Measuring Cortical Thickness and Neuronal Density in Stroke Patients

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Abstract

Ischemic stroke causes necrosis of the cortex leading to atrophy in directly affected tissue. However, little is known about what occurs to the cortex surrounding the lesion due to the indirect effects of stroke. This thesis aims to develop a method to assess cortical thickness and neuronal density in vivo in stroke patients using surface-based cortical thickness mapping from MRI and high resolution [18F]Flumazenil positron emission tomography respectively. No significant changes in cortical thickness were found in stroke patients’ affected hemispheres as compared to the contralateral hemisphere and control brains. [18F]Flumazenil binding was significantly reduced in the affected hemisphere in regions surrounding the infarct compared to the contralateral hemisphere and gradually increased farther from the lesion. With our methods, cortical thickness can reliably be measured in very close proximity to lesions and changes in neuronal density can be detected in stroke affected hemispheres as compared to the contralateral hemisphere.
Abrégé

L'accident vasculaire cérébral (AVC) ischémique cause une nécrose de la région du cortex cérébral directement affectée et mène à une atrophie du tissu touché. Cependant, les effets indirects causé par l'AVC dans la région péri-lésionnelle sont peut connus. Ce mémoire a pour objectif d'évaluer l'épaisseur corticale et la densité neuronale in vivo chez des patients ayant eu un AVC ischémique en utilisant l'analyse automatisée de l'épaisseur du cortex à partir d'imagerie par résonance magnétique ainsi que la tomographie par émission de positrons au $[^{18}F]flumazénil à haute résolution.

Aucun changement significatif n’a été détecté au niveau de l’épaisseur corticale entre l’hémisphère affecté par l’AVC et l’hémisphère contra-lésionnel ou en comparaison avec des sujets contrôles. Toutefois, la fixation du $[^{18}F]flumazénil était significativement réduite dans les régions adjacentes de l’hémisphère affecté en comparaison avec l’hémisphère contra-lésionnel et augmente graduellement en s’éloignant de la lésion. Notre méthode a permis de déterminer que l’épaisseur corticale peu être mesurée dans la région péri-lésionnelle et les changements de densité neuronale peuvent être détectés dans les régions cérébrales affectées par un AVC ainsi que dans les régions contra-lésionnelles.
Chapter 1 – Introduction

1.1 Rationale

A stroke is a sudden loss of brain function with ensuing loss of brain tissue caused by the interruption of blood flow to the brain (ischemic stroke) or the rupture of blood vessels in the brain (hemorrhagic stroke). It is the number two cause of death worldwide and may soon become the leading cause (Feigin, 2005; Murray & Lopez, 1997). Ischemic stroke, also known as cerebral infarction, causes necrosis of the cerebral cortex in the core of the ischemia but also has indirect effects on viable and structurally intact brain tissue surrounding the ischemic core and also in remote brain regions.

There are two different ways for cells to die: necrosis and apoptosis. Necrosis or ‘accidental cell death’ is cell death that occurs as a result of acute tissue injury (Chu et al., 2002). Apoptosis is mediated by an intracellular program and is therefore deemed programmed cell death (Kerr et al., 1972). In the aftermath of stroke necrosis is the major source of post-traumatic cell death however apoptotic neuronal cell death may also play a role in neuronal injury induced by ischemia (Chu et al., 2002; Yuan, 2009). Apoptosis is generally under active control and carried out in a beneficial and regulated manner, but when this complex cell suicide mechanism is triggered by ischemic stroke it can have deleterious effects.
After an ischemic stroke blood flow and therefore oxygen transport decreases in the area surrounding the ischemic event. This region neighbouring the infarct is called the penumbra. The penumbral brain tissue is functionally impaired due to ischemia but still viable (Astrup, 1981). Though it is not yet permanently damaged from the original insult it is at risk of undergoing delayed cell death. Delayed death of neurons can lead to the amplification of the initial damage caused by the infarction. Positron Emission Tomography (PET) studies have demonstrated viable tissue in the penumbra up to several hours after ischemic stroke rendering the rationale for therapeutic interventions (Heiss and Graff, 1994).

Another remote effect of ischemic stroke is diaschisis (from Greek, meaning “shocked throughout”) which was first described by von Monakow (1914). Diaschisis is a depression of regional neuronal metabolism and cerebral blood flow due to an acute focal disturbance in an anatomically separate but functionally related brain region (von Monakow, 1914). It is important to note that diaschisis is not tissue damage rather it is tissue dysfunction that is caused by damage upstream. Evidence for the process of diaschisis in stroke has come from studies using autoradiography (Ginsberg, 1989) as well PET (von Geisen et al., 1994) that showed reduced metabolism of deoxyglucose in distant brain regions.

An additional contributor to neuronal death in ischemic stroke is excitotoxicity (Schiene et al., 1996). Excitotoxicity is caused by suppressed function of γ-
Aminobutyric acid (GABA)\textsubscript{A} receptors that mediate inhibition making them molecular targets for the treatment of ischemic stroke (Liu et al., 2010).

Beyond the direct cell necrosis, these indirect effects on viable and structurally normal cortex may also play a role for an individual patient’s recovery potential (Mergenthaler et al., 2004). However, studying these effects in vivo in stroke patients is difficult because the spatial resolution of clinical MRI scanners is limited.

The most direct indicator of structural cortical integrity in the human cerebral cortex is a change in thickness. While dynamic changes in cortical thickness have been investigated in brain development and degenerative diseases, little is known about changes associated with focal brain lesions or the possibility of these changes being associated with recovery processes. Changes of cortical thickness following stroke may be observed in different directions: loss of neurons may cause a decrease in cortical thickness (atrophy), or neuroplastic processes (sprouting of dendrites, synaptogenesis) and/or also scar formation (glia cell proliferation) may cause increases in cortical thickness. Studies suggest that cortical thickness may be a suitable predictor in addition to functional activity for functional recovery in stroke patients (Schaechter et al., 2006).

In order to interpret those changes correctly, it is necessary to non-invasively measure a more direct (functional) parameter of neuronal integrity or neuronal density in vivo with sufficiently high resolution (Nedergaard et al., 1984). It is the purpose of this thesis to develop a non-invasive in vivo imaging strategy that allows the assessment of
these two parameters (cortical thickness and neuronal density) in acute stroke patients and to demonstrate its feasibility in a pilot sample.

1.2 Objectives

The overall goal of this study is to establish a method to evaluate cortical thickness and neuronal density \textit{in vivo} in the peri-infarct zone.

Specific Aims:

- To assess the accuracy of our surface-based cortical thickness (SCT) mapping method by comparing measures in regions of varying distance from the infarct.
- To employ the same region-based analysis in the peri-infarct region of positron emission tomography (PET) scans using $[^{18}\text{F}]$Flumazenil ($[^{18}\text{F}]$FMZ) as our marker for neuronal density.
- To propose a strategy to assess the relationship between SCT and $[^{18}\text{F}]$FMZ binding surrounding brain lesions.

