

Spinal Cord Trauma: An Overview of Normal Structure and Function, Primary and  
Secondary Mechanisms of Injury, and Emerging Treatment Modalities

Daniel Morin

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Stephen W. Eakin, M.D., H.F.S.  
Thesis Chair

---

Mark Blais, D.P.M.  
Committee Member

---

Jeffrey N. Lowes, D.C.  
Committee Member

---

James H. Nutter, D.A.  
Honors Director

---

Date

### Abstract

The structures of the spinal cord and vertebral column are designed to provide flexibility, while still providing ample protection for the spinal cord deep within. While it does offer remarkable protection against most routine trauma, the spinal cord is still vulnerable to high-force etiologies of trauma and may become damaged as a result. These events are referred to as primary injury. Following the initial injury, the body's own physiological responses cause a cascade of deleterious effects, known as secondary injury. Secondary injury is a major therapeutic target in mitigating the effects of spinal cord injury (SCI), and much research is currently being done to develop more effective treatment options.

## Spinal Cord Trauma: An Overview of Normal Structure and Function, Primary and Secondary Mechanisms of Injury, and Emerging Treatment Modalities

Spinal cord injury is a devastating condition that affects over a quarter of a million individuals in the U.S. alone, with up to 500,000 new cases occurring worldwide each year [1, 2]. While the cause of spinal cord injury (SCI) can be due to disease-related mechanisms, the World Health Organization estimates that around 90% of cases are attributable to trauma-related etiologies [2]. SCI is a complex disorder that can result in a plethora of different outcomes depending on the site and severity of the traumatic insult, the promptness and efficacy of acute and sub-acute phase treatment, and the success of therapy efforts. Manipulation of just one of these variables can potentially have an enormous influence on long-term prognosis.

### **Normal (Physiological) Spinal Cord Structure & Function**

The spinal cord itself is a masterpiece of engineering, massively intricate and complicated in both structure and function. Fundamentally, it can be thought of as a very large bundle of nerves, extending from the medulla oblongata portion of the brainstem and traversing the superior two thirds of the vertebral canal before terminating in the conus medullaris between the levels of T12 and L3 [3, 4]. Together with the brain, it comprises the central nervous system (CNS), and serves to connect the brain to the rest of the body. It is made up of numerous *tracts*, that is, smaller neural bundles each with a more narrow purpose [4]. Some of these spinal tracts bring information from the brain to the peripheral nervous system (PNS)—these are called *descending tracts*, while other tracts carry information from the periphery to the brain—these are referred to as

*ascending tracts* [4]. These pathways are analogous to a super-highway, with various lanes going in both directions, each with its own destination and purpose, and yet collectively integrated into one comprehensive structure.

As with any highway, the spinal cord also contains exits. At the level of each vertebrae, a pair of spinal nerves emanate from the cord which provide innervation and communication for the somatic structures at that level [3]. These step-wise regions of innervation are called *dermatomes* in reference to the skin and *myotomes* in reference to muscle tissue [3]. When these spinal nerves become impinged or severed, partial or complete paralysis and loss of sensation occurs in the tissues relying exclusively on that source of innervation. As we will discuss later on, trauma to the spinal cord itself can result in paralysis and loss of sensation in the entirety of the body inferior to the lesion location.

In light of this complex organization, it is easy to see just how devastating, and strangely enigmatic, damage to the spinal cord can be. But the spinal cord is not an easy structure to injure, as it has multiple layers of defense that protect it from damage in most physiological contexts.

### **Normal (Physiological) Vertebral Column Structure**

While the spinal cord itself is relatively delicate, it possesses an exceptionally protective surrounding structure in the various components of the vertebral column. These structural elements envelope the spinal cord in many layers of reinforcement and prevent damage from occurring as a result of day-to-day wear and tear. Immediately surrounding the cord are the spinal meninges. These meninges arise in three layers (from innermost to outermost): the pia mater, the arachnoid mater, and the dura mater [3]. They

provide a tri-layer connective tissue sheath that runs the length of the cord [3]. The pia mater is the deepest layer, an exceedingly thin membrane that directly encapsulates the spinal cord throughout its length [3]. Just superficial to the pia mater is the arachnoid mater. The arachnoid is avascular and made up of fibrous and elastic tissue [3]. Between the pia and arachnoid is the subarachnoid space [3]. The subarachnoid space is an additional protective feature. It is filled with cerebrospinal fluid (CSF), which cushions the entire CNS system, isolating it from injury by absorbing exogenous traumatic forces [5].

