to respond to GABA in an excitatory manner. The chloride ion gradient, 7-30 mM intracellular, 110-150 mM extracellular, fundamental to the functioning of GABA<sub>A</sub> receptors is maintained by multiple chloride extrusion mechanisms (Figure 1.12) such as K⁺-Cl⁻ cotransporters (KCC2), Sodium dependent Cl⁻/HCO₃⁻ exchangers, Cl⁻ ATPase and voltage-dependent Cl⁻ channels.¹²₈,¹²₉
Under physiologically normal conditions GABA acts as expected, serving to hyperpolarise cells through a passive influx of chloride through the receptor ion channel. The key fact to note is that the movement of chloride ions down a concentration gradient through GABA receptors is a passive process and dependant on this concentration gradient. Under certain cellular conditions, as in the neonatal brain or following brain damage for example, the chloride concentration is greater within the cell than the external milieu. As such GABA\textsubscript{A} receptor activation causes an efflux of chloride and a net excitatory effect, possibly contributing to the initiation of an action potential.\textsuperscript{119, 130, 131}

Using electron probe x-ray micro-analysis it has been shown that following oxygen-glucose deprivation intracellular chloride is raised in proportion to the extent of substrate deprivation.\textsuperscript{119, 132} This is supported by the in vitro observation that anoxia suppresses GABA-mediated IPSCs due to this shift in chloride gradients.\textsuperscript{119, 133} Mechanical trauma to hypothalamic neurons result in approximately 60\% of these neurons developing an excitatory response to GABA administration within minutes to days after trauma.\textsuperscript{119, 134}

The mechanism by which intracellular concentration of chloride is raised post-insult is expanded on in Figure 1.12. Compromised cellular membranes also contribute to raised intracellular chloride and therefore this phenomenon can be seen as particularly applicable to advanced ischaemic injury or traumatic brain injury.\textsuperscript{119}

Normalisation of GABA receptors begins as soon as 30 minutes after re-perfusion but the overall reduction in GABA inhibition has been measured as lasting up to 14 days after ischaemia.\textsuperscript{119} Long-term hyperexcitability as the result of a reduction in efficacy of GABA-mediated inhibitory postsynaptic potentials (IPSPs) has been observed in the murine neocortex for 6 - 17 months after cerebral ischaemia.\textsuperscript{119, 135} The proposed mechanism is thought to lie within the post-synaptic receptor, as the neurons themselves were structurally normal. This loss in GABAergic inhibitory efficiency was preceded by a loss of GABA\textsubscript{A} receptors in regions of ischaemic brain shortly after insult.\textsuperscript{119}

These phenomena alter the perception of the short term down regulation of GABA receptors after insult. In the damaged brain the persistent raised extracellular GABA will initially have an inhibitory effect. But as ionic gradients shift and chloride ions flood into the cellular environment and remain there, eventually GABAergic stimulation may have an excitatory effect. As such, down regulation of GABA receptors, with their passive ionic channel may in fact serve to keep intracellular concentrations of chloride raised, reducing excitation.
Figure 1.12: Major chloride transport mechanisms. Adapted from Schwartz-Bloom RD. Normal direction of ion flow is illustrated. Following insult chloride may accumulate due to (i) repetitive opening of GABA$_A$-gated Cl$^-$ channels (ii) increase exchange of Cl$^-$ for HCO$_3^-$ (iii) Activation of Na$^+$/K$^+$/2Cl$^-$ (NKCC-1) cotransporters. Corresponding to this increase in chloride influx, efflux regulatory mechanisms may also fail. Decreases in efflux occur through (i) inhibition of Na$^+$-dependent Cl$^-$/HCO$_3^-$ exchange (ii) inhibition of voltage-gated chloride channels (iii) reversal or functional impairment of KCC2 channels (iv) impaired chloride-ATPase activity.¹¹⁹
1.7 **RECOVERY**

Some degree of spontaneous recovery is usually encountered in acquired brain injury patients in the weeks-months after injury. The nature of these changes typically varies wildly between neurological regions and different patients, giving clinicians little in the way of standardised processes to investigate. As far as trends are concerned, what can be said is that most spontaneous recoveries occur within the first three months. After this window cognitive improvements are most likely to make themselves manifest as opposed to motor changes. Initial voluntary movement is usually seen between 6 and 33 days post insult.\textsuperscript{136, 137} Research conducted on damage to motor circuits, (stroke primarily) revealed that maximum movement is restored within 80\% of the sample population during the first three weeks of recovery, and by 95\% within nine weeks. A similar trend is true for speech recovery. Beyond these initial improvements, approximately 36\% of patients continue to show increasing functional improvements for years.\textsuperscript{136, 138} The first step of this discussion will be examining the changes associated with these time frames.

1.7.1 **Receptor Changes**

Figure 1.13 visualises the initial GABAergic changes discussed in the previous section. The progression of receptor alterations in the weeks following injury are clearly visible.

Following infarct recovery, application of bicuculline, a \textit{GABA}_A receptor antagonist at concentrations estimated to block 10 - 20\% of available receptors is sufficient to induce epileptiform activity in post infarct rats, providing further evidence that the brain recovering from stroke, enters a hyper-excitatory state due to, in part, GABA down-regulation.\textsuperscript{122} The same study by Redecker, et al. found an increase in the subunit \( \alpha_3 / \alpha_1, 2, 5 \) ratio.\textsuperscript{122} This establishes a ratio of receptor isoforms akin to that found during development. These changes have been theorised to facilitate increased neuroplasticity within the damaged brain. Additional changes associated with this altered GABAergic landscape is a reshaping of topographical cortical maps. Additionally wider regions of neuronal representation are found within the ipsilateral hemisphere with supplementary cortical areas often being recruited in primary activation.\textsuperscript{122, 139, 140}
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Figure 1.13. The immunochemical binding distribution of GABA$_A$ receptor subunits within uninjured control rats vs those with photothrombic infarcts. Adapted from Redecker C et al. Images are colour coded according to binding intensity, dark colours (black/blue) indicate no to small levels of binding, yellow-red indicate the largest amounts of binding. Arrowheads indicate site of infarct for a given column. Due to the free-floating immunohistochemical process, the centre of the ischaemic lesion is absent from the majority of slices. Due to the necrotic changes and non-specific staining in high-resolution microscopy, the strong immunoreactivity found in the rim of the necrotic site is most likely due to non-specific reactions, and not due to true GABA receptor binding. Control rats display a typical binding pattern for the subunits tested. Photothrombotic rats display bihemispheric regional reductions in immunostaining for all tested subunits within cortical, hippocampal and thalamic areas. This down regulation was most prominent in the ipsilateral hemisphere and was most prominent on days one and seven while still being present, on day thirty. Thirty days after infarct induction GABA$_A$ receptors are down regulated throughout the brain with an upregulation in the contralateral site corresponding to the original lesion.\cite{122}
Research into human stroke survivors provides insight beyond the first 30 days of recovery demonstrated in the previous murine study. Figure 1.14 shows the results of PET-CT scans conducted on stroke survivors (mean age 65) one month and three months into recovery. (18F)Flumazenil was used as a GABA<sub>A</sub> receptor specific tracer and computational techniques were used to isolate areas of deviation from the norm. Broadly speaking their results support the conclusion of brain wide reduction in GABAergic inhibition months into recovery, with a somewhat greater concentration of receptors persisting in the damaged hemisphere. Concentration of GABA<sub>A</sub> receptors were greatly raised within the infarct region one month post insult but normalised at the three month mark. The same group found a correlation between decreasing inhibition in the infarct site and functional restoration.

Figure 1.14: GABA receptor availability as seen in human stroke survivors using (18F)Flumazenil PET/CT. Adapted from Kim YK et al. Post processing shows changes in binding between two sets of readings (Pre/Post), illustrated using different colour schemes. Reds progressing to shades of yellow indicate greater extents of GABA receptor binding vs control. Yellows indicate the largest increase in binding as compared to controls. Light blue/teal through to darker blue (least amount of binding) indicate diminished GABA receptor binding vs control. (A) Images taken approximately 1 month after stroke. Significant reductions (blue) in GABA receptor availability was found in the ipsilateral thalamus, bilateral subcortical white matter in the parietal cortex. Increased receptor availability (red) was found in the ipsilesional cortical area and contralateral cerebellum with a large increase in perilesional tissue. (B) Images taken 3 months after stroke. Images taken follow a similar trend to those taken in (A), with a more widespread reduction in receptor availability in the cortical and subcortical white matter. The greater receptor availability in the contralateral cerebellum persisted but to a lesser extent. Perilesional activity was also raised, but was no longer significant above control values. (C) Results of B minus that of A. Comparison of
the two sets of scans reveal that GABA receptor availability generally decreases with recovery through both the neocortex and cerebellum during recovery, especially in the contralateral hemisphere.  

Based off the assumption that murine and human brains proceed through a similar series of phases after ischaemic damage, if not necessarily a similar timeline, the combined data from the human and animal imaging techniques presented here, provide unique insight into GABAergic changes after severe ischaemic damage. Initially there seems to be a drastic acute decrease in GABA receptors within the ischaemic core and surrounding tissue accompanied by globally decreased concentrations of GABA receptors. The first 30 days of recovery are marked by steadily increasing concentrations of GABA receptors but never approaching baseline levels. The clear exception to this statement is the lesion centre, inhibition here exceeds baseline levels of GABAergic inhibition 30 days into the recovery process. The second phase of recovery is marked by a secondary, lesser decrease in GABAergic inhibition across the brain.

Although it is tenuous to state inferred conclusions as fact without additional testing, it does seem that three unique phases of the post-anoxic/stroke brain can be identified through GABAergic alterations. (1) Immediate mass-down regulation in affected tissue, possibly to prevent paradoxical GABAergic contribution to excitotoxicity. (2) After re-oxygenation and any additional damage that may occur due to it has settled, the brain shifts focus to repair damage at a neuronal level, increasing GABAergic inhibition to spare recovering cells from excess metabolic demand. (3) Promoting circuit level repair through neuroplastic mechanisms and associated excitement's partial reliance on down-regulation of GABAergic inhibition. The eventual decrease of excessive inhibition within the ischaemic centre and diaschetic sites have been correlated to functional improvement.

The mechanisms underpinning these changes leading to functional gains include structural recovery in axons, dendrites and synapses, increased release of migration factors as well as accompanying changes in the extracellular matrix, glia and vitally, angiogenesis. On the molecular front (Table 1.2) there is an increase in binding to NMDA receptors with accompanying GABA downregulation. Cell-cycle proteins and nerve growth factor are expressed to greater levels than they are in control neurological tissues. The majority of these events occur with greater intensity within the peri-infarct region. With the exception of GABAergic inhibition which is one of the last lingering sequelae within the peri-infarction region and highly connected regions. Table 1.2 summarises the molecular underpinnings of these mechanisms.
Table 1.2: Repair-phase related molecular and cellular alterations after ischaemic damage. Adapted from Cramer SC. 136

<table>
<thead>
<tr>
<th>Alteration</th>
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<tbody>
<tr>
<td>Inflammatory markers</td>
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<tr>
<td>Growth-associated proteins</td>
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<td>Cell-cycle proteins</td>
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<tr>
<td>Growth factors</td>
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<tr>
<td>GABA receptor downregulation</td>
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<tr>
<td>N-methyl-D-aspartate receptor binding</td>
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<tr>
<td>Angiogenesis</td>
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<tr>
<td>Hyperexcitability, with facilitation of long-term potentiation</td>
</tr>
<tr>
<td>Synaptogenesis</td>
</tr>
<tr>
<td>Dendrite branching/spine density</td>
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<tr>
<td>Neuronal sprouting</td>
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<tr>
<td>Cortical thickness</td>
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</table>

Summary of repair-related molecular and cellular changes that have been described after a focal infarct in animal models. In most cases, these changes can be measured bilaterally, though ipsilesional is generally greater than contralesional extent. Some of these changes increase with exogenous treatment in parallel with enhanced behavioral gains, further supporting their importance as biological targets for promoting repair after stroke.

The behavioural improvement seen in the recovery phase is not solely evident on the molecular level, but also on a circuit level. Neurological reorganisation is a vital element of recovery and can be divided into three forms (1) increased activity in regions distant from but connected to the damaged area (2) increased activity in the contralateral mirror region (3) somatotopic shifts within intact cortical regions associated with increased excitability. Not all functional changes to manifest during this reorganisation are positive, diaschisis refers to the phenomenon by which there is altered activity in uninjured brain areas that are connected to the injured area. 136, 145, 146 In some cases recovery is associated not with overt recovery of function in the lesioned site, but restoration of diaschisis. 136
1.8 DIASCHISIS

Diaschisis has been reported as a potential phenomenon in nearly all forms of acquired brain injury where a distinct lesion is formed as a result of the traumatic insult, but not in progressive and diffuse neurodegenerative processes such as various dementias. Diaschisis has been shown to be partly responsible for the functional deficits associated with these forms of injury. Recovery from diaschisis typically begins in the days shortly after injury, but studies have shown that diaschetic regions remote from initial lesion, may remain hypoactive for months to years in certain patients. These same regions are in some patients subject to reactivation through zolpidem administration. The manifestations of the diaschetic phenomenon can be divided into two primary forms: diaschisis at rest and functional diaschisis.

Diaschisis at rest appears as a focal decrease in energy metabolism (usually measured as perfusion, SPECT, or glucose uptake, PET) at rest without stimulation or neural activation, occurring in anatomically intact regions distant from any lesions.

Functional diaschisis refers to focal anomalies in metabolism or neuronal activity within anatomically intact regions distant from any lesions, following activation or stimulation.

An example will perhaps clarify the difference between these two forms of the same class of phenomenon. During functional diaschisis, following a cortical lesion, there is an increase in the response to evoked potentials in the contralesional cortex without necessarily manifesting as an alteration of baseline metabolism at rest. Figure 1.15 illustrates this process.
Figure 1.15: At Rest vs Functional diaschisis. Adapted from Carrera E & Tononi G. Left pair: Pre-lesion. Right pair: Post-lesion. Focal lesion is illustrated as a black region. (1) Diaschisis at rest: a focal lesion induces a distant reduction of baseline metabolism (red region). (2) Functional diaschisis: Normal brain activation pattern (yellow regions). The normal activation pattern of a specific task is altered after a lesion. Activation of affected areas may be increased or decreased.\textsuperscript{147}

1.8.1 Diaschisis at Rest

A matter of particular relevance to zolpidem intervention for brain damage, is the problem of how does one detect diaschisis and any improvements there in. One vital element to the definition of diaschisis is a preservation of neurovascular coupling within the affected region. Neurovascular coupling refers to a decrease in metabolic demand within the brain being met by an associated reduction in perfusion. By this physiological process, regions with suppressed synaptic activity will be associated with a perfusion deficit.\textsuperscript{147} The latter process can be imaged by perfusion scanning methodologies such as fMRI, SPECT or PET,\textsuperscript{147, 151} the former through electrophysiological measurements such as EEG and MEG (magnetoencephalogram).\textsuperscript{147}
Although animal studies reveal that perfusion imaging is sensitive to at rest diaschisis, these imaging techniques do tend to underestimate the full extent of the changes. Electrophysiological measurements are more accurate when determining the extent of cortical diaschisis but are limited by only being able to detect superficial cortical changes, whereas deep brain electrophysiological readings represent a logistical challenge.\textsuperscript{147, 152-154} Mild diaschisis of the thalamus and striatum at rest, following cortical injury presents as non-significant perfusion deficits.\textsuperscript{152} Whereas the same pathology measured by intracerebral monitoring finds a similar pattern but with significantly larger changes.\textsuperscript{147, 153, 154}

The impact of diaschisis of rest varies with the location of the causative lesion. Subcortical lesions with associated cortical lesions present with clinical deficits similar to if the lesion had been in the affected cortical structures. These alterations are thought to be mediated through cortico-thalamic circuitry.\textsuperscript{147}

Following cortical lesions, a different pattern is identified. Crossed cerebellar diaschisis is a well documented phenomenon where a reduction in metabolism and perfusion is identified in the cerebellar hemisphere contralateral to the cortical lesion.\textsuperscript{147, 151} The primary structures mediating this process are thought to the cerebrocerebellar pathways. Problematically, the clinical correlates for this form of diaschisis are largely undefined. Additional forms of cortical diaschisis include hypometabolism and perfusion in the ipsilateral thalamus and striatum, as well as cortico-cortical diaschisis where the mirrored contralateral cortex is hypo-perfused due to interruption of transhemispheric pathways.\textsuperscript{147, 155, 156}

Diaschisis at rest has been shown to present on electrophysiological measurements as an increase in low frequencies in the homotopic contralateral cortical region after cortical lesioning. This is accompanied by a reduction in alpha peak frequency and raised delta frequency activity.\textsuperscript{147, 157-160}

1.8.2 Functional Diaschisis

Functional diaschisis often presents to electrical studies as an increase in the amplitude of evoked potentials in the contralesional cortex.\textsuperscript{147, 150, 161} Imaging reveals a decrease or absent neuronal activation in response to stimulation at sites distant from a specific lesion. Functional diaschisis may or may not overlap with areas of diaschisis at rest. To complicate matters, in a study testing four patients with lesions of Broca's area,\textsuperscript{162} a decrease in fMRI activation was observed in the undamaged posterior inferior temporal region during reading. In one of the patients, this region could be activated during a semantic exercise. The conclusion drawn is that functional diaschisis may be specific to the neural network attempting to activate a given cortical region.\textsuperscript{162} This has been defined as sub-type of functional diaschisis, given the name "dynamic diaschisis".\textsuperscript{147}
The concept of functional diaschisis introduces the important notions that (a) not all forms of diaschisis present as a decrease in neurological activity and (b) not all forms of diaschisis are visible to resting state perfusion scans.

1.8.3 Connectional Diaschisis

Defined as a selective change in neurological coupling due to lost afferent connections from a lesioned site within a defined network. As with functional diaschisis, two apparent manifestations can be identified, dependant on if the affected networks are specific to a certain functional context or widespread, figure 1.16 elaborates on this concept.