The hypotheses to be tested:

- Measurements of SCT from magnetic resonance imaging (MRI) can be made in the border zone surrounding cerebral infarcts.
- Changes in SCT and $[^{18}\text{F}]$FMZ binding in the stroke affected hemisphere can be detected in individual subjects during the period of post-stroke recovery compared to a group of age matched control subjects or the patients' own contralateral hemisphere.
By comparing results from the SCT mapping and the $[^{18}\text{F}]$FMZ PET, we can make inferences regarding the following cases:

1) If a decrease in SCT is observed it is either due to
   - A loss of neurons, in which case we expect $[^{18}\text{F}]$FMZ binding to be low
   - A change in the cytoarchitecture with preserved neurons, in which case we expect $[^{18}\text{F}]$FMZ binding to be normal

2) If an increase in SCT is observed it is either due to
   - Glial proliferation, in which case we expect $[^{18}\text{F}]$FMZ binding to be low
   - An increase in neuronal structures, in which case we expect $[^{18}\text{F}]$FMZ binding to be high

Chapter 2 – Background

2.1 Cortical Thickness

The cerebral cortex is a tightly folded sheet of gray matter encasing the brain. The study of its cellular composition, the field of cytoarchitectonics, was founded by Theodor Meynert. Other scientists who subsequently contributed to this field include Vladimir Alekseyevich Betz, Alfred Walter Campbell, Sir Grafton Elliot Smith, and Korbinian Brodmann. Staining slices of the cortex using Nissl stain to visualize neuronal cells allowed these neuroanatomists to produce descriptions of the laminar structure of the cortex in different species. Brodmann described the laminar organisation of the cortex
which is organised into six separate layers throughout the neocortex (the outermost layer of the cerebral cortex and newest part to evolve) and fewer in the allocortex (i.e. the olfactory cortex and the hippocampus) (1908). The layers are (from the pial surface towards the white matter): (I) the molecular layer, (II) the corpuscular layer, (III) the pyramidal layer, (IV) the granular layer, (V) the ganglionic layer, and (VI) the multiform layer. The differences between these layers are described based on pyramidal cell staining methods which reveal different density of pyramidal cells in the various laminae (Heiko, 1984).

The thickness of the human cerebral cortex is an important parameter to consider in the study of both normal and abnormal neuroanatomy. The measurement of cortical thickness has been of interest in the neurosciences since the turn of the 20th century. Cortical thickness varies by region (Economo and Koskinas, 1925), changes throughout the life-span (Thompson et al., 1998), and can be affected by disease (Thompson et al., 2004). One of the earliest studies of cortical thickness was performed by Constantin von Economo and Georg N. Koskinas in 1925 and is still used as the classic reference in the field. Economo and Koskinas provided detailed maps of cortical thickness as well as the thickness of each layer of cortex. They also defined 109 cortical areas on the basis of cytoarchitectonic criteria in their landmark work “Die Cytoarchitektonik der Hirnrinde des erwachsenen Menschen” ("Cytoarchitectonics of the Adult Human Cerebral Cortex") (Economo and Koskinas, 1925).
Using formalin-fixed and paraffin-embedded human brains, von Economo and Koskinas found that the average thickness over the whole brain is around 2.5 to 3mm and can vary from 1.5 up to 4.5mm (Economo, 1929). The cortex is thinnest in the post-central gyrus and the occipital lobes (about 2mm) and thickest in the anterior temporal lobes and the pre-central gyrus (about 4mm) (Economo, 1929; Zilles, 1990). Variations in thickness relate to differences in cell type (Economo, 1929; Geyer et al., 1999) and to functionally distinct areas (Brodmann, 1908; Economo & Koskinas, 1925).

Post-mortem studies on cortical thickness and neuronal density surrounding cerebral infarcts by Nedergaard et al. (1984 &1986), support the traditional view held by pathologists that a sharp transition exists between infarcted and normal brain tissue. They found that cortical thickness and density of histologically intact neurons was normal at a distance of 5mm from the infarct measured on the outer brain surface with a slightly elevated glial cell density observed in a few cases (Nedergaard et al., 1984). While the study of post-mortem cerebral anatomy is an established method it does not allow the examination of the number of brains necessary to make statistically meaningful inferences about neuroanatomy (Evans, 2002). Moreover, thickness measured directly using post-mortem brains is not considered an absolute metric due to inevitable tissue shrinkage (Kabani et al, 2001). Another considerable problem with single slice estimation of cortical thickness is that it is strongly dependent on the cutting
angle and slight shifts in this angle can create significant differences in the final measurement (Economo and Koskinas, 1925).

Literature on the thickness of the cortex in health and disease has grown owing to recent advances in brain imaging techniques. Prior to imaging, few studies investigated the thickness of the cerebral cortex due to the laborious nature of studying post-mortem brains. Today the morphometric analysis of MR images of the brain has become a commonly used method to examine neuroanatomical correlates of neurological disorders and brain development (Thompson et al., 2004). Current MRI techniques make it possible to measure thickness throughout the cortex in vivo in a quantitative and biologically meaningful way.

Many methodologies for measuring cortical thickness from MRI exist, (e.g. Zeng et al., 1999, Miller et al., 2000, Jones et al., 2000, Fischl & Dale, 2000, Lohmann et al., 2003, Yezzi & Prince, 2003, Thompson et al., 2004, Lerch et al., 2005, Barta et al., 2005, Scott & Thacker, 2005) yet there is still no gold standard to measure up to. Cortical thickness can be measured manually on image slices from the cortical sheet identifiable on MR images (Lerch and Evans, 2005). However, due to its three-dimensional structure this method is complicated and very time consuming. Consequently, several image processing methods that automate this procedure have emerged. Such methods have made it feasible to study very large numbers of subjects.
Automated methods can be either surface-based or voxel-based. Generally surface-based approaches involve generating three-dimensional surface models to fit the gray matter (GM) and white matter (WM) (Fischl & Dale, 2000; Jones et al., 2000; MacDonald et al., 2000; Miller et al., 2000; Zeng et al., 1999). Other surface-based techniques extract only the WM surface then this surface is expanded toward the GM/cerebrospinal fluid (CSF) intersection to create the GM surface (e.g. Lerch and Evans, 2005; Thompson et al., 2005). Next the distance between points on these surfaces is measured to determine cortical thickness. Voxel-based cortical thickness measurement methods involve spatially normalizing all the images to the same stereotactic space, extracting the gray matter from the normalized images, smoothing so that each voxel represents the average of itself and its neighbours, and finally performing a voxel-wise comparison of the local concentration of gray matter between two groups of subjects (Ashburner and Friston, 2000; Hutton et al., 2002; Jones et al., 2000; Yezzi and Prince, 2003). Voxel-based morphometry (VBM) studies have suggested that reductions in GM volume maps may represent changes in neuronal composition due to atrophy (Keller et al., 2002; Wessels et al., 2006) or neuronal loss (Mueller et al., 2006), however the nature of the GM changes identified by VBM are still poorly understood. In contrast to VBM methods, which provide relative GM densities, SCT mapping bears the advantage of providing a direct quantitative index of cortical morphology.
Thickenss changes in the cortex can be considered a sign of neuroplasticity. During normal brain development changes in cortical thickness are associated with the development of intellectual capabilities (Shaw P, 2006a). In adults, similar changes have been described which are related to practice and learning (Maguire EA, 2000). Changes in cortical thickness can be related to disease, such as focal atrophy in degenerative diseases (Lerch et al., 2005), or prognostic indicators for disease progression and clinical outcome (Shaw P, 2006b). Recently, a study by Schaechter et al. (2006), demonstrated an increase in cortical thickness and functional activation response in chronic post-stroke patients.

