THE ROLE OF ACUTE INTRASPINAL HEMORRHAGE IN SPINAL CORD INJURY

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ABSTRACT

Spinal cord (SCI) injury initiates a cascade of destructive and reparative processes, an understanding of which may help in designing strategies for minimizing tissue loss and enhancing endogenous repair. Vascular disruption and hemorrhage is a prominent characteristic of the acute lesion environment, and is central to many of the secondary pathological consequences of SCI, most notably lesion formation. However its role, if any, in reparative processes is not known. An important endogenous repair mechanism after SCI is the proliferation of NG2+ cells and their maturation into new oligodendrocytes. We created a rat model of collagenase-induced intraspinal hemorrhage (ISH), and investigated the spatial-temporal dynamics of lesion formation, astrogliosis, microglia/macrophage reactivity, NG2 cell proliferation, and mature oligodendrocyte numbers after injury. Lesion pathology was similar to a contusion injury; however, lesion size, shape and spread were different between ISH and contusion. The astrocyte and macrophage responses of ISH were also similar to that of a contusion. In addition, we determined that hemorrhage alone is sufficient to initiate NG2 cell proliferation as early as 1dpi and continuing up to a week. Furthermore, oligodendrocyte...
numbers decreased by hemorrhagic injury are rapidly restored to normal levels by 7dpi. We have established that acute intraspinal hemorrhage following a SCI is not only the central initiating force for secondary injury cascades, but that it also plays a role in stimulating acute NG2 proliferation following injury.

INTRODUCTION

Traumatic spinal cord injury (SCI) is comprised of the primary injury due to the mechanical trauma itself, and secondary injury cascades that are responsible for the expansion of the primary injury and continued tissue damage and cell death\cite{1-4}. The injury microenvironment is highly dynamic. The full extent of the lesion develops over the course of 2 weeks; and the chronic lesion consists of a lesion cavity bordered by an astroglial scar, and activated macrophages residing within the lesion. Because the full extent of lesion evolves over time with secondary injury cascades, there is an early therapeutic window that can be harnessed to prevent further damage. In addition, it has recently been demonstrated that conduction can be restored to non-conducting demyelinated axons as late as 6 months post-injury\cite{5}, indicating that spared axons can recover function even at chronic time points. Hence, there is potential for functional recovery at various times after injury, depending on the state of the injured tissue at that time. Regardless of what mode of therapy is pursued, whether the goal is to protect tissue from further damage due to secondary processes, or to replace lost cells/tissue through transplantation, or to restore function to damaged tissue, the spatial-temporal dynamics of the lesion environment need to be understood in great detail for any therapeutic approach to be successful.
One of the most prominent characteristics of the acute lesion environment after SCI is intraspinal vascular disruption and hemorrhage. The forces generated from the primary insult typically result in the disruption of vasculature and petechial hemorrhage within the spinal cord parenchyma immediately after impact. Over the course of a few hours, the hemorrhagic petechiae increase and coalesce, and hemorrhage expands both radially, and rostral and caudal to the epicenter\textsuperscript{3, 4, 6-8}. In this manner, the full extent of intraparenchymal hemorrhage evolves over the course of 24h, accompanied by progressive hemorrhagic necrosis\textsuperscript{1, 9}. The degree of hemorrhage depends on the severity of the injury and the precise nature of the insult. It usually subsides by 3dpi and most red blood cells are degraded by 3-5dpi. Intraspinal hemorrhage initiates and plays a role in many of the secondary pathological cascades following SCI, such as cell necrosis, increased blood-brain barrier permeability, free radical formation, excitotoxicity, immune cell infiltration, reduced blood flow and ischemia\textsuperscript{1-3}. Most notably, the formation of a cavitated lesion at chronic times correlates closely with areas where intraparenchymal hemorrhage was present acutely\textsuperscript{10, 11}. Hence, intraspinal hemorrhage is typically studied in the context of secondary injury cascades and spinal cord pathology following injury. Its role, if any, in reparative processes is not known.