An illustrative example of connectional diaschisis is the decrease in interhemispheric functional connectivity between homotopic cortical areas of motor cortex following purely subcortical strokes. These changes are typically maximal early after stroke and diminish during recovery with a strong correlation to functional impairment. Inspection of effective connectivity reveals a decreased influence of ipsilateral motor cortex on the contralateral which improve with recovery. The reduction in motor ability is correlated to the extent of which this functional connectivity is compromised.
Figure 1.16: Subtypes of connectional diaschisis. Adapted from Carrera E. & Tononi G.
(1) Connectional diaschisis, distant strength and directions of interconnected neuronal network specific to a certain function or action may be altered. (2) Connectomal diaschisis, a lesion induces widespread changes in multiple neurological networks. Blue indicates normal activation, green indicates increased activation and red indicates a decrease in activation.¹⁴⁷
1.9 CONCLUSION

Having examined a large body of evidence for post-insult changes, both acutely and in the initial phases of recovery, what can be said for the environment with which zolpidem administered for rehabilitation is expected to act?

Vulnerability to damage increases with metabolic demand, the hippocampus, basal ganglia and frontal lobe are most likely to sustain damage from non-specific insult. The cerebellum and brain stem have a favourable survival profile.

Neurons may be completely lost from the centre of lesioned site. In the wake of this, cortical maps become less specific in an effort to compensate. To facilitate recovery of neuronal networks and associated function by driving neuroplastic mechanisms, NMDA receptors are upregulated, GABA receptors are down regulated. Repair related molecular analogues are upregulated.

Within regions of focal neuronal damage, GABA receptors are upregulated. Diaschetic phenomena result in functional impairment at sites distal to focal trauma.

The following chapter will review the current theories pertaining to zolpidem’s paradoxical action to restore function in late-recovery phase, brain damaged individuals.
1.10 REFERENCES


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Chapter 2

Explaining zolpidem’s paradoxical action
2.1 PHYSIOLOGICAL RESPONSES TO ZOLPIDEM

Before proceeding with the examination of current hypotheses regarding zolpidem’s ability to restore function in brain damaged patients, it would be pertinent to review how zolpidem affects certain aspects of normal physiology. Following this, the analysis will delve into how different authors have interpreted the existing body of evidence and combined it with their unique backgrounds to form novel and varied explanations of ‘the zolpidem effect’.

2.1.1 Effect of Zolpidem on Cerebral Blood Flow

There is intimate coupling in the brain between regional metabolism and blood flow. As discussed in chapter one, this mechanism is regulated via vascular carbon dioxide reactivity and cerebral perfusion pressure. In the significantly damaged brain these auto-regulatory mechanisms are compromised in proportion to the extent of damage. As brain vasculature recovers from injury these functions are rapidly restored.¹

Zolpidem administration to neurologically normal sleeping volunteers modifies absolute cerebral global metabolic rate (cGMR), decreasing the metabolic rate of the majority of neuroanatomical regions during non-REM sleep, in a direct relationship to the plasma concentration of zolpidem. The effects on neurological perfusion are non-uniform and tend to be greatest in subcortical and midline cortical areas as compared with lateral cortical areas.²

Regions found to have statistically significant reductions in perfusion after zolpidem administration are the neocortex, anterior cingulate gyrus, medial frontal gyrus, posterior cingulate gyrus, frontal white matter, caudate, putamen, thalamus, insula and hippocampus.²,³

This perfusion decrease can readily be explained through zolpidem’s neuroinhibitory action. As a GABA agonist, in regions with susceptible receptor conformations, zolpidem can be expected to reduce the rate of synaptic firing through its hyperpolarising effect. A reduction in synaptic activity places less strain on metabolic pathways to replenish neurotransmitters and cycle receptors. The end result is a decrease in metabolic demand and an associated decrease in regional perfusion.¹,⁴

As expected there is a correlation between these affected regions and the receptor distribution for zolpidem throughout the brain. Brain regions expressing zolpidem receptors can be seen in Table 2.1.
Table 2.1: GABA\textsubscript{A} \textit{α}\textsubscript{1} subunit distribution.
The regions listed in this table reflect the anatomical locations of significant distributions of α\textsubscript{1} subunits. Ventral anterior nucleus (VA), Ventral lateral nucleus (VL), Ventral posterolateral nucleus (VPL).

<table>
<thead>
<tr>
<th>Anatomical Region</th>
<th>Parent Structure</th>
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<tbody>
<tr>
<td>Neocortex</td>
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<tr>
<td>Globus Pallidus</td>
<td>Basal Ganglia</td>
</tr>
<tr>
<td>Substantia Nigra</td>
<td>Basal Ganglia</td>
</tr>
<tr>
<td>Subthalamic Nucleus</td>
<td>Basal Ganglia</td>
</tr>
<tr>
<td>Ventral Thalamic Complex (VA, VL, VPL)</td>
<td>Thalamus</td>
</tr>
<tr>
<td>Substantia Innominata</td>
<td>Basal Forebrain</td>
</tr>
<tr>
<td>Dentate Gyrus</td>
<td>Hippocampal Formation</td>
</tr>
<tr>
<td>Cerebellum</td>
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</table>

This specificity towards a given receptor subunit pair, and the associated neuroanatomical regions expressing this subunit conformation, is thought to underlie many of the beneficial effects zolpidem has had on a wide variety of neurological pathologies. Figure 2.1 clarifies this statement, elaborating on and connecting the specific regional binding profile to the possible functional improvements that may occur after zolpidem administration in related pathologies.
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Figure 2.1: Regional distribution of zolpidem binding and the clinical entities which may theoretically be improved through region specific interactions. Adapted from Hoque R & Chesson AL, Jr. Despite zolpidem's strict preference for α₁ receptor subunits it readily interacts with much of the brain due to the α₁ subunit's prodigious expression throughout the brain. Given zolpidem’s many potential binding sites, the improvement noted across a range of neurological disorders are difficult to localize to binding at a single anatomic location. Through understanding of how damage to specific neurological regions cause certain kinds of functional deficits, a speculative interaction map can be drawn.⁸
2.1.2 Zolpidem Receptor Modification

GABA_A receptor polypeptides are believed to fold and insert into the cellular membrane, taking on a conformation with an extracellular n- and c-terminus, 4 hydrophobic α-helices, spanning the cell membrane and a large hydrophilic intracellular loop region between membrane spanning domains 3 and 4. The intracellular loop region is the least conserved between different subunit assemblies. The GABA_A receptor family is subject to significant enzymatic modification stemming from consensus phosphorylation sequences for cAMP-dependent protein kinase (PKA) which can be found on the intracellular loop regions of α4 & 6 and β1-3 subunits. Although these specific alpha subunits aren’t applicable to zolpidem binding, beta protein modification may have an effect the regulation of clusters of receptors specific to zolpidem.9,10

Additional consensus sequences for Ca^{2+}/phospholipid-dependent protein kinase (PKC) have been identified on the intracellular loop of α_6, β_{(1-3)}, γ_2 and π subunits. Phosphorylation of the γ_2 subunit is of particular relevance to zolpidem pharmacology. Ca^{2+}/Calmodulin-dependent protein kinase II (camkII) also phosphorylates γ_2 subunits.9,10 Lastly, extracellular N-linked glycosylation sites are present on all GABA_A receptor subunit N-terminal sequences 5 & 6.10

In summary, through the variety of β-subunits, GABA_A receptors are subject to PKA phosphorylation. The γ_2 subunit consensus regions are the target of PKC & camkII, while the N-terminal sequences are targets of n-linked glycosylation. The majority of these modifications regulate the intracellular trafficking of GABA_A receptors.10

2.1.3 Conclusions:

1) Zolpidem administration selectively affects various regions of the brain in a manner that’s not only dose dependant but also likely to be influenced by non-homogenous receptor density throughout the brain (Chapter 1). In physiologically normal patients, zolpidem decreases neurological perfusion in a variable pattern, proportional to the α1γ2 receptor distribution throughout the brain.

2) Zolpidem specific receptors (GABA_A α1β_{(1-3)}γ2), are subject to various enzymatic modifications through the majority of their subunits.
2.2 NEURODORMANCY / ALTERED RECEPTOR THEORY

Central Hypothesis - Zolpidem normalises hypersensitive suppressive receptor systems.\textsuperscript{11}

The exact mechanism by which zolpidem is able to transiently restore neurological function is still a debated topic within the literature. It is agreed that zolpidem promotes reactivation of anatomically intact but functionally compromised ‘dormant’ neural networks; yet how this is achieved remains a point of contention between the theories analysed within the discussion to follow.\textsuperscript{12} The Neurodormancy Hypothesis was the first put forward in an attempt to explain zolpidem’s effect on disorders of consciousness.\textsuperscript{6}

The initial case which led to the development of this hypothesis was a chronic vegetative patient, who would be roused to consciousness shortly after drug administration, only to return to a sub-conscious state as the drug wore off. SPECT (single photon emission computed tomography) scintigraphy revealed significant perfusion deficits (10 - 25\%) in the left occipital, parietal and posterior frontal lobes as well as the basal ganglia. On administration of the drug, these deficits improved to 5\% in most regions.\textsuperscript{6} Continued administration of the drug over the following years did not result in any attenuation of the effect, quite to the contrary, the patient was able to remain awake for 6 - 8 hours after a single dose as opposed to the original 2 - 4.\textsuperscript{13} Cognitive function, notably memory and communication skills, continued to improve over the following years.\textsuperscript{13}

Considering that (a) after three years without any over neurological changes the patient was assumed to have plateaued in his recovery and was deemed unlikely to rouse from his vegetative state (b) drug response improved over time (c) there was a sudden continuation of functional recovery; it would appear that via action through an unspecified mechanism, zolpidem is able to transiently reactivate regions of the brain. During these active periods, increased functionality stimulates neuroplastic recovery, assisting functional restoration.

2.2.1 Neurotransmitters - Production & Impairment

Brain injury is associated with metabolic disturbances which impair the production of neurotransmitters. The magnitude of these disturbances is related to the extent of initial impairment or loss of consciousness. Brain damage also results in alterations to the expressed subtypes of GABA\textsubscript{A} receptor isoforms as well as the regional expression pattern of associated genes. It is suspected that vegetative state after brain insult is not solely related to tissue destruction, but also to changes in the GABA\textsubscript{A} receptor.\textsuperscript{14}
Neurotransmitters are highly localised by the impermeable nature of the blood brain barrier, therefore the brain needs to synthesise any neurotransmitters required from substrate materials that are able to traverse the blood brain barrier. These restrictions limit the possible reserves of neurotransmitters.15-18

GABA biosynthesis is reliant on an O$_2$ dependent conversion of glutamate. Although GABA production is the most costly O$_2$ dependant biosynthetic pathway, other neurotransmitter productions pathways are also dependent on O$_2$. Tyrosine requires O$_2$ for conversion to dopamine (which may in turn be converted to noradrenaline).11, 19-21 Additionally, tryptophan’s conversion to 5-hydroxy-L-tryptophan (5-HTP), a serotonin precursor, is also an O$_2$ dependant pathway. The supply of glutamate stems from intracellular reserves and local production where NH$_3$ combines with alpha-ketoglutarate (AKG), derived from the Kreb’s cycle (part of the oxidative metabolism pathway), forming glutamate.15, 22 Alternatively aspartate may undergo transamination, yielding glutamate and oxaloacetate.15, 23, 24 Glutamate can also be derived from deamination of glutamine which releases NH$_3$.15, 22, 25 See Figure 2.2 for a summary of these processes.

In ischaemic scenarios, other sources of glutamate are used by the brain e.g. aspartate. This is supported by decreased levels of aspartate which occur in damaged or diaschetic sites, improving when the related functional impairment resolves.15, 26-29 The increase of anaerobic metabolic markers in non-functioning viable tissue, lead Clauss et al. to consider anoxia tolerant vertebrates, where these markers are commonly found to be raised during times of oxygen deprivation. In these animals the onset of ischaemia also triggers the release of GABA, with an associated decrease in neural metabolism and blood flow.15

If ischaemic damage is beyond repair neurons die, this is a risk for all compromised neurons unless they can initiate changes in their metabolic demand. Anoxia tolerant vertebrates circumvent this problem by increasing GABA release in a regulated fashion.15, 30 GABA mediated inhibition facilitates a reduction in the demand of neurotransmitters, lightening the energetic load on a cell.11, 31, 32

A similar process may be encountered when axonal integrity is compromised. Axonal transport is vital to supply enzymes, organelles and substrate for both neurotransmitter production as well as receptor trafficking.11, 33-35 Intracellular calcium is one of the regulators of axonal transport and abnormal calcium homeostasis can trigger massive axonal and neuronal dysfunction.11, 36-40 Axonal damage is a common finding in head trauma where axonal injury can either be focal or diffuse.11, 41 It has been shown that synaptic neurotransmitters such as aforementioned Glutamate and GABA are manufactured in the pre-synaptic region and are dependent on axonal substrate transport.11, 42-44 Therefore synaptic damage or glucose deprivation could also force reliance on depletable secondary substrates for neurotransmitter production as is encountered in ischaemia.11, 23
Figure 2.2: Metabolic pathways detailing CNS neurotransmitters production. Adapted from Clauss RP. Oxygen is a vital substrate for the renewal of all neurotransmitters. Of particular relevance is the reliance of both GABA and glutamate oxygen and glucose. The implications of this oxygen dependence is that during ischaemia, even if local glucose can be maintained, cells cannot utilise it for lack of oxygen-facilitated oxidative metabolism. As neurotransmitters are exhausted, normal neurological function, including consciousness is compromised. Ammonia (NH3), 5-hydroxy-L-tryptophan (5-HTP), L-3,4-dihydroxyphenylalanine (L-DOPA).11,15
2.2.3 Inhibitory Modification

If resource supply is not rapidly reinstated, the available amount of neurotransmitters will rapidly diminish. In these conditions, affected regions need to instigate new pathways by which to maintain inhibition in the face of resource deficits. It is speculated by Clauss et al. that where anoxia tolerant vertebrates achieve this through increased GABA inhibition, in the absence of large amounts of GABA reserves in humans, this is achieved through an increase in the sensitivity of inhibitory receptor systems. Thereby allowing diminished GABA release and a smaller number of GABA receptors to maintain an inhibitory state, promoting resource conservation.\textsuperscript{15, 45} These processes could potentially involve (1) messenger feedback systems specific to ischaemia, (2) readjustment of expressed receptor isoforms, or (3) allosteric shift in existing isoforms to those with greater inhibitory effect.\textsuperscript{15, 45}

After ischaemia resolves and/or axonal damage is repaired, normal regions return to their expected functional levels. Yet in some regions, in certain patients, an unidentified process locks inhibited regions into this dormant state. It is proposed that zolpidem binding to these modified and hypersensitive GABA\textsubscript{A} receptors causes an allosteric shift which transiently reverses hypersensitivity.\textsuperscript{11, 15, 26} It is this post-recovery, ‘lack of functional activity’ state that has been coined “neurodormancy”. Clinical manifestations include unconsciousness, coma, diaschisis and deficits in function specific to inhibited damaged and inhibited areas.\textsuperscript{15}

“Neurodormancy” has been defined by Clauss et al. as “the continued suppression of neurons due to hypersensitive receptors in the face of reduced resource supply”.\textsuperscript{15} For the purpose of this review a modified definition of neurodormancy will be used so that it can be applied to all theories which will be examined. The proposed definition of neurodormancy is “the cessation of normal regional neuronal function, presenting as a reduction in metabolism within neuronal populations which are considered anatomically intact”.

There are distinct similarities between neurodormancy and diaschisis, and in many cases the terms could almost be used interchangeably, there are however some technical differences. Neurodormancy is an umbrella term for decreases in function in anatomically normal parts of the brain, be they remote or around a central insult. Diaschisis refers to remote alterations in function, increased or decreased, distant from a central lesion. The neurodormancy hypothesis asserts that persistent diaschisis is related to neurodormancy and directly or indirectly due to GABA\textsubscript{A} receptor modification.\textsuperscript{11, 15}
Clauss et al.\textsuperscript{15, 46} suspect that it is this unique combination of altered receptor function and zolpidem’s highly specific binding which underlie its rehabilitative action. Stating that “the alteration in the metabolic balance of neurotransmitters can be partially or completely, albeit transiently reversed by the highly selective GABA\textsubscript{A} receptor agonist zolpidem.” Several single-photon emission computed tomography (SPECT) studies have shown that post-zolpidem administration there is a reversal of suppressive functional diaschisis.\textsuperscript{12} Figure 2.3 illustrates one such study.\textsuperscript{47-49} Further case studies report three patients presenting with diaschisis where both the central lesion and the contralateral diaschetic sites improved after drug administration.\textsuperscript{48}

**Figure 2.3:** Neurological perfusion changes as imaged with SPECT scintigraphy in a zolpidem responder patient. Adapted from Clauss RP et al. *The slices presented here are taken at basal ganglia level. The image pair on the left illustrate perfusion before zolpidem administration. Right is post-administration. Note the improvement in all basal ganglia structures. Caudate nucleus (C), Lentiform Nucleus (L), Thalamus (T).*\textsuperscript{6}
In an attempt to narrow down the list of possible receptor modifications behind this phenomenon, Du et al. suggest that phosphorylation mediated through protein kinase A & C might be likely candidates to explain this effect. Phosphorylation does indeed affect GABA$_A$ receptor function, but whether it is relevant in this context is uncertain. Their logic follows from - the expression of the GABA$_A$ receptor $\alpha_1$ subunit is decreased after treatment with flunitrazepam, a non-selective GABA$_A$ agonist. This reduction in subunit expression can be completely reversed by treatment with the protein kinase inhibitor staurosporine. Therefore, GABA$_A$ receptor phosphorylation may be one of the mechanisms that underlie fast structural changes in the receptor. Highly $\alpha_1$ selective agents may lead to structural changes which expose tryptophan or lysine residues on the GABA$_A$ receptor or related proteins that are targets for protein kinases. This may disrupt the altered metabolism resulting in termination of dormancy.

Taken together, the ideas presented here raise two very pertinent questions: (1) what could the nature of this proposed receptor super-sensitising modification be, and; (2) how does the action of zolpidem reverse it?