What complicates the cortical thickness literature is the difficulty finding a consistent definition of cortical thickness. Overall, the thickness of the cortex is defined by the distance between the inner and outer extents of cortical GM, with the gray-white intersection as the inside boundary and the pial surface as the outside boundary. Where studies differ is in determining how to identify where these boundaries are. Most in vitro studies stain for neurons however, Economo was unsatisfied with this method due to the fact that the appearance of neurons does not end abruptly where the WM begins, rather there are some interspersed in the WM (Economo and Koskinas, 1925). Therefore, he chose to stain for fibre bundles that run horizontally along the WM border so as to not overestimate the extent of GM. Even with Economo’s improved silver-stained preparations, the boundary between GM and WM was diffuse and difficult to
delineate precisely in some cortical areas like the primary motor cortex. According to Economo and Koskinas (1925) the blurred nature of the boundary caused differences of up to 0.5mm and greater in the estimation of thickness in similar locations by different raters.

In vivo computation of thickness is always based on some measure of distance between two points, one on the WM/GM surface and the other on the GM/CSF surface. Differing definitions of correspondence and/or the distance measurement between these two points results in several studies offering varying estimates of thickness.

2.2 Neuronal Density

GABA is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). GABA<sub>A</sub> receptors have been identified electrophysiologically and pharmacologically in all regions of the brain and are widely expressed on cerebral neurons (Olsen, 1981). They are located in axo-somatic and axo-dendritic synapses on neurons that are innervated by inhibiting interneurons (Richards, 1987). Benzodiazepine (BZ) binding sites copurify with GABA<sub>A</sub> receptors (Sigel, et al., 1983) and are immunoprecipitated with antibodies developed to recognize the protein containing the GABA<sub>A</sub> binding site (Fritschy, et al., 1995). This indicates that BZ sites co-localize with GABA<sub>A</sub> receptors on cortical neurons (Frey et al., 1991) and are an integral part of the GABA<sub>A</sub> receptor - Cl<sup>-</sup> channel complex. BZ sites are located on and are markers of dendrite membranes of projecting neurons. Therefore, BZ site concentration mainly
depends on the presence of dendrites from projecting neurons in the cortex and consequently may serve as a marker for neuronal density and neuronal integrity (Heiss et al., 1998).

Until around the mid 1980’s, attempts to map the distribution and density of BZ sites in the CNS were dominated by radiohistochemical techniques that evaluated \textit{in vitro} or \textit{in vivo} binding of radioligands in tissue sections (see Kuhar et al., 1986). Young and Kuhar (1979) were the first to report autoradiographic localisation of BZ sites in mammalian CNS using light microscopy and carried out the first receptor autoradiography in human brain tissue. Limited resolution and other limitations of such techniques prompted the use immunohistochemistry (Richards, 1986). Later work to visualize BZ sites in the human post-mortem brain was done by Dennis et al. (1988) and Zezula et al. (1988). These groups used receptor autoradiography to map BZ sites with [3H]Flunitrazepam and/or [3H]Zolpidem and documented the distribution of BZ sites in detail. They found no major differences in GABA\textsubscript{A} receptor densities throughout the neocortex (Zezula et al., 1988) except for in the sensorimotor cortex where the density is low and in the primary visual cortex where the density is high (Zezula et al., 1988; Dennis et al., 1988). This suggests that despite significant variation in individual cortical layers the proportion of GABA neurons in the neocortex is considered to be relatively homogenous. Meaning that differences in overall numbers of neurons are reflected in the number of the GABA neurons, thus measuring the number of GABA
neurons should allow inferences on the number of total neurons in the neocortex.

In the early to mid 1980s developments in *in vivo* ligand binding techniques and PET allowed the analysis of the gross distribution of BZ sites. Although radioligand binding studies *in vitro* offer several advantages (controlled binding conditions, metabolic stability of ligands, circumvention of the blood-brain barrier, possibility to study post-mortem human brains and the affinity of different ligands in adjacent sections), *in vivo* studies provide the only means of investigating receptor occupancy by drugs and their metabolites, after systemic administration, under physiological conditions (Richards, 1986). Flumazenil (FMZ) was the first radioligand developed for visualizing the distribution of GABA_A receptors *in vivo* in the human brain and is still the most widely used tracer for this purpose (Frey et al., 1991; Salmi et al., 2008). FMZ acts as an antagonist at the benzodiazepine binding site of GABA_A receptors containing α1, α2, α3, or α5 subunits (Hammers, 2004). Early studies using [11C]FMZ demonstrated that different areas of healthy human brain showed approximately 10-fold variation in tracer binding, which corresponded to the previously known distribution of benzodiazepine receptors in these regions (Odano, 2008).

Recently the synthesis of [18F]FMZ was reported (Ryzhikov et al., 2005), the use of which might be preferable to [11C]FMZ due to the longer half-time of 18F and the slightly higher image quality in terms of spatial resolution due to the shorter positron range of 18F as compared to 11C. Therefore, the combined use of [18F]FMZ and a high
resolution research tomograph (HRRT) would offer a PET image of GABA distribution with the highest resolution available to date.

Changes in this inhibitory neurotransmitter system in the CNS have been found in various neurological and psychiatric disorders, such as dementia (Ihara et al., 2004; Meyer et al., 1995), congenital syndromes like Prader-Willi-syndrome (Lucignani et al., 2004), anxiety disorders (Malizia et al., 1998), alcoholism (Litton et al., 1993), and stroke (Heiss et al., 1997). In addition, previous studies have demonstrated that permanently and irreversibly damaged cortex can be distinguished from viable brain tissue as early as three hours after stroke using $[^{11}\text{C}]$FMZ PET and that this method might be of relevance for the selection of individual therapeutic strategies (Heiss et al., 1998; Heiss et al., 2001; Heiss et al., 2004).

To measure cortical thickness in this study we will use an automated surface-based approach as opposed to a voxel-based method in order to obtain quantitative and biologically meaningful cortical thickness values. To investigate neuronal density we will acquire PET images on an HRRT using $[^{18}\text{F}]$FMZ as our marker for neuronal density in order to obtain data with the highest resolution possible.
Chapter 3 – Methods

3.1 Participants

A total of 13 patients (9 male, 4 female) were recruited from the stroke unit at the Sir Mortimer B. Davis Jewish General Hospital in Montreal participated in this study. Patient logs from the emergency department very screened multiple times per week in search of possible participants. Potential participants were screened for MRI compatibility (i.e. no pacemakers or metal implants). The inclusion criteria stipulated that patients needed to have suffered from a first ever ischemic stroke. All participants underwent thorough consent procedures before commencing the study. The study was approved by McGill’s Research Ethics and Compliance Institutional Review Board (IRB study number A12-M142-06B and A06-M64-07A).