Continuing out from the arachnoid mater, the superficial-most layer of the meninges is the spinal dura mater. The spinal dura is a continuation of the cranial dura mater, which covers the brain, and serves as its spinal counterpart [3]. It originates superiorly at the foramen magnum of the cranium and lines the entire interior of the vertebral canal [3]. In this way, it forms a sort of membranous capsule around the spinal cord, portions of the spinal roots, and the deeper meningeal layers, and is referred to in this regard as the *spinal dural sac* [3]. The dura mater membrane has a tougher, more fibrous composition than the deeper meninges, and provides a resilient outer jacket for the spinal cord itself [3]. Surrounding the dura mater is a layer of epidural fat [3]. Together, the spinal meninges, the CSF contained within, and the epidural fat layer provide the deepest line of defense for the spinal cord amongst the structures of the vertebral column.

The vertebral column itself is designed with spinal cord protection as a primary consideration. Each vertebra (especially those in the thoracic and lumbar regions) features a relatively large, rounded body, which fulfills the majority of the weight-bearing

component of the bone's responsibility [3]. This ensures that the spinal cord will be subjected to minimal compressive force, as the bulk of these compressive forces will be borne by the vertebral bodies. Furthermore, extending posteriorly from the body of each vertebra is a thick semilunar band of bone called the vertebral arch [3]. Together, this arch and the posterior aspect of the vertebral body combine to form the walls of the vertebral foramen [3]. The vertebral foramina of all the vertebrae, taken together, make up the vertebral canal. In addition to the protection afforded by the vertebral arch, the vertebral foramen is further reinforced to the posterior by the spinous process of the vertebra, and on the lateral aspects by the transverse processes of the vertebra [3]. All of these bony features defend the spinal cord from penetrating and blunt forms of trauma. Lastly, the articular processes of the vertebrae interact with one another in such a way as to positively limit the flexibility of the spine and promote the maintenance of its alignment [3]. The limitations imposed by these articular processes protect the spinal cord from being placed in positions where it is subjected to excessive bending and twisting forces [3].

Beyond this, surrounding the vertebrae are various ligaments that increase the protective capacity of the vertebral column even further. The anterior longitudinal ligament can be found running along the anterolateral aspect of the vertebral column, where it limits spinal hyperextension [3]. Its counterpart, the posterior longitudinal ligament traverses the posterior surface of the vertebral bodies within the vertebral canal [3]. In addition to these, other ligaments, such as the ligamenta flava and supraspinous ligaments, serve to reinforce the joints between individual vertebrae [3]. The combined effect of these structures helps protect the spinal cord from tensile and sheering forces.

Finally, we come to the muscular layers of protection. While a comprehensive examination of the musculature of the back is beyond the scope of this thesis, the system can be understood in an elementary way as consisting of intrinsic (deep) muscles and extrinsic (superficial) muscles [3]. The deep muscles of the back have various functions. While some of them, chiefly the muscles of the *erector spinae* group, have dynamic function, many of the other, smaller muscles have a more postural and proprioceptive function [3]. The extrinsic muscles of the back, such as the *trapezius* group, *latissimus dorsi*, and the *rhomboids*, are not spinal muscles, proper, but instead perform dynamic actions in the posterior region of the body [3]. Regardless of their particular function, however, all of these muscles and their associated fascia provide an additional layer of support and protection for the spine and, together, serve to shield the vertebral column from external sources of trauma by absorbing shock and excessive forces on a superficial level [3].

All things considered, from the pia mater to the superficial fascia of the back, the spinal cord is remarkably well-defended from injury by a diverse and numerous set of protective structures. It is this set of protective structures which keeps us safe from injury on a daily basis; despite the many trips, falls, and bumps we experience in our lifetime, relatively few of us will ever suffer remarkable neurological consequences. With that being said, however, these protective structures are not impervious; if and when they are subjected to extreme mechanisms of trauma they may be breached, exposing the vulnerability of the spinal cord within. In the next section, we will briefly explore what is termed *primary injury*, the initial traumatic insult to the spinal cord tissue.

### **Primary Injury: An Overview**

Any given spinal cord injury is really a composite of two types of injury: *primary injury* and *secondary injury*. As was mentioned above, primary injury refers to the initial damage incurred in the acute trauma event—it is probably what most people would think of when presented with the concept of spinal cord injury. Secondary injury, which will be the focus of much of this thesis, is as much a disease process as it is a mechanism of injury. It refers to the cumulative deleterious effects of the body's physiological responses to primary injury, which result in lesion expansion and exacerbation of functional losses. For clinical purposes, secondary injury is actually a much more relevant topic to study [6]. After all, very little can be done in immediate response to primary injury—in most cases it happens instantaneously, and by the time emergency services personnel arrive little can be done to alleviate it [6]. There are a few exceptions to this, including cases where surgery is required to remove a primary injury implement, such as is the case with some combat-related wounds, but for the most part, primary injury is not a feasible target for treatment [6].