2.2.4 Commentary

Before delving in to these questions, some aspects of the cited literature need to be addressed. The basis for the concept of GABAergic inhibition in this theory is rooted in the role of GABA within anoxia-tolerant animals, such as certain turtles and the Crucian carp (Carassius carassius). Within these water vertebrates raised levels of GABA is utilised to suppress neurological function in low oxygen conditions. Ionic homeostasis is maintained, and ATP demand is reduced allowing supply-demand coupling to remain intact.

Important differences which question the suitability of applying the same GABAergic model to humans, are that (1) In both of these animals unique metabolic pathways exist to cope with anoxic-ATP production, including buffering lactic acid with shell and bone calcium, or in the case of carp, converting lactate to ethanol and excreting it. (2) The postulate that massive GABA release shortly after focal trauma or ischaemia also serves a protective inhibitory role in humans is more nuanced than one might expect. In these animals ionic homeostasis is maintained due to viable methods of low-oxygen ATP production. Therefore GABA release will act according to the predicted normo-physiological model and inhibit neurological function and reduce energy demand.
In the damaged brain, as discussed in Chapter 1, ionic homeostasis is completely dysregulated within severe and/or focal injuries. Under these conditions GABA release is most likely an unregulated process facilitated by uncontrolled calcium stimulation. Within both marine-vertebrate models of anoxic survival, calcium remains tightly regulated (see Figure 2.4 for differences). More importantly, during failure of ionic homeostasis, GABA becomes excitatory, contributing to neuronal damage within sufficiently damaged regions. GABA will however remain neuroprotective in undamaged regions of brain, thereby slowing the spread of ischaemic lesions.51,52

Considering: (1) the disparities between these regulatory responses during ischaemia and (2) the unlikelihood of a highly controlled hibernative response taking place after focal insult, due to the initial excitatory action of GABA, there seems to be little evidence to point to immediate localised supersensitisation of GABA receptors during the acute phase of injury. Despite this, localised inhibition within the human brain after trauma / ischaemia, specific to regions most significantly affected and their projections is a well described phenomenon which potentially serves to regulate metabolic demand during neuronal repair.53
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![Diagram showing neurological function and perfusion in mammals, turtles, and Crucian carp](image)

**Figure 2.4:** Differences in the major factors determining neuronal survival in mammals, anoxia-tolerant turtles and Crucian carp. Adapted from Nilsson GE & Lutz PL. In general the mammalian brain is poorly adapted to ischaemia and the fluctuations pictured here are typically the result of catastrophic dysregulation. The variations between the turtle and carp reflect different strategies for anoxic survival. Within the turtles neuronal activity is maximally supressed, resulting in a coma like state. Carp neuronal suppression is catered towards continual activity.⁹⁰
2.2.5 Receptor Supersensitisation

The question of the mechanism behind the hypothesised GABA\(_\text{A}\) receptor supersensitisation in this context, has not been adequately described or tested within the literature. Concerning cellular expression of GABA receptors, it’s unlikely that an expressed receptor will be assembled in a “supersensitive conformation”. The primary regulators of inhibitory postsynaptic potentials (IPSPs) are processes which determine the insertion of GABA\(_\text{A}\) receptors into neuronal membranes or modify existing membrane bound receptors.\(^5^4\)

Cellular GABA\(_\text{A}\) receptor trafficking and modification is primarily limited to (1) controlling the number of expressed receptors, through up/down regulation of gene expression and regulation of the intracellular pool of GABA receptors and (2) specific subunit expression patterns, determining the location of the receptor (synaptic vs. extrasynaptic) or agonist binding patterns (3) enzymatic modification of the receptor.\(^5^5\)

A likely causative agent for any form of supersensitisation also needs to interact with zolpidem or vice versa to be relevant to this line of study, limiting receptor modulators to those affecting receptors containing the \(\alpha_1\gamma_2\) subunit pair or related proteins. For the sake of completion, a brief rundown of likely candidates will follow.

Protein Kinase C (PKC)
The hypothesis suggesting that zolpidem induces an allosteric shift, exposing target amino acid residues, resulting in the PKC mediated internalisation of excessively inhibitory receptors is a problematic fit in this context.\(^1^4\)

The major intracellular domains of the GABA\(_\text{A}\) receptor family do indeed contain consensus phosphorylation sites for serine, threonine and tyrosine protein kinases.\(^5^6\) However, although PKC plays a role in GABA\(_\text{A}\) receptor trafficking (whole-receptor internalisation), chloride conductance (decrease) and sensitivity to allosteric modulators (increase), this is not achieved through modification of external receptor domains. Instead, PKC targets GABA\(_\text{A}\) intracellular domains and receptor related proteins.\(^5^7\)-\(^5^9\) PKC phosphorylation consistently causes a reduction in the amplitude of IPSPs in the majority of neuronal cell lines.\(^6^0\)

Neurosteroids
There is complex indirect interplay between PKC and neurosteroids. Neurosteroids such as allopregnanolone are powerful modulators of GABA\(_\text{A}\) receptors, increasing both the frequency of ion channel opening as well as the duration. This action is dependent on G-proteins or protein kinases, as blocking either of these pathways abolishes GABA potentiation by allopregnanolone. The effect of neurosteroids overwrites the anti-GABAergic effect of PKC. Based on these results co-regulation of GABA\(_\text{A}\) receptors by allosteric modulators and phosphorylation can be proposed.\(^6^1\)-\(^6^3\)
Two theories are put forward to explain this co-regulation (1) Constitutively high receptor phosphorylation is a pre-requisite for binding of allosteric modulators; or (2) Agonistic allosteric shift exposes phosphorylation sites to constitutively active PKC which exists in association with the receptor or with receptor associated proteins. Considering that PKC acting in isolation has an antagonistic effect, the second of these seems more likely.64 This would also provide a connection between agonist binding and receptor internalisation, as PKC activation is associated with receptor internalisation through clathrin coated vesicles.58, 65, 66

**Protein Kinase A (PKA)**
The majority of authors seem to agree that PKA phosphorylation acts through the β1/β3 subunits, reducing receptor function through by chloride currents through the receptor.64, 67, 68

**Protein Tyrosine Kinase (PTK)**
Protein tyrosine phosphorylation is fundamental to various cellular signalling pathways and has been shown to modify neuronal functioning through phosphorylation of β2/β3 subunits. Inhibition of these by membrane-permeable PTK inhibitors produce a reversible reduction in the amplitude of inhibitory currents produced by GABA<sub>A</sub> receptors. In contrast to this, intracellular application of PTK yields a progressive increase in GABAergic current amplitude.56

**Extracellular ATP**
ATP release into the pericellular space serves a neurotransmission function through activation of purinergic-2 receptors. It has previously been reported that extracellular ATP inhibits NMDA receptors through allosteric shifts following direct binding.69 Very recent research into ATP as a neurotransmitter has shown that ATP, ADP and AMP also potentiate GABAergic action.70

High levels of ATP exist in the extracellular space as the result of both normal synaptic release as well as due to rupture of cellular membranes following neurological insult. There is growing evidence that normal synaptic GABA release is accompanied by the release of ATP within the same synapse.71-73 The effect of ATP/ADP/AMP on postsynaptic GABA<sub>A</sub> receptors is transient and reversible, requiring concentrations of above 100 μM. For reference, following head trauma extracellular levels of ATP have been shown to rise to 3.46 mM.74 Direct binding followed by allosteric shift is the favoured mechanism due to the fact that neither ADP nor AMP are able to substitute ATP in phosphorylation reactions.70
Before this recent discovery can be added to the accepted literature regarding extracellular GABA\textsubscript{A} potentiation, additional research is required to identify the exact residue(s) to which ATP may bind and what the nature of the allosteric shift is. Until these steps have been elucidated it cannot be ruled out that extra cellular ATP modifies GABA\textsubscript{A} receptors through established intracellular mechanisms. Adenosine phosphates are however unlikely candidates for long term suppression of neurological territories, due to the fact that excessive release is associated with initial trauma. Therefore extracellular ATP levels are expected to return to near normal during constant-state brain damage disorders.\textsuperscript{70, 75}

### Brain-Derived Neurotrophic Factor (BDNF)

The exact effect of BDNF on GABA receptors is subject to conflicting reports. Groups studying the effect of BDNF on GABA\textsubscript{A} receptors found a decrease in surface-expression in cultured neurons.\textsuperscript{76-79} Contrary to this researchers using tissue samples with preserved neuronal activity found a potentiation of GABAergic function in the presence of BDNF.\textsuperscript{80-82} These studies found that BDNF enhances the strength of GABAergic synapses on cultured hippocampal neurons. When these neurons are cultured along with astrocytes the combined action of BDNF and tyrosine Kinase B is sufficient to increase the number of postsynaptic GABA\textsubscript{A} receptor clusters.\textsuperscript{80}

### Calcium/calmodulin-dependent kinase II (camkII)

Increase in camkII activity directly increases the phosphorylation of the \(\alpha_1\) subunit. The net result is an increase in allosteric-modulator binding. In addition to potentiation of benzodiazepine binding, chloride current is also enhanced.\textsuperscript{83}

### Zolpidem Modulation

With regards to post agonist binding phosphorylation (via PKA&C), the end result of which is downregulation of GABA\textsubscript{A} receptors. Theoretically this process could reduce GABAergic inhibition after administration of an agonist, but in practice it has been shown that the uncoupling mechanism following a single brief exposure only shows any measurable effect twelve hours after initial binding.\textsuperscript{84}
2.2.6 Conclusion

So where does this leave the two vital questions surrounding this theory?

(1) What could the nature of this proposed receptor super-sensitising modification be?

According to the literature reviewed here, kinase mediated internalisation and down-regulation of inhibitory receptors is too slow to be a likely candidate behind hypersensitisation. Allosteric hypersensitivity caused by neurosteroids can conceivably be reversed through agonist binding mediated allosteric shift or binding competition. Especially since the α-subunit is involved in neurosteroid binding. There is however no experimental evidence to support this conclusion.85,86

(2) How can the action of zolpidem reverse theoretical hypersensitivity? From the literature reviewed here there seems to be little evidence for a short term (within minutes) response elicited by zolpidem which can effectively reduce inhibition. Whether this conclusion represents the truth of the situation or stems from a flaw in the review process or insufficient literature existing on the problem is difficult to determine.
2.3 FUNCTIONAL DESYNCHRONISATION

Central Hypothesis - Zolpidem normalises pathological synchronous activity.\textsuperscript{26}

Once again the initial conclusions used to form this hypothesis were drawn from experimental evidence taken from a single case report. This particular patient was diagnosed as having plateaued in recovery after a focal stroke until he was found to be responsive to zolpidem.\textsuperscript{26}

2.3.1 Evidence

Pre-zolpidem administration the patient had relatively fluent speech but presented with difficulty comprehending certain words as well as problems with word-finding and semantic paraphasia. In addition to these problems of speech processing, unilateral somatosensory diminution and abnormal gait were also evident. Post-administration the majority of these symptoms resolved spontaneously, accompanied by an objectively measured increase in IQ.\textsuperscript{26}

In what remains one of the most thorough published investigations of a zolpidem responder, Hall \textit{et al.} performed numerous investigations both on and off zolpidem, with the aim of elucidating the exact mechanism behind the miraculous changes. These investigations included SPECT, Magnetic Resonance Imaging (MRI), Magnetic-Resonance-Spectroscopy (MRS) and magnetoencephalography (MEG). The results of the majority of these investigations are presented in Figures 2.5 and 2.6.\textsuperscript{26}

Data processing for the MEG results were performed using the Synthetic Aperture Magnetometry (SAM) method which identifies the spatial distribution of oscillatory power changes between set time periods. This was used in conjunction with MRI to guide measurement locations. Zolpidem was found to have a powerful desynchronizing effect on the enhanced theta and beta activity seen in the ipsilateral cortex in the language-associated and sensorimotor areas (Figure 2.6).\textsuperscript{26}

To clarify, the electrophysiological measurements presented here are not comparable to a standard, unprocessed EEG, where complex voltage fluctuations are measured in real time. These post-processing MEG measurements give the summed amplitude of all measured waveforms during a given time period for each frequency band. The purpose of this is to give the researcher insight into which neuronal frequencies dominate throughout different anatomical regions, within a set time period. In this context, “synchronisation” refers to large numbers of neurons oscillating at the same frequency, and will be measured at greater amplitude on the Y-axis.\textsuperscript{26}
Zolpidem - effect on neurological function & perfusion in brain damage

**Figure 2.5: Multiple anatomical and physiological imaging modalities utilised to determine effects of zolpidem administration.** Adapted from Hall SD et al. SPECT: (a) Pre-administration a significant perfusion deficit is noted in the temporal lobe. (b) Improved perfusion noted in the left temporal lobe after administration. MRI: (c) Stroke foci manifests as a large lesion in posterior portion of the left hemisphere of brain, impinging on language and motor areas. (d) Markings indicate three regions from which MRS and MEG readings were taken. Namely, centre of infarct “A”, perimeter of infarct “C” and region from the contralateral hemisphere mirrored to the infarct centre “B”. MRS: (e) “A” Infarct - absence of typical markers of functional neurological metabolic activity (N-acetyl-aspartate (NAA), creatine (Cr), choline (Ch), myo-inositol (mI)) slight lactate peak is indicative of altered metabolism in remaining tissue. (f) “B” Control - normal metabolic markers. (g) “C” Peri-infarct - Reduction of normal metabolic markers, significant lactate peak is indicative of anaerobic metabolism. MEG: (h) “A” Infarct - No cortical electrical activity. (i) “B” Control - relatively even spread of frequency amplitudes across all bands. (j) “C” Peri-infarct - drastically altered summed frequency amplitudes in the 0 - 10 Hz range, particularly in the theta band (8 Hz peak). Pathological frequencies were prevalent across the entire ipsilateral hemisphere of the lesion, including the dorsolateral prefrontal cortex, parietal lobe, superior temporal lobe and sensorimotor cortex and were not evident in the contra lateral hemisphere. Additionally the ipsilateral sensorimotor regions adjacent to the lesion had an elevated beta frequency oscillation (25 Hz).26

Zolpidem administration caused desynchronisation in peri-infarct regions and no significant effects on the contralateral hemisphere. Placebo testing had no effect. Zopiclone (a non-subunit-selective GABA<sub>A</sub> agonist) administration saw a striking bilateral broadband increase in oscillatory activity in the beta and theta frequency range, enhancing pathological activity.26
Figure 2.6: Effect of zolpidem and zopiclone on at rest cortical neuron firing frequency as measured through spatial distribution of oscillatory power changes. Adapted from Hall SD et al. Blue hues indicate a reduction in oscillatory power, red hues, an increase. Zolpidem (selective GABA<sub>A</sub> agonist) - (a) Theta desynchronisation. (b) Beta desynchronisation. (c) Desynchronisation in peri-infarct dorsolateral prefrontal cortex (DLPFC). (d) Negligible change in contralateral DLPFC. (e) Desynchronisation in ipsilateral supplementary motor region (SMC). (f) No significant change in contralateral SMC theta synchronisation. Zopiclone (non-selective GABA<sub>A</sub> agonist) - (k) Increase in bilateral beta synchronicity within the frontal cortex and (i) SMC. (g-j) Pre- & post- changes following zopiclone administration. Increased synchronicity within the 0 - 30Hz band within all cortical regions.26

From the gathered data, the suppression of pathological slow wave activity, coincident with measured and reported improvements in cognitive and motor function formed the key observations used to propose a new theory.26 It was inferred that the action of zolpidem observed in this patient is related to its unique dose-dependent selectivity for GABA<sub>A</sub> receptors containing the α<sub>1</sub>γ<sub>2</sub> subunit combination. This conclusion is emphasized by the fact that non-selective GABA<sub>A</sub> receptor modulators such as lorazepam, diazepam and zopiclone do not desynchronize resting neural network activity. Instead oscillatory power is enhanced.26, 87

2.3.2 Desynchronisation

Hall et al. speculate that the desynchronizing effect of zolpidem may reflect the differential distribution of α<sub>1</sub>γ<sub>2</sub> subunit containing GABA<sub>A</sub> receptors between specific interneuron subtypes which sub-serve oscillatory activity. Neurons with greater numbers of zolpidem specific receptors will experience greater degrees of inhibition vs. those with fewer α<sub>1</sub>γ<sub>2</sub> GABA<sub>A</sub> receptors. Variable resistance to depolarisation will force desynchronisation of neuronal populations.26, 88
Considering that information transfer in the brain is coded within the unique frequency of different synaptic connections at any given moment, synchronisation across extensive neuronal populations can result in a marked reduction in information transfer. It has been shown that broad elevation in slow-wave synchronous activity between neuronal populations is a common finding in neurological pathologies. Broad elevation in the mutual frequency between cortical regions will reduce the capacity for computational processing via a reduction in complexity of information coding. This would provide an explanation for the cognitive decline observed under pathological conditions.\textsuperscript{26, 89-92}

Event-related desynchronisation (ERD) refers to the decrease in synchronicity between anatomically adjacent neurons when a specific task requires processing, for example being requested to make a specific movement. This is a central phenomenon in normal brain activity\textsuperscript{26, 93} and ERD has been established as a fundamental feature of sensorimotor\textsuperscript{26, 94} and cognitive processing.\textsuperscript{26, 95}

In this patient the high power and persistent nature of pathological oscillations appears to represent an obstacle to adequate ERD. Inability to desynchronise may present a barrier to effective computation in neural networks. Drug induced suppression of this barrier may allow for a return to cognitive performance.\textsuperscript{26}

### 2.3.4 Conclusion

Hall \textit{et al}\textsuperscript{26} do annotate this deduction with the disclaimer that even though their data do support this line of thinking, it is also entirely possible that the observed reduction in synchronous power is an epiphenomenon of increases in neural activation similar to that observed in ERD, and the desynchronisation is simply a manifestation of cortical reactivation through other means. This argument does however only emphasize the interpretation that normalization of the MEG signal permits regular processing; albeit by a different mechanism.
2.4 MESOCIRCUIT THEORY