An important endogenous repair mechanism after SCI is the proliferation of NG2\textsuperscript{+} cells and their maturation into new oligodendrocytes\textsuperscript{12-15}. These newly formed oligodendrocytes can contribute to functional recovery after SCI by remyelinating damaged axons\textsuperscript{16}. The immediate vicinity of the lesion is an area of high and sustained oligodendrocyte renewal capacity, especially within the highly vascularized gray matter\textsuperscript{12, 13, 17}. NG2 cells begin dividing within the first day post-injury (dpi), and start to differentiate into mature oligodendrocytes within the first 3dpi\textsuperscript{12-14}. Mechanisms of acute NG2 cell proliferation are currently unknown. The time course of acute
NG2 cell proliferation after injury coincides with the evolution and duration of intraspinal hemorrhage. In turn, the borders of intraparenchymal hemorrhage spatially correlate with areas of proliferating NG2 cells. Based on these spatial-temporal dynamics of acute lesion environment properties and cell proliferation, we hypothesize that vascular disruption and the resultant hemorrhage may play a role in stimulating NG2 proliferation and oligodendrocyte renewal after SCI.

To study the role of acute intraspinal hemorrhage in both the secondary histopathological processes after SCI, and in repair processes such as oligodendrocyte renewal, we created a model of intraspinal hemorrhage (ISH) by adapting the collagenase-induced intracerebral hemorrhage model to the rat spinal cord. We investigated the spatial-temporal dynamics of lesion formation, astrogliosis, microglia/macrophage reactivity, NG2 cell proliferation, and mature oligodendrocyte numbers after injury. We confirmed that acute hemorrhage is central to much of the lesion pathology observed after SCI. In addition, we determined that hemorrhage alone is sufficient to initiate NG2 cell proliferation as early as 1dpi. Furthermore, oligodendrocyte numbers decreased by hemorrhagic injury are rapidly restored to normal levels by 7dpi. This is the first time that intraspinal hemorrhage has been linked to spinal cord repair processes. This is an important step in understanding the mechanisms involved in initiating NG2 cell proliferation and oligodendrocyte maturation after SCI.

**METHODS**

**Spinal Cord Injuries**
Adult female Sprague-Dawley rats (230-250 g) were anesthetized with ketamine (80mg/kg i.p.) and xylazine (10mg/kg i.p.). The spinal cord was exposed via a laminectomy at the T8 vertebral level and the animal secured in a stereotactic frame via the T7 and T9 dorsal processes.

*Intraspinal hemorrhage (ISH)*

A beveled glass pipette with a 20-40µm tip was positioned 0.7mm lateral to the midline and inserted 0.9mm ventral to the surface of the spinal cord. A pneumatic picopump microinjection device (DKI, Tujunga, CA) was used to inject 0.5µL of 0.04U collagenase (type VII,Sigma) over 3min. The pipette was left in place for 3 min and then raised half of the distance to the spinal cord surface. After another 1min pause, the pipette was completely removed. The location of the microinjection was marked on the surface of the cord with charcoal.

*Hemicontusion*

A laminectomy was performed at T8 as above. The stereotactic frame was secured in the IH SCI device and a 1 mm probe was positioned approximately 0.2mm lateral to the midline. The probe was lowered and the lesion site flooded with saline up to the tip of the probe. The desired force was set at 200 kdynes and the computer controlled probe was rapidly driven into then removed from the spinal cord. The recorded actual forces were between 264 and 356 kdynes. Displacements ranged between 846 and 1393 mm (n=6).

*Post-operative Care*

Muscles were sutured and the incision closed via wound-clips. Animals were given 5cc saline subcutaneously, and their cages placed on slide warmers overnight. Animals were checked daily for the duration of the studies. Animals underwent manual bladder expression once a day for the
first 5 days following injury until spontaneous bladder control was regained. Bladder control was usually regained by 3dpi in both injury groups.

*Bromo-deoxyuridine Injections*

Bromo-deoxyuridine was administered daily (i.p. 50mg/kg). Animals in each survival group received a specific BrdU pulsing regimen as outlined in Table 1.