The average age of the patients included in the study was 62 ± 14 years and ranged from 31-80 years of age. 9 patients had suffered a middle cerebral artery (MCA) stroke and 4 of the strokes were in the posterior cerebral artery (PCA) territory. Individual participant characteristics are listed in Table 1.
<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
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<td>50</td>
<td>Male</td>
<td>Right</td>
<td>Left</td>
<td>MCA</td>
</tr>
</tbody>
</table>

Table 1. Individual patient characteristics including age, gender, handedness, stroke side, region and size.

A total of 26 healthy, age-matched controls scans (2 per stroke patient) were obtained from the Montreal Neurological Institute’s International Consortium for Brain Mapping extended MRI database. The average age of the controls included in the study was 64 ± 11 years and ranged from 31-78 years of age.

### 3.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a non-invasive medical imaging technique used to produce high quality images of detailed structures inside the body. It can distinguish between different soft tissues making it particularly useful in neurological imaging. MRI is based on the absorption and emission of energy in the radio frequency range. It uses powerful magnets and radio waves to influence hydrogen atoms in the body. When placed in a strong magnetic field, hydrogen atoms align with the direction of the field. A
radio frequency pulse of the appropriate frequency excites hydrogen atoms causing
them to absorb energy and displace from their aligned position. When the pulse finishes
this energy is released in the form of a radio frequency signal which is detectable by the
scanner (Bloch et al, 1946; Lauterbur, 1973). This signal can be manipulated by
additional magnetic fields to accumulate sufficient information to construct an image of
the brain. Different pulse sequences can generate images with varying image contrasts.
T1-weighted contrast is one of the basic types of MR contrast where fluid-containing
tissues are dark and fat-containing tissues are bright. T1-weighted brain scans provide
good gray matter/white matter contrast. MRI can be used to differentiate pathologic
tissue such as a stroke from normal tissue. One MRI technique in particular that can
provide helpful information about damage to parts of the nervous system is called
diffusion weighted imaging (DWI). DWI produces images in which each voxel has an
intensity that reflects a measurement of the rate of water diffusion at that location.
These images have an increased sensitivity to early changes after a stroke as
compared to traditional MR images. Following a stroke, areas of cerebral infarction have
decreased apparent diffusion resulting in increased signal intensity on the DW images.
It is thought that increases in restriction to water diffusion, as a result of cellular
swelling, are responsible for the increase in signal on a DWI scan.

Owed to their good gray matter - white matter contrast, T1-weighted anatomical scans
were used in this study as a basis for extracting the gray and white matter surfaces
necessary for the surface-based cortical thickness mapping. The T1-weighted scans were also used to discern where the stroke was and to demarcate infarct borders in order to precisely label the infarct on the image. The role of the DWI scans was to corroborate the localization and boundaries of the infarct on the T1.

3.3 Magnetic Resonance Imaging Acquisition

Patients were scanned on a 1.5 Tesla Siemens Sonata MRI scanner at the McConnell Brain Imaging Centre of the Montreal Neurological Institute (BIC). MRI volumes were obtained using a 3D fast-field echo sequence (repetition time = 22ms, echo time = 9.2ms, matrix size = 256×256mm$^2$, slice thickness = 1mm, flip angle = 30°) resulting in images with a voxel size of 1.0×1.0×1.0mm. DWI volumes were also acquired (6 directions, b-value=0, b0=1000, voxel size = 2.0×2.0×2.0mm, TR = 10800 ms, TE = 98ms) while patients were in the MRI scanner.

3.4 Magnetic Resonance Imaging Analysis

Images were analyzed using CIVET, a fully-automated image-processing pipeline (a sequence of programs) for cortical surface extraction and cortical thickness evaluation which performed the following steps (Ad-Dab'bagh et al., 2006). The native MRIs are registered into MNI-Talaraich stereotaxic space using a 12-parameter linear affine transformation to the MNI ICBM 152 6th generation non-linear target (Collins et al., 1994, Grabner 2006), after being corrected for intensity non-uniformity artifacts (signal intensity varying smoothly across an image) using the non-uniform intensity
normalisation package N3 (Sled et al., 1998) and masked using the Brain Extraction Tool (BET) to eliminate the skull and meninges (Smith, 2002). The registration step brings all image volumes into the same spatial coordinate system (MNI-Talaraich stereotaxic space) by aligning them all to a template brain (MNI ICBM 152) so that data from similar locations in different brains can be compared. Once in stereotaxic space, a final correction for intensity non-uniformity artifacts is performed (using N3) and a new mask is recomputed (using BET). After pre-processing, a non-linear registration to a probabilistic atlas is performed then CIVET discretely classifies (segments) the corrected and registered volumes into WM, GM, cerebrospinal fluid (CSF) and background using INSECT and an artificial neural network (ANN) (Zijdenbos et al., 2002), from which partial volumes of the tissue classes are estimated (Tohka et al., 2004). The partial volumes for CSF are used to create a skeletonized CSF surrounding the pial surface, important for the production of cortical surfaces.

A Constrained Lapacian-based Anatomic Segmentation using Proximity algorithm (CLASP) fits a deformable polygonal model to the inner surface of the cortex that is produced by the interface between GM and WM (MacDonald et al., 2000; Kim et al., 2005). Then it expands outward through the cortex to find the GM-CSF intersection outlined by the CSF skeleton (Lerch & Evans, 2005). Since this surface is an expansion from the WM surface, each vertex on the new surface is 'linked' to its vertex of origin on the WM surface. Thus, surface cortical thickness (SCT) is defined as the distance
between these linked vertices (t-link). SCT is evaluated in native space, using the t-link approach (Ad-Dab'bagh, Y., et al 2005) and thickness data are blurred on the surface using a diffusion-smoothing kernel of 20mm FWHM to enhance the strength of the signal (Chung, 2004) The cortical thickness measures in native space provide measurements closer to the real dimensions of the cortex (unlike SCT in stereotaxic space). The surfaces generated by CLASP are aligned to the MNI ICBM 152 surface template through non-linear surface registration and are resampled in native space (Robbins, 2004; Lyttelton et al., 2007). SCT is reinterpolated onto the resampled surfaces from the original CLASP surfaces. The number of polygons (81920 triangles per hemisphere) in the model will always be identical across subjects, creating a surface coordinate system that can be used to run statistical analyses of cortical thickness at every vertex (40962 per hemisphere) of the surface across subjects.

![Figure 1. Main stages from CIVET pipeline](image)

Figure 1. Main stages from CIVET pipeline a) Native T1-weighted MRI  b) MRI after preprocessing (non-uniformity correction and linear registration to stereotaxic space) and classification into GM, WM, and CSF  c) and d) WM and GM surfaces generated by CLASP’s deformable model approach  e) Cortical thickness is measured across
corresponding points of an inner WM/GM surface and an outer GM/CSF surface (Lepage, 2008).