Central Hypothesis - Zolpidem normalises suppressive neurological networks.\textsuperscript{96}

2.4.1 Anatomical Pathology

The core conclusions of this hypothesis are based on observations made from the anatomical pathologies associated with varying degrees of disorders of consciousness. Autopsies performed on patients with both traumatic and non-traumatic injuries (which lead to impaired consciousness) consistently find widespread neuronal death throughout the thalamus.\textsuperscript{96, 97} Despite these findings, even severe thalamic compromise does not guarantee concomitant diffuse neocortical cell death despite implicating impairment of consciousness. Diminished consciousness after focal thalamic insult only serves to underline thalamic integrative function’s importance in maintaining a functional neurological state.\textsuperscript{96}

This post-injury, thalamic damage is not a homogenous occurrence. Specific nuclei are more prone to insult-induced cell loss than others. The central thalamic nuclei (intralaminar nuclei & paralaminar nuclei) are most typically involved. There appears to be a correlation between grading of insult to these structures and the degree of functional impairment following the injury (Figure 2.7).\textsuperscript{98-100}

This unique pattern of injury-outcome coupling is most like due to the unique geometry of the connections of the central thalamic components. The neurons within these subnuclei have wide point-to-point connectivity across the neocortex and are likely to integrate information from large swathes of neurons. This implies that damage to these structures affects large cortical territories through a relatively small amount of thalamic injury.\textsuperscript{101, 102}

The disproportionate impact due to injury to a relatively small numbers of neurons stems from the vital functional connections the thalamus provides. Neuroimaging and electrophysiological studies reveal selective activation of the central thalamus and related neocortical areas during tasks which entail short-term shifts of attention, sustained vigilance or cognitively demanding tasks or even during prolonged execution of working memory.\textsuperscript{103-106}

In addition to involvement in these forebrain functions, the thalamus is also densely innervated by (1) ascending projections from the brainstem’s arousal systems which modulate the activity of many thalamic, and through it, cortical neurons during sleep-wake cycles (2) feedback loops between itself and the cortex, between itself and the cerebellum, as well as mediating information transfer between these structures (3) descending projections from the neocortex which facilitate goal-directed behaviour and adjust levels of cortical arousal.\textsuperscript{96, 102-104, 107, 108}
Figure 2.7: Comparison of localised thalamic damage and degree of impairment. Adapted from Schiff ND. Red Circle: Damage to the anterior intralaminar structures results in moderate functional impairment. Green Circle: Cell loss extending to the central and medial aspects of the posterior intralaminar nuclei causes severe disability. Blue Circle: Permanent vegetative state was found to arise after the broad loss of central thalamic neurons, including portions of the posterior intralaminar group. Central lateral nucleus (CL), Lateral dorsal nucleus (LD), Lateral posterior nucleus (LP), Medial dorsal nucleus (MD), Centromedian nucleus (CM), Parafascicular nucleus (Pf).96,99

The neurons found within the central thalamus are even more specialised, both anatomically and physiologically. They have diffuse projections to the supragranular layers of the neocortex as well as projections to striatal neurons.109-112 This can be evidenced through the activation of the anterior and posterior intralaminar nuclei along with the mesencephalic reticular neurons which project monosynaptically to the aforementioned neurons during the short-term shifting of attention associated with a forewarned reaction-time test.103 In addition to this, activity in the thalamus has been shown to vary along with activity in the anterior cingulate cortex, pons and mesencephalon.96,104
The central thalamic nuclei are intimately connected with the frontal lobe in a topographically mapped distribution. Almost every cortical processing centre has a corresponding cluster of thalamic neurons linked through corticothalamic connections coupled with indirect links via the frontal cortical-striatopallidal-thalamocortical loop systems. The behavioural fluctuations following insult to either the thalamus or frontal lobe show strong interspecies similarities, both quantitative and qualitative. In rodent lesion studies, damage to the thalamus typically results in severe functional impairment in tasks related to connected cortical regions, whereas lesions to unilateral cortical structures have somewhat more non-specific presentation.

2.4.2 Structural Changes and Recovery

It has been shown that structural remodelling continues long past what is considered conventional windows of recovery. MRI studies on a patient that had been in a minimally conscious state for 19 years after a severe TBI revealed structural alterations in language areas associated with his recovery of expressive and receptive language abilities.

Studies were conducted on this patient using diffusion tensor imagining (DTI), a technique utilised to quantify and visualise the anisotropy (directional scatter) of proton diffusion within the brain. By quantifying the direction and rate of scatter, white matter tracts can be visualised and their integrity determined. Extensive white matter injury and concomitant cerebral tissue loss was noted in both the brainstem and frontal lobes. Repeated longitudinal assessments showed a process of continuous change over time, particularly within the midline cerebellar white matter. A cohort study of severely neurologically injured individuals over the year after initial injury detected similar, continuous remodelling.

The implications of this are that normal recovery after injury is not solely limited to the formation of glial scars and repair to cells which managed to evade apoptosis, but includes an aspect of structural remodelling of white matter connections.

2.4.3 Hypothesis

By combining the anatomical evidence presented throughout this section of the review and others, three broad causative events can be said to behind large scale neocortical dysfunction: (1) widespread destruction of cortical neurons, (2) widespread deafferentation and associated disconnection of neurons, (3) circuit disconnections, where functional disturbances arise due the lack of communication between different processing structures.
As reviewed throughout this text, the first method is clearly irreversible at this stage of scientific progress. Despite these late axonal alterations (presented here) related reconnections may continue to occur after long periods of time. Disturbances at the circuit level are what the mesocircuit hypothesis focuses on. The primary result of circuit compromise is to effectively produce a widespread decrease in background synaptic activity and excitatory neurotransmission. The disturbances referred to here are the same as those mentioned earlier in the text, diffuse axonal injury, global anoxia, multi-focal infarction etc.  

On the neuronal level medium spiny neurons of the striatum have a key role in maintaining activity of the anterior forebrain - their GABAergic inhibitory projections to the globus pallidus interna (GPi), which itself inhibits the thalamus, forming part of a feedback cortical loop where the thalamus once again projects to cortical regions before they themselves project towards effectors. Activation of these medium spiny neurons within the striatum leads to a disinhibition of central thalamic neurons via cessation of GPi inhibitory input. Thalamic activation causes an outflow of thalamocortical transmission and could, in theory, promote rebound of high frequency thalamocortical activity due to the looped nature of these connections. Thalamocortical projections from the central thalamus strongly excite frontal cortical neurons which then in turn project back to the striatum.

A major proponent of this method of cortical activation is recent research revealing that thalamocortical projections to the neocortex have a stronger excitatory effect than connections between cortical neurons themselves. Following this evidence it is not unreasonable to believe that downregulation of thalamic output can be expected to have profound effects across cortical networks.

Neurons from the thalamus itself also drive this excitatory loop. Central lateral and parafascicular nuclei neurons project to the medium spiny neurons of the striatum and diffusely innervate the structure. These thalamic excitatory projections use glutamate as their primary neurotransmitter and along with cortical innervation provide large amounts of excitation to the striatum.

The relevance of this dual excitatory input is that the striatum medium spiny neurons have a high threshold to depolarisation. This locks them into an inactive state in the absence of significant levels of cortical and thalamic input. It is suspected that diffuse brain injury affecting these inputs compromises whole brain function far beyond what the extent of trauma would predict. Figure 2.8 summarises this process.
The primary application of this model is to disorders of consciousness following diffuse brain trauma. The implications of the events illustrated in Figure 2.8 are that frontocortico-striatopallidal-thalamocortical loops are particularly vulnerable to disruption. If one aspect of this loop fails, many structurally sound elements may accompany it. In this manner, even if the thalamus is spared, diffuse neurological injury may still compromise consciousness or significantly impair functional processing. It is precisely in these cases where zolpidem may play a role in restoring consciousness.\textsuperscript{96,123}

Figure 2.8: Proposed mesocircuit model of cortical dysfunction following diffuse brain injury. Adapted from Schiff ND. Medium spiny neurons (MSN) are dependent on significant amounts of input from the thalamus and cortex to reach firing threshold. Regulation of this excitatory system is driven largely by glutamate and dopamine. Activation of the striatum inhibits the globus pallidus interna (GPI) which releases thalamic neurons from GPI inhibition.\textsuperscript{96}

2.4.5 Conclusion

Observations in zolpidem responders report minimally conscious patients who recover language, eating and ambulatory abilities after drug administration. Where EEG analysis reveals removal of pathological low frequency components,\textsuperscript{124} PET reveals frontal and thalamic reactivation (Figure 2.9).\textsuperscript{118}
Although the presented data is somewhat cherry-picked to support this model, while also being limited to widespread diffuse injuries, the theorised role of zolpidem in this framework is compelling. Under normal circumstances medium spiny neurons disinhibit the central thalamus through the reviewed pathway. As MSN activity is compromised due to injury, activity in the central thalamus follows suit and large functional deficits ensue. Zolpidem administration is suspected to directly inhibit the GPi in to a much greater extent than any other structures in this loop. Thereby acting as an artificial striatum, deactivating GPi inhibition and reactivating the thalamus.\textsuperscript{96, 125}

\textbf{Figure 2.9: Changes in cerebral metabolism after zolpidem administration.} Adapted from Brefel-Courbon C et al. \textit{Top: Before Bottom: After. This particular patient was roused from minimal consciousness to full consciousness after zolpidem administration. Note the bilateral improvement in frontal cortex, both thalami and the striatum.}\textsuperscript{118}
There is good evidence to support this conclusion as well: (1) The GABA\textsubscript{A} \(\alpha_1\) subunit is expressed in much larger quantities within the GPi than in any other components of this loop.\textsuperscript{126} (2) The MSNs are uniquely vulnerable to ischaemic damage.\textsuperscript{127} (3) Several zolpidem responders within the literature suffered ischaemic brain damage.\textsuperscript{118, 124, 128, 129} (4) Therapeutic doses of zolpidem have been shown to selectively reduce inhibitory GABA concentrations within the thalamus.\textsuperscript{96, 130}

According to this model, withdrawal of zolpidem as the drug is metabolised and excreted will result in a “circuit breaker” effect and a return to the functionally deficient state. Although in practice this is a slight exaggeration of the rate at which patients return to the impaired state. What is reported in case reports is more of a gradual slide. This can be explained by replacing the circuit breaker analogy with the idea of diminishing feedback. As zolpidem wears off, the crutch which maintains thalamo-cortical loops is removed, but circuit activity will continue for some time, steadily decreasing as feedback loops lose greater amounts of signal with each pass.\textsuperscript{118, 124, 128, 129, 131} This continues until there is no longer enough background stimulation from cortical and thalamic neurons to maintain the “on” state within the striatum’s MSN and the patients slide back to their “off” drug state.\textsuperscript{96}
2.5 CONCLUSION

Presented here are three different theories, which at the time of writing have yet to be proven conclusively wrong or right. Due to each group of authors targeting a slightly different subpopulation of zolpidem responders it is not necessarily the case that the three theories are mutually exclusive. It is entirely possible that aspects of each may be at play in those patients who experience a return of cognitive abilities after zolpidem administration.
2.6 REFERENCES


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Chapter 3

Experimental design
3.1 HYPOTHESES

After review of the literature concerning zolpidem’s paradoxical method of action, it becomes clear that there is little consensus as to how the drug is able to reawaken dormant regions of brain. To complicate this matter, the existing body of evidence is limited to a small number of research/diagnostic tools which have been repeatedly used in numerous studies. An illustrative example of this is that at the time of writing, the changes in neurological activity following zolpidem administration have only ever been numerically quantified through a very limited set of investigations. These investigations include approximate quantification through measures of functional decline or improvement, e.g. Glasgow Coma Scale, Coma Recovery Scale, Rancho Los Amigos Scale, and the Tinetti Falls Efficacy Scale or measurement through digital tools; such as cerebral state monitoring or EEG and its derivatives. The hypotheses tested in this research project aimed to provide novel supporting numerical evidence to aid in determining which theoretical framework is most likely to be correct.

Despite having been well documented, the changes in neurological perfusion after zolpidem administration have only ever been qualitatively studied in humans via SPECT, PET, and fMRI analysis. Numerical quantification of these same perfusion changes have yet to enter the published body of literature. This leads to the primary tested hypothesis around which the experiment was designed:

H₁: Zolpidem administration is followed by a quantifiable increase in brain perfusion in neurologically compromised patients.

H₀: There is an association between zolpidem administration and quantifiable improved perfusion in neurologically compromised patients.

Contingent on measurement and verification of zolpidem’s paradoxical effect within this sample, a secondary hypothesis can be substantiated through additional analysis of data arising from testing the primary hypothesis:

H₁: Post-zolpidem administration changes in perfusion show preference to specific neuroanatomical regions.

H₀: Post-zolpidem administration perfusion changes show no regional specificity.
Due to the sparse nature of zolpidem responders (see Chapter 1 for review), it has been a recurring problem for single research teams to gather sample groups of sufficient magnitude to make generalisations as to which neurological pathologies are most likely to respond to zolpidem facilitated/assisted rehabilitation. As a result of this, the majority of zolpidem responder literature takes the form of single case reports. The largest study to date used in excess of 100 patients but only reported generalised findings - a dangerous practice when the minority of your sample set is likely to show any improvement. The final tested hypothesis was:

**H₁:** Zolpidem responders can be isolated to specific diagnostic groups.

**H₀:** Zolpidem responders aren't confined to clearly delineated diagnostic groups.
3.2 MATERIALS AND METHODS

3.2.1 Project Overview

This retrospective, analytical study consisted of multiple components, combining newly generated data with that from previous studies (Figure 3.1). Over the course of more than a decade, in excess of 400 patients have been examined by a team in private medical practice, lead by Dr H.W. Nel. These consultations were offered free of charge in hopes that zolpidem might offer improved management of wide varieties of brain damage, leading to new medical discoveries. After consultation and the discussion of the potential risks/rewards, the vast majority of these patients were administered zolpidem and the effects of the drug were recorded.

Selected patients (primarily responders) were given the choice to enter into a second research project which would entail two SPECT scans, before and after zolpidem administration. Dr Nel’s case studies have not previously been published in any fashion. The SPECT scans were published by Nyakale et al.\textsuperscript{13} combining qualitative brain scan analysis with functional testing in form of the Tinetti Falls Efficacy Scale.\textsuperscript{14}

These two data pools were combined in this clinician-blinded retrospective analytical study. Both the original unpublished patient response reports as well as a new semi-quantitative re-analysis of the SPECT scans were examined in unison to yield original data.
Figure 3.1: A schematic outline of this research project. Yellow box indicates original research component. “Unusable scans” includes any patient in which a complete set of Pre- and Post- scans weren’t available or data had become corrupted during storage. “Missing scans” are defined as those patient scans which were no longer retrievable from Pretoria Academic Hospital’s central archive.
3.2.2 Patient Records

After having successfully cleared an ethical audit, Dr H.W. Nel and his family practice in Springs have been collecting private records on the effects of zolpidem administration to brain damaged patients for over a decade. These patient logs numbering more than 400 have not been used in other research studies, and due to the time constraints of running a private practice combined with the data existing in physical paper form, they were likely to remain unprocessed and unpublished without external assistance.

Of these 400 patients, 40 are of particular relevance to this M.Sc. project. These patients were examined by the private practice team as part of a SPECT study performed in 2008 by the Department of Nuclear medicine, Pretoria Academic Hospital. Ethical clearance for use of the patient data was granted by the University of Pretoria’s Faculty of Health Science (80-2008). The full data set (n = 400) will be analysed and reported on separately as the largest published zolpidem case study following the completion of this M.Sc. project.

All patients examined as part of this original zolpidem data collection process were required to sign an informed consent form. This includes the 40 patients of interest. These 40 also signed a second informed consent form allowing the Department of Nuclear Medicine to utilise their data for research purposes.

Dr H.W. Nel gave this project written consent to access his records. New consent was not obtained from individual patients as the original consent signed for Dr HW Nel’s study combined with his written permission as well as the ethical clearance granted to the 2008 study as well as this project’s own ethical clearance (46-2014) was determined to be sufficient to legally and ethically utilise the data at hand.

3.2.3 Phase 1: Data Capture

The first phase of this study entailed collecting, digitising and capturing data from this vast case report archive. 400 patient files were digitised by a research team coordinated by the primary investigator (M.Sc. candidate). Once the data was in digital format, the research team began the process of capturing this data. Specific data points captured can be found within Table 3.1. The data capture phase aimed to intentionally capture as much data as possible. This inevitably resulted in data being captured which was superfluous to this study’s particular research goals, however it was decided that this process would save both time and the financial resources of future studies that may wish to utilise this data source in a different manner.
Zolpidem - effect on neurological function & perfusion in brain damage

Table 3.1: Data Capture Categories.
Asterisk “*” Indicates data not directly relevant to this project’s research questions.

<table>
<thead>
<tr>
<th>Data Capture Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Surname and Initials*</td>
</tr>
<tr>
<td>Date of Birth</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Coma State</td>
</tr>
<tr>
<td>Date of Incident</td>
</tr>
<tr>
<td>Date of First Visit*</td>
</tr>
<tr>
<td>Date of First Administration</td>
</tr>
<tr>
<td>Initial Dosage</td>
</tr>
<tr>
<td>Initial Reaction</td>
</tr>
<tr>
<td>Other Medication - Pre</td>
</tr>
<tr>
<td>Date of Follow-up*</td>
</tr>
<tr>
<td>Current Dosage</td>
</tr>
<tr>
<td>Current Reaction</td>
</tr>
<tr>
<td>Other Medication - Post</td>
</tr>
<tr>
<td>SPECT Scan performed</td>
</tr>
<tr>
<td>Date of First Scan</td>
</tr>
<tr>
<td>Other Investigations Performed*</td>
</tr>
<tr>
<td>Date of Other Investigations*</td>
</tr>
</tbody>
</table>

Patient Surname and Initials
This is logged purely to keep track of individuals through the different components of the study. This was the most efficient way to refer to a specific patient in the event that additional information needed to be requested. Patient names also provide a starting point from which to search for information on Pretoria Academic Hospital’s database, since many of the files captured did not note this information.