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<th>Table1. Post-Injury BrdU Pulsing and Survival Time Points</th>
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*Tissue Processing*

Rats were transcardially perfused with heparin-treated (2U/L) saline until the blood was cleared, followed by 4% paraformaldehyde (PFA) for 10min at a speed of ~30mL/min. Spinal cords were dissected and post-fixed for 2h, then placed in 0.2M phosphate buffer overnight. The cords were then transferred to a 30% sucrose solution for 2-3 days. The spinal cords were cut into 1.5cm pieces centered on the microinjection/ contusion site, which were embedded in OCT compound and frozen using dry ice. Tissue blocks were stored at -80°C until they were cut on a cryostat
into 10µm transverse sections at a rostral to caudal orientation and collected sequentially positively charged glass slides.

**Immunohistochemistry**

Immunohistochemistry was performed as described in Schonberg et al. (2007). Briefly, sections were rinsed with 0.1M PBS, and non-specific antigen binding was blocked using 4% bovine serum albumin/0.1% Triton X-100/PBS (BP+). For NG2 staining, slides were incubated with primary antibody for 18h at RT. All other primary antibody incubations were overnight at 4C. Antibodies used included the following: anti-NG2 (1:400; US Biological, Swampscott, MA); CC1 (antibody clone for oligodendrocytes, also called APC, 1:800 Abcam, Cambridge, MA); anti-CD11b for microglia and macrophages (1:2000, Ox42 clone; Serotec, Raleigh, NC); anti-gliafibrillary acidic protein for astrocytes (1:4000; Sigma-Aldrich); anti-BrdU for proliferating cells (1:200 G3G4; Developmental Studies Hybridoma Bank) and anti-neurofilament for axons (1:2000; Developmental Studies Hybridoma Bank, Iowa City, IA). To visualize myelin along with axons, neurofilament (NF) labeling was combined with Eriochrome Cyanine staining, as described in Schonberg et al. (2007).

**Tissue Analysis**

*Lesion Area*

Digitized images of EC/NF labeled cross-sections were captured using MCID image analysis software. The lesion area, defined as lack of myelin or axon staining, was outlined and the area measured.

*Cell Quantification*
Cells were manually counted in transverse spinal cord cross-sections directly at 40x using a counting reticle. Through initial cell counts we determined that the contralateral spinal cord was not different than vehicle controls (not shown). Thereafter, the ipsilateral half of the cross-section was used for all cell quantifications.

RESULTS

Collagenase-induced intraspinal hemorrhage produces distinct focal lesions

Collagenase microinjections into the intact thoracic rat spinal cord were targeted to the lateral white matter bordering the intermediate gray matter. Vehicle injections caused no pathology or behavioral deficits. Collagenase microinjections produced mild behavioral deficits on the ipsilateral hindlimb such as missed steps, lack of toe clearance, and rotated paw position. Trunk instability was also observed. These hindlimb locomotor deficits were observed by 1d post-injury (dpi), and progressively recovered to normal by 7dpi (data not shown). At the acute time points 1 and 3dpi, hemorrhage was clearly observed on the ipsilateral side of the spinal cord during tissue collection, and the rostral-caudal length of observable hemorrhage at the acute time points was consistent across different animals (Fig.1K, L). Hemorrhage was not visible in vehicle, 7dpi or 7d hemicontusion spinal cords.

Our laboratory has shown that the response to SCI can be different between gray and white matter. For example, oligodendrocyte genesis is more robust in the spared gray matter compared to white matter. In contusive SCI models it is only possible to study spared gray matter in sections distal to the epicenter, or in mild injuries, as the gray matter is usually completely destroyed at the lesion epicenter in moderate or more severe injuries. We therefore wanted to create an injury in an area with sufficient surrounding gray and white matter. Our
microinjection paradigm produced distinct focal lesions that were mainly localized to the centrolateral white matter, with varying degrees of gray matter damage between subjects (Fig.1B-E). Acute lesions (1 and 3dpi) contained myelin debris and damaged axons. By 7dpi, myelin debris had been cleared and the lesion area was mostly devoid of both myelin and axons (Fig.1F-J).

7dpi hemicontusion lesions occupied more than twice the cross-sectional area as ISH lesions and composed nearly the entire ipsilateral cross-section. When the percent lesion areas were compared along the rostral-caudal axis, ISH lesions maintained a relatively stable lesion size at long distances, whereas hemicontusion lesions rapidly decreased in size distal to the epicenter (Fig.2A-J). ISH lesions were more than twice as long as hemicontusion lesions (Fig.2K).