Nevertheless, despite the fact that primary injury is the less clinically-significant subject, it is essential to developing a full understanding of SCI as a whole. Primary injury severity is the single greatest predictor of overall SCI prognosis and discerning the mechanism of primary injury in any given case is an important consideration for clinicians seeking to develop a treatment protocol in the emergency setting. Therefore we will give the topic of primary injury a brief treatment as a part of this thesis. For a much more detailed look at primary injury mechanisms, see the article, *Mechanism and pathophysiology of spinal and spinal cord injury* (Braakman, 1991).

In terms of primary injury causation, data from The National Spinal Cord Injury Statistical Center (NSCISC) lists “Vehicular Accidents” as the most common cause of SCI, contributing 39.08% of all cases [7]. This is followed by “Falls” at 29.54% of cases, “Violence” at 14.41% of cases, and “Sports and Recreation” at 8.39% of cases [7]. It is perhaps notable that the number one single cause, automobile accidents, has only existed since the invention of the automobile in the late 1800’s [8]. Looking at these common causes should serve to underscore the fact that SCI is actually not at all easy to do. On the contrary, overcoming the built-in defenses of the vertebral column requires a tremendous amount of force directed at the spine; force that can typically only be amassed in vehicles or through falls, sports collisions, or violence.

Just as we have reviewed a brief primer in SCI *causation*, we will now delve into a cursory look at SCI *mechanism*. When discussing vertebral column structure above, several different types of forces were mentioned which come into play as we look at the essential mechanisms of spinal trauma. Iencean (2003), describes them more comprehensively in the following in four ways: 1. Axial deformation, 2. Torsion or axial rotation, 3. Segmental translation, including shearing version, and 4. Combined mechanism, simultaneous or successive [9].

The first of these is *axial deformation*. This essentially means a disruption to the structure of the spinal cord in the axial plane. This takes in both compression of the spinal cord and elongation of the spinal cord. Iencean continues to break both of these down further into centric and eccentric variations [9]. Centric compression refers to compression in a directly axial plane, causing forced shortening of the spinal cord [9]. Centric elongation refers to stretching of the spinal cord in a directly axial plane, also

referred to as *distraction* [9]. The eccentric forms of these mechanisms refer to instances where compression and/or elongation occur in conjunction with bending trauma. To illustrate this, imagine what happens when you attempt to bend a metal rod. Inevitably, the aspect of the rod on the side of the bend will experience compression, while the side opposite the bend will likely experience some degree of elongation. This same principal applies to the spinal cord in instances when it is subjected to bending forces that exceed physiological parameters (such as in cases of hyperflexion and hyperextension). At the location where the trauma was sustained, part of the cord will experience some compression, or crushing, while the opposite face will exhibit some elongation, or stretching, with the overall degree of axial deformation varying based on the intensity of the injury [9].

The second essential mechanism of spinal trauma described by Iencean is *torsion*, or *axial rotation* [9]. This occurs in conjunction with twisting injuries, where the spinal cord is rotated beyond its physical limits, often associated with catastrophic damage to the bony structures of the spinal column [9]. The third type of trauma outlined is *segmental translation* [9]. Segmental translation occurs when opposing forces are exerted on two or more sequential vertebrae, causing them to slide across one another in opposite directions. This process results in the pinching off of the interior of the vertebral canal and often results in severe acute damage to the spinal cord [9].

The final class of mechanism Iencean describes is the *combined mechanism*, in which traumatic forces result in a mechanism pattern which includes, to some extent, components of two or more of the mechanisms described above [9]. In this way, the combined mechanism can practically be understood as a complex comprised of a set of

more simple mechanisms, which may all occur simultaneously in one instant or may present sequentially to one another [9]. Due to the intense, unpredictable nature of many SCI etiologies, Iencean states that the combined mechanism is the trauma mechanism most commonly observed in real-life cases [9]. Understanding each of these mechanisms and the plethora of ways they can compound upon each other is helpful in classifying and grading spinal cord insults in the clinical setting.

The other primary factors of consideration in primary spinal cord injury are the site and severity of the insult. Site is critical; since nervous function is affected throughout the entirety of the body below the point of trauma, damage at inferior levels of the spine conserves more function and sensation than damage at superior levels. Trauma in much of the cervical spine, for example, often results in *quadriplegia*—paralysis in all four limbs, while trauma below the cervical spine often results in preserved upper limb function, referred to as *paraplegia* [10]. As a general rule, in cases where mechanism and completeness of the lesion is the same, lower lesion location results in more favorable outcomes in terms of conserved function and reduced morbidity [10].