Date of Birth
Age is known to affect the pharmacokinetics of drugs. Age is also a determining factor in the recovery from brain damage and could influence the end result of zolpidem intervention.

Gender
Capturing gender information allowed for the comparison to be made between how zolpidem affects recovery from neurological insult in male vs. female populations.

Date of Incident
By logging the date of the initial incident we are able to develop a clearer picture of the required time window after which zolpidem administration becomes feasible for use in the management of neurological pathologies.
Date of First Visit / First Administration
Combined with the date of incidence this data can be used to establish how long after initial incident zolpidem intervention becomes effective.

Initial / Current Dosage
Required for comparison with the reaction following administration.

Initial / Current Reaction
The changes in patient signs and symptoms following initial administration. Both patient and clinician reported. Some cases include external specialist reports.

Other Medication Pre / Post
This information allows for an avenue of inquiry if anomalous results arise from the data.

SPECT Scan Performed & Date of Scan
Vital administrative information for the second leg of the study.

Other Investigations Performed / Date of Other Investigations
Although not of direct interest to this study, capturing this data will generate additional lines of inquiry for future researchers.

Inclusion criteria for patient selection from the available sample:

Primary Criteria
- Patients that signed the informed consent form. Failure to have done so resulted in automatic exclusion.
- Patients that had a SPECT scan performed. Patients whom signed the necessary documentation and had a SPECT scan done are automatically included in the data capture phase.

Secondary Criteria
- All fields marked with a section sign (§) were designated as vital. Any given patient file had to fulfil the primary selection criteria as well as having sufficient data to complete at least one of the vital fields. If there wasn’t enough data or the data given wasn’t of significance these patient files will also be omitted from the study.
3.2.3. Phase 2: Nuclear Medicine Component

Where typical anatomical imaging techniques such as X-ray, traditional MRI and CT scanning techniques typically only image the anatomical morphology of the brain, they are limited when it comes to providing data regarding physiological activity. This enquiry was focussed on metabolic changes and their accompanying perfusion fluctuations. As such a scanning methodology is required that enables the researcher to track blood flow.

Brain SPECT (single photon emission computed tomography) is a useful tool in the evaluation of a wide spectrum of neurological disorders. What sets SPECT apart from conventional imaging techniques is the ability to provide an estimate of regional cerebral blood flow. Modern processing software even allows for quantification of recorded changes.15

Neurological activity/metabolism and therefore resource utilisation are closely linked to regional blood flow (Chapter 1). As the resource demand of a particular region goes up, blood flow increases. By taking advantage of this physiological principle SPECT enables physicians and researchers to obtain information that is generally unobservable using conventional imaging methodologies.15 Cerebral areas which receive poor afferent signals or have diminished efferent activity become hypofunctional. This processes is associated with a decrease in metabolism and thus perfusion. The end result is a decrease in tracer uptake in SPECT imagery.16

To cross the blood brain barrier a neurological tracer needs to be relatively small (<500 Daltons), lipophilic and have no significant charge.17 The typical radiopharmaceutical (also known as a tracer) used for neurological SPECT scans is technetium 99m exametazime (99mTc HMPAO). Tracers usually consist of a radioactive isotope bound to a radioligand. In this case these entities refer to Technetium-99m (radioisotope) and exametazime (radioligand) (Figure 3.2). Exametazime is chemically known as hexamethylpropyleneamine oxime (HMPAO) and is a lipophilic amine. Under normal vascular conditions it is able to cross the blood brain barrier and distribute according to regional cerebral blood flow.15 Once across the blood brain barrier, environmental pH favours its conversion to a charged conformation thus temporarily trapping the tracer on the brain’s side of the blood brain barrier. This principle results in variable accumulation of tracer in the brain, with greater accumulation where blood flow is the greatest.15
Radioactive decay of the tracer emits gamma rays which gamma cameras detect by measuring scintillations which arise due to gamma sensitive crystals connected to photomultipliers. Eventual processing is achieved through signal amplification and reconstruction by which a perfusion map is constructed. This unique property of the tracer allows for mapping of the perfusion and metabolism of gray matter in the brain. Activity is delineated within the cerebral hemispheres, cerebellum, thalamus and basal ganglia and thus their activity can be approximated.

Traditional indications for brain SPECT imaging include: suspected dementias, schizophrenia, traumatic brain injury, localisation of epileptic foci and the evaluation of cerebral vascular disease.

The majority of zolpidem perfusion studies rely on qualitative clinician reports to evaluate perfusion changes. At the time of writing there does not seem to be a concerted study which has attempted to quantify perfusion changes following zolpidem administration using post-processing of SPECT scan data. Quantitative comparisons, be they absolute or relative, allow a metric for different research groups to compare their findings. Quantification also provides another measure through which the beneficial effects of zolpidem (or lack thereof) can be established.
Study Design

This portion of the study aimed to extract novel data from existing information gathered as part of a previous research project, namely the previous nuclear medicine study. The scans of arising from the initial were conducting using the following inclusion/exclusion criteria:

Inclusion criteria
- Patients with brain damage or that are semi-comatose.
- Brain damaged and semi-comatose patients that have been in their present state for 18 months or longer.
- Patients which are currently taking zolpidem.

Exclusion criteria
- Patients that cannot complete a brain SPECT scan. Typically this is due to uncontrolled muscle activity or anxiety.
- Pregnant or breast-feeding females.
- Healthy volunteers.
- Patients participating in other research studies.
- Patients under the age of 18 years.
- Patients whom have exceed their safe annual radiation exposure.
- Patient presenting with any of zolpidem’s known contraindications. Contraindications for zolpidem include: obstructive sleep apnoea, acute pulmonary insufficiency, respiratory depression, myasthenia gravis, severe hepatic impairment, pregnancy and breast-feeding.

3.2.4 Previous Nuclear Medicine Contribution

The initial experiment carried out by the Department of Nuclear Medicine, took the form of a prospective study which was performed to observe the neurological and scintigraphic changes that occur following the administration of zolpidem to brain damaged and semi-comatose patients.

Referring physicians were notified in writing by the principal investigator of the study with the aim of requesting the referral of patients which were deemed eligible for the study.

Once patients indicated an interest in being part of the study as well as being deemed eligible, the protocol was explained to them and their guardian/caretaker and informed consent was obtained by a member of the research team.

Each patient underwent full neurological and clinical examination before and after drug administration.
Neurological evaluation

Neurological evaluation included assessment of cognitive and emotional status, speech and communication capabilities, function of the cranial nerves, motor, sensory and coordinative functions as well as autonomic reactions.

Clinical Evaluation

General clinical investigation included a complete history of various disorders such as: heart, blood pressure, respiratory system, digestive system, kidneys, bladder, reproductive organs, nervous or mental complaints, eyes ear, nose or throat disorders, disorders of the skin, muscles, bones, joints limbs or spine, diabetes, cancer or tumour growth of any kind as well as a history of tropical diseases.

Patient history of previous tests and examinations such as X-rays, ECG, EEG, MRI, CT scans were taken into account, especially when considering if additional radiation exposure would entail a health risk. History obtained included previous hospitalisations and drug use.

Final patient examination include operation scars or skin lesions, thyroid and lymphatic glands, ear disease and inspection of any deformation or physical abnormalities. Assessment of cardiovascular-, respiratory-, gastrointestinal-, central nervous- and genitourinary system were also performed.

If a patient presented with any significant negative clinical changes or side effects following the study that patient was removed from the study, treatment discontinued and symptoms managed.

Study Procedure at Each Patient Visit

Off zolpidem scan: After fasting overnight, patients arrived in the Nuclear Medicine Department and were examined as laid out above. If the patient was deemed fit for the study an intravenous injection of 370 MBQ of tracer ($^{99}$mTc-HMPAO) was given. Patients were required to refrain from ingesting caffeine or alcohol for 24 hours before the scanning procedure.

Scans were conducted in a quiet, dimly lit room 1 hour after tracer injection with the patient in a supine position using a Siemens signature series Ecam Dual Head Gamma Camera. The imaging parameters were a 128 x 128 matrix, 360 degrees with 3 degree stops, 20 seconds per frame and less than 19 cm radial distance at a 140 keV photo-peak symmetrical 20% window using low energy high resolution collimators.

On zolpidem scan: The fasting patient arrived in the Department and a clinical and neurological evaluation followed. 10 mg was given per os. After an hour the same procedure as laid out previously was repeated.
Images arising from the scanner were digitally reconstructed with filtered back projection using a Metz filter. All images were reformed into axial, coronal and sagittal views and viewing was conducted using the software provided by Siemens. Specialists performing the scan analysis were blind as to which scans were pre and which was post during the analytic phase of the initial study. Scan analysis entailed identification of any perfusion changes between the presented pair of scans.

Patient follow up after the procedure was in the form of regular routine follow up with the family physician.

3.2.5 M.Sc. Study Contribution

This component of the study aimed to provide novel data in the form of software computed semi-quantification of the perfusion changes associated with zolpidem intervention. This can then be combined with the relevant patient data.

The Siemens software suite accompanying the Signature series dual head camera has the capability to calculate perfusion intensity (using scintigraphic concentration for a given region). This feature was used to provide a relative or semi-quantitative measure of perfusion. Although this method does not allow for objective or absolute perfusion measurements as more invasive techniques would, it does allow relative quantification perfusion changes to be calculated between pre and post scans.

Inclusion Criteria
- Patients that had SPECT scans performed according to the original Nuclear Medicine Protocol.
- Complete scan data remained available on the Pretoria Academic Hospital network (both Pre and Post).

Exclusion Criteria
- Stored patient scan data is corrupt, incomplete or missing.
- Clinical private practice patient data was not available or complete.

The existing database of scans was reprocessed by a single technician (M.Sc. candidate) to circumvent inter-observer error. Measurements were made after training by- and under supervision from- the staff of the Department of Nuclear Medicine. The sequence of events involved in this form of processing entails spatially orientating the pre/post scans to achieve the closest matched configuration possible. Following this, raw images are run through a Metz filter and necessary image parameters are adjusted to where the two images become comparable.
The original specialist reports were used to identify specific regions of interest. Fine anatomical alignment of the pre/post scans allows for comparison of the same region pre/post scan. Prior to measurement the exact anatomical region of interest was located using three dimensional perfusion maps. The software measurement tool was used to measure the clinician identified lesions (Figure 3.3) by using fixed shape measurement tools to draw regions of interest (ROIs) at the most visible point of individual lesions within the transverse slice. Great care was taken to measure the same anatomical region pre/post. Table 3.2 lists the data gathered from each measurement.

Figure 3.3: Illustrative measurement of two regions of interest on a pre-scan. NOTE: MRI scan (greyscale) is for anatomical illustrative purposes only. Top MRI image’s localisation line is slightly inferior to the true anatomical site presented on the corresponding SPECT image.

During the measurement process, the size of the measured ROI will be noted to ensure that equally large segments of brain are being measured between sets of scans.

Average perfusion values for a given region will be utilised as the primary measure of perfusion. Minimum and maximum perfusion values are subject to enormous variation due to the fact that if a single voxel presents as an outlying value in either direction, the maximum or minimum value will simply be representative of that one voxel, as opposed to the whole region measured. Utilising average values presents a truer reflection of the actual perfusion changes within a given region.
Table 3.2: SPECT Data captured with each measurement.  
Region of interest (ROI).

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>Clinician Findings</th>
<th>Pre or Post scan</th>
<th>Whole brain perfusion at ROI</th>
<th>Minimum perfusion value within ROI</th>
<th>Maximum perfusion value within ROI</th>
<th>Average perfusion value within ROI</th>
<th>Standard deviation of perfusion within ROI</th>
<th>Size of ROI</th>
</tr>
</thead>
</table>
3.3 VARIABLES

3.3.1 Variables of Patient Preparation

Patient Preparation Protocol
There is good reason for the strict preparation protocol followed in the original study. 
$^{99m}$Tc-HMPAO completes the majority of its distribution to the brain with the first two
minutes after administration. It's most rapid decay occurs in the first hour after
administration where remaining activity is at 84% of the initial quantity. After the first
hour of decay, a mere 10% of radioactivity activity is lost within the following 23
hours. By waiting an hour before initiation of the scanning protocol, studies can be
conducted within this stable window. Decreasing both inter- (between different
patients) and intra- (different readings within the same patient) variation. Tracer
concentration was kept constant throughout the study as measured through effective
dose of radiation.\textsuperscript{17}

Drug Administration
Zolpidem administration needs to occur before tracer administration due the fact that
once the tracer has distributed through neurological tissue it remains fixed. Since
initial tracer distribution is activity dependent, any modifiers to neurological activity
need to be administered prior to tracer administration. By this same logic tracer
administration needs to occur when the administered modifier, in this case, zolpidem
has reached maximum effect. This is also the why sedative administration after
tracer distribution has occurred will not affect the resultant scintigraphic
measurement.\textsuperscript{17}

Natural Variations in Brain Perfusion
The purpose of the darkened, quiet room in which patients are prepared for the
scanning procedure is to minimise neurological activation after tracer administration.
Cortical processing will deviate blood to the activated region, skewing tracer
uptake.\textsuperscript{17}

Whole brain perfusion is also subject to natural variation. Intra-subject whole brain
perfusion has been reported as varying between perfusion scans by 1.3%\textsuperscript{23} and
3%,\textsuperscript{24} for normalised measurements, or 8.3%\textsuperscript{25} and 14.8%\textsuperscript{23} for non-normalised
measurement. Normalisation refers to the process of comparing all measurements to
what is presumed to be a stable portion of brain within each individual data set. This
is discussed in greater depth under "normalisation" (to follow).
3.3.2 Scan Acquisition Variables

Movement
Patient movement is an important factor in virtually any imaging procedure. Sedation was employed in patients where their neurological insult prevented them from remaining still for the time within the camera.

Distance between camera heads and patient
Distance of the camera head from the patient affects the resultant image generation. The closer the heads can be positioned to the patient the higher the quality of the final image. As such, radial distance was kept under 19 cm and closer wherever possible. This is a source of inter-patient variation, but kept relatively constant for each patient between the two sets of scan.26

3.3.3 Processing Variables

Processing Induced Variables
The measurement of scintigraphic intensity (perfusion) is not significantly affected by the post processing applied on the users end. To confirm that this was not a notable variable which needed to be accounted for, the effect of user-processing was tested using wildly different post-processing parameters to ascertain the extent of possible user induced error with regards to processing preferences.

A single measurement area was identified and a set of readings were taken. The scan was reprocessed with increasingly more extreme post-processing adjustments. Provided the same filtering methodology (Metz) was utilised, the resultant measured change between differently processed scans utilising the same ROI were found to be <1% (Appendix A). Similarly processed scans were found to induce virtually no appreciable error in the final comparison. As such, this rules out any subjective errors that may occur due subtle differences in scan processing between pre and post scans.

Normalisation
Within the field of nuclear medicine it is common practice when conducting semi-quantification studies to utilise a stable region of the brain as a control within each scan. This allows for the majority of inter-scan variables, e.g. variation in camera head distance, tracer uptake, natural perfusion fluctuation to be ruled out by expressing the final measurement as the ROI divided by the Control Region. The fundamental assumption is that the control region’s perfusion will remain stable, or nearly so, throughout the study. The cerebellum is the usual choice in these studies, with most authors assuming it to be relatively free of pathological changes particular to their study. Using whole brain perfusion as a suitable control has also been proposed.27, 28
This is where this study design ran into a problem. As shown in Chapter 1, the distribution of GABA receptors throughout the brain is anything but equal. Therefore zolpidem administration will affect perfusion to different extents throughout the brain. This results in differential degrees of inhibition between the cerebellum and other cortical areas. This problem is compounded by the fact that in zolpidem responder patients, cortical reactivation may be associated with varying degrees of distal normalisation. A particularly problematic variant of this is cerebellar normalisation.

Recent research has shown that the classical view of the cerebellum as purely an adjuvant to motor processing is a gross oversimplification of its full role within neurological networks. Neuronal inputs and projections have been found between the cerebellum and the thalamus, as well as the cerebellum and nearly all cortical processing areas. So much so that cortical projections are topographically mapped within the cerebellum. Through these connections, any normalisation or “reactivation” of the cortex or basal ganglia can be expected to reactivate associated regions of the cerebellum. If the cerebellum is used as a control in these cases, ratioing it and the region of interest will result in a diminished reported change.

A similar argument can be made for using whole brain measurements as the control. In cases which may respond with widespread reactivation of cortical networks comparing, whole brain perfusion can also be expected to increase. Therefore comparison between whole brain and ROI may also reduce the overall sensitivity of the testing procedure.

The workaround to this problem is that data will be presented in the following chapter from both forms of normalisation as well as without any normalisation.

### 3.3.4 Data Processing

Combining the two data sets will allow for both quantitative, in the form of measured perfusion and qualitative self- and clinician-reported changes to be combined. This process provided two distinct forms of insight into the dataset being used for this research project.

By combining the two phases of this study, namely the patient database with the SPECT scans, it enabled the investigation of the connection between zolpidem administration, and any neurological changes/symptomatic improvements accompanying it.

Within the observational patient reports, patients were examined both before and after zolpidem administration, first to establish a baseline and secondly to observe what changes occur following administration.
3.4 ETHICAL CONSENT

The principal investigator (M.Sc. candidate) has provided the University of Pretoria’s, Faculty of Health Science’s, Research Ethics Council and M.Sc. Committee with all documentation they require. Both committees were kept abreast of any changes the other required and up to date protocols were maintained with both committees. Clearance from the necessary authority within Pretoria Academic Hospital was sought before the study commenced and this documentation was submitted to the Ethics Council.

Due to the unconventional ethical aspects of this study, in that two different existing datasets were used to generate new data, combined with the fact this data stemmed from a vulnerable population - this protocol was submitted for ethical comment and clearance prior to submission to the M.Sc. committee.

The final result of these procedures is that this study was granted full Ethical and Masters committee clearance.