3.5 Definition of Regions of Interest

The patients’ infarcts were localized and masked on each slice of the native T1 MR images by manually delineating the infarcted tissue with the help of the diffusion weighted images using Display, an interactive brain visualization tool that allows manual segmentation of MRIs, aka ‘brain painting’ (Figure 2.a.). (Figure 2.a.). The mask of the infarcted tissue was transposed onto the resampled, surface-registered cortical surface in native space using the program volume_object_evaluate (Figure 2.b.). In order to fill in small holes in the mask due to painting errors the borders of the infarct masks were expanded along the surface by two vertices in all directions then eroded back by two vertices to their original size using the program dilate_surface. Next, 3 ROIs were generated in the peri-infarct zone at increasing distances from the masked infarcted tissue to assess whether SCT was altered in these penumbral regions. To create the first ROI ring around the infarct the mask was blurred on the surface using an 8mm fwhm filter then thresholded at 7.5% maximum intensity. Two more ROIs were created the same way each using the blurred and thresholded ROI from the previous step to carry out the next round of blurring (8mm fwhm) and thresholding (7.5%). The resulting ROIs were combined to create ROIs with vertex values of 1-4 (4 = infarct; 1 = outermost ROI) (Figure 2.c.). The ROIs were transferred into the patient’s contralateral
hemisphere (Figure 2.d.). Then the ROIs were displayed on a sphere in brain-view (a graphical user interface application from the BIC used in this study to view models of the brain surface along with data associated with each vertex on that surface such as SCT, BPND, and ROI labels) to ensure that they were contiguous and properly followed the outline of the infarct masks (Figure 2.e.).

All tools used in the definition of the regions of interest (ROI) are part of software packages created at the BIC (www.bic.mni.mcgill.ca/ServicesSoftware/HomePage).

Figure 2. Flow chart of the main steps involved in defining the peri-infarct ROIs and an
example of the ROIs depicted on the surface of patient with accompanying example images from patient 3.

3.6 Positron Emission Tomography

Positron emission tomography (PET) is a nuclear medicine imaging technique that uses small amounts of radioactive labelled radiopharmaceuticals to quantitatively study physiological processes in the body. The process to be studied during a PET scan determines which radioactive chemical tracer (radiotracer) is used. The radiotracer is produced by attaching a radioactive atom or molecule to a compound of interest. Then very small amounts are injected into the patient’s bloodstream, from which it can be taken up into the brain. As the radiotracer undergoes radioactive decay it releases positrons which collide with electrons, resulting in pairs of gamma rays which are detected by pairs of scintillation crystals in the PET scanner. The information gathered by thousands of scintillation pairs arranged in several detector rings around the patients head, represents projections of the activity distribution in the brain. Using computer algorithms, similar to those applied in clinical CT-scanners, three-dimensional images of the spatial radioactivity distribution in the brain are reconstructed. During a scanning session, multiple such image volumes are acquired, representing radiotracer concentration within the brain over time. With this approach, one can estimate the binding and the distribution of particular receptors in the living human brain.
3.7 Positron Emission Tomography Acquisition

$[^{18}\text{F}]$FMZ was synthesized using the method published by Massaweh et al. (2009). $[^{18}\text{F}]$FMZ PET scans were obtained from 4 of the 13 patients (patient 3, 5, 6, and 10) using the high resolution research tomograph (HRRT), which acquires data in list mode (Siemens Medical Solutions, Knoxville, TN, USA). This fully 3D high resolution brain scanner, with a field of view of 25.2 cm (axially) by 31.2 cm (diameter), has a spatial resolution of between 2.3 and 3.4 mm at full width at half maximum (FWHM) and enables data acquisition with high spatial resolution combined with high sensitivity. In addition, the use of two crystal layers (LSO/LYSO) permits photon detection with depth-of-interaction information. After a transmission scan for attenuation correction (137Cs source), approximately 370 MBq $[^{18}\text{F}]$FMZ was injected intravenously as a slow bolus over 60 seconds. List-mode data were acquired for 60 minutes after the injection start time and were subsequently binned into fully 3D sinograms for a total of 17 time frames (40 sec, 20 sec, 2 x 30 sec, 3 x 60 sec, 4 x 150 sec, 3 x 300 sec, and 3 x 600 sec). All studies were reconstructed 3D filtered backprojection (3D-FBP) algorithm, both including a frame based motion correction. All reconstructed data were corrected for scatter, random coincidences, scanner normalization, attenuation, decay, and dead-time and resulted in a time-series of 3D images (each of $256 \times 256 \times 207$ voxels). The voxel dimensions of the reconstructed images were $1.22 \times 1.22 \times 1.22$ mm$^3$. 
3.8 Positron Emission Tomography Analysis

The last three frames from the PET scan were extracted and used to generate an average image (30-60 mins after tracer injection). The average images were normalized using the white matter in the semioval center (Hara et al., 2002). The normalized PET volumes were linearly coregistered to the native T1 MRI scans. Then the PET data were transposed onto the native surface that was produced during the MRI analysis to extract the PET surface data at the same 40,962 vertices using volume_object_evaluate.

![Flowchart summarizing the main steps in the PET analysis.](image)

3.9 Positron Emission Tomography Regions of Interest

With BPND [18F]FMZ PET data now associated with each vertex on the patient’s surface, the identical ROIs generated for SCT analysis were transposed onto the surfaces to extract the PET data from each ROI in the peri-infarct zone.

3.10 Estimation of Region of Interest Width

A distance map moving away radially from the border of the infarct was created in the
MRI volume using the program mincchamfer from the BIC. Chamfer maps were plotted on the surface so that vertices in the border zone around the infarct were assigned a distance from the edge of the infarct in millimetres. Sample vertex numbers taken from the perimeters of the ROI rings in 2 patients were used to locate the ROI border locations on the chamfer maps. Chamfer distance measures were recorded and used to estimate each ROI’s distance from the infarct and to approximate the width of each ROI.

3.11 Statistical Analysis

Unless otherwise noted, all statistical tests were carried out using SigmaPlot (Systat Software Inc.). Values of P<0.05 were considered significant in this study. The average and standard deviation of SCT in the ROIs were calculated for each patient in both hemispheres using an inhouse customized script, lobe_stats, to get the distribution of SCT at each vertex on the surface. The SCT values from the patients’ stroke affected hemisphere were compared to their contralateral hemisphere using a two-way repeated measures ANOVA evaluating the effect of hemisphere (affected, contralateral) and ROI (inner, middle, outer). Another two-way repeated measures ANOVA was carried out with the same factors but this time with the standard deviation of SCT as the dependent variable to assess the variance of SCT in the ROIs in each both patient hemispheres.

The average and standard deviation of SCT in the ROIs was calculated using lobe_stats in each control, in the appropriate hemisphere based on the patient’s stroke affected
side, to obtain the distribution of SCT across all relevant vertices on the control surfaces. This data was compared to each patient’s average ROI data using a two-way ANOVA to test for differences comparing group (patient, control) and ROI (inner, middle, outer). Then an additional two-way ANOVA comparing group (patient, control) and ROI (inner, middle, outer) was used to assess the difference in variance of SCT between the two groups.

Next a program from the BIC, called vertstats_average, was used to average the thickness measurements from all of the controls to create our study’s population average brain. Then z-scores at each vertex for each patient were computed (patient SCT - control μSCT / σSCT control) using vertstats_math, another statistical analysis tool from the BIC, and compared to the control population average. The average and standard deviation of the z-scores in each of the patients’ ROIs was calculated using lobe_stats to compare variance of z-scores across patients. Z-score surface maps were plotted in brain-view in order to visualize whether regions in the patient brains varied significantly from the control population average.