The last piece of primary injury which we will cover is the severity, also referred to as the *completeness*, of the injury. Incomplete injuries are characterized by lesions which do not completely impede the passage of impulses between the brain and periphery. Individuals in these cases often spontaneously regain some level of function below the level of injury and usually are more responsive to physical therapy efforts [10]. Individuals who experience complete or nearly complete injury are much less likely to

spontaneously regain significant function and have a poorer prognosis, even with rigorous therapy [10].

Together, these primary injury characteristics—cause, mechanism, site, and completeness—form an objective picture of spinal cord injury. By piecing together an accurate history of the injury event and using radiology to image the trauma site, practitioners can glean a fairly comprehensive understanding of the injury. This is useful in preliminary classification of the injury and can help with forming an appropriate treatment plan in the acute and early sub-acute phases of the injury process. As time goes on, however, practitioners shift to a more standardized measure of SCI severity, a universal classification scale known as the International Standards of Neurologic Classification of Spinal Cord Injury (ISNCSCI) [10]. The ISNCSCI is a testing protocol published by the American Spinal Injury Association (ASIA) that measures sensory and motor function throughout the body through a numerical scoring system that classifies the injury quantitatively [10, 11]. This evaluation protocol is typically conducted seven to ten days post-injury, with the intent of allowing sufficient time for physiological responses to stabilize [10].

### **Secondary Injury: An Overview**

Now that we have laid the foundation of understanding primary injury more clearly, we can begin to delve into deeper exploration of *secondary injury*. Secondary injury, as we have already defined above, consists of the cumulative deleterious effects of the body's physiological responses to primary injury [6, 10]. In other words, the incidence of a primary injury initiates a cascade of physiological responses at and around the site of the traumatic lesion. Some of these responses are beneficial to the healing

process, but many of these responses only serve to cause further damage. Deleterious secondary injury processes exacerbate nervous tissue death, further expanding the lesion and resulting in increased functional losses, ultimately leading to a poorer prognosis overall [6, 10]. The remainder of this thesis will focus on these secondary injury processes, with a special emphasis on two key mechanisms, their biochemical backgrounds, and current research in therapies to mitigate their effects. We will conclude with a look forward to the promising field of multi-modal therapy, which seeks ways to maximize optimal outcomes by targeting multiple secondary injury mechanisms at once.

There are many different modalities of secondary injury. One source lists up to 25 individual contributing mechanisms [6]. These processes begin within seconds of the injury event, and may continue, to some degree, for months following onset [6]. While all twenty-five mechanisms are contributing factors, six of these mechanisms have been highlighted by the National Institute of Neurological Disorders and Stroke in their feature, *Spinal Cord Injury: Hope Through Research* [10]. Namely, they are: changes in blood flow and leaky vasculature in and around the injury site; inflammation due to overactive immune response; spontaneous apoptotic pathway/demyelination; scarring of injury site due to glial cell activity; toxic release of neurotransmitters from damaged cells, especially glutamate; and, lastly, free radical chain reaction [10]. Together, these physiological processes contribute significantly to the exacerbation of neuronal damage following primary injury. The first four of these mechanisms will be given a brief discussion, in order to convey a working understanding of their biochemical and cellular basis, while the final two will be featured in more depth, with a short analysis of research in these areas.

### **Secondary Injury Mechanism 1: Blood flow and vascular leakage**

Disruption of the spinal cord vasculature begins with primary injury [12]. Trauma to the affected region causes damage to the microvasculature in that immediate region, resulting in both local bleeding (hemorrhage) as well as reduced perfusion (ischemia) [6, 12]. This reduced perfusion may be attributed to either clotting or vasospasm in the damaged microcirculation [6]. Secondary to this vascular damage, edema has been noted to develop, which puts further pressure on the damaged region and exacerbates detrimental effects [6, 10]. All of these disease processes cause necrosis of cells surrounding the immediate site of trauma, and cause expansion of the lesion within the first several-to-48 hours following injury [6, 10, 12]. It is also important to realize that these vascular disruptions also fundamentally affect the blood-spinal cord barrier (BSCB). The deterioration of this barrier stems, on a cellular level, from the disruption of the endothelial cells, pericytes, and astrocytes surrounding the spinal microvasculature [12]. In a normal physiological context, these cells protect and nourish the neural tissue, and breaking the barrier thus results in a two-fold negative effect. On one hand, it exposes the neural tissue to harsh blood-borne molecules that accelerate necrosis, and on the other hand it causes the neural tissue to starve due to lack of available nutrients [12].

### **Secondary Injury Mechanism 2: Inflammation due to immune cell infiltration**

In a normal physiological state, somatic immune cells are restricted from entering the CNS by the blood-CNS barrier. With this barrier broken, as has just been discussed, these immune cells are no longer prohibited access to the spinal cord and begin to infiltrate the injury site in large numbers [6, 10]. This begins with neutrophils, followed by monocytes, which develop into macrophages. While these cells produce some positive

effects, they also contribute considerably to the negative secondary injury process by causing a rapid escalation of inflammation.