3.4.1 Patient Risk - Data Capture

This study is entirely retrospective and used existing records in a novel manner. Therefore the only risk to participants lies in the data capturing phase of the study, in the form of a breach of confidentiality. As such all reasonable measures were taken to ensure that patient files were kept secure within the Department of Physiology at all times, and that access to the files was limited to individuals assisting with the project. Access to the records was contingent on signing a non-disclosure agreement.

Prior to inclusion in the sample, all patients signed two consent forms -
   i) before being examined by Dr. Nel.
   ii) prior to being entered into the nuclear medicine study.

In compliance with the Declarations of Helsinki the initial nuclear medicine study took care to inform the patient or the patient’s next of kin/guardian both verbally and in writing of the nature of the study, its aims, methods, anticipated benefits as well as their right to abstain from participation or withdraw at any time without fear of prejudice in the use of health services.
The patients or legal/authorised guardians of patients who were willing to participate in the study were required to sign a consent form which was written in comprehensible English and explained to patients in local vernacular. This properly executed written informed consent form complied with the good clinical practice guidelines and was obtained from the parent/guardian before entering any participants into the study. A signed copy is presently maintained within each patient’s file.

3.4.2 Patient Risk - SPECT

It is important to note that this section is only relevant to the previous SPECT study and due to the retrospective nature of this project direct physical risks to the patients are not applicable. To reiterate, this study examines existing patient data in a new way, or for the first time (in the case of Dr Nel’s patient files). As such this study does not present any physical risk to patients. This section is included to cover the original risks associated with the research this study was based on.

The administration of zolpidem may result in side effects such as diarrhoea, nausea, vomiting, headache, confusion, hallucinations and amnesia (Chapter 1). If these or any other side effects were reported, subjects were removed from the study and treatment was considered based on the specific side effect.

The principal investigator only utilised the processing stations within the Nuclear Medicine Department at pre-arranged times ensuring zero interruption to the workflow of the specialists within the Department. Conscious effort was made to ensure that the presence of the researchers did not hinder staff duties.

As far as the ethical aspects involved with the previously collected data are concerned:

The administration of radiopharmaceuticals involves a small injection through an intravenous line. Insertion of the intravenous line may feel like a pinprick. The patient does not feel anything related to the radiopharmaceutical itself. The radiation dosage is small enough not to have an effect on the normal physiological processes of the body. Radiation exposure associated with $^{99m}$Tc HMPAO is very low, an effective dosage of 5.6 mSv. To relate this radiation dose to more conventional imaging techniques, this figure is roughly equivalent to a barium enema and less than a chest CT. $^{34}$

Claustrophobic patients may feel some discomfort while lying within the enclosed space of the camera. There are no excessively loud noises associated with this scanning methodology. Patients may experience some discomfort while lying in the same position for the duration of the scanning process.
All necessary precautions were taken with thorough quality assurance of the radiopharmaceuticals and gamma cameras to minimize any discomfort to the patients.
3.5 REFERENCES


Chapter 4

Results & Discussion
4.1 INTRODUCTION

Forty patients had data of sufficient completion to qualify for the SPECT analysis leg of this research project. No exclusions were made based on incomplete patient data yet some were made at the scan processing stage due to corrupted scan data. These exclusions will be mentioned in the relevant discussions.

Statistical analysis was conducted utilising IBM’s SPSS software by Dr L. Fletcher and Mr A. Masenge from the Department of Statistics, University of Pretoria. Various statistical techniques have been employed within the analytic process including, Descriptive Statistics, Crosstab tables, Wilcoxon Signed Ranks Test and in one case Student’s Test. The primary factors influencing which test was chosen for a given data set were the nature of the distribution of the given data (non-normally distributed unless otherwise indicated) as well as the available number of samples.

4.2 SAMPLE DESCRIPTION

Table 4.1 contains the information pertaining to the patients within the studied sample as a whole. It cannot be sufficiently stressed that the sample is in no way random with regards to individual response to zolpidem. A clear sampling bias was found within the clinician gated phase of the study. Considering that only 6 - 10%¹⁻³ (Chapter 1) of a random population of brain damaged individuals can be expected to react favourably to zolpidem, the 34 out of 38 (89.5%) patients (2 patients not included due to unlisted zolpidem reaction) with a known positive response to zolpidem confirms sampling bias. Randomness can still be assumed within this sample when analysing the quantitative perfusion changes within each patient. This factor was entirely unknown prior to entry into the study.

This bias in patient selection shifts the focus of the study from detecting the differences in the reaction to zolpidem in responder patients vs. non-responders, to analysing the different reactions to zolpidem administration within the brains of responders. The implication of this unique sample is that this project involved a larger number of zolpidem responders than any other published work to date, with the possible exception of Du, B. et al. 2014,⁴ who do not explicitly state the number of responder patients involved in their study.

The majority of the patients in this sample could be grouped into three categories by their specific aetiological mechanism of brain injury (Table 4.1), Trauma (n = 6), stroke (n = 15) and anoxia (n = 4). The remaining patients had brain damage arising from a diverse array of sources and were grouped together as “Miscellaneous” (n=13). Within this group only two aetiologies were represented by more than one patient, two patients diagnosed with Parkinson’s disease and two with Cerebral
Palsy. All patients within the sample had at least six months or more elapse since the onset of their neurological symptoms or the primary damaging event. The mean age of the sample was 39.7 years (Standard deviation = 24.9) and the distribution of the sexes was found to be primarily male, 80% vs. 20% female.

Unlike many other zolpidem studies\(^2, 4, 5\) (see Whyte, J. et al. 2014 for review\(^5\)), this research project did not specifically focus on patients with overt disorders of consciousness (\(n = 2\)). The vast majority of the sample group were fully conscious individuals whom responded to zolpidem not necessarily with elevations in levels of consciousness, but rather functional improvements.

**Table 4.1: Sample population sorted by diagnostic code.**
Zolpidem responders indicated with a superscript plus (\(^+\)). Mean age = 39.7 (SD = 24.9). Sex distribution: 80% Male (1) 20% Female (0). Diagnostic Coding: 0 represents miscellaneous pathologies defined as <2 cases in the sample group. 1 represents trauma patients. 2 represents stroke. 3 represents anoxic injury. For clarification on zolpidem Reaction coding see Table 2. Level of consciousness (LoC), Reaction (Rxn), Fully conscious (FC), Minimally conscious state (MCS), Waterhouse–Friderichsen syndrome (WFS), Reye Syndrome (RS), Motor Neuron Disease (MND), Hydrocephalus (HC), Intra-uterine (IU), Herpes Encephalitis (HE), Febrile Convulsions (FC), Motor Vehicle Accident (MVA).
<table>
<thead>
<tr>
<th>Designation</th>
<th>Age at first scan</th>
<th>Sex</th>
<th>LoC</th>
<th>Cause of Injury</th>
<th>Diagnostic Code</th>
<th>Zolpidem Rxn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1*</td>
<td>16</td>
<td>0</td>
<td>FC</td>
<td>Brain Haemorrhage</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Patient 7*</td>
<td>52</td>
<td>0</td>
<td>FC</td>
<td>Multiple sclerosis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Patient 8*</td>
<td>10</td>
<td>1</td>
<td>FC</td>
<td>WFS</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Patient 9*</td>
<td>47</td>
<td>1</td>
<td>FC</td>
<td>Multiple Sclerosis</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Patient 11</td>
<td>42</td>
<td>1</td>
<td>FC</td>
<td>Psychiatric disorder</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>Patient 13</td>
<td>46</td>
<td>1</td>
<td>FC</td>
<td>Dystonia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient 21*</td>
<td>30</td>
<td>1</td>
<td>FC</td>
<td>Cerebral Palsy</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Patient 23</td>
<td>10</td>
<td>1</td>
<td>MCS</td>
<td>RS &amp; Brain Haemorrhage</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient 26*</td>
<td>65</td>
<td>1</td>
<td>FC</td>
<td>MND</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient 27*</td>
<td>4</td>
<td>1</td>
<td>FC</td>
<td>HC &amp; Brain Haemorrhage</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Patient 28*</td>
<td>91</td>
<td>1</td>
<td>FC</td>
<td>Parkinson's</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Patient 34*</td>
<td>71</td>
<td>1</td>
<td>FC</td>
<td>Parkinson's</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Patient 40*</td>
<td>4</td>
<td>0</td>
<td>FC</td>
<td>Cerebral Palsy</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Patient 2*</td>
<td>23</td>
<td>1</td>
<td>FC</td>
<td>Trauma MVA</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Patient 3*</td>
<td>29</td>
<td>1</td>
<td>FC</td>
<td>Trauma MVA</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Patient 14*</td>
<td>41</td>
<td>1</td>
<td>FC</td>
<td>Trauma</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Patient 29</td>
<td>11</td>
<td>1</td>
<td>FC</td>
<td>Trauma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Patient 31*</td>
<td>63</td>
<td>1</td>
<td>FC</td>
<td>Trauma</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Patient 33*</td>
<td>6</td>
<td>0</td>
<td>FC</td>
<td>Trauma</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Patient 4*</td>
<td>51</td>
<td>0</td>
<td>MCS</td>
<td>Stroke - Brain Stem</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient 5*</td>
<td>61</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Patient 10*</td>
<td>52</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Patient 12*</td>
<td>22</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient 15*</td>
<td>35</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient 18*</td>
<td>25</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient 19*</td>
<td>9</td>
<td>1</td>
<td>FC</td>
<td>IU Anoxia, Later Stroke</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient 22*</td>
<td>43</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient 24*</td>
<td>36</td>
<td>0</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient 25</td>
<td>71</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Patient 32*</td>
<td>58</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient 35*</td>
<td>77</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Patient 36</td>
<td>61</td>
<td>0</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Patient 37*</td>
<td>68</td>
<td>0</td>
<td>FC</td>
<td>HE &amp; Stroke</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Patient 39*</td>
<td>64</td>
<td>1</td>
<td>FC</td>
<td>Stroke</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Patient 6*</td>
<td>27</td>
<td>1</td>
<td>FC</td>
<td>FC + Anoxia</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Patient 16*</td>
<td>24</td>
<td>1</td>
<td>FC</td>
<td>Anoxic Brain Injury</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Patient 30</td>
<td>1</td>
<td>1</td>
<td>FC</td>
<td>Anoxia</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Patient 38*</td>
<td>4</td>
<td>1</td>
<td>FC</td>
<td>Anoxia</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Patient 17</td>
<td>65</td>
<td>1</td>
<td>FC</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Patient 20</td>
<td>73</td>
<td>1</td>
<td>FC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A unique scale was implemented to allow for comparison of the effects of zolpidem administration on different members of the sample population (Table 4.2). Due to the inconsistencies relating to measures of functional outcome utilised within the literature,\textsuperscript{1, 2, 5} combined with a purely observational approach used in the clinician phase, a simplified response scale was developed. This scale crudely ranks patients according to their reaction to the drug, ranging from side effects requiring medical intervention “-2” through to emergence from a state of impaired consciousness “4”.

Despite being accounted for within the scale, no patients within this sample experienced any significant side effects beyond increased somnolence, nor were there any cases of “reawakenings”, referring to drastic increases in level of consciousness. Twelve patients (30%) presented with an improvement in the negative side-effects of their specific brain injury, twenty patients (50%) were shown to regain some functionality, primarily motor or cognitive after zolpidem administration. The remainder of the sample either had no response (n = 4), succumbed to the sedative effect of the drug (n = 1) or in one case regained the ability to walk in a controlled fashion (n = 1).

<table>
<thead>
<tr>
<th>n</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4 Return to Consciousness</td>
</tr>
<tr>
<td>1</td>
<td>3 Major Improvement (restoration of a previously lost ability)</td>
</tr>
<tr>
<td>12</td>
<td>2 Functional Improvement (improvement in any ability, or a large decrease in uncontrolled actions)</td>
</tr>
<tr>
<td>4</td>
<td>0 No Change</td>
</tr>
<tr>
<td>1</td>
<td>-1 Negative Change (excessive somnolence)</td>
</tr>
<tr>
<td>0</td>
<td>-2 Serious Negative Change (reduction in function, or side effect requiring medical attention)</td>
</tr>
</tbody>
</table>

Table 4.2: Zolpidem reaction scale.

4.3 CIRCADIAN PERFUSION FLUCTUATIONS

In an effort to account for diurnal variation in neurological perfusion as a possible variable (Table 4.3), all scans were performed within a set window between 09:00 and 12:00. The mean difference in time of day between the two sets of scans was found to be 28 minutes. This is well within acceptable limits to account for diurnal variation, which is typically associated with day / night changes.\textsuperscript{6-8}
Table 4.3: Time Difference Between Scans.
Descriptive statistics are included at the bottom of each column. Difference is recorded in minutes. Time of Scan (ToS), Standard Deviation (SD).

<table>
<thead>
<tr>
<th>Designation</th>
<th>Time Pre-Scan</th>
<th>Time Post-Scan</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>10:27</td>
<td>10:31</td>
<td>00:04</td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 3</td>
<td>10:30</td>
<td>09:52</td>
<td>00:38</td>
</tr>
<tr>
<td>Patient 4</td>
<td>10:16</td>
<td>09:45</td>
<td>00:31</td>
</tr>
<tr>
<td>Patient 5</td>
<td>10:37</td>
<td>10:22</td>
<td>00:15</td>
</tr>
<tr>
<td>Patient 6</td>
<td>10:38</td>
<td>10:29</td>
<td>00:09</td>
</tr>
<tr>
<td>Patient 7</td>
<td>10:32</td>
<td>10:05</td>
<td>00:27</td>
</tr>
<tr>
<td>Patient 8</td>
<td>09:54</td>
<td>09:52</td>
<td>00:02</td>
</tr>
<tr>
<td>Patient 9</td>
<td>10:30</td>
<td>10:36</td>
<td>00:06</td>
</tr>
<tr>
<td>Patient 10</td>
<td>09:52</td>
<td>10:40</td>
<td>00:48</td>
</tr>
<tr>
<td>Patient 11</td>
<td>10:12</td>
<td>09:43</td>
<td>00:29</td>
</tr>
<tr>
<td>Patient 12</td>
<td>10:23</td>
<td>10:33</td>
<td>00:10</td>
</tr>
<tr>
<td>Patient 13</td>
<td>10:44</td>
<td>10:31</td>
<td>00:13</td>
</tr>
<tr>
<td>Patient 14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 15</td>
<td>11:12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 16</td>
<td>10:40</td>
<td>10:12</td>
<td>00:28</td>
</tr>
<tr>
<td>Patient 17</td>
<td>10:58</td>
<td>10:44</td>
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</tr>
<tr>
<td>Patient 18</td>
<td>10:25</td>
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<tr>
<td>Patient 19</td>
<td>10:44</td>
<td>10:09</td>
<td>00:35</td>
</tr>
<tr>
<td>Patient 20</td>
<td>10:12</td>
<td>10:13</td>
<td>00:01</td>
</tr>
<tr>
<td>Patient 21</td>
<td>10:18</td>
<td>10:30</td>
<td>00:12</td>
</tr>
<tr>
<td>Patient 22</td>
<td>09:59</td>
<td>10:33</td>
<td>00:34</td>
</tr>
<tr>
<td>Patient 23</td>
<td>10:37</td>
<td>10:18</td>
<td>00:19</td>
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4.4 SPECT SEMI-QUANTIFICATION

Data gathered from the general patient workup and zolpidem testing phase were used to identify how individual patients reacted to zolpidem administration as well as identifying the aetiology of neurological deficit.

Seven patients were discarded through this phase of the study, (Patients: 4, 8, 12, 13, 20, 23, 28) due to the original SPECT data being irretrievable. Therefore the final sample size used for semi-quantification was 33 patients, of which 29 were known zolpidem responders. Average changes across the different Regions of Interest (ROI) were used to calculate descriptive statistics for the sample (Table 4.4).

4.4.1 Reference Region

The process of semi-quantification is dependent on a reference region to which any reading taken can be compared. This comparison (ROI / Reference) allows for some of the innate variability arising from spontaneous differences in tracer uptake or poor injection technique to be ruled out. Variability between choice of reference region in studies has been deemed a potential cause for discrepancies between nuclear medicine studies conducted at difference centres, as such needs to be very carefully selected.9

Two different in-scan references, referred to here as “controls”, were used during the recording of the data, leading to three sets of measurements being taken: (1) no control, (2) cerebellar control, (3) whole-brain-at-slice control. The cerebellar control (Figure 4.1) readings were measured across 3 difference transverse slices of the cerebellum and averaged. Whole brain readings were taken from the transverse slice from which the given ROI was measured. In patients where a segment of the cerebellum displayed decreased perfusion and was thus identified as a region of interest, the opposite half of the cerebellum was used as control.

Figure 4.1: Illustrative example of cerebellar control readings. White rings indicate measured regions from which readings are averaged to generate a control parameter for comparison.
4.4.2 Indiscernible Perfusion Fluctuations

Natural variations in neurological perfusion and tracer uptake / distribution were accounted for by utilising established ranges for these parameters (Chapter 3). Tracer uptake within uncontrolled semi-quantitative measurements has been shown to average between 8.3% and 14.8%. Therefore for non-controlled readings, differences in pre / post perfusion could only be deemed to exceed natural variation if they were found to be greater than 12 %. A similar limit was established using data for controlled samples, but of reduced magnitude. Studies indicate that even within self-controlled studies, perfusion reproducibility varies by an average of 1.3 - 3%. Therefore a change of 2.2% or greater was required to distinguish controlled readings from possible variations in tracer uptake. The middle point was chosen as the critical value for these ranges due to further attenuation of expected changes to arise from the utilisation of specific references regions.

Although these approaches to controlling the data do increase the validity of the final result, they are still subject to the fundamental problem associated with semi-quantification in this context. Due to the differential spread of GABA<sub>1</sub> receptors within the human brain, the resultant degree of inhibition (or reactivation) can be expected to vary with receptor density. As demonstrated in Chapter 1, the cerebellum also contains GABA receptors. Therefore neither the whole brain nor cerebellum can truly be seen as inert under the influence of zolpidem.