**Similar to contusion injury, astrocytes are activated and macrophages accumulate after ISH**

Similar to traumatic SCI models, astrocytes in proximity to the lesion became reactive, including hypertrophied processes and increased GFAP immunoreactivity (Fig.3B-E). At 7dpi, they clearly delineated the lesion border as they began forming an astroglial scar (Fig.3D,E). They were completely absent inside the lesion. It is also of note that at this time, astrocyte reactivity was more prominent at the gray matter lesion border (Fig.3B-E).

At 1dpi, ramified and amoeboid reactive microglia were found within the lesion (Fig.3G). Same as a contusion injury, there were activated macrophages within the lesion at 3-7dpi (Fig.3H-J). In CNS injury, accumulation of activated macrophages within lesion sites is usually accompanied by iron accumulation, both of which persist within the lesion. To determine if this occurred in our model, we used the Perl’s Prussian Blue Stain Reaction to visualize ferric ions. Indeed, iron accumulated within the lesion at 3dpi, and was present at least through 7dpi
Iron staining was present on round, amoeboid-shaped cells that were presumably macrophages based on their morphology (Fig.3H-J,M-O). Since double-staining is not possible with the Perl’s Reaction, we were not able to confirm the identity of iron-containing cells. It is interesting to note that in ISH, both the density of activated microglia/macrophages and iron staining were more prominent along the lesion edges that border spared gray matter (Fig.3G-I,M,N). Furthermore, the areas of the highest staining intensity overlap for both stains (Fig.3G-J,M-O).

**NG2+ cells proliferate after ISH**

We previously demonstrated that NG2+ proliferation is increased in response to contusion injury within the first 3dpi\(^1\). Increased NG2 proliferation was also demonstrated at 24hrs post-injury in a dorsal hemisection model\(^2\). To investigate whether NG2 cells are able to proliferate acutely after intraspinal hemorrhage, we injected rats with BrdU at 4hrs and 1d post-injury, and sacrificed them at 1dpi. Indeed, NG2+/BrdU+ cells were significantly increased in the ipsilateral cross-sectional area compared to vehicle and naive (Fig.4K). This indicates that NG2 cells to respond to hemorrhage in the absence of mechanical tissue disruption, suggesting that something related to vascular disruption or blood stimulates acute NG2 cell proliferation.

When animals received BrdU at 4hrs and 1-3dpi and were sacrificed at 3 and 7dpi, the number of NG2+/BrdU+ cells was increased (Fig.4K), indicating elevated NG2 cell proliferation within the first 3dpi. Similar results were obtained when animals received BrdU in the latter half of the week (4-7dpi) and were sacrificed at 7dpi (Fig.4K). The number of NG2+/BrdU+ cells was similar between all of the groups investigated, suggesting that the majority of acute cell proliferation occurs within the first day after ISH. The number of proliferating NG2 cells in hemicontusion animals which received BrdU at 4hrs and 1-3dpi, and survived to 7dpi were not
significantly different than the corresponding ISH group, despite the fact that more of the ipsilateral spinal cord was comprised of lesion, suggesting that similar mechanisms affect acute NG2 proliferation in both models.

When the tissue was examined for the overall distribution of BrdU+ profiles, there were differences between animals that received BrdU at the acute vs. sub-acute half of the week after injury. Overall there were few BrdU+ profiles in animals that received BrdU acutely (0-3dpi), and the profiles that were observed were more distal to the lesion compared to the sub-acute group. BrdU+ profiles were not present within the lesion, and there was no apparent difference in distribution between wm and gm. This was true for animals that were sacrificed at 1 and 3dpi, and those that survived to 7dpi. In contrast, animals that received BrdU subacutely (4-7dpi) had an accumulation of BrdU+ profiles within the lesion, most-likely macrophages, and most BrdU+ profiles were in closer proximity to the lesion. Furthermore, BrdU+ cells both within and bordering the lesion were more abundant closer to the gray matter lesion border (Fig. 4D).