3.12 Ratio Maps

In order to visualize whether there was a region of affected tissue surrounding the infarct the BP<sub>ND</sub> of [<sup>18</sup>F]FMZ was divided by SCT at each vertex to produce ratio maps. The quotient was mapped onto the surface and viewed in brain-view. These maps were
designed as an alternative way to visualize our data and verify the region-based results not as a means of primary analysis.

Chapter 4 – Results

4.1 Surface-Based Definition of ROIs

ROI rings followed the contour of the infarcts properly irrespective of the infarct location (MCA vs PCA) (Figure 4). The ROIs were approximately the same width all the way around. In some cases the ROIs were difficult to see on the brain surface due to the folding of the cortex so they were also viewed on a sphere (Figure 4).
Figure 4. Three-dimensional maps of inner (blue), middle (green) and outer (red) peri-infarct regions of interest in patients 6 (MCA) and 10 (PCA) (from top to bottom) depicted on the native brain surfaces and on a sphere.

4.2 Region-Based Analyses of Surface-Based Cortical Thickness (SCT)

The average SCT in all three ROI rings across all patients’ affected hemispheres was 3.2mm (range: inner = 2.7-4.0, middle = 2.7-3.9, outer = 2.7-3.8) with a standard deviation of ±0.47, ±0.46, ±0.43 respectively (Table 2). The average SCT in all three ROI rings across all patients’ contralateral hemispheres was 3.2mm (range: inner = 2.7-4.1, middle = 2.8-4.0, outer = 2.7-3.9) with a standard deviation of ±0.39, ±0.41, ±0.43 respectively (Table 3).

In control brains the average SCT in the inner ROI was 3.1mm with a standard deviation of ±0.4. Whereas the average SCT in both the middle and outer control ROIs was 3.2mm with a standard deviation of ±0.38, ±0.40, ±0.42 respectively (Table 4).
### Table 2. Average and standard deviation of SCT in the inner, middle, and outer ROIs in individual patient’s stroke affected hemisphere as well as the mean SCT in each ROI.

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<tr>
<th>Patient</th>
<th>SCT in Patients' Affected Hemisphere [mm]</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Inner ± Middle ± Outer ±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>1</td>
<td>3.1 ± 0.35 ± 3.1 ± 0.37 ± 3.0 ± 0.35</td>
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<tr>
<td>2</td>
<td>4.0 ± 0.50 ± 3.9 ± 0.50 ± 3.8 ± 0.50</td>
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<tr>
<td>3</td>
<td>2.9 ± 0.41 ± 2.8 ± 0.39 ± 2.9 ± 0.41</td>
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<tr>
<td>4</td>
<td>2.9 ± 0.49 ± 2.9 ± 0.46 ± 2.9 ± 0.47</td>
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<tr>
<td>5</td>
<td>3.2 ± 0.57 ± 3.1 ± 0.53 ± 3.2 ± 0.54</td>
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<tr>
<td>6</td>
<td>3.7 ± 0.67 ± 3.6 ± 0.60 ± 3.5 ± 0.59</td>
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<td>±</td>
<td>±</td>
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<tr>
<td>1</td>
<td>3.0 ± 0.33 ± 3.1 ± 0.33 ± 3.2 ± 0.29</td>
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<tr>
<td>3</td>
<td>2.8 ± 0.28 ± 2.8 ± 0.27 ± 2.8 ± 0.32</td>
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<tr>
<td>7</td>
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Table 3. Average and standard deviation of SCT in the inner, middle, and outer ROIs in individual patient’s contralateral hemispheres as well as mean SCT in each ROI.

<table>
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<th>Patient</th>
<th>SCT in Control Hemispheres [mm]</th>
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<td>3.1 0.38</td>
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Table 4. Average and standard deviation of SCT in the inner, middle, and outer ROIs in control brains as well as the control average SCT in each ROI.

Two separate two-way repeated measures ANOVAs showed no statistically significant differences in SCT or variance of SCT between the ROIs (inner, middle, outer) in the patients’ stroke affected hemisphere and the equivalent ROIs in the patients’ contralateral hemispheres.

A two-way ANOVA comparing SCT in the ROIs in the patients’ affected hemisphere and in controls did not uncover any statistically significant differences.
No significant differences in the variance of SCT were found in patients’ affected hemispheres compared to the control hemispheres using a two-way ANOVA.

4.3 Region-Based Analyses of Non-Displaceable Binding Potential (BP\textsubscript{ND}) of Flumazenil

Results

The average BP\textsubscript{ND} of \textsuperscript{[18}F\textsuperscript{]}FMZ in the patients’ affected hemispheres in the inner ROI was 9.0 ± 3.88 (range 5.6-10.6) (Table 5). In contrast average BP\textsubscript{ND} of \textsuperscript{[18}F\textsuperscript{]}FMZ in the patients’ contralateral hemispheres in the inner ROI was 13.7 ± 2.82 (range 11.2-16.4) (Table 6).

The average BP\textsubscript{ND} of \textsuperscript{[18}F\textsuperscript{]}FMZ in the patients’ affected hemispheres in the middle ROI was 10.1 ± 3.53 (range 7.4-11.7) (Table 5). Whereas the average value of BP\textsubscript{ND} of \textsuperscript{[18}F\textsuperscript{]}FMZ in the patients’ contralateral hemispheres in the middle ROI was 13.7 ± 2.86 (range 11.4-16.5) (Table 6).

The average BP\textsubscript{ND} of \textsuperscript{[18}F\textsuperscript{]}FMZ in the patients’ affected hemispheres in the outer ROI was 11.0 ± 3.45 (range 9.4-12.8) (Table 5). While the average BP\textsubscript{ND} of \textsuperscript{[18}F\textsuperscript{]}FMZ in the patients’ contralateral hemispheres in the outer ROI was 13.5 ± 2.93 (range 10.8-15.7) (Table 6).
Table 5. Average $\text{BP}_{\text{ND}}$ of $[^{18}\text{F}]$FMZ and standard deviation of $\text{BP}_{\text{ND}}$ in inner, middle, and outer ROIs in patient’s stroke affected.

<table>
<thead>
<tr>
<th>Patient</th>
<th>FMZ Binding in ROIs in Affected Hemisphere $[\text{BP}_{\text{ND}}]$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner $\pm$ Middle $\pm$ Outer $\pm$</td>
</tr>
<tr>
<td>3</td>
<td>10.2 4.57 11.7 3.91 11.9 3.72</td>
</tr>
<tr>
<td>5</td>
<td>9.6  2.25 9.8  2.20 9.8  2.27</td>
</tr>
<tr>
<td>6</td>
<td>5.6  3.82 7.4  3.73 9.4  3.41</td>
</tr>
<tr>
<td>10</td>
<td>10.6 4.87 11.6 4.29 12.8 4.39</td>
</tr>
<tr>
<td>Mean</td>
<td>9.0  3.88 10.1 3.53 11.0 3.45</td>
</tr>
</tbody>
</table>

Table 6. $\text{BP}_{\text{ND}}$ of $[^{18}\text{F}]$FMZ and standard deviation of $\text{BP}_{\text{ND}}$ in inner, middle, and outer ROIs in patient’s contralateral hemispheres.