Figure 4.2: Illustrative example of whole brain control reading. Whole brain measurements were made from the same transverse slice from which regions of interest were measured. Peripheral white ring indicates measured regions, inner selections represent regions of interest.
To further complicate this matter, recent research has shown that the cerebellum is not purely an adjuvant to motor processing (Chapter 3), but contains topographically mapped cortical inputs (mainly via the thalamus & basal ganglia) from virtually every higher processing centre, with corresponding loops back to the thalamus and cortical centres.\textsuperscript{12, 13} As such any cortical or basal ganglia reactivation encountered after zolpidem administration will cause an associated increase in activity within the connected portion of the cerebellum. If the cerebellum is used as a control in this instance the result will be somewhat diminished compared to what may be measured via absolute perfusion.

A similar phenomenon is expected to arise by using a whole brain control. Reactivation of areas of tissue can be expected to cause perfusion changes within connected regions on the same slice level. In addition to this, the region which is theorised to show increased perfusion after administration is also included in the whole brain at slice measurement.\textsuperscript{14-17} These processes will result in some attenuation of the measured change when whole brain is used as control.

In light of these arguments it could be proposed that no control be used and the suggested natural perfusion range be implemented as barrier as to what can be considered an intervention mediated change in perfusion. However, as revealed in Table 4.4, this range is so aggressive for uncontrolled data that seven patients with a mean improvement in perfusion cannot be ranked as such due to these changes being of insufficient magnitude to exclude normal fluctuation.

By comparing the means of each data set (Table 4.4) for the three different control methodologies additional insight can be gained as to how these uncertainties affect real world measurements. Regardless of reference region used, all techniques identified a mean increase in perfusion across the sample. The cerebellar reference region yielded 18 patients with mean increased perfusion as compared to 11 for no control and 13 for whole brain.

The overall means for each sample reflect a similar trend. Using the cerebellum as a reference region found the largest mean increase across the sample, +4.1907\% (P = 0.358), while no control found a mean perfusion increase of +3.6558\%, (P = 0.642) after one extreme outlier was excluded (Patient 3) and whole brain +1.7752\% (P = 0.82) after one extreme outlier was excluded (Patient 17).
In light of these findings it becomes evident that using no reference region leads to too many cases being discarded due to inability to differentiate anything but large changes from natural variation. The high statistical insignificance and small mean change of the whole brain reference when compared to the other two standards, suggests, but does not prove, that whole brain perfusion, as measured from specific slices containing ROI’s, is notably increased after zolpidem administration. By utilising whole brain as a control, a portion of the increased perfusion within the region of interest is indeed obfuscated. This is supported by the actual mean perfusion change for the difference between all individual whole brain readings (mean increase = 3.2622% (P = 0.510)). Despite being statistically insignificant and thus not a generalisable rule, it is the within sample effect of this increase which is relevant to the data presented here.

Surprisingly, the mean cerebellar differences, when averaged, were found to be relatively stable (mean change = -0.099 (P = 0.468)). Once again, although statistically insignificant, it is the effect on the sample at hand which is important. These findings suggest, albeit tentatively so, that whole brain perfusion (within an ROI’s slice) is likely to increase to a greater degree than cerebellar perfusion following zolpidem administration in responder patients. Based on these results the cerebellum was selected as the primary reference region for further analysis.

**Table 4.4: Comparison of mean values.**
Comparison of the mean perfusion across all regions of interest for each patient. Blue cells indicate decreased perfusion. Red cells indicate increased perfusion. Significance determined using Wilcoxon Signed Ranks Test due to non-parametric distribution of data. Purple cells indicate no discernible change. Cerebellar (CB), Whole Brian (CB), Reaction (Rxn).
### Zolpidem - effect on neurological function & perfusion in brain damage

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| Total        | 33       | 33       | 33       | 33       |

Wilcoxon Signed Ranks Test: P=0.642, P=0.358, P=0.82
4.5 ANALYSIS OF RESULTS

A problem which prevents achievement of statistical significance within this sample is that of “noise” within the data. Zolpidem has previously been shown to increase neurological perfusion in specific regions of interest within responder patients.\textsuperscript{1, 18} In non-responders or non-responsive regions of the brain, administration of the drug decreases perfusion in accordance with its inhibitory action.\textsuperscript{19} This effect will result in diminished apparent means when averages are used in data analysis. A notable finding is that even within the brain of a zolpidem responder, certain regions of interest display improved perfusion while other present with a decrease. A whole brain perfusion increase does not seem to be the norm. This manifests as not only a much smaller mean positive change for patients with multiple lesions, but prevents significance from being achieved across the sample as whole.

By drawing inspiration from the methodologies of macroscopic zolpidem studies, a workaround to this problem was identified. The issue of conflicting changes within a sample has been encountered within other large scale zolpidem studies.\textsuperscript{2, 3, 5} In these studies the zolpidem response is usually drowned out due to the small number of responders within a truly random sample of brain damaged individuals. Therefore the standard approach has been to isolate zolpidem responders within a given dataset and conduct analysis on the responder group to determine the full extent of changes and determine significance thereof.

This same concept can be applied to quantitative data gathered from multiple regions of interest behaving in a similarly opposing manner. Within the literature, the damaged region within each brain with the greatest scintigraphic increase after zolpidem administration is typically associated with the functional improvements arising after administration.\textsuperscript{20-22} In contrast to this, the majority of lesions show no change or a decreased perfusion response to zolpidem administration. By isolating the primary “responder lesions” (Figure 4.3), or rather the lesions which improved or were most resistant to zolpidem mediated perfusion decrease, a second set of analysis can be conducted on this newly formed secondary-sample mean.
Zolpidem - effect on neurological function & perfusion in brain damage

Figure 4.3: SPECT image of zolpidem responder patient before and after administration. Note highly visible change within frontal structures at the same slice level after drug administration.

4.5.1 Perfusion Change within Primary Region of Interest

By completing separate data analysis for the single primary region of interest within each responder patient (Table 4.5 & Figure 4.4), regardless of whether perfusion increased or decreased, a mean perfusion improvement of 12.759% (P < 0.01) is identified. The combination of increased mean perfusion as compared to the original sample mean, coupled with high significance provides evidence for the conclusion that within zolpidem responder patients, zolpidem is indeed able to increase perfusion within select regions of interest. The idea that this effect is not universal across all damaged regions with the brain is also supported by the overall means as well as the appearance of statistical significance within this specific analysis.

Figure 4.4: Primary responsive lesion’s reaction to zolpidem administration.
### Table 4.5: Analysis of primary region of interest.

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<tbody>
<tr>
<td>Patient 1</td>
<td>0.6471</td>
<td>0.5483</td>
<td>-15.2588</td>
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</tr>
<tr>
<td>Patient 2</td>
<td>0.4052</td>
<td>0.5693</td>
<td>40.508</td>
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</tr>
<tr>
<td>Patient 3</td>
<td>0.8266</td>
<td>1.0847</td>
<td>31.2310</td>
<td>2</td>
</tr>
<tr>
<td>Patient 5</td>
<td>0.5630</td>
<td>0.6415</td>
<td>13.9319</td>
<td>1</td>
</tr>
<tr>
<td>Patient 6</td>
<td>0.7928</td>
<td>1</td>
<td>26.1292</td>
<td>1</td>
</tr>
<tr>
<td>Patient 7</td>
<td>0.9593</td>
<td>0.6160</td>
<td>-35.7874</td>
<td>1</td>
</tr>
<tr>
<td>Patient 9</td>
<td>0.9060</td>
<td>0.9845</td>
<td>8.6553</td>
<td>2</td>
</tr>
<tr>
<td>Patient 10</td>
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<td>0.4212</td>
<td>4.8259</td>
<td>1</td>
</tr>
<tr>
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<td>0.8524</td>
<td>0.9270</td>
<td>8.7557</td>
<td>2</td>
</tr>
<tr>
<td>Patient 15</td>
<td>0.4683</td>
<td>0.4585</td>
<td>-0.955</td>
<td>2</td>
</tr>
<tr>
<td>Patient 16</td>
<td>0.9853</td>
<td>0.9794</td>
<td>-0.5944</td>
<td>2</td>
</tr>
<tr>
<td>Patient 17</td>
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<td>1.0093</td>
<td>12.4648</td>
<td>2</td>
</tr>
<tr>
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<td>0.2181</td>
<td>0.2420</td>
<td>10.9593</td>
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</tr>
<tr>
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<td>0.6826</td>
<td>0.9244</td>
<td>35.4245</td>
<td>2</td>
</tr>
<tr>
<td>Patient 21</td>
<td>1.2484</td>
<td>1.2375</td>
<td>-0.8679</td>
<td>1</td>
</tr>
<tr>
<td>Patient 22</td>
<td>0.6291</td>
<td>0.7534</td>
<td>19.7716</td>
<td>2</td>
</tr>
<tr>
<td>Patient 24</td>
<td>0.5887</td>
<td>0.6864</td>
<td>16.6079</td>
<td>2</td>
</tr>
<tr>
<td>Patient 26</td>
<td>1.0465</td>
<td>0.9481</td>
<td>-9.4041</td>
<td>2</td>
</tr>
<tr>
<td>Patient 27</td>
<td>0.9547</td>
<td>1.1355</td>
<td>18.9448</td>
<td>2</td>
</tr>
<tr>
<td>Patient 30</td>
<td>0.7098</td>
<td>0.7827</td>
<td>10.2781</td>
<td>1</td>
</tr>
<tr>
<td>Patient 31</td>
<td>0.6839</td>
<td>0.7505</td>
<td>9.7328</td>
<td>2</td>
</tr>
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<td>Patient 32</td>
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<td>0.5168</td>
<td>22.0170</td>
<td>2</td>
</tr>
<tr>
<td>Patient 33</td>
<td>1.1434</td>
<td>1.001</td>
<td>-12.4537</td>
<td>3</td>
</tr>
<tr>
<td>Patient 34</td>
<td>0.8951</td>
<td>1.0181</td>
<td>13.7482</td>
<td>1</td>
</tr>
<tr>
<td>Patient 35</td>
<td>0.5896</td>
<td>0.6166</td>
<td>4.5842</td>
<td>1</td>
</tr>
<tr>
<td>Patient 37</td>
<td>1.1652</td>
<td>1.2515</td>
<td>7.40129</td>
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</tr>
<tr>
<td>Patient 38</td>
<td>0.8689</td>
<td>1.0033</td>
<td>15.4662</td>
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</tr>
<tr>
<td>Patient 39</td>
<td>0.3364</td>
<td>0.4917</td>
<td>46.1479</td>
<td>2</td>
</tr>
<tr>
<td>Patient 40</td>
<td>0.7541</td>
<td>1.0835</td>
<td>43.6858</td>
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</tr>
<tr>
<td>Mean</td>
<td>0.7463</td>
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</tr>
<tr>
<td>SD</td>
<td>0.2563</td>
<td>0.2604</td>
<td>17.7278</td>
<td></td>
</tr>
</tbody>
</table>

Wilcoxon Signed Ranks  
At 0.01 Significance level.
4.5.2 Non-Responsive Lesions

Increases in perfusion are not the only variable of interest the data allows insight into. By identifying lesions which did not respond to zolpidem administration we can establish a guideline figure for the nature of perfusion changes within non-responsive regions of the brain. All ROI’s which did not respond with a perfusion increase of greater than 2.2% were used for this analysis. As illustrated in Figure 4.5, non-responsive lesions reacted to zolpidem administration with a decrease in perfusion of approximately 11% (P < 0.01). A small but statistically significant perfusion difference.

![Mean perfusion change within “non-responsive” lesions.](image)

**Figure 4.5: Mean perfusion change within “non-responsive” lesions.** Mean perfusion before and after zolpidem administration in n = 44 “non-responsive” lesions. A cerebellar reference was used for all perfusion measurements. Mean change was noted to be -14.49%. P < 0.01.
4.5.3 Functional Restoration and Perfusion Changes

An additional element of analysis can be incorporated by identifying the magnitude of perfusion changes within degrees of functional restoration. Due to insufficient samples within the population this can only be done for zolpidem responders falling within the categories of “1 - Slight Improvement” and “2 - Functional improvement” (Figure 4.6).

![Figure 4.6: Mean percentage perfusion differences between Class 1 and Class 2 responders. Red bars correspond to Rank 1 responders (n = 12), Pink bars correspond to Rank 2 responders (n=12). The first pair utilises data gained from averaging all regions of interest (ROI) within each patient. Data for this approached a normal distribution and Student’s T-Test was utilised. P-value for difference between class 1 and 2 responders = 0.283 (95% Confidence Interval). The second set consists of the average mean when only the primary ROI is taken into account within each patient and when compared P = 0.101 (95% Confidence Interval).

Although not statistically significant due to limited sample size (Figure 4.7), there does seem to be an established trend between Class 1 and Class 2 responders. Regardless of analytic convention used, Class 1 responders present with a much smaller perfusion increase compared to Class 2. This finding suggests that larger degrees of functional improvement in zolpidem responders is associated with a perfusion increase often times orders of magnitude larger than slight improvers.
Zolpidem - effect on neurological function & perfusion in brain damage

![Bar chart showing perfusion changes by responder class](chart.png)

**Figure 4.7: Individual illustration of perfusion changes by responder class.** Each bar represents one patient. Blue indicates Class 1 responders, Red indicates Class 2 responders. The spread of data points does seem to favour the conclusion that class two responders do in general regain more perfusion than Class 1. The similarities in trend pictured here does clarify the need for a larger sample before this conclusion can truly be proven.

### 4.5.4 Zolpidem Responsiveness by Aetiology

Through inspection of the qualitative data a clearer picture can be formed of the nature of the which different forms of brain damage as responsive to zolpidem administration (Table 4.6). With individual sample sizes being too small reach statistical significance for this specific analysis, only observations will be reported.

It is of great interest to note that within the three main pathologies found within this sample, trauma patients had the largest proportion of greater clinical responses to zolpidem, with only one patient not having a noticeable reaction. Four patients showed functional improvement while one regained a lost function altogether.

Stroke patients represent the largest discreet aetiological entity in the study and the response to zolpidem was also very impressive. 60% of stroke responders showed signs of functional improvement after administration with an additional 26.7% displaying symptomatic improvement.
Table 4.6: Crosstab between aetiology of brain damage and observed qualitative response to zolpidem administration.

<table>
<thead>
<tr>
<th>Response</th>
<th>Aetiology</th>
<th>Ischaemia</th>
<th>Miscellaneous</th>
<th>Stroke</th>
<th>TBI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1.00</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.8%</td>
</tr>
<tr>
<td>.00</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.1%</td>
</tr>
<tr>
<td></td>
<td>0.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30.6%</td>
</tr>
<tr>
<td></td>
<td>33.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>52.8%</td>
</tr>
<tr>
<td></td>
<td>66.7%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.00</td>
<td>n</td>
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<td></td>
<td></td>
<td></td>
<td>2.8%</td>
</tr>
<tr>
<td></td>
<td>0.0%</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>n</td>
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<td></td>
<td></td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>100.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.5.5  Perfusion Change by Anatomical Region

An attempt was made to identify which regions of interest, as sorted by anatomical location were most responsive to changes following zolpidem administration (Table 4.7). In total 85 regions of interest were measured across the sample population.

Table 4.7: Crosstab between nature of perfusion change and gross anatomical regions.

Quantitative responses grouped for simplicity. “-1” refers to any negative change in perfusion exceeding previously established guidelines. “0” represents a perfusion changes between -2.2% and +2.2%. “1” encompasses all perfusion changes greater than the established limits.

<table>
<thead>
<tr>
<th>Anatomical Code</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebellum</td>
</tr>
<tr>
<td>Mean % Change</td>
<td>22.1%</td>
</tr>
</tbody>
</table>

Change within ROI (Cerebellar Control)

<table>
<thead>
<tr>
<th>% of ROI Group</th>
<th>0.0%</th>
<th>38.5%</th>
<th>7.7%</th>
<th>19.2%</th>
<th>19.2%</th>
<th>15.4%</th>
<th>100.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Anatomical Group</td>
<td>0.0%</td>
<td>33.3%</td>
<td>33.3%</td>
<td>35.7%</td>
<td>23.8%</td>
<td>36.4%</td>
<td>30.6%</td>
</tr>
<tr>
<td>N/A</td>
<td>-15.8%</td>
<td>-14.4%</td>
<td>-16.9%</td>
<td>-6.7%</td>
<td>-15.0%</td>
<td>-13.8%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of ROI Group</th>
<th>3.7%</th>
<th>25.9%</th>
<th>14.8%</th>
<th>14.8%</th>
<th>33.3%</th>
<th>7.4%</th>
<th>100.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Anatomical Group</td>
<td>33.3%</td>
<td>23.3%</td>
<td>66.7%</td>
<td>28.6%</td>
<td>42.9%</td>
<td>18.2%</td>
<td>31.8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of ROI Group</th>
<th>6.3%</th>
<th>40.6%</th>
<th>0.0%</th>
<th>15.6%</th>
<th>21.9%</th>
<th>15.6%</th>
<th>100.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Anatomical Group</td>
<td>66.7%</td>
<td>43.3%</td>
<td>0.0%</td>
<td>35.7%</td>
<td>33.3%</td>
<td>45.5%</td>
<td>37.6%</td>
</tr>
<tr>
<td>+22.1%</td>
<td>+19.1%</td>
<td>N/A</td>
<td>+13.8%</td>
<td>+27.6%</td>
<td>+10.5%</td>
<td>+18.6%</td>
<td></td>
</tr>
</tbody>
</table>

Total

<table>
<thead>
<tr>
<th>% of ROI Group</th>
<th>3.5%</th>
<th>35.3%</th>
<th>7.1%</th>
<th>16.5%</th>
<th>24.7%</th>
<th>12.9%</th>
<th>100.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Anatomical Group</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Although these data are too coarse and sample size much too limited for the number of variables to attempt reaching statistical significance, the crosstab description of the data does allow for speculation as to trends behind zolpidem response. Table 4.7 reveals that the majority of patients within the sample sustained injury to temporal (n=30) or parietal (n=21) cortical structures. Of note were the slight smaller number of cases representing damage to the frontal cortex (n=14) and basal ganglia (n=11). The basal ganglia (45.5%) and the temporal cortical structures (43.3%) appear to respond most readily to zolpidem mediated reactivation. Of the 6 regions of interest falling within the occipital lobe, none succeeded in producing a noticeable increase in perfusion. The cerebellar sample size is too small to identify any speculative trends.
Beyond these trends an additional noteworthy pattern seems to arise within Table 4.7, between quantitative responses and the nature of the reaction within the larger sampled anatomical regions (i.e. excluding the cerebellum and occipital lobe). The number of responsive lesions reacting to zolpidem administration either through increased or decreased perfusion seems to be similar to each other for the above two categories. The magnitude of the mean change within these divisions also seems similar.

