A Multi-Modal Quantitative Analysis of Neurodegeneration in Spinocerebellar Ataxia Type 1 using Magnetic Resonance Imaging and Spectroscopy

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BY

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Abstract
Spinocerebellar Ataxia Type 1 (SCA1) is a hereditary neurodegenerative disorder that is predominantly characterized by degeneration of the cerebellum and the brainstem. Symptoms of SCA1 include worsening gait and a progressive loss of motor coordination, as well as a host of other symptoms. Magnetic resonance imaging (MRI) and spectroscopy (MRS) offer a means to measure the structural and chemical changes that occur in the brain in a reliable, quantitative, and non-invasive manner. This thesis aims to use multiple modalities of magnetic resonance, including MRS, structural MRI, and diffusion MRI, in order to quantitatively understand how the chemical, macrostructural, and microstructural characteristics of the brain are affected in SCA1. The information obtained from each of these modalities will then be used to outline a model of SCA1 that aims to describe the process of neurodegeneration from the cellular level, to the structural level, and finally to the symptomatic level. The results from this thesis may prove useful in understanding the process of neurodegeneration in SCA1, as well as offering a means of evaluating brain pathology for future clinical trials.
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Chapter 1

Introduction

Project Overview

Spinocerebellar Ataxia Type 1 (SCA1) is a hereditary neurodegenerative disorder that is characterized by degeneration of the brainstem and the cerebellum [1]. The term “ataxia” is defined as the presence of abnormal or uncoordinated movements [2]. As such, patients with SCA1 experience worsening gait and a loss of motor coordination, as well as some non-motor symptoms, including impaired speech and executive (cognitive) dysfunction [3]. SCA1 affects 1 or 2 in every 100,000 people worldwide, with patients typically surviving between 10 and 30 years after symptoms begin [4, 5].

The current method for measuring SCA1 progression is the SARA clinical scale (Scale for the Assessment and Rating of Ataxia) [6]. While SARA can be quickly and easily administered, test-retest reliability is limited, and it cannot detect early changes in the brain prior to onset of symptoms. Therefore, objective and non-invasive neuroimaging markers could have a high impact on the development of potential therapies at pre-symptomatic and symptomatic stages of SCA1. Such neuroimaging markers can be obtained through multiple modalities of magnetic resonance, including magnetic resonance spectroscopy (MRS), magnetic resonance imaging (MRI), and diffusion tensor imaging (DTI).

The objective of this thesis was to use the chemical information from MRS, information of brain macrostructure from MRI, and information of brain microstructure from DTI to
understand and model the process of SCA1 neurodegeneration in the brain. This project consisted of analysis of existing MR scans and SARA data from SCA1 patients and healthy controls. Figure 1.1 below shows the structure of data acquisition and analysis for this project.

![Figure 1.1: Structure of the thesis project.](image)

As shown above, this project consists of multiple modalities, including SARA, structural MRI, MRS, and DTI. The SARA measure provides information regarding the clinical status of the subjects. From MRS, one can obtain information regarding the chemical makeup of the brain. Structural MRI is able to provide information on the volumes of certain brain regions (volumetry), as well as the thickness of regions of the cerebral cortex (cortical thickness). Finally, DTI provides information regarding the diffusion of
water molecules in neuronal pathways in the brain, thus describing some of the microstructural features of the brain.

The analysis of the data provided by these modalities was split into two “arms.” In the “MRS vs. Volumetry vs. SARA” arm, the goal was to determine which modalities were more sensitive to SCA1 progression. In the “Cortical Thickness vs. DTI” arm, the goal was to determine whether there was any relation between changes in cortical thickness and white matter tracts due to SCA1. Based on the results from the two arms, a qualitative model of SCA1 progression was constructed, which linked the molecular, microstructural, and macrostructural findings with the known symptoms of SCA1.

**Thesis Structure**

**Chapter 2** goes over the relevant background information for this project. Section 2.1 goes over the basics of the human nervous system, including the neuron and the central nervous system. Section 2.2 covers the background of spinocerebellar ataxia type 1. Section 2.3 goes over the history and relevant physics of magnetic resonance imaging. Section 2.4 is about the physics and applications of magnetic resonance spectroscopy. Section 2.5 covers the physics and applications of diffusion imaging.

**Chapter 3** focuses on the methods of the project. Section 3.1 gives an overview of the structure of analysis, as well as the sample sizes for each study. Section 3.2 contains the imaging parameters that were used to acquire the relevant data. Section 3.3 discusses the analysis techniques used to obtain the volumetry and cortical thickness data. Section 3.4 discusses the methods of analysis used to obtain chemical information from MRS data.
Section 3.5 goes over the basics of diffusion tractography, and how tractography was used in this study to analyze white matter tracts in the brain.

Chapter 4 describes the results from this project. Section 4.1 covers the results from the “MRS vs. Volumetry vs. SARA” arm, while Section 4.2 discusses the results of the “Cortical Thickness vs. DTI” arm. In Section 4.3, the results from the two arms were used to create a model for SCA1 neurodegeneration. The potential applications for the model were also discussed.
Chapter 2

Background

2.1: The Human Nervous System

Overview

The human nervous system is the master control and communication system of the body. The primary cells of the nervous system are “neurons,” which receive and transmit signals by means of chemical neurotransmitters and electrical potentials. The three primary functions of the nervous system are sensory input (the collection of signals to monitor changes both inside and outside the body), integration (the processing and interpreting of the information), and motor output (the activation of muscles or glands) [7]. The nervous system is separated into two main components: the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists of the brain and spinal cord, while the PNS primarily consists of the nerves that extend from/to the brain and spinal cord (Figure 2.1) [7]. For the purpose of this project, the central nervous system (more specifically the brain) will be the primary focus of attention.
The Neuron

The neuron is a signal-propagating cell that is considered to be the basic unit of the nervous system. Neurons can communicate with other neurons, as well as tissues and organs [7]. As a result, the nervous system contains several pathways and connections. For example, the human brain contains about 86 billion neurons, resulting in trillions of possible connections [8]. While neurons come in many shapes and sizes, they all share certain unique characteristics.
Figure 2.2: Basic model of a neuron. Source: Wikimedia Commons.

Figure 2.2 is a basic model of a neuron. The dendrite receives the incoming signal. The signal is then propagated in the form of an action (ion) potential, down the axon. Eventually, the signal is transmitted to another neuron or tissue through the axon terminal, using neurotransmitters. The axon is covered by myelin sheaths, which are insulation envelopes that aid in signal transmission. Myelin sheaths are created by “glial cells,” which are non-neuronal cells that provide support and protection for neurons. In between each myelin sheath are the “nodes of Ranvier,” which is where the action potential is regenerated and “jumps” across the axon [7].

The Brain

The brain consists of multiple parts: the cerebrum, the cerebellum, and the brainstem. The cerebrum (Figure 2.3) is responsible for the integration of sensory and neural functions, as well as the initiation and coordination of voluntary activity. It is divided into two
hemispheres, with each hemisphere comprised of four distinct lobes: the frontal, parietal, temporal, and occipital lobes [7].

Figure 2.3: The human brain, consisting of the cerebrum (with colored lobes), cerebellum, and brainstem. Source: Gray’s Anatomy of the Human Body, 20th edition

The outer part of the cerebrum is the “cerebral cortex,” a highly-folded structure of 2-4 mm thickness, which is comprised of grey matter (the dendrites and axon terminals of neurons) [7].

Figure 2.4: Coronal cross-section of the brain, consisting of the cortex (light-colored) and white matter (dark-colored). Source: Wikimedia Commons.

The inner region of the cerebrum is made up of “white matter,” which is comprised of the axons of neurons (the myelination of the axons result in the distinctive light appearance).
The axons in the white matter are bundled into neuronal pathways, or “tracts,” which help direct signals from one region of the nervous system to another [7]. Figure 2.5 shows some examples of major tracts within the cerebrum.

Figure 2.5: Major white matter tracts of the cerebrum.
Source: Gray’s Anatomy of the Human Body, 20th edition

The cerebellum sits just below and behind the cerebrum (see Figure 2.3). In general, the cerebellum receives information from the cerebrum regarding planned movements, compares the planned movements with the body’s current position and movements, and sends information back to the cerebral cortex on how to compensate for any differences between the intended movements and the current position [7].

Figure 2.6: Sagittal cross-sectional slice of the cerebellum
Source: Gray’s Anatomy of the Human Body, 20th edition
The brainstem (Figure 2.7) connects the brain to the spinal cord, and is comprised of three parts: the medulla oblongata, pons, and the midbrain. The medulla oblongata is the most inferior (lowest) part of the brainstem, and is responsible for unconscious activities, such as breathing, heart contraction, salivation, etc. The pons is located in the middle of the brainstem, and forms a bridge between the brainstem and the cerebellum. The most superior (highest) portion of the brainstem is the midbrain, and is involved in vision, hearing, eye movement, and body movement [7].

![Figure 2.7: The brainstem, consisting of the medulla, pons, and midbrain. Source: Wikimedia Commons](image-url)
2.2: Spinocerebellar Ataxia Type 1

Overview

Spinocerebellar Ataxia Type 1 (SCA1) is a genetic disorder that is characterized by neuronal death and degeneration of the cerebellum and brainstem, with subsequent loss of motor control and coordination. While clinical signs vary between different patients, symptoms generally include the loss of control of gait, stance, and limbs, unclear articulation of speech, and eye movement abnormalities. Later stages of this disease are also associated with difficulty swallowing, repetitive and jerking movements, muscle spasms, vocal cord paralysis, and executive (cognitive) dysfunction [4]. The age of symptom onset for SCA1 varies between 4 and 74 years of age, though it is most common in the fourth decade. Individuals with SCA1 tend to survive an average of 20 years after symptoms first appear [3, 9].