<table>
<thead>
<tr>
<th>Patient</th>
<th>FMZ Binding in ROIs in Contralateral Hemisphere $[\text{BP}_{\text{ND}}]$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner $\pm$ Middle $\pm$ Outer $\pm$</td>
</tr>
<tr>
<td>3</td>
<td>14.2 2.73 13.9 3.10 14.1 3.41</td>
</tr>
<tr>
<td>5</td>
<td>11.2 2.08 11.4 2.20 10.8 2.25</td>
</tr>
<tr>
<td>6</td>
<td>13.1 2.88 13.1 2.82 13.3 2.73</td>
</tr>
<tr>
<td>10</td>
<td>16.4 3.58 16.5 3.30 15.7 3.32</td>
</tr>
<tr>
<td>Mean</td>
<td>13.7 2.82 13.7 2.86 13.5 2.93</td>
</tr>
</tbody>
</table>

A two-way repeated measures ANOVA revealed a statistically significant overall interaction between hemisphere and ROI ($P = 0.014$). The $\text{BP}_{\text{ND}}$ of $[^{18}\text{F}]$FMZ in the affected hemisphere ROIs was significantly different from the ROIs in the contralateral hemisphere ($P = 0.031$). Within the affected hemisphere, $\text{BP}_{\text{ND}}$ of $[^{18}\text{F}]$FMZ in the inner ROI was significantly different from both the middle ($P = 0.017$) and the outer ROIs ($P = 0.001$) (Figure 5). The inner ROIs were significantly different in the affected hemisphere compared to the contralateral hemisphere ($P = 0.011$), as were the middle ROIs in the affected hemisphere compared to the contralateral hemisphere ($P = 0.026$) (Figure 5).
Figure 5. Average BP\textsubscript{ND}\textsuperscript{[18F]}FMZ in inner (blue), middle (green) and outer (red) peri-infarct regions of interest in 4 patients’ affected compared to contralateral (contra) hemispheres showing statistically significant results and the significance level.

A two-way repeated measures ANOVA testing for differences in the variance of BP\textsubscript{ND} of \textsuperscript{[18F]}FMZ showed a significant difference between ROIs in the affected hemisphere compared to the contralateral hemisphere (P = 0.050). Within the affected hemisphere, variance of BP\textsubscript{ND} of \textsuperscript{[18F]}FMZ in the inner ROI was significantly different from both the middle (P = 0.044) and the outer ROIs (P = 0.040). Variance within the inner ROIs were
significantly different in the affected hemisphere compared to the contralateral hemisphere ($P = 0.011$).

### 4.4 Chamfer Maps

The average width of the ROIs estimated using a chamfer distance transform in the MRI volumes was found to be 3.5mm (range 3.2-3.6mm) (Table 7).

![Chamfer maps](image)

Figure 6. Chamfer maps plotted on the native surfaces of patient 3 (left) and 10 (right) showing the distance from the infarct border in millimetres (0-20mm+).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Inner [mm]</th>
<th>Middle [mm]</th>
<th>Outer [mm]</th>
<th>Average ROI [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.4</td>
<td>3.6</td>
<td>3.3</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>3.7</td>
<td>3.5</td>
<td>3.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Mean</td>
<td>3.6</td>
<td>3.6</td>
<td>3.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Table 7. ROI width measurement estimates from 2 patients in the inner, middle, and outer ROIs as well as the mean ROI width for each ROI and the overall average ROI width.
4.5 Ratio Maps of BP\textsubscript{ND} of [$^{18}$F]FMZ / SCT

The BP\textsubscript{ND} [$^{18}$F]FMZ /SCT ratio is lower in the peri-infarct region as seen on the ratio maps below (blue ‘rings’ around the infarct) nicely support our region-based results of decreased [$^{18}$F]FMZ binding surrounding the stroke (Figures 7-10). Focal increases in SCT seen around the infarct and in the mesial temporal lobe of patient 10 are thought to be due to methodological errors discussed further in section 5.3.

![Figure 7. Surface maps of BP\textsubscript{ND} of [$^{18}$F]FMZ, SCT, and BP\textsubscript{ND} of [$^{18}$F]FMZ divided by SCT depicted in patient 3’s contralateral and affected hemispheres with the infarct](image-url)
shown in white.

Figure 8. Surface maps of BP_{ND} of [^{18}\text{F}]FMZ, SCT, and BP_{ND} of [^{18}\text{F}]FMZ divided by SCT depicted in patient 5’s contralateral and affected hemispheres with the infarct shown in white.
Figure 9. Surface maps of $BP_{ND}$ of $[^{18}F]$FMZ, SCT, and $BP_{ND}$ of $[^{18}F]$FMZ divided by SCT depicted in patient 6’s contralateral and affected hemispheres with the infarct shown in white.
Figure 10. Surface maps of $\text{BP}_{\text{ND}}$ of $[^{18}\text{F}]$FMZ, SCT, and $\text{BP}_{\text{ND}}$ of $[^{18}\text{F}]$FMZ divided by SCT depicted in patient 10’s contralateral and affected hemispheres with the infarct shown in white.
Chapter 5 – Discussion

The present study provides the first demonstration of cortical thickness measured using SCT mapping in acute stroke patients as well as the first region-based analysis of $BP_{ND}$ of $[^{18}\text{F}]$FMZ in the peri-infarct tissue. As predicted in the apriori hypothesis, measurements of cortical thickness from MRI can be made in the border zone surrounding cerebral infarcts using the SCT mapping method integrated in the CIVET pipeline at the BIC. However, only changes in $[^{18}\text{F}]$FMZ binding, and not in SCT, were detected in the stroke patients.

There are three main findings of this thesis: 1) SCT is the same in stroke patients’ peri-infarct region and in the corresponding region in their contralateral hemisphere. 2) Measures of SCT in the peri-infarct region of stroke patients are equivalent to those from matching regions in healthy controls. 3) Stroke patients have decreased $[^{18}\text{F}]$FMZ binding in the peri-infarct compared to the analogous region in their contralateral hemisphere. 4) There is a visible peri-infarct zone on the ratio maps.

5.1 Unchanged SCT in Stroke Patients

Using this SCT mapping method, cortical thickness can be measured in close proximity (<3.5mm) to infarcted tissue and still obtain reliable results.

Our average SCT results within the individual patients’ ROIs ranged from 2.7-4.0mm in the affected hemisphere and from 2.8-4.1 in the contralateral hemisphere which corresponds well with the average cortical thickness findings in the work of
Economo and Koskinas. They found that the average thickness over the whole brain is around 2.5 to 3.0mm and can vary from 1.5 up to 4.5mm (Economo, 1929).

Our SCT results support the conventional view held by pathologists that a sharp transition exists between infarcted and normal brain tissue. Nedergaard et al. found that this abrupt change occurred at a distance of approximately 5mm from the infarct in post-mortem brains (1986) and similarly our results indicate that this switch takes place <3.5mm to the lesioned tissue.