**Oligodendrocytes lost after hemorrhagic injury are replaced by 7dpi**

Since NG2 cells can mature into oligodendrocytes, we next investigated whether there was an increase in mature oligodendrocytes after ISH. We quantified the total number of oligodendrocytes (CC1+ cells) in the ipsilateral cross-sectional area compared to vehicle and naïve controls. At 1 and 3dpi there was a significant decrease in CC1+ cells compared to controls, indicating a loss of oligodendrocytes to the hemorrhagic injury (Fig. 5C). However, by 7dpi CC1+ cell numbers had returned to control levels, indicating the lost cells were replaced. The number of CC1+ cells in the epicenter of 7d hemicontusion animals was significantly less than 7d ISH animals, most likely due to much larger lesion size in hemicontusion animals. This
is consistent with our previous work on contusion injury showing that CC1+ cell numbers were still well below naïve at 7dpi, and recovered to naïve levels by 14dpi\textsuperscript{12}.

**DISCUSSION**

The two most commonly used models of intracerebral hemorrhage are the autologous whole blood model\textsuperscript{22} and the collagenase model\textsuperscript{23-25}. In the autologous whole blood model, the animals’ own blood is taken, typically through tail-vein draws, and injected freshly into the brain. The advantage of this model is that it directly tests blood effects. The main disadvantage is that it is not a good replication of what occurs after an injury, as the blood is injected all at once instead of bleeding over the course of hours, and all of the blood is pooled at the injection site instead of expanding over a distance. In addition, injecting a volume of fluid into the tissue may cause an increase in pressure and thus result in compression effects. In the collagenase model, collagenase injected into the brain disrupts the integrity of capillaries by digesting collagen in the basement membrane, causing capillaries to leak and then degenerate in a feed-forward mechanism that ultimately causes full-blown hemorrhage over the course of hours\textsuperscript{25}. In addition, the collagenase model also incorporates the ischemic component of vascular disruption, and thus mimics acute injury better. Therefore, we used collagenase to model acute hemorrhage after SCI because it is a more accurate representation of the time course of vascular damage and hemorrhage over time following trauma\textsuperscript{23-25}. In this way we modeled the acute injury microenvironment without the biomechanical properties of tissue disruption, and this enabled us to separate processes related to primary injury vs. related aspects of secondary injury. We were also able to test whether or not vascular disruption and hemorrhage is sufficient to induce NG2 cell proliferation and oligodendrocyte renewal after injury.
We used a hemicontusion model in which the goal was to replicate the lesion size and location of the ISH model. Although we succeeded in creating unilateral thoracic lesions via hemicontusion, the lesion area, location, and distribution was different between the two models. This demonstrates for the first time that lesion morphology is very different between a purely vascular injury and one induced by a dorsal contusion to the spinal cord. This highlights the importance of injury biomechanics in lesion formation and injury microenvironment\textsuperscript{26,27}. Despite the differences, we used the hemicontusion as a comparison to ISH because the lesion size and injury location were much more similar to ISH than the usual midline contusion. The lesion areas and locations of hemicontusion cross-sections at \(~\)1mm distal to the epicenter were actually quite similar to epicenter ISH lesions. In this study we compared the epicenters between the two models even though lesion area and spared tissue was different at that distance, because the effect of primary injury in the hemicontusion would be highest at the epicenter. Further studies will compare cross-sections of similar lesion sizes.

Although it is a long-held assumption, we provide for the first time strong evidence that most of the spinal cord pathology observed after SCI is due to secondary damage incurred by processes related to vasculature damage and hemorrhage. Blood and ischemia form a highly toxic and necrotic microenvironment, hence leading to lesion centers devoid of both cells and axons at 1dpi. The lesion is later populated by activated microglia and macrophages. ISH lesions are much more focalized and have sharper lesion outlines compared to contusion lesions, properties most likely due to greater tissue disruption from mechanical forces in the hemicontusion.