SCA1 is an “autosomal dominant” genetic disorder, meaning that if an individual (regardless of sex) has at least one copy of the SCA1 gene (out of two possible copies), then he or she will be affected by the disorder, and will have a 50% chance of passing that gene on to offsprings [9]. From the perspective of molecular genetics, individuals with SCA1 have a CAG trinucleotide repeat expansion on the ATXN1 gene (meaning that the DNA sequence of that gene contains expanded repeats of cytosine, adenine, and guanine, in that order). The number of CAG repeats for SCA1 patients are typically between 40 and 81, and is inversely correlated with the age of onset and the severity of the disease [9].
SCA1 currently affects 1-2 individuals in 100,000 worldwide. While there are certain therapies and medications that may alleviate some symptoms of SCA1 (such as physical therapy and medications to reduce bladder urgency, depression, pain, etc.), there is currently no known treatment or cure for this disease [4].

**Measuring the Progression of SCA1**

One of the most prominent clinical scales for determining the progression of ataxia is the “Scale for Assessment and Rating of Ataxia” (SARA), first proposed by Schmitz-Hübsch et al. in 2006. When tested using the SARA scale, the subject is asked to perform certain tasks to evaluate neurological functions, such as walking parallel to a wall, perform fast alternating hand movements, speaking clearly during conversation, etc. From these tests, the subjects are rated on a scale from 0 to 40, with 0 being asymptomatic, and 40 being the most severe [6]. Although SARA can be easily administered in the clinical setting, it suffers from potential subjectivity and limited test-retest reliability; if a subject were tested under the SARA scale twice on the same day, the SARA results may be different. As a result, if one were to perform clinical trials using SARA, a larger number of subjects would be needed (which is especially difficult to achieve for rare diseases) than if a more reliable tool were used. Furthermore, SARA cannot assess the effectiveness of therapies in the preclinical stage, when neuroprotective approaches are likely to be the most effective.

Therefore, objective and noninvasive neuroimaging markers will have a high impact on the development of neuroprotective therapies. Prior work in Öz lab demonstrated that chemical markers measured using magnetic resonance spectroscopy (MRS), such as $N$-
acetylaspartate (NAA), myo-inositol, and glutamate show strong correlations with disease status [10]. In addition, they have shown that the same MRS markers are sensitive to progression and reversal of pathology in an animal model of SCA1 [11-13]. Magnetic resonance imaging (MRI) analysis of brain volumes has also been shown to be more sensitive in detecting disease progression in SCAs relative to the SARA clinical scale [14]. Several other neuroimaging markers may also show sensitivity to SCA1, including cortical thickness measurements and white matter microstructure integrity estimates from diffusion tensor imaging (DTI).
2.3: Magnetic Resonance Imaging

Background

Magnetic resonance imaging (MRI) is a medical imaging modality that uses nuclear magnetic resonance (NMR) to create three-dimensional visualizations of the human brain's structure and function, most commonly for the purpose of medical diagnosis.

The basic theory of NMR is the following: when an charged particle (such as the hydrogen proton) is exposed to a strong magnetic field, the magnetic moment of the particle precesses about an axis at an angular frequency that is proportional to the strength of the magnetic field (usually in the radio frequency range). If a group of such particles is placed in a magnetic field and is subject to radio waves at the same frequency of precession, a resonance effect occurs.

NMR was first described by Isidor Isaac Rabi in 1938 as a technique for measuring the magnetic characteristics of atomic nuclei. In 1946, Felix Bloch and Edward Purcell independently discovered the NMR phenomenon in liquids and solids. Five years later in 1951, Erwin J. Hahn’s “spin-echo” experiment quantified molecular diffusion in liquids using magnetic resonance (a concept that laid the foundation for diffusion MRI). In 1966, Richard R. Ernst developed NMR spectroscopy, a method that uses the NMR phenomenon to identify and differentiate chemical compounds. Later in 1973, Paul Lauterbur and Peter Mansfield independently described the use of magnetic field gradients to localize NMR signals, which set the physical foundation for MRI [15].

By utilizing the effects of NMR, MRI can non-invasively produce images of the human anatomy (structural MRI), the relative concentrations of metabolites within the body (MR
spectroscopy), or even the diffusion of water molecules within organized (anisotropic) cellular structures (diffusion MRI).

**Physical Model**

As briefly mentioned above, magnetic resonance is based around the principle that when an atom is exposed to a strong magnetic field, the magnetic moment of the nucleus will precess at a frequency known as the “Larmor frequency” \( (\omega_0) \), which is directly proportional to the strength of the magnetic field \( (B) \):

\[
\omega_0 = \gamma B_0
\]

In the above relation (also known as the Larmor formula), “\( \gamma \)” is the gyromagnetic ratio, which is the proportionality constant between the magnetic field strength and the angular frequency of the nuclei. For the proton nucleus of \(^1\text{H}\) (Hydrogen-1) in a water molecule (\(\text{H}_2\text{O}\)), the focus of MRI, the gyromagnetic ratio is approximately \(2.68 \times 10^8 \text{ rads/s/T}\). As a result, for magnetic fields ranging from 1.5 T to 7 T (the typical range for modern MRI machines), the resulting frequency range sits well within the radio frequency range [16].

In a natural environment (i.e. no external magnetic field), all the protons of a given sample are confined to one energy state. As such, there is no preference for the orientation of their magnetic moments (Figure 2.8, left). However, if the sample is exposed to a strong external magnetic field \(\vec{B}_0\), the magnetic moments will align along the direction of \(\vec{B}_0\), in either the low energy (parallel to \(\vec{B}_0\), or “spin-up”) or high energy (antiparallel to \(\vec{B}_0\), or “spin-down”) states (Figure 2.8, right). At equilibrium in a magnetic field, there are slightly more protons in the spin-up orientation than spin-down [16].
Figure 2.8: The orientation of water protons without (left) and with (right) an external magnetic field $\vec{B}_0$. Source: Lenglet, C., *Geometric and Variational Methods for Diffusion Tensor MRI Processing* [17].

The Zeeman effect dictates that the difference in energy between the spin-up and spin-down states ($\Delta E$) is directly proportional to the strength of the magnetic field:

$$\Delta E = \frac{\gamma h B_0}{2\pi}$$

Where “h” is Planck’s constant (6.626 x 10$^{-34}$ J-s). The Zeeman effect shows that while there is a very small energy difference between “spin up” and “spin down” nuclei, this difference is much greater (and measurable) at stronger magnetic fields (Figure 2.9).

Figure 2.9: The difference in energy between spin up/down protons in a magnetic field. Source: Wikimedia Commons.
Excitation

The spin of the magnetic moments within a given sample creates a net magnetization, which can be decomposed into two main components: Longitudinal magnetization \((\vec{M}_z)\), and transverse magnetization \((\vec{M}_{xy})\). Longitudinal magnetization is parallel to \(\vec{B}_0\), while transverse magnetization is perpendicular to \(\vec{B}_0\). At equilibrium (Figure 2.10), since there are more protons in the spin-up (low energy) state, longitudinal magnetization will point in the \(+z\) direction, while transverse magnetization will cancel out to zero [16].

![Net Magnetization Vector and Precession Trajectory](image)

**Figure 2.10:** Equilibrium magnetization of the protons in a magnetic field. Source: Lenglet, C [17].

When a radiofrequency (RF) pulse at the Larmor frequency is emitted orthogonal to \(\vec{B}_0\), the protons will absorb the energy of the pulse and change from their low-energy position to their high-energy position (which is why this pulse is called an “excitation pulse”). As a result, their magnetic moments will start to move away from the direction of \(\vec{B}_0\) by an amount that is proportional to the duration of the pulse. Depending on the type of RF
pulse emitted, the direction of net magnetization will change as well. For example, a 90-degree RF pulse will cancel out $M_z$ and create a net magnetization in the direction of $M_{xy}$ (Figure 2.11). On the other hand, a 180-degree RF pulse will invert the net magnetization towards the $–z$ direction [16].

![Figure 2.11: Excitation of the protons, where the energy given by the RF pulse flips the net magnetization by angle $\alpha$ (in this case, it is 90 degrees). Source: Lenglet, C. [17]](image)

**Relaxation**

Once the RF pulse has stopped, the protons will eventually release the absorbed energy and go back to their equilibrium state in a process known as “relaxation.” During relaxation, $M_{xy}$ decreases back to zero, while $M_z$ increases back to its equilibrium magnitude. The energy signal that is released from relaxation can be determined by measuring the induced current in the location where the RF pulse was applied. The frequency of the current is the Larmor frequency of the nucleus, and its amplitude is directly linked to the amount of magnetization in that plane [16].
Two prominent structural imaging methods, T1 and T2-weighted imaging, measure different relaxation characteristics. T1-weighted imaging measures the time \((T_1)\) it takes for the \(\overline{M}_z\) to increase to 63% of its equilibrium magnitude \((\overline{M}_0)\). It is modeled by the following relation:

\[
\overline{M}_z = \overline{M}_0 (1 - e^{-t/T_1})
\]

\textbf{Figure 2.12:} Longitudinal relaxation and T1 relaxation time. Source: Lenglet, C. [17]

Longitudinal magnetization is considered to have reached its equilibrium magnitude after 5 \(T_1\) periods. On the other hand, T2-weighted imaging measures the time \((T_2)\) it takes for \(\overline{M}_{xy}\) to lose 63% of its maximum magnitude, as is modeled by the following relation [17]:

\[
\overline{M}_{xy} = \overline{M}_0 e^{-t/T_2}
\]
Since the magnetic fields and the Larmor frequencies are not constant throughout the region being observed, the precession of $\mathbf{M}_{xy}$ will induce an oscillating current that is known as the “free induction decay” (FID, Figure 2.13) [17].