These cells release various cytokines, including inflammatory interleukins, as well as other pro-inflammatory molecules and byproducts, which ramp up recruitment of additional immune cells and cause the wholesale activation of inflammatory pathways in the region surrounding the lesion [6, 10, 13] These upregulated cytokines include tumor necrosis factor (TNF)  $-\alpha$ , interleukin (IL)-6, and IL-1. The combined effect of these processes cause the large-scale death of neurons not originally affected in the initial trauma [6, 10].

### **Secondary Injury Mechanism 3: Apoptosis/Demyelination**

In a poorly-understood response to the influx of non-nervous immune cells and the upregulation of inflammatory cytokines, neurons and oligodendrocytes in the general vicinity of the lesion have been observed to undergo apoptosis in the acute and sub-acute phases following the primary injury [6, 10, 13]. This process is typically associated with an intracellular influx of the  $\text{Ca}^{2+}$  ion, an explanation which makes sense in the cells immediately surrounding the lesion site [6, 13]. Oddly, however, apoptosis is also observed to occur in neurons and oligodendrocytes further away from the injury locus, beyond the range of any influence from the localized spike in calcium [6]. It is theorized that apoptosis in these cells may be stimulated by other components of the secondary injury process [6].

In addition to apoptosis, progressive demyelination has also been observed in the vicinity of the lesion [6, 10]. This occurs secondarily to the apoptosis of oligodendrocytes described above, as it is these oligodendrocytes which provide the myelin sheath [6, 10].

Loss of these cells results in the deterioration of the myelin covering in surviving neurons, which exposes them to free radical degradation, inflammatory damage, etc. [6]. Destruction of these exposed axons is a major contributing factor to the progressive loss of communicative patency along the spinal cord in the sub-acute phase [6, 13].

#### **Secondary Injury Mechanism 4: Astroglial scarring**

In the period following SCI, reactive astrocytes begin to congregate on the margins of the spinal cord lesion [10, 14]. These astrocytes undergo hypertrophy and proliferation to create a dense fibrous network, separating the damaged region from the healthy regions of tissue [14, 15]. In so doing it re-establishes the BSCB and protects the healthy tissue regions from further degradation [16]. This is very beneficial effect in the short-term, but it leads to difficulties in the long term [10, 14, 16-17]. As acute processes stabilize, this astroglial scar prevails, and has been shown to impede the regrowth of axons back through the lesion site, leading to the inhibition of functional recovery [10, 16, 17].

#### **Secondary Injury Mechanism 5: Glutamate excitotoxicity**

##### **Mechanism**

Finally, it is time to take a deeper look at two of the key mechanisms of secondary injury. The first of these is glutamate excitotoxicity. Glutamate is an excitatory neurotransmitter that has a major role in the CNS [6, 18, 20]. In normal physiological states, it can be found in storage vesicles at the axon terminal, and in transiently-occurring, minute amounts within the synaptic cleft [18, 20]. When released, glutamate stimulates various receptors on the target neuron. There are a few classes of cell-surface receptors that respond to glutamate. The first three are referred to as ionotropic receptors,

these are the *N*-methyl-d-aspartate receptor (NMDA), the kainic acid (KA) receptor, and the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor (AMPA) [20]. The fourth class of glutamate receptor is the metabotropic glutamate receptor, a G-protein coupled class of receptor that mediates downstream processes [20]. While glutamate is an extremely potent neurotransmitter, it does not typically spend much time in the synapse, as it is rapidly taken up by glutamate transporters in the surrounding neural and glial cells following its release [18, 20]. This moderates the duration for which the target neuron is under the influence of glutamate and prevents the accumulation of glutamate in the synaptic cleft [18, 20].

In SCI conditions, various processes cause an excessive release of glutamate into the extracellular space. Necrosis of damaged cells causes spillage of glutamate stores into the surrounding extracellular fluid, and ion gradient fluctuations resulting from other secondary injury mechanisms can reverse the action of glutamate transporter proteins, such that the very transporters that are supposed to remove glutamate from the extracellular space actually cause the release of additional neurotransmitter [18, 20]. As these drastically excessive glutamate levels remain unchecked, the neurotransmitter diffuses through the extracellular space, causing indiscriminate and constant stimulation of all cells with glutamate receptors in that region of tissue [18-20]. Especially at risk are cells with high numbers of NMDA and AMPA receptors, which include neurons and oligodendrocytes [6, 20]. These cells receive a deadly amount of excitatory stimulation. This stimulation causes the opening of cation channels, resulting in an influx of  $\text{Ca}^{2+}$  ions which initiate a feedforward response which brings about the rapid death of the cell [6, 18-20].