Recently, Schaechter et al. used a surface-based approach to measure cortical thickness in chronic stroke patients (2006). Their SCT measurements focussed on sensorimotor cortical areas regardless of the lesion location. They reported that cortical thickness in the ventral post-central gyrus was greater in the patients relative to controls and that there was no generalized increase in thickness across the cortical mantle compared to controls. We did not note any focal or generalized increases in SCT in the peri-infarct regions compared to our control SCT measurements.

Finding no gross morphological change does not rule out neuronal loss. Nedergaard et al. (1986) observed a slightly elevated glial cell density in a few of their case studies on post-mortem stroke brains, therefore it is possible that there may be an increased number glial cells thickening the cortex enough that it gives the impression of structurally normal cortex.
5.2 Decreased $BP_{ND}$ of $[^{18}F]FMZ$ in the Peri-infarct Region

The accuracy of PET for measuring regional radiotracer concentrations in the brain is limited by the finite resolution of the scanner used and the resulting partial volume effects (PVEs) (Rousset et al., 1998). An effect of poor spatial resolution is the contamination of activity from neighbouring tissues or spillover effect (Kessler et al., 1984). There are PVEs affecting our data however we expect them to be low based on the resolution of the HRRT scanner that was used to acquire the data. The lack of binding in the infarcted tissue may be diluting the signal in our innermost ROIs, but it would not affect the middle or outer ROIs so there we see a true decrease in $[^{18}F]FMZ$ binding.

We found an overall decrease in $BP_{ND}$ of $[^{18}F]FMZ$ in the affected hemisphere ROIs. Heiss et al. found that reduced $[^{18}F]FMZ$ binding was an indicator of permanently and irreversibly damaged cortex (1997). This suggests that the area surrounding the infarct in our stroke patients may be undergoing necrosis without showing decreases in cortical thickness just yet.

Postischemic GABA$_A$ receptor downregulation has been described in the peri-infarct region of rats (Sommer et al., 2003). This may be another possible explanation for the decrease in $[^{18}F]FMZ$ binding in the peri-infarct region with a gradual increase in binding with increased distance from the infarct that we observed within the affected as
compared to the contralateral hemisphere. This increase in binding was significant from the inner to the middle ROI, but not from the middle to the outer ROI. The outer ROI had binding close to that found in the contralateral hemisphere (11.0 ± 3.4 [affected] compared to 13.5 ± 2.9 [contralateral]).

Our BPND[^18F]FMZ findings corroborate the findings of Nedergaard et al. who noted that the density of histologically intact neurons changed from affected to normal at a distance of about 5mm from the infarct (1984). Our findings would suggest that this change happens at around 6.5-7.0mm since the width our ROIs was approximately 3.5mm and the inner and middle ROIs saw significantly reduced binding relative to the contralateral hemisphere.

5.3 Ratio Maps

Ratio maps were not intended as a means of primary analysis rather they were shown as graphical way to represent the

In the peri-infarct region, the ratio of BPND[^18F]FMZ /SCT was lower. The regions of low ratio values discernable on the ratio maps nicely replicate the regions investigated in the region-based results. These low ratio peri-infarct regions seen in the ratio maps (in blue) taper off approximately where our outer ROIs regions were situated where we no longer found significant results.

An older patient (5) had a more marked ratio decrease around the infarct than what was seen in the youngest patient (3).
The ratio seems to have been influenced by the location of the infarcts as there was a more marked reduction visible surrounding the MCA lesions (patients 3 and 5) compared to the PCA infarcts (patients 6 and 10).

Focal increases in the mesial temporal area, similar to what is seen in patient 10’s SCT map, have been described previously by la Fougere et al. and are thought to be due to an issue with the algorithm that defines the surfaces for SCT measurement (2009). An explanation for the focal increases that are close to the infarcted tissue may be that the infarct mask was not properly delineated and that there was some lesioned tissue included in the analysis that caused an overestimation of the gray matter.

5.4 Limitations

There are few limitations that have to do with using these methods in infarcted brains since we exclude the infarcted region from the analysis. However, a major limitation is associated with the accuracy with which the infarcts were defined by manual segmentation. If lesioned tissue was missed during the masking and therefore not eliminated from inner ROI this would affect the SCT and $[^{18}\text{F}]\text{FMZ}$ binding by overestimating SCT and underestimating binding in the peri-infarct region.

From the combined results of our SCT mapping and $[^{18}\text{F}]\text{FMZ}$ PET we can only speculate what might be happening in the peri-infarct tissue. We are unable to identify the basis for decreased $[^{18}\text{F}]\text{FMZ}$ binding that we see without follow up data. If in the follow-up data we find that SCT remains the same and $[^{18}\text{F}]\text{FMZ}$ binding increased back
to normal in the peri-infarct region then we can conclude that the decreased binding in the acute phase is due to receptor downregulation. If SCT stays the same or decreases and $[^{18}\text{F}]$FMZ binding is still reduced then this would be indicative of necrosis.

PVEs are a limitation in the current study, however this is something that could be corrected for in the future. Another limitation of the methods presented here, but that is not a principle limitation as it too can be incorporated into the methods in the future is that the infarct is not masked prior to the tissue classification. If infarct voxels that appear hypointense on the MRI scan were included during the tissue classification process it could have resulted in an overestimation of gray matter.

5.5 Future Directions

The next important next step for this research will be analyzing follow up scans from these stroke patients. This will allow for insight into the underlying reason for the decreased BP$_{\text{ND}}$ $[^{18}\text{F}]$FMZ. Another strategy to be used in the future could be to map z-scores on the surface and scale the map from 2 standard deviations above the mean to 2 standard deviations below the mean to visualize areas of individual difference compared to a control average. This technique would be an interesting tool to do individual analyses as opposed to group analyses. Below are two z-score maps produced for two of the patients from this study as an example of what they look like.
Chapter 6 – Conclusion

The present study provides the first demonstration of cortical thickness measured using SCT mapping in acute stroke patients as well as the first region-based analysis of $BP_{ND}$ of $[^{18}F]FMZ$ in the peri-infarct tissue. Surface-based cortical thickness mapping proved to be an accurate method to investigate SCT in close proximity to infarcted tissue. The region-based analysis is a useful approach to investigating tissue at varying distances from infarcts in a systematic way.

The thickness of the cortex surrounding the lesions studied (3.2mm) was not different from that in the patients’ contralateral hemisphere (3.2mm) as well as in healthy age-matched controls (3.2mm). There was decreased $[^{18}F]FMZ$ in the peri-
infarct region that gradually increased in tissue more distant from the infarct. Potential correlates of the observed changes in neuronal density include a downregulation of GABA\textsubscript{A} receptors or neuronal death. Important contributions to the causes of the [\textsuperscript{18}F]FMZ binding changes will come from studies that look at the follow-up PET and MRI scans.

These non-invasive methods of studying cortical thickness and neuronal density have proven to be valuable ways to investigate these parameters in stroke brains. The exact cause of these lesion-related cortical thickness and neuronal density findings, however, is still not known.
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