Different types of tissue have different T1 and T2 relaxation times (Table 1.1). As a result, T1 and T2-weighted imaging can differentiate and emphasize certain anatomical characteristics. For example, while fluid-filled compartments of the brain will look dark on T1-weighted imaging, those same compartments will look bright on T2-weighted imaging.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>T1 (ms)</th>
<th>T2 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrospinal Fluid</td>
<td>2200-2400</td>
<td>500-1400</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>920</td>
<td>100</td>
</tr>
<tr>
<td>White Matter</td>
<td>780</td>
<td>90</td>
</tr>
<tr>
<td>Fat</td>
<td>240-250</td>
<td>60-80</td>
</tr>
<tr>
<td>Blood (deoxygenated)</td>
<td>1350</td>
<td>50</td>
</tr>
<tr>
<td>Blood (oxygenated)</td>
<td>1350</td>
<td>200</td>
</tr>
<tr>
<td>Muscles</td>
<td>860-900</td>
<td>50</td>
</tr>
</tbody>
</table>

**Table 1.1:** T1 and T2 relaxation times for common biological structures at 1.5 Tesla [17].
Gradients

If a sample were in a homogeneous magnetic field, a single-frequency radio pulse would excite all the protons in the sample, which would make spatial localization difficult. To account for this, a gradient magnetic field has to be induced, which changes the magnetic field linearly as a function of position in space, and thus changes the Larmor frequency as a function of space. In three dimensions, if the magnetic field $B_0$ linearly changes as a function of the position $(x, y, z)$ by a factor of $G_x$, $G_y$, $G_z$, one would see that the expression for the magnetic field $B$ is:

$$B = B_0 + G_x x + G_y y + G_z z$$

Using the Larmor equation, one can therefore calculate the frequency as a function of the position:

$$\omega = \omega_0 + \gamma G_x x + \gamma G_y y + \gamma G_z z$$

Therefore, in order to measure the signal from a certain region of a sample, one would have to emit an RF pulse at the range of Larmor frequencies that correspond to the position in space [18].
A pulse sequence is a series of RF pulses and/or magnetic field gradients applied to a sample to produce a specific form of MR signal. Figure 2.14 shows an example of a basic pulse sequence. As can be seen, there are three gradients, $G_z$, $G_y$, and $G_x$, which correspond to the z, y, and x directions in space respectively. The first gradient, $G_z$, is in the direction of the magnetic field $B_0$, and is used for selection of a slice that is orthogonal to $B_0$ (usually 1-10 mm in thickness). Using the Larmor relation as previously described, an RF pulse is then emitted at the specific frequency of the noted slice. Following this, two transient gradients are applied to encode the x and y dimensions in the plane of the slice. First, during the “phase encoding” step, the $G_y$ gradient is created to induce a phase shift related to the y-axis position. Next, during the “frequency encoding” step, the $G_x$ gradient is created to induce a precession frequency that is variant along the x-axis. The function of the last two gradients is for acquisition of the plane data (slice) in the frequency space (k-space), which will be discussed in the next section [18].
Pulse sequences are also characterized by the type and duration of the RF pulses, as well as the time delay between similar RF pulses (known as the repetition time). For example, T1 weighted imaging is enhanced by “Inversion Recovery,” which consists of a 180 degree pulse, followed (after a specific “inversion time”) by a 90 degree pulse. T2 weighted imaging relies on a “Spin-Echo” pulse sequence (pioneered by Edward Hahn), where a 90 degree pulse is initially emitted, and is followed by a 180 degree pulse (after half an “echo time,” or TE), which refocuses the transverse magnetization and allows for direct measure of T2 decay [18].

**Image Reconstruction**

When linear gradient fields are applied to a predetermined slice (by convention, in the x-y plane), the precessional frequencies of the protons also linearly vary as a function of position (as dictated by the Larmor equation). As a result, each excited spin accumulates a phase $\theta$ based on its location (in x-y space) and the integrated area of the gradients ($G_x$ and $G_y$) [18]:

$$\theta = 2\pi (k_x x + k_y y)$$

Where $k_x$ and $k_y$ are a function of the integrated area (da) of the gradients [18]:

$$k_x = \gamma \int G_x da$$

$$k_y = \gamma \int G_y da$$

The signal $s$ in a receiver coil is the integrated signal from all spins, and is expressed via Fourier transformation as follows:
\[ s = \int f(x, y)e^{i2\pi[k_x x + k_y y]} \, dx \, dy \]

Where \( f(x, y) \) is the net magnetization signal across the excited slice as a function of the x-y position. If one can sample \( s \) for sufficient values of \( k_x \) and \( k_y \), one can take the “inverse” Fourier transform of \( s \), and thus display \( f(x, y) \) as an MRI image. As a result, the signal \( s \) is measured as a function of \( k_x \) and \( k_y \), in what is known as “k-space.” One can sample values of \( s \) for different \( k_x \) and \( k_y \) values by manipulating the gradient areas as the receiver coil current is being measured. The changing values of \( k_x \) and \( k_y \) define a “trajectory” through k-space, whose speed and acceleration are proportional to the gradient hardware’s amplitude and slew rate, respectively (Figure 2.15) [18].

**Figure 2.15:** A collection of signals in k-space (left), and their corresponding image (right). Source: Johansen-Berg & Behrens [18].

Due to the decay of the signal over time, it is typical for excitation pulses to be repeated at a constant time interval (the repetition time, or TR). Using time measurements such as \( T_1 \), \( T_2 \), TE, TR, etc. one can determine the signal form each pixel of the MRI image. For example, for a spin echo pulse sequence, the signal from each pixel would be [18]:
\[ f(x, y) = M_0 \left( 1 - e^{-\frac{TR}{T_1}} \right) e^{-TE/T_2} \]

In summary, by utilizing the principles of magnetic resonance, it is possible to generate signals from the water protons within the human body. By mapping them into k-space and subsequently performing a Fourier transformation of the signal, one will be able to reconstruct an image of the human body.
2.4: Magnetic Resonance Spectroscopy

Overview

Magnetic Resonance Spectroscopy (MRS) is a technique that uses nuclear magnetic resonance (NMR) to measure chemical concentrations within a living sample (i.e. \textit{in vivo}). Unlike MRI, MRS does not focus on measuring the amount of water in a sample. Rather, it focuses on measuring the relative amounts of other molecules (far fewer in concentration compared to water) within each sample [19].

![Molecular models of water, benzene, and N-acetyl Aspartate.](image)

**Figure 2.16:** Molecular models of water, benzene, and \textit{N}-acetyl Aspartate.

Figure 2.16 describes the chemical structures of three different molecules: water, benzene, and \textit{N}-acetyl aspartate. Each molecule has a unique structure; consequently, the attached hydrogen atoms of each molecule (not labeled) are surrounded by varying “clouds” of electrons. The size of each electron cloud for each hydrogen proton may differ, based on factors such as nearby protons, the electronegativity of adjacent atoms, and the distribution of electrons throughout the molecule. When the hydrogen proton is exposed to a magnetic field $B_0$, the electron cloud will generate a field $B_i$ in the opposite direction, in a phenomenon known as “electron shielding” [16].
Figure 2.17: The shielding effect from an electron, creating an opposing magnetic field.

$B_i$ is proportional to a factor $\sigma$, such that:

$$B_i = B_0 \sigma$$

As a result, the net magnetic field $B_{\text{eff}}$ is:

$$B_{\text{eff}} = B_0 - B_i = B_0(1 - \sigma)$$

Using the Larmor equation, the Larmor frequency $\omega_0$ of each proton is therefore:

$$\omega_0 = \gamma B_0(1 - \sigma)$$

Therefore, in order to obtain signals from various chemicals in a given sample, an RF pulse will have to be emitted at a wide range of frequencies. As shown in Figure 2.18, the resulting signal will be a sum of multiple free induction decays. Though that signal by itself may not yield useful results, a Fourier transformation will result in a separation of peaks based on the resonant frequency of each proton [16].
Each peak of the post-transformation plot represents the signal generated by each proton at their respective Larmor frequency. The area under each peak is the relative amount of protons of that same type (i.e. the same electron cloud environment) within the sample.

As was shown for the Larmor frequency equation above, the resonant frequency is directly proportional to the strength of the magnetic field. However, this poses an issue, since the resulting output would change, depending on the type of scanner used. To account for this, the frequency scale is converted to a “chemical shift” scale, with units of $\delta$ (in ppm). The chemical shift value $\delta_i$ for each peak is calculated as:

$$\delta_i = \frac{\omega_i - \omega_r}{\omega_r} \times 10^6$$

There are two important points that can be inferred from the equation above. First, the chemical shift is independent of the strength of the magnetic field (though a stronger magnetic field would yield an increased contrast). Secondly, this is achieved by relating the frequency to a reference frequency $\omega_r$. For the purpose of NMR/MRS, the reference is the frequency of tetramethylsilane (TMS), which consists of twelve equivalent protons [16].

![Chemical structure of TMS](image.png)

**Figure 2.19**: Chemical structure of TMS, consisting of 12 equivalent protons.

However, since TMS does not appear in biological samples, MRS uses water as a reference, since its frequency is already known for the magnetic field. Once the water
signal is acquired, one can then convert the chemical shift scale to be relative to the TMS reference [16].

In summary, by sending an RF pulse across a wide range of frequencies to a given sample, one will receive signals from a variety of protons, with frequencies that depend on the chemical (and electronic) environment of the proton. By performing a Fourier transform of the signal, one can separate the signal into individual peaks, based on frequency. The area under each peak represents the relative (not absolute) amount of that specific type of proton, which would allow one to quantify the type of molecule and its relative amount within the sample. Figure 2.20 is an example of an MRS spectrum one would expect to see [16].