### **Therapeutic Targets**

There are currently two major avenues of therapy thought to combat glutamate excitotoxicity. The first involves the use of glutamate receptor antagonists [6, 20]. These compounds act by binding competitively to glutamate receptors and preventing stimulation from occurring [6, 20]. These have been shown to be partially effective in animal models, but have not historically had great success in human trials [20]. It is suspected that receptor antagonists may not be as effective in clinical scenarios, where the trauma has already happened, and glutamate has already infiltrated and overwhelmed the tissue [20].

The second potential therapy for excitotoxicity which is currently under research are glutamate scavengers [21, 22]. The theory behind these drugs is that it is possible to modulate glutamate levels in the CNS indirectly by manipulating glutamate levels in the blood. Glutamate-pyruvate transaminase (GPT) and glutamate–oxaloacetate transaminase (GOT), two blood resident glutamate scavenging enzymes, have both been tested for this use in animal models, with positive outcomes [21, 22]. The treatment concept follows the principle that glutamate will follow its concentration gradient. In cases of SCI, glutamate levels in the blood and CNS, once separated by the BBB, come to an equilibrium which allows the concentration of glutamate to build up in the brain. By introducing a blood glutamate scavenger, glutamate levels are dramatically lowered in the blood, and CNS glutamate follows its concentration gradient out of the CNS into the bloodstream, relieving the damaged neural tissue from excitotoxic stimulation [21, 22].

## **Current Research**

Glutamate scavenging is a therapy that has been consistently shown to be effective in animal models over the past several years [21-23]. The majority of new publications on the subject are review articles stressing the need for future human trials [21, 24, 25].

### **Secondary Injury Mechanism 6: Free radical damage**

#### **Mechanism**

The final mechanism we will consider is damage resulting from tissue exposure to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Under normal physiological conditions, these reactive species are produced in limited quantities to enable essential reactions in the cell. These molecules include superoxide, hydroxyl radical, and hydrogen peroxide, among others [26]. While these molecules are very volatile and dangerous in high quantities, their production within the cell is limited to only what is necessary for optimal function [26]. This is regulated by the mitochondria and through the presence of various naturally-occurring antioxidant molecules and enzymes [26].

In spinal cord injury states, however, these physiological safeguards are overrun, causing an uncontrolled spike in ROS and RNS in the intracellular and extracellular spaces [26]. It is suspected that the glutamate cascade and calcium ion influx described above have a role to play in this, stimulating dysregulation of the mitochondria and upregulating the generation of free radicals. Also contributing to this spike is increased phagocyte activity; a byproduct of the neutrophils and macrophages that have infiltrated the tissue in the absence of the BSCB. Finally, a third major source of these reactive

species is tissue necrosis, which results in the uncontrolled dumping of lysosomal and peroxisomal contents into the extracellular fluid [26]. Altogether, these sources, along with a few other minor ones, combine to create an extracellular environment teeming with ROS and RNS.

ROS and RNS are defined as having a much higher reactivity than their integral element in its ground state. This characteristic makes them extremely volatile oxidizing reagents. They have the ability to cause major oxidative damage and secondary protease-mediated damage to proteins and nucleic acids, with quite severe consequences; however, they are *especially* devastating in their destruction of membrane lipids [6, 26-28]. They accomplish lipid peroxidation by performing oxidation on a membrane lipid molecule [6]. This electrostatic alteration causes instability and results in a chain reaction that literally rips apart the membrane, ending in spontaneous cell death [6, 27]. ROS also can act as intracellular messengers, causing direct activation of inflammatory and necrotic pathways [6, 27]. The fatty acid composition of neuronal membranes leaves them especially vulnerable to the deleterious effects of reactive oxidative species, and makes these deadly molecules an especially important target in developing effective SCI treatments [26].

### **Therapeutic Targets**

The strategy behind mitigating free radical damage in SCI relies on the use of antioxidants; which antioxidants to use and how to use them is not a simple thing to determine, however. Z Jai, et al. (2012) lists several antioxidant compounds currently under varying extents of investigation, including Cu,ZnSOD, vitamin E, 21-aminosteroids (especially U-74006F), 3H-1,2-dithiole-3-thione, among many others [26].

The goal is to supplement the body's grossly overwhelmed natural antioxidant supply by administering exogenous antioxidant throughout the critical window of time in which oxidative stress is the most extreme.