This is most likely a simple apophenic error of interpretation in response to this specific method of data visualisation. The alternative does present an interesting avenue for speculation though. Is this trend an illusion, or does it represent a theoretical ceiling to zolpidem’s maximum functional suppression (as a hypnotic) and its neuro-activation ability in brain damage? Possibly indicating a reversal of the mechanism behind the former, causing the latter. The discrepancy in the well sampled parietal lobe’s response suggests that although interesting, this is most likely a fluke resulting from chance distribution of a small sample.

4.5.6 Clinician Noted Change vs. Measured Change

A point which has yet to be addressed and can be seen illustrated in Table 4.4 is that of the discrepancy between clinician noted and measured changed. A simple crosstab (Table 4.8) summarises the issue.

<table>
<thead>
<tr>
<th>Measured Change</th>
<th>Clinician Noted Changed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1.00</td>
<td>.00</td>
</tr>
<tr>
<td>-1.00</td>
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<td>7</td>
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<td>.00</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>1.00</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>22</td>
</tr>
</tbody>
</table>

Thirty-eight of the quantitative measurements aligned perfectly with the assessment made by the inspecting clinician, leaving 47 measurements to account for. In the cases where the mismatch was off by one degree (n = 28), such as one group reporting a perfusion increase or decrease while the other reported no change, and vice versa, this can be explained by semi-quantification elucidating minor changes not particularly visible to the naked eye. Most likely minor perfusion alterations not of sufficient magnitude to form the opinion in an analysing clinician that there was a notable change.
Within a fraction of the sample \((n = 19)\) there was direct conflict between clinician recorded readings and those recorded by semi quantification. This conflict could arise from a number of processes: (1) as with one degree miss-alignments of findings, self referenced controls may serve to swing subtle changes in a particular direction, causing conflicting readings, (2) unexpected variation in tracer uptake in either of the scans producing a false change which was corrected through utilisation of self referencing controls in the quantitative phase, (3) experimental error during the drawing of regions of interest, a possible source of error in a study design where two different sets of individuals are responsible for generating final data, (4) processing errors during either phase, inaccurate processing may lead to clinicians forming false impressions, or over aggressive filtering parameters could conceivably skew measured readings, (5) although highly unlikely, the possibility does exist of error arising due to misallocation of scans as pre/post zolpidem administration at any point during the SPECT phase of the study. The SPECT processing workstation software provided by Siemens records dates in the American format “Month/Day/Year”. The hospital archive and clinician notes recorded dates in the British format, “Day/Month/Year”. Although great care was taken to avoid errors due to administrative confusion of this nature, it is not inconceivable that this could be a minor source of error.

4.5.7 Qualitative zolpidem reaction vs. Observed Perfusion Change

A final point of discrepancy is illustrated in Tables 4.4 and 4.5, perfusion changes in lesions do not pair perfectly with the observed response in the patient, although the fit can be improved by focussing on primary regions of interest. There are some patients \((n=11)\) whom responded well to zolpidem administration but perfusion decreases were identified through quantification. In these patients it is very likely that the site of improvement was not localised to the primary lesion. As such subtle or widespread reactivation of various cortical networks could plausibly be responsible for the functional restoration.

In a few patients \((n = 2)\) the opposite is true, and a perfusion increase could not be coupled to functional improvements. The literature and limited evidence presented here, support the conclusion that neurological reactivation needs to pass a critical threshold before functional restoration can be achieved.\(^2\) The magnitude of this threshold is dependent on the nature, location and extent of neurological damage.\(^2\)
4.6 CONCLUSION

The findings presented here are in coherence with the bulk of published literature regarding the action of zolpidem in neurologically compromised, responder patients. Zolpidem does indeed increase neurological perfusion in some patients. This effect on perfusion within the brain found to be of sufficient magnitude to be quantifiable within both responder and non-responder patients. Within the final chapter, these findings will be linked to the specific research questions this project set out to answer.
4.7 REFERENCES


Chapter 5

Conclusion
5.1 CONCLUSIONS

Having presented the data produced in this project in the previous chapter, it is pertinent to shift focus to the implications of these findings in relation to the set hypotheses. To recap:

Hypothesis 1

\( H_1 \): Zolpidem administration is associated with a quantifiable increase in brain perfusion in neurologically compromised patients.

\( H_0 \): There is no association between zolpidem administration and quantifiable improved perfusion in neurologically compromised patients.

Hypothesis 2

\( H_1 \): Zolpidem associated changes in perfusion show preference to specific neuroanatomical regions.

\( H_0 \): Zolpidem associated perfusion changes show no regional specificity.

Hypothesis 3

\( H_1 \): Zolpidem responders can be isolated to specific diagnostic groups.

\( H_0 \): Zolpidem responders aren’t confined to clearly delineated diagnostic groups.

5.1.1 Effect of zolpidem on neurological perfusion (Hypothesis 1)

The sample used in this study was found to consist almost entirely of zolpidem responder patients. The implications of this are that the perfusion response in neurologically normal patients could not be quantified. However, as was shown, non-responsive lesions can still be used to provide contrast between responsive and non-responsive regions of brain.

Within the group of responder patients (n = 29), 22 patients (~76%) presented a significant increase in perfusion within at least one lesion with a range of 4.5 - 46.1% (mean = 11.9%). In opposition to this finding non-responsive lesion perfusion decreased with a significant mean change of -14.5%. For both sets the p-value was determined to be <0.01. Of all lesions measured (n = 85) 32% displayed increased perfusion after zolpidem administration, whereas 30.6% presented with a perfusion decrease.
In light of these findings, the null hypothesis can indeed be rejected but not without modification to the tested hypothesis. In brain damaged zolpidem-responsive patients, administration is indeed associated with an increase in neurological perfusion. This perfusion increase is primarily limited to a central responsive lesion and connected regions in the majority of cases. In a small minority, diffuse perfusion increases may occur with or without specific lesional increases. In the majority (62.6%) of studied neurological lesions, zolpidem administration either appears to have no effect, or causes a reduction in perfusion.

This evidence shows that zolpidem responder patients do not present with a unique response to zolpidem across all cortical structures. Instead zolpidem continues to act as it otherwise would with the exception of responder regions and any reaction within their associated neural networks.

5.1.2 Effect of zolpidem on different anatomical regions (Hypothesis 2)

A coincident discovery while testing this hypothesis was the general lack of damage to the cerebellum and occipital lobe within the sample. Out of 85 identifiable lesions, only \( n = 3 \) and \( n = 6 \) belong to these two anatomical regions respectively. Whether these findings arise from an innate resistance to injury or simply a skewed sample population is uncertain. No lesions within the cerebellum responded negatively to zolpidem administration, whereas no lesions responded positively within the occipital lobe. Yet due to insufficient sample numbers these two regions can’t be cited as evidence in argument for or against this hypothesis.

Of the remaining anatomical regions, the largest average increase was isolated to the Parietal region (+27.6%) which also presented with the smallest perfusion decrease in non-responsive regions. However, in the absence of statistical significance it is impossible to decisively say that zolpidem shows preference for rehabilitative action in the parietal cortex. Beyond the scope of the two excluded regions and the parietal cortex, zolpidem was shown to have similar degrees of perfusion enhancement in the temporal and frontal lobes as well as the basal ganglia. The same can be said for the perfusion decreasing effect in non-responsive lesions.

Based off the data presented here it is impossible to reject either hypothesis, but the data do seem to lean in favour of the null hypothesis, that zolpidem mediated perfusion increases are not limited to specific anatomical parts of the brain.
5.1.3 Zolpidem Response by Aetiology (Hypothesis 3)

Within the context of the final hypothesis, the null cannot soundly be rejected without the use of a placebo control group in unison with a much larger sample size. To make a conclusive decision on this hypothesis, a collection of random samples, each confined to specific neurological pathologies would need to be challenged with zolpidem. These results could then be compared, to accurately identify which forms of brain damage warrant zolpidem-trial inclusion as part of a standard rehabilitative regime. A study of this magnitude would however far exceed the resources available to an M.Sc. project and would likely have to take the form of a national or international collaborative effort.

Despite this, the unique sample pool presented here, consisting almost entirely of zolpidem responders does still allow for commentary on the nature of zolpidem response across different forms of brain damage.

An important aspect to consider is that the 40 patients involved in this project were drawn from a random collection of ~400 patients whom approached Dr Nel’s practice in hopes of a rehabilitative aid for poorly recovered or recovering brain damage. Due to large numbers of incomplete quantitative (observational) data points it cannot be determined exactly how many zolpidem responders there were in the original group, but estimates can be made. Factoring in the standard zolpidem response rate of ~6 - 10%\(^1\)\(^-\)\(^5\) (Chapter 1) it can be assumed that (a) the majority of responder patient’s had SPECT scans performed and (b) the number of responder patients which underwent SPECT scintigraphy but were not included in the studied sample is relatively small.

In light of this an important conclusion regarding zolpidem response rates can be drawn. The true number of zolpidem responders within the overall sample is suspected to err on the larger side (→10%) of the population estimate generated from literature analysis in chapter one.

Through examination of the general population statistics for forms of brain damage (Table 5.1 & Figure 5.1) insight can be gained into the distribution of the zolpidem responders studied here. The largest sampled pathology within this study was that of stroke patients (n = 15), followed by traumatic injury (n = 6), ischaemic injury (n = 3), Parkinson’s disease (n = 2) and multiple sclerosis (n = 2). The common thread between each of these is their classification as “acquired brain injury” involving specific cell death.\(^6\),\(^7\)
If zolpidem responsiveness in all forms of brain damage was equal, an estimated sample of 400 patients would present with variations in responder figures approximately following a ratio of stroke 47%, TBI 32%, MS 20% Ischaemic damage 1% (Figure 5.1.1). The observed distribution was Stroke 58%, TBI 23%, MS 8% and Ischaemic damage 11%. The deviation from the expected distribution was significant only for MS and Ischaemic damage (Figure 5.1.2). Parkinson’s disease has been excluded from the figures to clarify illustration.

Considering this it would seem that zolpidem’s restorative action shows preference to: (1) brain damage arising due to cell loss, (2) due to cell loss arising from insult, be it in the form of physical trauma or resource deprivation as opposed to gradual innate forms of cell loss. Despite there being limited evidence in this study, zolpidem administration may still of use to individuals falling within the latter group as well.

If we examine this theory in the context of the full sample, there appears to be some truth to the matter. Aside from the patient presenting with dystonia, the aetiology of which was unknown, all responsive patients in this sample presented with pathology related to various forms of neurological cell loss as opposed to dysfunction (including the patient with MND, which affected upper motor neurons). A much larger sample would be needed to conclusively prove this, but this study does provide data in support of the statements.

Although evidence is insufficient to discard the null hypothesis, evidence provided in this study is in support of zolpidem being of use, primarily in the rehabilitation of neurological disorders arising from acquired neurological damage.

**Table 5.1: Incidence of 5 most frequently occurring brain pathologies within sample group.**
Bracketed abbreviation indicates source of statistic. United States (US), United Kingdom (UK), International (Int).

<table>
<thead>
<tr>
<th>Brain Pathology</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson's Disease</td>
<td>2 168 / 100 000 (US)</td>
</tr>
<tr>
<td>Stroke</td>
<td>342 / 100 000 (US)</td>
</tr>
<tr>
<td>Traumatic Brain Injury</td>
<td>235 / 100 000 (Int)</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>140 / 100 000 (UK)</td>
</tr>
<tr>
<td>Near Drowning</td>
<td>&gt;7 / 100 000 (Int)</td>
</tr>
</tbody>
</table>
Figure 5.1: Brain pathology sample distributions. (1) Expected natural distribution for the examined pathologies, based on epidemiological data. (2) Distribution of pathologies within the sample obtained. Parkinson’s was discarded from visualisation due to its large expected occurrence skewing the visualisation.

The relative absence of Parkinson’s disease, especially when considering its high prevalence in the general population, cannot be commented on with any degree of accuracy. Considering that Parkinson’s disease is well managed by current medical practices as compared to the disorders presented here, it is possible that there are simply fewer patients actively looking for novel methods to manage their particular disorders.

To summarise the findings of this study - the data presented here are in accordance with the published literature\(^1\)\(^-\)\(^3\),\(^5\) which suggests zolpidem’s use as a neuro-rehabilitatory agent. Perfusion increases, as associated with functional improvements were successfully semi-quantified. The distribution of specific sites of action seems to be as varied as the distribution of the zolpidem specific GABA\(_A\) receptor isoform itself. The responsive types of neurological pathology responsive to zolpidem administration appear to favour acquired types of brain damage.
5.1.4 Mechanism of Action

A final point to address is how these findings interact with the theories presented in Chapter 2. If the data were isolated to single responsive regions of interest, with the primary result of zolpidem action being coupled to functional increases, as it is for the majority of the sample, both the neurodormancy and mesocircuit hypotheses would be a good fit to explain the mechanism of action. However, factoring in the finding that in seven patients, observed improvements were not associated with positive perfusion changes within the region of interest, the mesocircuit or functional desynchronisation theories begin to appear a better fit. In its current form, the neurodormancy hypothesis is also incompatible with the finding that zolpidem responders include patients with non-traumatic sources of injuries. I.e. their pathologies aren’t limited to forms of brain in which trauma or resource deprivation trigger cellular death through excitotoxic events which may be associated with survival through entering a dormant state.

There is however no evidence within this dataset to force selection of one single hypothesis among the three presented. It cannot be conclusively said that zolpidem’s rehabilitative action is via a constant mechanism in all cases. It is entirely possible that improvement in different pathologies arise from entirely separate mechanisms. The highlighted perfusion differences in a small number of responders could be interpreted as evidence in support of this.

It is tempting then to speculate that within the seven responder patients whom presented with a decrease in perfusion in the central region of interest, this exact perfusion decrease might in fact be the root cause of functional increase and diffuse perfusion changes. In the absence of EEG or MEG it cannot be shown that the same pathological increase in neurological low frequency oscillations was present in these patients as was found in the study by Hall et al.\textsuperscript{16, 17} That said, it does seem plausible that pathological oscillations may impede the function of surrounding or efferent neurons. By silencing pathological oscillations in these neurons there could theoretically be a functional increase in both intra- and inter-hemispheric connected populations.

5.1.5 Conclusion

This project has succeeded in providing additional insight into the nature of perfusion changes within zolpidem responders, including the surprising finding that perfusion does not necessarily need to increase within a central lesion to facilitate positive drug reaction. A step has been taken towards isolating which neurological pathologies are maximally responsive to zolpidem administration and it has been shown that zolpidem action is not uniform within the brains of responders.
5.2 FUTURE WORK

Despite the thorough data analysis presented here, work on this project does not end with this dissertation. Following completion of this project the findings presented here are to published as multiple articles in peer-reviewed journals specific to the various sub-fields involved in this project. A simultaneous spin-off project will be initiated to work with Dr HW Nel and his practice to facilitate sufficient completion of the captured patient records to allow for peer-reviewed publication of his life’s work in the form of the largest zolpidem case study published to date.
5.3 REFERENCES


APPENDIX A: PROCESSING ERROR

Before the study could begin, it was paramount to rule out, or at the very least determine the extent of, variation induced by the scan processing procedure itself, specifically the filtering parameters. To determine this, a single brain scan was selected at random, and an easily identifiable anatomical landmark (cerebellum, right hemisphere) was selected. This region was repeatedly measured using equally sized, standard, circular regions of interest. Each measurement was performed using increasingly drastic processing parameters and a Metz filter. (Table A.1).

For this simple verification, judgement of SPECT image suitability was made by eye. Columns highlighted as green in Table A.1 mark the range of parameters which would have been deemed suitable to utilise for quantitative comparison. The resultant images produced by the other parameters would have been deemed too coarse and reprocessed using a different set of filtering parameters. The possible measurement error was found to be up to 0.3% within appropriately processed scans. Unsuitable filtering parameters, even when slightly outside of an optimal range, were found to produce sets of images easily identified as such by observation.

When compared, catastrophically poorly processed images, at the opposite ends of the filtering spectrum (Table A.1, column 5 and 6), were found to induce an error of 2.2%.

Contrast and scatter corrections were found to cause no change to measurements.

**Table A.1: Measurements made under different filtering conditions.**

Measurements 2 and 3 correspond to what falls within a visually appropriate range. As colours approach red the resultant SPECT image becomes less compatible with any form of diagnostic imaging. Parameters utilised for readings 5 and 6 are completely un-interpretable. Minimum (Min), Maximum (Max), Average (Avg).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point Spread</td>
<td>3.25</td>
<td>4.45</td>
<td>5.8</td>
<td>7.3</td>
<td>2.65</td>
<td>4</td>
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<tr>
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<td>5.41</td>
<td>6.22</td>
<td>2.17</td>
<td>8.11</td>
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<tr>
<td>Total Count</td>
<td>5638</td>
<td>5534</td>
<td>5610</td>
<td>4903</td>
<td>5678</td>
<td>55.69</td>
</tr>
<tr>
<td>Min</td>
<td>23</td>
<td>27</td>
<td>34</td>
<td>36</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Max</td>
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<td>85</td>
<td>84</td>
<td>79</td>
<td>77</td>
<td>94</td>
</tr>
<tr>
<td>Avg</td>
<td>58.124</td>
<td>58.872</td>
<td>59.053</td>
<td>59.793</td>
<td>57.939</td>
<td>59.244</td>
</tr>
</tbody>
</table>

In light of these findings, errors due to filtering parameters were deemed small enough to assume negligible.