![Figure 2.20: Example MRS spectrum, consisting of multiple biomolecules. Source: Barker, Bizzi, & Stefano. Clinical MR Spectroscopy: Techniques and Applications [20].]
MRS Signal Acquisition

In order to obtain a proper MR spectrum from a single voxel, the pulse sequence must be able to select the voxel in three dimensions using gradients. Two prominent MRS pulse sequences are STEAM (stimulated echo acquisition mode) and PRESS (point-resolved spectroscopy). Though the types of RF pulses and the duration between each pulse are different, they both contain key similarities. Below is an example of a STEAM pulse sequence [19]:

\[
90^\circ_{G_x} \xrightarrow{TE/2} 90^\circ_{G_y} \xrightarrow{TM} 90^\circ_{G_z} \xrightarrow{TE/2} \text{Acquire}
\]

Where “TE” is the echo time, and “TM” is the delay in which the magnetization is longitudinal (as opposed to transverse). For both types of pulse sequences, the x, y, and z-gradients are applied respectively for the first, second, and third RF pulses, allowing for voxel selection [19].

Since water molecules outnumber brain metabolites 8000:1, its signal may drown out signals from other biomolecules of importance. As a result, the MRS pulse sequence must also be able to suppress water signals. The most common approach for water suppression is to emit multiple 90°RF pulses at the Larmor frequency of the water protons, just prior to the excitation pulses. As a result, the proton spins of the water will dephase, thus removing its signal during MRS signal acquisition. Signals from other common biomolecules (such as lipids) can be suppressed in a similar manner [20].
Figure 2.21 is an example of a complete STEAM pulse sequence, with a suppression “module” shown to the left of the dotted line.

Figure 2.21: Diagram of a water suppression pulse (left of the dotted line), followed by a STEAM pulse sequence (right of the dotted line). Source: Gadian [19].
**MRS and Brain Metabolites**

Metabolites are molecular compounds that are either the intermediates or products of cellular metabolism. There are several neuronal and glial metabolites that can be detected using MRS. Table 1.2 describes some common metabolites, their associated chemical shifts, and their correlations with neuronal health.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Chemical Shift (ppm)</th>
<th>Associated changes in Neuron Health</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetylaspartate (NAA)</td>
<td>2.01</td>
<td>Neuronal Health/Viability</td>
</tr>
<tr>
<td>Choline (Cho)</td>
<td>3.20</td>
<td>Demyelination, Increased cell membrane turnover</td>
</tr>
<tr>
<td>Creatine (Cr)</td>
<td>3.03</td>
<td>Metabolism Defects</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.31</td>
<td>Neuron Oxygenation</td>
</tr>
<tr>
<td>Myo-inositol (mI)</td>
<td>3.5-3.6</td>
<td>Gliosis (Morphological changes or proliferation of glial cells)</td>
</tr>
<tr>
<td>Glutamate (Glu)/Glutamine (Gln)</td>
<td>2.1-2.4, 3.7 (similar peaks for both compounds)</td>
<td>Health/ Viability of Neurons (Glu) Or Glial Cells (Gln)</td>
</tr>
</tbody>
</table>

**Table 1.2:** Common cerebral metabolites, their chemical shifts, and their associations with neuronal health [20].

One can use this information to infer changes in cell density, cell type and biochemical composition in the patient’s nervous system. For example, an increase of Lactate may indicate a decrease in tissue oxygenation. Therefore, if a patient were to suffer from hypoxia (deficiency of oxygen), one would expect to see an increase of the Lactate peak in the MRS signal. Such correlations can be used for other conditions and diseases as well, including SCA 1 [20].
2.5: Diffusion Imaging

Overview

Molecular diffusion is a process that can best be described as random motion of a particle due to its thermal energy. This type of motion is called “Brownian motion,” named after the scientist who first discovered the phenomenon in 1827. In 1905, Albert Einstein found that the average square displacement of all molecules in a sample \( \langle r^2 \rangle \) is linearly related to the elapsed time “t,” with a constant of proportionality known as the “diffusion constant” (D) [16].

\[
\langle r^2 \rangle = 6Dt
\]

Figure 2.22: Plot of molecular diffusion, showing its average increase as a function of time. Source: Johansen-Berg & Behrens

The diffusion of a given sample of molecules (such as water in the case of diffusion MRI) depends on the spatial characteristics of the medium. For example, for a sample where
diffusion is not restricted (i.e. an isotropic sample), the distribution of $\langle r^2 \rangle$ is Gaussian in nature, with equal probability for displacement in any direction in three dimensions [16].

![Gaussian distribution of molecular diffusion](image1)

**Figure 2.23:** Gaussian distribution of molecular diffusion (in one dimension), with an average radius of $\sqrt{2Dt}$. Source: Johansen-Berg & Behrens

As a result, the average diffusion of the molecule is spherical in nature. However, in the case of a sample where diffusion is restricted to a particular direction (an anisotropic sample), one will see the average diffusion having a preferred direction (Figure 2.24).

![Diffusion in isotropic and anisotropic medium](image2)

**Figure 2.24:** Diffusion in an isotropic (left) and anisotropic (right) medium. Source: Johansen-Berg & Behrens [16]

In diffusion MRI, an example of anisotropic medium are the white matter tracts of the cerebrum (Figure 2.5). As a result, diffusion MRI can be used to obtain information of the neuronal microstructures of the brain.
Although diffusion MRI cannot directly measure the diffusion coefficient of a sample, one can measure the average displacement of the water molecules within that sample to indirectly calculate the coefficient, which is thus called the “apparent diffusion coefficient,” or ADC. Due to the structural complexity of three-dimensional white matter tissue, Gaussian diffusion has to be characterized by a “diffusion tensor,” which is a 3x3 symmetric matrix with values corresponding to the x-y-z coordinates in space [16]:

$$
D = \begin{pmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{xy} & D_{yy} & D_{yz} \\
D_{xz} & D_{yz} & D_{zz}
\end{pmatrix} = E^T \begin{pmatrix}
\lambda_1 & 0 & 0 \\
0 & \lambda_2 & 0 \\
0 & 0 & \lambda_3
\end{pmatrix} E
$$

The diffusion tensor models diffusion as a three-dimensional Gaussian distribution with an ellipsoid shape (although true diffusion may take a different form). As shown above, the diffusion tensor can be described in terms of the eigenvalues $\lambda$, where $E$ is the matrix of the three eigenvectors, and the superscript $T$ indicates a matrix transpose. Several important diffusion values can be extracted from the diffusion tensor, using the eigenvalues. For example, mean diffusivity (MD) is a measure of the overall mean-squared displacement of molecules, and is calculated as the average of the three eigenvalues of the diffusion tensor [16]:

$$
MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}
$$

Axial diffusivity (AD) is a measure of diffusivity along the longest axis of the diffusion tensor. For example, let’s say that for a known tensor, the x-axis is the longest axis. Therefore:

$$
AD = \lambda_1
$$
Radial diffusivity (RD) is a measure of diffusivity along the minor axes of the diffusion tensor, and is calculated as the average of the other two eigenvectors:

$$RD = \frac{\lambda_2 + \lambda_3}{2}$$

Fractional anisotropy (FA) is a measure of the directional dependence of the molecular diffusion, and is calculated as the following [16]:

$$FA = \sqrt{\frac{3}{2} * \frac{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

FA is a scalar value with a range of 0 to 1, where 0 is a perfectly isotropic medium, and 1 is a perfectly anisotropic medium. The magnitude of each value of the diffusion tensor can also describe the overall direction of the diffusion, as shown in the figure below:

Figure 2.25: Effect of varying values of the diffusion tensor matrix on the shape of the diffusion. Source: Johansen-Berg & Behrens [16]
Diffusion Signal Acquisition

Magnetic resonance signals can be made sensitive to diffusion through the use of a pair of sharp magnetic field gradient pulses. For example, Figure 2.27 represents a diffusion pulse sequence being applied to a group of protons. In the first scenario (left), there is no diffusion of the protons. Initially, all protons in the sample precess at the same frequency, as they are in a homogeneous magnetic field (a). Next, a sharp gradient is applied to the group of protons, causing them to precess at different frequencies, as per the Larmor equation (b). When the first gradient is turned off, all the protons will precess at the same frequency, but at different phases (c). As a result, there is no signal emitted from the protons. Next, a second gradient of equal magnitude is applied in the opposite direction, causing a change in the Larmor frequencies of the protons along that direction, and cancelling out the previously added phase changes (d). When the second gradient is turned off, the protons will once again precess in phase, causing a strong signal output that can be detected using the receiver coils of the MRI (e). In the second scenario, the protons are allowed to diffuse via Brownian motion (f). In this case, regardless of the strength of the gradient, its duration, or its frequency (g), the diffusion of the protons results in a residual phase difference, leading to a reduction of the signal strength (h).

As can be seen, the more reduced the diffusion within a sample is, the stronger its signal will be. Figure 2.26 is an example of a diffusion-weighted image of a patient who suffered from an obstruction of a blood supply to the brain (a cerebral infarction). In this figure, the right hemisphere of the brain (the left side of the image) is higher in intensity, thus indicating a region of reduced diffusion resulting from of the infarction.
Figure 2.26: Diffusion-weighted image of a patient suffering from a cerebral infarction. Source: Radiopaedia.org
Figure 2.27: Effect of diffusion pulse sequence on protons with (left) and without (right) diffusion restriction. Source: Dr. Klioze at YouTube.com/user/Klioze
Using similar concepts as described for structural imaging, some pulse sequences can be modified to be sensitive to diffusion. After excitation of a slice and before signal acquisition, a bipolar gradient is applied to induce diffusion sensitivity. Figure 2.28 above is an example of a basic diffusion pulse sequence in the z-direction (though any direction can be used, as is described by the dotted lines), where the applied gradient has a magnitude “G”, the duration of the gradient is “δ”, and the separation of the diffusion gradients is “Δ”[18]. In order to find the approximate apparent diffusion coefficient (ADC), one will first need to measure the signal without any gradient (S₀), the signal after the diffusion pulse sequence is applied (S₆), and a factor b, so that:

\[
\text{ADC} \approx \frac{\log \left( \frac{S_0}{S_D} \right)}{b}
\]
Where \( b \) is [18]:

\[
b = (\gamma G \Delta)^2 \left( \Delta - \frac{\delta}{3} \right)
\]

It is important to note that the ADC calculation above is for just one point, in one direction. Therefore, multiple measurements must be taken at different directions (using a similar pulse sequence) in order to obtain the full diffusion tensor [18].