### **Current Research**

There are currently many researchers working on finding viable neuroprotective antioxidant therapies. In one study, Wang Y, et al (2014) tested the antioxidant effects of  $\alpha$ -Lipoic acid-plus (LAP) using chelating intralysosomal iron as an oxidative reagent [29]. The intent was to test LAP as a possible therapy for sub-arachnoid hemorrhage (SAH), using rats as a model. They administered LA and LAP orally once a day for 72 hours [29]. Upon analyzing the results, they found that LA and LAP treatment was correlated with significantly positive outcomes, including reduced cerebral edema and BBB breakdown, lower incidence of cortical apoptosis, and less functional impairment following the SAH [29].

Another study, performed by Zhang T, et al (2014), tested the neuroprotective effects of ursolic acid (UA) in the same rat SAH model. Rats from each test group were subjected to the injury, sacrificed after two days, and evaluated [30]. They found that the UA treatment was associated with a significant reduction in early brain injury (EBI), cerebral swelling, BBB breakdown, neural cell apoptosis, and neurological deficits [30].

Yet another study, Ohnishi M, et al (2012), explored the neuroprotective capacity of another antioxidant, sesamin, specifically as a microglial inhibitor and MAP kinase antagonist [31]. A rat intracerebral hemorrhage model was once again used [31]. The sesamin therapy was successful at suppressing nitric oxide (NO) production and

inhibiting p44/42 MAP kinase activation [31]. It also prevented the activation of microglial cells in response to the hemorrhage [31].

A fourth study, conducted by Zhang X, et al (2014), investigated the neuroprotective effects of the carotenoid, astaxanthin [32]. The researchers utilized a SAH model in two different species, rats and rabbits [32]. The treatment was evaluated using two modalities, intracerebroventricular injection and oral administration [32]. Not only did the antioxidant perform well in virtually all of the parameters in which it was tested, researchers observed that it actually upregulated the levels of endogenous antioxidant in the rat model [32].

Overall, these studies only serve to underscore the immense potential that appears to be associated with exogenous antioxidant therapies. As time goes on, further studies on animal models will hopefully guide researchers to developing protocols for human trials.

### **A Comprehensive Approach: Multi-modal Therapy**

Today, many researchers have come to see SCI trauma treatment as a complex and multi-faceted undertaking [6, 33]. As discussed above, secondary spinal cord injury is a phenomenon that comprises more than 20 individual pathological processes. It is not a pathology that will respond optimally to a one-size fits all type of treatment. It is also not a phenomenon that will be treated with maximum effectiveness by focusing on just one or two of the specific, isolated elements of secondary injury at the exclusion of the many others at work. It is a constellation of processes, and, as such, effective treatment will increasingly entail a many-pronged approach that can be tailored to the individual patient.

Approaching secondary injury comprehensively and using a combination of treatment modalities has the potential to promote a degree of therapeutic synergy, leading to better overall outcomes than could be achieved with single-modality treatments alone. As researchers continue to develop new, more effective strategies for approaching each individual facet of the condition, treatment protocols will become increasingly more elegant and efficacious. One day, we will hopefully be able to develop a treatment protocol that addresses each aspect of the complexity in an individualized manner and results in an optimization of qualitative outcome.

### **Other Current Fields of Research**

The focus of this thesis is primarily on primary and secondary injury, with a special focus on research that surrounds improving treatment of the latter. Before concluding, it is worthwhile to mention some of the other areas of SCI treatment research that are being pursued currently. In 2013, when the NIH originally released the publication “Spinal Cord Injury: Hope through Research,” which has already been referenced several times in this thesis, they listed four distinct categories into which the general body of SCI research could be classified: neuroprotection, regeneration, cell replacement, and retraining/plasticity [10]. Conceptually, these four categories represent the four primary avenues of SCI treatment, as it is currently understood. Although this is merely one classification scheme, it is a helpful one, and we will use it here to help make more sense of the broad array of current research endeavors.

All of the research that we have discussed to this point falls under the category of neuroprotection research. This describes the effort of finding ways to mitigate ongoing neuronal damage following the initial insult by regulating secondary injury processes,

ultimately promoting neuron sparing. As we have already discussed much of this research at length, we will not take additional time to explore it here.

The second major focus category of research, regeneration, deals with the regrowth and remyelination of severed axons through the site of the SCI lesion [10]. This is a challenging outcome to achieve, as damaged neurons within the CNS are notorious for their ineptitude at growing back in physiologically viable ways, assuming they ever even grow back at all. With that being said, there have been a number of studies that have seen some success in stimulating regeneration, however. For example, after SCI, a number of proteins and cytokine molecules are upregulated at the site of the lesion, many of which either directly or indirectly inhibit axonal growth [10]. One line of research has been to look for pharmacological agents that can serve as antagonists to the action of these growth inhibitors [10]. Numerous promising molecules have been identified [10]. Additionally, research has also been done to identify cellular pathways and neural growth factors that can positively stimulate regeneration, as well. One study in 2015 demonstrated exceptional regeneration outcomes using the neurotrophic factor artemin [34]. More recent research has demonstrated exciting potential in the activation of the Wnt/ $\beta$  catenin signaling cascade [35,36]. This pathway is involved in embryogenesis and has been shown to promote axonal regeneration in CNS trauma models, as well [35, 36].