**Diffusion Measures and Nervous System**

The diffusion measures mentioned above (FA, MD, AD, and RD) have a direct relation with the region of the human brain being analyzed. For example, in the lateral ventricles of the brain, where freely diffusing cerebrospinal fluid is in abundance, the FA is expected to be low, and the MD is expected to be high. However, along the white matter tracts of the brain (where diffusion is restricted along the axons), the FA would be greater, while the MD would tend to be lower. In the case of neurodegenerative conditions, neuron degradation frequently involves axonal degradation. As a result, one would expect water diffusion to be less restricted in one particular direction (a reduction of FA), with an increase of diffusion in other directions (an increase of MD). As can be seen by this example, mapping the changes in the diffusion parameters along certain white matter pathways can provide a better understanding of the mechanism of neurological disease progression in the brain [21].
Chapter 3

Methods

3.1: Project Structure

Overview of Analysis

Figure 3.1: Diagram of the different MR modalities used in this thesis project, and the general direction of the project.

Figure 3.1 describes the structure of the thesis project. As can be seen, the modalities are grouped based on the type of measure. For instance, there are clinical measures (SARA), chemical measures (MRS), structural measures (volumetry and cortical thickness), and measures of brain microstructure (DTI).
Since the volumetry, SARA, and MRS values were measured over multiple time points (three visits over the course of three years), the results from the analysis of these three measures were used to characterize the temporal evolution of SCA1. Furthermore, the results from the analysis of the DTI and cortical thickness measures were used to look for a potential correlation between white matter tract degeneration and changes in cortical thickness (atrophy). Based on the findings from these analyses, one may be able to achieve a better understanding of both the chemical and physical changes that occur in the brain due to SCA1 progression.

**Subject Sample Sizes**

For the volumetry and spectroscopy arms of this project, 16 patients with SCA1 (confirmed through genetic analysis) at early-to-moderate stage were recruited, alongside 24 age-matched healthy controls. Each subject was scanned with spectroscopy and T1-weighted sequences. Subjects were scanned three times over the course of three years: once during the initial visit (Visit 1), about 18 months after the first visit (Visit 2), and about 36 months after the first visit (Visit 3). Prior to each scan, patients were tested using the SARA scale. The T1-weighted images from this set of scans were used to obtain cortical thickness measurements as well. Throughout Visits 2 and 3, a subset of the subjects from the spectroscopy arm (9 controls and 8 SCA1 subjects) was scanned with diffusion-weighted imaging. Diffusion subjects were usually scanned within 1-2 days of their spectroscopy scan.

The table below (Table 3.1) lists the number of patients from each part of the study (spectroscopy, volumetry, cortical thickness, and diffusion). As can be seen, some of the
subjects from Visit 1 did not appear for subsequent visits, thus resulting in reduced sample sizes for both Visits 2 and 3. Although cortical thickness data was taken from the T1-weighted scans of the spectroscopy study, some of the initial scans had poor sensitivity, due to the use of a surface coil for the first few subjects. While the surface coil resulted in acceptable signal from the back of the head (near the cerebellum and brainstem), the signal attenuated when progressing towards the cerebrum, making whole brain cortical thickness analysis difficult. To avoid inconsistencies, scans that used the surface coil were therefore removed from analysis.

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Cohort</th>
<th>Visit</th>
<th>Number of Subjects</th>
<th>Age ((\bar{x} \pm \sigma))</th>
<th>SARA ((\bar{x} \pm \sigma))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectroscopy/Volumetry/SARA</td>
<td>Controls</td>
<td>1</td>
<td>24</td>
<td>53.3±15.2</td>
<td>0.06±0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>21</td>
<td>54.7±16.3</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>15</td>
<td>53.1±14.3</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>SCA1</td>
<td>1</td>
<td>16</td>
<td>50.3±11.5</td>
<td>8.9±3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>16</td>
<td>51.9±11.5</td>
<td>10.1±4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>12</td>
<td>56.5±10.5</td>
<td>10.2±5.4</td>
</tr>
<tr>
<td>Cortical</td>
<td>Controls</td>
<td>1</td>
<td>18</td>
<td>53.2±16.9</td>
<td>0.03±0.12</td>
</tr>
<tr>
<td>Thickness</td>
<td></td>
<td>2</td>
<td>20</td>
<td>53.7±16.1</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>12</td>
<td>51.2±15.0</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>SCA1</td>
<td>1</td>
<td>11</td>
<td>54.6±9.8</td>
<td>8.5±3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>16</td>
<td>51.9±11.5</td>
<td>10.1±4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>12</td>
<td>56.5±10.5</td>
<td>10.2±5.4</td>
</tr>
<tr>
<td>Diffusion</td>
<td>Controls</td>
<td>2+3</td>
<td>18</td>
<td>54.5±16.6</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>SCA1</td>
<td>2+3</td>
<td>8</td>
<td>49.6±14.3</td>
<td>8.7±4.2</td>
</tr>
</tbody>
</table>

Table 3.1: Table of the sample sizes of Control and SCA1 subjects for each MR study, with the associated age and SARA scores.
3.2: Scan Parameters

Magnetic Resonance Spectroscopy

MRS was obtained using the semi-LASER pulse sequence (see Öz and Tkáč, Magnetic Resonance in Medicine, 2011 for more information), with an echo-time (TE) of 28 milliseconds, and a TR of 5000 milliseconds. Spectra were taken of the cerebellar vermis, the cerebellar hemispheres, and the pons. For each region, 64 transients were averaged to obtain the final spectra for analysis.

Structural Imaging (Volumetry & Cortical Thickness)

The type of T1-weighted scan that was obtained was the “MPRAGE” scan (see Brant-Zawadzki et al., Radiology, 1992 for more information). The following parameters were used in this study (Table 3.2).

<table>
<thead>
<tr>
<th>MPRAGE Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>TR</td>
<td>2530 ms</td>
</tr>
<tr>
<td>TE</td>
<td>3.65 ms</td>
</tr>
<tr>
<td>Flip Angle (FA)</td>
<td>7°</td>
</tr>
<tr>
<td>Slice Thickness</td>
<td>1 mm</td>
</tr>
<tr>
<td>Number of Slices</td>
<td>224 Slices</td>
</tr>
<tr>
<td>Field of View (FOV)</td>
<td>256x176 mm²</td>
</tr>
<tr>
<td>Matrix Size</td>
<td>256x256</td>
</tr>
</tbody>
</table>

Table 3.2: MPRAGE scanning parameters
Diffusion Imaging

The following parameters were used for diffusion imaging (Table 3.3).

<table>
<thead>
<tr>
<th>Diffusion Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voxel Size</td>
<td>1.8x1.8x1.8 mm³</td>
</tr>
<tr>
<td>$b$</td>
<td>1500s/mm²</td>
</tr>
<tr>
<td>Number of Gradients</td>
<td>128</td>
</tr>
<tr>
<td>Number of additional b=0 volumes</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 3.3: Diffusion Scanning Parameters

3.3: Structural Imaging Analysis

FreeSurfer

When obtaining information regarding the volumes of cortical structures in certain regions of the brain, there exists a need for a computational method that can accurately label anatomical components of each MRI slice (a process called “segmentation”). This is especially difficult, as various brain regions have similar intensities in T1-weighted scans.

In order to achieve proper segmentation of the cerebellum, brainstem, and the cerebral structures, a software tool known as “FreeSurfer” was used. FreeSurfer is a completely automated tool, allowing the user to run multiple datasets in a time-efficient manner. When FreeSurfer is run for a particular set of scans, two separate “streams” are run: the surface-based stream, and the volume-based stream. Both streams involve transferring each scan to a predefined coordinate system based on a template scan (an “atlas”), in a
process called “registration.” The atlas used in FreeSurfer is MNI305, an average of 305 T1-weighted scans from the Montreal Neurological Institute.

During the surface-based stream, there are generally three steps that occur for analysis (Figure 3.2). First, the skull is stripped from the rest of the brain (Figure 3.2, left). Next, the voxels are classified as either white matter, or anything other than white matter (Figure 3.2, center). Finally, with the additional aid of the intensity gradients from the image (bias field correction), the white matter and cortex are separated from each other into distinct regions (Figure 3.2, right). From this, FreeSurfer is able to compute the cortical thickness at each location of the cortex, and over 70 different regions (average) of the cerebral cortex (35 for each hemisphere).

![Figure 3.2: The three major steps that FreeSurfer uses to analyze cortical thickness. Source: FreeSurfer Website at Freesurfer.net](image)

During the volume-based stream, the subcortical structures are labeled by finding the segmentation that maximizes the probability of matching the previously hand-labeled atlas (from the MNI305 template). From these labels (as seen in Figure 3.3), FreeSurfer is then able to measure the volumes of multiple anatomical regions.
Volumetry and Cortical Thickness Analysis

The goal of the “MRS vs. volumetry vs. SARA” arm of the study was to compare the change-over-time of the volumes of several brain structures with the metabolite concentrations measured using MRS. Using FreeSurfer, the volumes of the brainstem (the medulla oblongata, pons, midbrain, and superior cerebellar peduncles) and the cerebellum (cerebellar cortex and cerebellar white matter, for both hemispheres) were obtained for both Controls and SCA1 subjects for all three visits (see Table 3.1 for sample sizes). During the early stages of this project, a software called “TurtleSeg” was initially used to create segmentations of the pons. However, TurtleSeg required manual input to create the segmentations, which led to increased variability in the measurements of the pons volume (especially at the anatomical boundaries). As a result, in order to maintain consistency and reduce variability, a recently available algorithm in FreeSurfer was used to obtain brainstem volumes, including the pons [22].
The goal of the “cortical thickness vs. DTI” arm of the study was to see if there was a
correlation between changes in cerebral cortical thickness and differences in diffusion
values along major white matter tracts, in order to understand the effects of SCA1 at the
macrostructural and microstructural levels. FreeSurfer was used to obtain cortical
thickness values for both Controls and SCA1 subjects (see Table 3.1 for sample sizes).
Cortical thickness analysis was performed in two ways: the “average” method, and the
“surface-based” method. The average method compared the average cortical thickness
over each cortical region between patients with SCA1 and control subjects for each visit.
In contrast, the surface-based method compared cortical thickness over each “point” of
the cortex, between SCA1 and Controls. Figure 3.4 shows a visualization of a surface-
based comparison. The brain is “inflated,” so that the outer grooves (gyrus) and the inner
grooves (sulcus) of the brain are on the same surface.

Figure 3.4: Example of an inflated brain for surfaced-based thickness analysis.
3.4: Spectroscopy Analysis

LCModel

As mentioned earlier, the brain has a variety of metabolites, each of which have characteristic NMR peaks. The area under each peak in the spectra represents the relative concentration of each type of proton in the metabolite. As a result, when an MR spectrum is taken from a biological sample, one can approximate the relative concentrations of each metabolite by creating a fit line that is a sum of the metabolite spectra. From these relative concentrations, the absolute concentration can be estimated, since the water content of the tissue is already known and an unsuppressed water spectrum is obtained from the same voxel as the metabolites. LCModel is a software which automatically creates a fit and outputs concentrations of brain metabolites from the spectra [23].

For each metabolite fit, there is an associated estimated percent standard deviation, as determined by “Cramér-Rao lower bounds.” As the name suggests, Cramér-Rao lower bounds set the lower bound on any unbiased estimator. Although the resulting standard deviations are only the minimum, they are still useful when examining reliability. The estimated standard deviations must be multiplied by 2 in order to obtain the 95% confidence interval (the range that would contain the true value about 95% of the time). For example, if the percent standard deviation is 50%, the metabolite concentration could be anywhere between zero to twice the estimated value. As a result, the metabolite is practically undetectable. Usually, a percent standard deviation less than 20% is considered to be a reliable estimate.
The figure below (Figure 3.5) shows an example output from LCModel. In the plot, the black line is the obtained spectrum, and the red line is the estimated spectrum. The table to the right shows metabolite name (4th column), the estimated relative concentrations (1st column), the concentration ratio to creatine (3rd column), and the percent standard deviation estimate (2nd column). The values in blue are metabolites with reliable concentration estimates, while the values in red are metabolites with practically undetectable concentrations.

Figure 3.5: Example LCModel Output. Source: LC model website at s-provencher.com/pages/lcmodel.shtml
Spectroscopy Analysis

For the “volumetry vs. spectroscopy” arm of the study, patient enrollment, MRS acquisition, and metabolite analysis were performed independently by Öz Lab. MR spectra was obtained from the pons, cerebellar hemispheres, and cerebellar vermis of both SCA1 and Control subjects for each visit (see Table 3.1 for subject sample sizes). Matlab was used for processing the spectra, and LCModel was used to obtain the metabolite concentrations from each acquired spectra.
3.5: Diffusion Imaging Analysis

Basics of Diffusion Tractography

Diffusion Tractography is an analysis method by which certain white matter pathways of the brain are mapped using diffusion parameters (such as FA, MD, etc.). Tractography can be used either to reconstruct white matter pathways (exploratory tractography), or to compare measures of anisotropy or diffusivity of certain tracts between groups of subjects (tractography of known pathways) [24].

Tractography methods are generally defined by their assumptions of the tensor orientation (deterministic versus probabilistic), and the method of pathway fitting (local versus global). In terms of tensor orientation, deterministic tractography assumes a single tensor orientation at each voxel, while probabilistic tractography assumes a probability distribution of tensor (or more general models) orientations. The main difference between these two methods is the output; deterministic tractography outputs three-dimensional lines (either there is a connection, or no connection at all), while probabilistic tractography shows three-dimensional maps, where each voxel is assigned a number that indicates the confidence as to whether there is a connection going through that voxel (Figure 3.6) [24].
As can be seen in Figure 3.6 above, while deterministic tractography (left) results in more “distinct” white matter tracts, probabilistic tractography shows a probability distribution of white matter tract orientation (right).

In terms of pathway fitting (Figure 3.7), local tractography fits the pathway step-by-step, using local diffusion orientations at each step. In contrast, global tractography fits the entire white matter pathway, using the diffusion orientation at all voxels along pathway length. Local tractography is best suited for an exploratory study of connections, while global tractography is best suited for reconstruction of known white matter pathways (with known endpoints) [24].
While there are several available tools for diffusion analysis, the main tool in this experiment is TRACULA (Tracts Constrained by Underlying Anatomy). TRACULA is a global probabilistic tractography tool that utilizes prior knowledge of the subject’s anatomy. In other words, by using processed T1-weighted images (analyzed using Freesurfer), TRACULA constrains the possible white matter pathways based on prior anatomical knowledge. As a result, TRACULA is able to reconstruct 18 different white matter pathways (Figure 3.8). From these pathways, one can extract key diffusion parameters along each point of the white matter tract [25].
Diffusion Analysis

The goal of the diffusion arm of this study was to compare diffusion scans for SCA1 subjects and healthy controls, in order to determine whether there were any significant differences in diffusion values (FA, MD, AD, etc.) for certain white matter tracts. This study used diffusion-weighted scans from 8 SCA 1 and 9 control subjects (acquired during Visits 2 or 3). This study was cross-sectional, meaning that only one time point was used for comparison.

TRACULA was run on each subject in order to obtain information of all 18 white matter pathways. Since the subject group for the diffusion study was a subset of the spectroscopy/volumetry study, the previously analyzed T1 scans from that study were used for TRACULA analysis. Each scan took approximately eight hours for complete processing by TRACULA. Once TRACULA processing was completed for each subject, diffusion values for each tract was obtained and used for analysis (See: Chapter 4).
Chapter 4

Results

4.1: Comparison of MRS, Volumetry, and SARA

Overview

Spectroscopy, volumetry, and SARA data were obtained from subjects during three visits, over the course of approximately three years. To compare the magnitude of the changes of each measure over time, an “effect size” was calculated, where:

$$\text{Effect Size} = \frac{\text{Mean (\% Change per Year)}}{\text{Standard Deviation (\% Change per Year)}}$$

For this arm of the study, the first step was to find the MRS and volumetry measure that was most sensitive to change, i.e. had the largest effect size (since both methods provided multiple measures). From there, MRS, volumetry, and SARA were compared with each other to determine which modality was the most sensitive to SCA1 progression.

Spectroscopy

Spectroscopy measurements were taken of the cerebellar vermis, cerebellar hemispheres, and the pons, with each measurement yielding concentrations of glutamate (Glu), myo-inositol (Ins), total choline (tCho), total creatine (tCr), and total N-acetyl aspartate (tNAA). The ratio of tNAA/Ins was also part of the analysis, as it is a commonly used measure for neurodegenerative disorders (Data courtesy of Dr. Dinesh Deelchand).
From these measurements, the rate of change per year for each metabolite was calculated for each of the three brain regions. The figures below show the average rate of change of the metabolites for each anatomical region.

**Figure 4.1:** MRS-measured changes in metabolite concentrations of the pons (Mean ± SD). (*) indicates a statistically significant difference, where p<0.05.

**Figure 4.2:** MRS-measured changes in metabolite concentrations of the cerebellar vermis (Mean ± SD). (*) indicates a statistically significant difference, where p<0.05.
Figure 4.3: MRS-measured changes in metabolite concentrations of the cerebellar hemispheres (Mean ± SD). (*) indicates a statistically significant difference, where $p<0.05$.

The figures above are summarized in Table 4.1 (below), which lists the mean rate of change of each metabolite concentration for each region, the associated standard deviation, effect size, and the results of an unpaired, two-tailed Student’s $t$-test comparison of % change per year between SCA1 and Control cohorts (listed as $p$-values). The $p$-values marked with a “*” indicate statistical significance, where $p<0.05$. The Student’s $t$-test is a test of the null hypothesis (that there is no difference between SCA1 and Control cohorts). A $p$-value less than 0.05 indicates strong evidence against the null hypothesis, so the null hypothesis would be rejected. Therefore a $p<0.05$ indicates that the percent change per year in SCA1 is “significantly different” from that of controls.
<table>
<thead>
<tr>
<th>Metabolite</th>
<th>SCA1</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (%change/year)</td>
<td>SD (%change/year)</td>
<td>Effect Size</td>
</tr>
<tr>
<td>Cerebellar Vermis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>2.36</td>
<td>6.70</td>
<td>0.3518</td>
</tr>
<tr>
<td>Ins</td>
<td>1.19</td>
<td>6.31</td>
<td>0.1891</td>
</tr>
<tr>
<td>tCho</td>
<td>-1.11</td>
<td>-5.82</td>
<td>-0.1915</td>
</tr>
<tr>
<td>tCr</td>
<td>0.54</td>
<td>5.33</td>
<td>0.1006</td>
</tr>
<tr>
<td>tNAA</td>
<td>-1.24</td>
<td>-7.08</td>
<td>-0.0337</td>
</tr>
<tr>
<td>tNAA/Ins</td>
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<td>-2.01</td>
<td>-0.7278</td>
</tr>
<tr>
<td>Cerebellar Hemispheres</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>1.55</td>
<td>6.50</td>
<td>0.2382</td>
</tr>
<tr>
<td>Ins</td>
<td>0.79</td>
<td>3.37</td>
<td>0.2348</td>
</tr>
<tr>
<td>tCho</td>
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<td>-4.71</td>
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<td>tCr</td>
<td>1.10</td>
<td>3.32</td>
<td>0.3317</td>
</tr>
<tr>
<td>tNAA</td>
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<td>-3.04</td>
<td>-0.6065</td>
</tr>
<tr>
<td>tNAA/Ins</td>
<td>-2.41</td>
<td>-3.70</td>
<td>-0.6516</td>
</tr>
<tr>
<td>Pons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>6.63</td>
<td>21.54</td>
<td>0.3077</td>
</tr>
<tr>
<td>Ins</td>
<td>0.84</td>
<td>5.02</td>
<td>0.1676</td>
</tr>
<tr>
<td>tCho</td>
<td>-1.66</td>
<td>-5.13</td>
<td>-0.3235</td>
</tr>
<tr>
<td>tCr</td>
<td>-1.02</td>
<td>-4.50</td>
<td>-0.2276</td>
</tr>
<tr>
<td>tNAA</td>
<td>-3.30</td>
<td>-5.74</td>
<td>-0.5736</td>
</tr>
<tr>
<td>tNAA/Ins</td>
<td>-4.00</td>
<td>-4.79</td>
<td>-0.8364</td>
</tr>
</tbody>
</table>