Other studies have looked for ways to clear obstructive glial and myelin debris from within the lesion using chondroitinases, which are specialized enzymes that degrade proteoglycan elements. A bacterial enzyme, chondroitinase ABC I (cABC I), has especially shown potential in this capacity [10]. Current research is now seeking out ways to augment the enzyme's stability so that it can be used more effectively in a therapeutic

context [37, 38]. Additionally, researchers are also seeking out ways to incorporate bioengineering in the field of axonal regeneration, through the use of *axon bridge* elements, which are physical implements that are inserted into the site of the lesion to essentially serve as scaffolding for the purpose of properly guiding and facilitating the growth of newly developing axons [10]. These elements are often used in combination with many of the pharmacological therapies described above [39, 40].

The third focus of research that the NIH report highlighted was that of cell replacement. This is a vast area of research with many promising prospects [41]. One of the chief focuses is that of stem cell research. The fundamental concept is that, by transplanting pluripotent or multipotent cells into the injury site, healthy new nervous tissue can be developed to fill the lesion zone. Any attempt to provide an adequate discussion of current stem cell research in spinal cord injury would require its own paper, and is thus far beyond the scope of this one, but for an excellent overview of the topic, see the reviews by Oh and Jeon (2016) and Vismara, et al (2017) [42, 43]. One of the major challenges still facing researchers in this field is the puzzle of determining the identity and influence of the many neurotropic growth factors that play into normal nervous system differentiation and development [10]. A deeper understanding of these processes will be necessary in order to maximize therapeutic benefits in the future.

The fourth and final area of research is in the field of plasticity and retraining the surviving neurons in the post-trauma CNS to take on functionalities that were initially lost due to the injury. Equally notable as the CNS's inability to grow back significant amounts of new tissue is its incredible ability to repurpose existing, intact tissue in adaptive ways. Researchers continue to explore ways to harness this capability to help

provide some degree of restoration in motor and sensory function in incomplete SCI cases [10]. Additionally, recent research has also sought to assess the health benefits of exercise on SCI patients, who often become more vulnerable to morbidities such as obesity and cardiovascular disease following their injury, due to a more sedentary lifestyle, on average [10]. For a good survey of research findings on this topic, see the recent article by van der Scheer, et al, in *Neurology* [44].

Beyond this, much is being done in the ever-advancing field of technology-based therapies, such as electrical stimulation techniques and robotic-assisted therapy [10]. As these technologies continue to emerge and develop, there is hope that they will one day allow us to bypass the lesion site entirely and restore function to paralyzed regions of the body via biotechnological interfaces [10].

### **Conclusion**

In conclusion, the structures of the spinal cord and vertebral column are truly a masterpiece of design. Every element is constructed from the inside out to provide an optimal balance of protection and mobility. When trauma occurs that overcomes these defenses, it sets into motion a constellation of physiological responses that exacerbate the severity and accelerate the expansion of the spinal cord lesion. These secondary mechanisms of injury cause latent functional losses and can worsen the prognosis in many spinal trauma cases.

However, while secondary injury does present major challenges in the effective management of spinal cord injury, it also presents great opportunity. Whereas there is typically no opportunity for clinical intervention to mitigate primary injury, past and current research suggests that we are merely scratching the surface in our ability to take

full advantage of opportunities to treat secondary injury. This means that we are currently not achieving optimal outcomes in the majority of spinal trauma cases—there is enormous room for growth in this field. While virtually all other areas of current SCI-related research deal with going back and repairing the damage incurred throughout the early-to-middle injury processes, secondary injury therapy has, perhaps more than anything else, the potential to substantially limit the amount of damage incurred in the first place. In light of this, we have a responsibility, both to learn more about the mechanisms contributing to secondary SCI and to become better equipped at managing it strategically and effectively, thereby bringing about qualitatively more optimal outcomes.

Additionally, the fact that secondary injury is so complex and multi-faceted presents a remarkable opportunity, because it gives us the ability to approach treatment in many different ways. A breakthrough in our understanding of any one of these facets has the potential to revolutionize how we conceive of and treat SCI.

For millennia, spinal cord injury has been a grave condition. Where it has not taken life, it has invariably stolen away quality of life, leaving its survivors with physical disability, psychological and emotional pain, and economic dependency. Now, finally, we may actually be on the cusp of a paradigm shift where secondary spinal injury can be managed in such a way as to bring about more favorable prognoses than ever before.

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