**Table 4.1:** The mean, standard deviation, and effect size of the changes in the metabolite concentrations for each measured region for both SCA1 and Control subjects. (*) indicates statistical significance between %change/year in SCA1 vs. Control, where p<0.05.

As can be seen, there are statistically significant differences in the change per year of tNAA/Ins in patients vs. controls for all three regions. In addition, the tNAA change is statistically significantly different between SCA1 and controls for the cerebellar hemispheres. Out of all the significant results, the tNAA/Ins values for the pons yielded the greatest effect size magnitude (-0.8364 for SCA1, compared to -0.0817 for Control). This would imply that out of all the spectroscopy measurements, the pontine tNAA/Ins values are the most sensitive to SCA1 progression.
Volumetry

FreeSurfer provides volumes for 39 different regions (74 when including both hemispheres). However, since prior literature specifically noted degeneration of the cerebellum and pons, the main focus of the volumetry analysis was that of the brainstem (consisting of the pons, medulla oblongata, superior cerebellar peduncles, and the midbrain) and the cerebellum (consisting of white matter and the cortex for each hemisphere) [12].

In order to correct for various cranial sizes, all brain volumes were measured as a percentage of the intracranial volume (%ICV). From these measurements, the rate of change per year for each cerebellar and brainstem structure was calculated. The figures below show the average rate of change of the volumes for each listed region.

**Figure 4.4:** Percent changes in the brainstem volumes for SCA1 and Control subjects (Mean ± SD). (*) indicates statistical significance, where p<0.005.
Figure 4.5: Percent changes in the cerebellar volumes for SCA1 and Control subjects (Mean ± SD). (*) indicates statistical significance, where p<0.005.

The figures above are summarized in Table 4.2 (below), which lists the mean rate of change of the volume of each region, the associated standard deviation, effect size, and the results of an unpaired, two-tailed Student’s t-test comparison between SCA1 and Control cohorts (listed as p-values). The p-values marked with a “*” indicate statistical significance, where p ≤ 0.005 after Bonferroni correction. Of note, the Bonferroni correction is a statistical correction to account for multiple comparisons, and is calculated as the initial p-value threshold (p=0.05) divided by the number of comparisons (in this case, there were 10 comparisons for each analyzed region). The Bonferroni correction is the strictest form of correction for multiple comparisons, and is useful for minimizing type I (false positive) errors. However, this comes at the expense of an increased likelihood of type II (false negative) errors [26].
The analysis showed statistically significant differences between SCA1 vs. controls in the change per year of the volumes of the pons, midbrain, the total brainstem, the left and right cerebellar cortices, and the total cerebellum. Of those regions, the pons had the greatest effect size magnitude (-1.8365 for SCA1, compared to -0.0988 for Controls). Therefore, this implies that out of the volumetry measurements, the pons volume seems to be the most sensitive to SCA1 progression, which agrees with the findings from Reetz et al [12].

**SARA**

Unlike the volumetry and MRS measurements, the SARA scale consisted of a composite measure per subject. Table 4.3 shows the change per year, along with the associated standard deviations and effect sizes of the SARA score for both SCA1 and Control...
cohorts. As evidenced by the p-value of 0.003, there is a significant difference (p<0.05) between the percent change of SARA scores of the SCA1 and Control cohorts. While the SCA1 cohort had an effect size of 0.6, the Control cohort did not have an effect size because the mean and standard deviation of the change/year were effectively zero for controls.

<table>
<thead>
<tr>
<th>(%) change/year</th>
<th>SCA1</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>7.8</td>
<td>0.0</td>
</tr>
<tr>
<td>SD</td>
<td>13.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Effect Size</td>
<td>0.60</td>
<td>N/A</td>
</tr>
<tr>
<td>p-value</td>
<td>0.005 *</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.3: Mean, standard deviation, and effect sizes of changes in SARA scores for SCA1 and Control subjects. (*) indicates statistical significance, where p<0.05.

Comparison of SARA, Volumetry, and MRS

![Figure 4.6: Comparison of the %Change/year of the pons volume, pontine tNAA/Ins, and SARA scores.](image)

<table>
<thead>
<tr>
<th>Effect Size Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>SARA</td>
</tr>
<tr>
<td>Pons Volume</td>
</tr>
<tr>
<td>tNAA/Ins (Pons)</td>
</tr>
</tbody>
</table>

Table 4.4: Comparison of effect sizes from MRS, volumetry, and SARA measurements.
Table 4.4 above shows the effect sizes of the most sensitive measurement from each of the three modalities. From this table, one can conclude that both volumetric MRI and MRS measurements of the pons at 3 Tesla are more sensitive to progressive neurodegenerative changes in SCA1, compared to the clinical SARA assessment. Furthermore, these results show that the pons volume is more sensitive to progression of neurodegeneration in SCA1 compared to MRS.

One finding of note is the results from pre-symptomatic subjects. The figures below are the MRS, SARA, and volumetry results from Controls (in blue) and SCA1 subjects (in black). The red and green lines are from two pre-symptomatic subjects with the SCA1 mutation. In each graph, there is a clear separation of the SCA1 subjects from the Control subjects. However, while the pre-symptomatic subjects are indistinct from the control subjects for the SARA score and between control and SCA1 subjects on the volumetry scale, one subject (in green) has an observably lower tNAA/Ins concentration that already overlaps with symptomatic SCA1 subjects. This finding might suggest that while MRI was most sensitive to disease progression, MRS might be more sensitive to detect early disease and subtle alterations due to the onset of SCA1.
Figure 4.7: Longitudinal changes of SARA scores (top), pons volume (middle), and tNAA/Ins ratio (bottom). SCA1 (means ± SD of all subjects with SCA1 except for the two pre-symptomatic volunteers) are marked as black, and Controls are marked as blue. Two pre-symptomatic subjects with SCA1 are marked as green and red.
4.2: Comparison of Cortical Thickness and Diffusion Tensor Imaging

Cortical Thickness

Cortical thickness analysis was performed using the “average” method and the “surface-based” method (see Chapter 3.3). For both methods, the SCA1 cohort was compared with the Control cohort at each visit, resulting in a total of three cross-sectional comparisons for each method (see Table 3.1 for sample sizes from each visit).

Unlike volumetry, which was primarily focused on the brainstem and cerebellum, there was no particular region of interest for cortical thickness, so all cortical regions were analyzed. As a result, with the average method, FreeSurfer was used to compute the average thickness of 70 cortical regions (35 for each hemisphere).

![3D reconstruction of a brain with various cortical regions labeled.](image)

Using these data, an unpaired two-tailed Student’s t-test was performed between SCA1 and Control groups for each cortical region and visit. To account for multiple comparisons, a Bonferroni correction was performed for the 35 regions, such that the p-
value threshold was \( \leq 0.0014 \) for statistical significance (just as with volumetry, this mitigated potential type I errors, while increasing the likelihood of type II errors) [26].

The figures below show the cortical regions where the average thickness for SCA1 patients significantly differed from that of Controls. While Visit 2 and Visit 3 both showed regions of significant difference, Visit 1 did not yield any such regions. This is presumably due to the higher variability of cortical thickness values for Visit 1 controls (since Visit 1 had the largest control sample size of all visits). Another possibility is that Visit 1 consisted of SCA1 patients at less-advanced stages of the disease, so the changes in cortical thickness became more pronounced over time.

**Figure 4.9:** Cortical regions of significant difference between SCA1 and Control groups during Visit 2 \( (p<0.0014) \).
Figure 4.10: Cortical regions of significant difference between SCA1 and Control groups during Visit 3 (p<0.0014)

As can be seen in Figures 4.9 and 4.10, both Visits 2 and 3 showed cortical thickness differences in right paracentral cortex, the left and right precentral cortex, and the left superior frontal cortex. The paracentral cortex is associated with motor and sensory control, while the precentral cortex (which is part of the motor cortex) is involved in the planning, control, and execution of motor functions. While the function of these two cortical regions may be associated with motor decline in SCA1, the superior frontal cortex is primarily associated with self-awareness [7]. Differences between Visits 2 and 3 include the hemisphere of the caudal middle frontal cortex (left in Visit 2, versus right in Visit 3), which is associated with executive function. Furthermore, Visit 3 showed an additional difference in the left postcentral cortex, which is the main sensory area for touch. The differences between Visit 2 and Visit 3 may be due to progression of SCA1, though this may only be verifiable by analyzing at the rate of change of cortical thickness over time (a potential topic for future study).
For the surface-based method, FreeSurfer was used to compare the cortical thickness at each location of the cortex. False discovery rate (FDR) correction was performed to correct for multiple comparisons, with p<0.05 for statistical significance (See Glickman et al. for more information regarding FDR correction) [27]. Unlike the average analysis, only Visit 3 yielded regions where cortical thickness was significantly different between SCA1 and Control groups. This is presumably due increased variability of cortical thickness at each cortical location (which would have been mitigated when averaged over larger regions). Figure 4.11 below shows the results of the surface-based comparison for Visit 3.

Figure 4.11: Surface-based comparison between SCA1 and Control groups during Visit 3. All four images are different views of the same comparison.
The surface-based analysis in Figure 4.11 showed the largest bilateral (left and right hemisphere) group differences throughout the rostral middle frontal cortex, caudal middle frontal cortex, precuneus, and pars triangularis. Both the rostral and caudal middle frontal cortices are associated with executive function. The precuneus is associated with aspects of visuospatial processing (alongside the superior frontal gyrus), short-term memory, and consciousness. A rather interesting finding is the cortical thinning of the pars triangularis, which is involved in semantic tasks and speech production [7]. This might correlate clinically with the symptoms of speech disturbances observed in SCA1 patients [4].

Overall, the findings from the cortical thickness analyses demonstrate significant cortical thinning for patients with SCA1 in areas that are associated with motor function, executive function, self-awareness, and speech production. Cortical thickness is a topic that has not been investigated for SCA1 before. These findings might provide a better understanding of SCA1 neurodegeneration, especially since they correlate with known symptoms of SCA1, such as reduced motor coordination, disturbances of gait, speech disturbances, as well as executive (cognitive) dysfunction [4].

**Diffusion Tractography**

TRACULA, a diffusion analysis software based on FreeSurfer, was used to compute diffusion parameters for 18 pre-defined white matter tracts. Out of the 18 tracts, it was hypothesized that the corticospinal tract (CST) would be the most affected by SCA1. This is because the corticospinal tract is primarily involved in the movement of muscles in the body, which might suggest some correlation with SCA1 progression [7].
The first step in the analysis was to look at the average FA, MD, AD and RD along the entire tract for each subject, and to see which averaged diffusion parameter significantly differed between the Control and SCA1 cohorts. An unpaired two-tailed Student’s t-test was performed, with p<0.05 required for statistical significance. The results for the left CST are displayed in the figures below.

**Figure 4.13:** Comparison of the average FA between SCA1 and Control groups.

**Figure 4.12:** The pathway of the corticospinal tract. Source: Gray’s Anatomy, 20th edition.
Figure 4.14: Comparison of the average diffusivity measures (MD, AD, RD) between SCA1 and Control groups.

As can be seen by the figures above, the averaged diffusion measures across the entire CST did not yield statistically significant differences. However, the outcomes may be different when comparing groups at each point along the tract, since only certain locations in the CST may be affected by SCA1. After comparing each group along each position of the CST, significant differences were found along the left CST. The figures below show the FA, MD, AD, and RD along each point of the left CST. For each figure, the x-axis starts at the lower end of the CST (0 mm), and moves upward to the superior (upper-most) portion of the CST (124 mm). The shaded regions indicate regions of significant differences, where p<0.05.
Figure 4.15: Fractional anisotropy along the left CST. Shaded regions indicate regions of significant differences between SCA1 and Control groups, with p<0.05.

Figure 4.16: Mean diffusivity (MD) along the left CST. Shaded regions indicate regions of significant differences between SCA1 and Control groups, with p<0.05.
Figure 4.17: Axial diffusivity (AD) along the left CST. Shaded regions indicate regions of significant differences between SCA1 and Control groups, with p<0.05.

Figure 4.18: Radial diffusivity along the left CST. Shaded regions indicate regions of significant differences between SCA1 and Control groups, with p<0.05.

Based on the four figures above, the greatest group difference occurs with FA, MD, and AD. With regards to the FA measurements (Figure 4.15), there seems to be lower FA in
the mid-to-lower region of the CST in SCA1 than controls, and higher FA in the mid-to-upper region of the CST. Normally, degeneration along white matter pathways would lead to lower FA (since there are more directions for the water molecules to diffuse). However, this does not seem to be the case in this scenario. The most probable reason for this is the effect from “crossing fibers.” As shown in Figure 4.19, in a single “bundle” of axons (a), the FA is usually high. During axonal degeneration, the diffusion will be less restricted to a single direction, so the FA will decrease (b). However, in the case where two bundles cross each other (c), although diffusion is still restricted along certain paths, the signal from those water molecules would be as if it is in an isotropic medium, thus leading to a lower FA. When degeneration occurs along one bundle (d), it is as if the diffusion has become more “ordered,” although the opposite is the case. The more superior (higher) along the pathway, the more likely that the tracts will cross one another. This is a possible explanation for the higher FA in patients with SCA1, especially since the significant difference is seen around the location of the semiovale, an anatomical region where the CST crosses with the superior longitudinal fasciculus (another white matter tract)[28].
Throughout the lower-middle to upper-middle CST, the MD appears to be higher for SCA1 patients compared to controls. This is expected, since white matter tract degeneration would permit water molecules to diffuse more freely (in single or multiple fiber configurations). While the MD for SCA1 seems to be significantly lower than controls at lower positions, it only seems to span ~4mm, and thus may be a product of variability in measurement.

The axial diffusivity for SCA1 patients appears to be significantly greater than controls throughout middle and upper-middle CST. One might consider this to be an unusual finding, as axonal degeneration would seem to reduce the axial diffusivity along the major axis of the white matter tract. However, studies have shown higher AD in certain pathways for other conditions, such as the CST for ALS [26]. Therefore, there is reason
to believe that such a correlation might exist for SCA1 as well. However, the structural and biophysical basis for the change in AD is still unknown at this point.

Comparing Cortical Thickness and Diffusion

In this arm of the study, cortical thinning was primarily observed in the precentral and paracentral cortex during both Visit 2 and 3. Since both regions are part of the primary motor cortex, it was presumed that there was possible involvement of the corticospinal tract (since the CST extends from the primary motor cortex to the brainstem and spinal cord). Analysis using diffusion tractography showed significant changes in fractional anisotropy, mean diffusivity, and axial diffusivity for SCA1 patients, when compared to controls. These results suggest a potential link between cortical thinning and degeneration for the left corticospinal tract due to SCA1 progression. In the future, a longitudinal analysis may provide the answer to whether cortical thinning occurs prior to CST degeneration, or vice versa.
4.3: Understanding SCA1 Neurodegeneration

Overview

Based on the results from the spectroscopy, volumetry, cortical thickness, and diffusion tensor imaging methods, it is possible to create a model that describes the “big picture” of SCA1 neurodegeneration. The purpose of this is to understand what occurs at the molecular, cellular, and structural level that eventually leads to the symptoms seen in patients with SCA1. Since only the “MRS vs. volumetry vs. SARA” arm of the analysis was longitudinal, a time-based model could not be made, and thus the conclusions from this analysis are qualitative in nature. Nevertheless, such a model is still useful, as it describes SCA1 pathology throughout various regions of the brain, and shows how they could potentially be linked with one other. Such a model may also prove useful for evaluating degenerative pathology during clinical trials. With the clinical assessments that are currently used, potential drugs may improve symptoms, but not slow the progression of pathology in the brain. However, with an understanding of multiple aspects of degeneration in the brain, future clinical trials could evaluate whether treatments reverse the pathology of the brain itself, which will aid in finding more effective treatments for SCA1.
The Proposed Model for SCA1 Neurodegeneration

The proposed mechanism for SCA1 neurodegeneration is as follows: first, the transcription of the CAG repeat on the ATXN1 gene leads to cytotoxicity for various neuronal structures in the brain. This reduction of neuron viability leads to a notable decrease of the tNAA/Ins ratio throughout the cerebellum, cerebellar vermis, and the pons (the last of which is the site of the greatest metabolite change). The changes in the metabolites are subsequently followed by atrophy of the brainstem, with the most degeneration occurring in the pons. Around this time, changes in the cerebral cortex occur as well, with notable cortical thinning occurring around the motor cortex, along with cortical regions responsible for speech production, executive function, and self-awareness. The structural changes occurring in the brainstem and the cerebral cortex are linked by notable degeneration along the corticospinal tract, which connects the motor cortex to the brainstem. The changes from the structural, chemical, connectivity aspects of the brain eventually lead to the visible symptoms of SCA1, including reduced motor coordination, gait and speech disturbances (all of which are currently quantified using SARA).
Chapter 5

Conclusion

In this thesis, multiple modalities of magnetic resonance, including MRS, structural MRI, and diffusion MRI were used in order to quantitatively understand how the chemical, macrostructural, and microstructural characteristics of the brain are affected in SCA1. A comparison between volumetry, MRS, and SARA found that volumetry of the pons was the most sensitive measure to SCA1 progression, although MRS of the pons could potentially detect early stages of the disease and subtle neurochemical changes due to disease onset. Analysis of DTI and cortical thickness results also found a potential link between cortical thinning around the motor cortex and changes in the left corticospinal tract (as evidenced by changes in key diffusion parameters). The results from these two arms of the thesis project were used to create a qualitative model of SCA1 neurodegeneration that described the progression of SCA1 from the molecular level to the symptomatic level. Future analysis may include looking at the progression of cortical thickness and white matter tract changes over time, so that a more direct sequence of events can be formulated for the proposed model. Potential applications of the findings from this thesis include using the objective and quantitative characteristics of MRI/MRS analysis to reduce sample sizes for future clinical trials. Furthermore, the proposed model of SCA1 neurodegeneration may aid in evaluating brain pathology during clinical trials, which could help in finding more effective therapies to help treat both the brain pathology and the symptoms of SCA1.
References