An advantage of the ISH model is that we controlled the stereotactic location of the lesion, a degree of manipulation not possible with the contusion paradigms. This allowed us to create a localized, focal lesion in such a way that we could study the response of both spared white and
gray matter at the lesion border. Indeed, we observed striking qualitative differences between the two different lesion borders. Astrocyte reactivity at 7dpi was more pronounced at the gray matter lesion border. At 1dpi, activated microglia within the lesion were preferentially found at areas bordering or within the gray matter. At 3 and 7dpi activated CNS macrophages within the lesion accumulated more near gray matter lesion borders, and iron accumulation mirrored that pattern. In animals that received BrdU at 4-7dpi before being sacrificed at 7dpi, BrdU+ cells within the lesion accumulated predominantly in areas bordering or within the gray matter. Furthermore, NG2+/BrdU+ profiles were more abundant at the gray matter lesion borders at that time point. These observations expand previous results from our lab where we demonstrated that oligodendrocyte renewal was higher and protracted at the lesion borders, with a more robust effect in the gray matter at all time points\textsuperscript{12}. It is likely that these elevated responses within the gray matter are related to the fact that the gray matter has a higher capillary density than the white matter. Taken together, the divergent pattern of spatial-temporal cellular dynamics between the acute (1-3dpi) and sub-acute (4-7dpi) time points suggest that the factors stimulating NG2 proliferation may be different between acute and more chronic times after injury.

We have previously shown that macrophages activated with the TLR4 ligand LPS induce oligodendrocyte renewal through an iron-dependent mechanism\textsuperscript{28}. Macrophage and iron accumulation within the cord at 3dpi and beyond have been documented consistently by us and others\textsuperscript{29}. This is relevant to oligodendrocyte renewal, as iron is required for the production of myelin\textsuperscript{30, 31} and the iron-storage protein ferritin stimulates NG2 proliferation and oligodendrocyte renewal (Schonberg et al., 2012, in press, JNeurosci). Therefore, we postulate that macrophages containing iron and ferritin comprise a distinct mechanism for NG2 proliferation and oligodendrocyte renewal at more chronic time points after injury.
The number of proliferating NG2/BrdU cells in the ipsilateral cord was similar between ISH and hemicontusion for animals receiving BrdU 0-3dpi and surviving to 7dpi, despite much larger lesion areas in hemicontusion. This suggests that factors independent of lesion size, spared tissue, or mechanical injury affect NG2 proliferation in both models. Conversely, hemicontusion animals had significantly less mature oligodendrocytes at 7dpi. This is most likely due to differences in lesion size, tissue damage, and gray matter sparing. There was less gray matter sparing in the ipsilateral cord of hemicontusion. If higher tissue damage and larger lesion area at the epicenter resulted in more oligodendrocyte loss within the ipsilateral cord in the hemicontusion compared to the ISH, but NG2 cells were proliferating at the same level in both models, then the level of oligodendrocyte maturation in hemicontusion may not have been sufficient to restore oligodendrocyte numbers to normal levels by 7dpi. This can be confirmed by comparing the numbers of newly formed (i.e. BrdU expressing) oligodendrocytes between both groups at 7dpi, which we are doing in ongoing studies. Future studies will also compare oligodendrocyte numbers at more chronic times to determine if oligodendrocyte numbers in hemicontusion eventually return to naïve levels as they do in the ISH model.

In conclusion, we have established that acute intraspinal hemorrhage following a SCI is the central initiating force for both secondary injury cascades and acute NG2 proliferation following injury. Future studies will focus on what it is about vasculature damage and/or blood within the spinal cord that stimulates NG2 cell proliferation. It is possible that factors within the blood entering the parenchyma following injury are acting directly on NG2 cells. It is also possible that indirect effects from other cells of the CNS, like astrocytes and microglia, or cells of the vasculature such as endothelial cells or pericytes play a role. Understanding the molecular mechanisms of NG2 proliferation will enable the development of pharmacological therapies that
stimulate this response. In this way, it can be possible to enhance and modulate the endogenous oligodendrocyte renewal response to injury both spatially and temporally, thereby increasing the efficiency of remyelination and improving functional recovery after injury.

FIGURES

**Fig.1: Collagenase-induced intraspinal hemorrhage produces distinct focal lesions. (A-E)** Cross-sections stained for eriochrome cyanine (myelin) and neurofilament (axons) reveal areas of myelin debris, myelin loss and axon loss that comprise a distinct focal lesion (* and dotted line). Cross-sections are representative of the mean of proportional lesion area at the epicenter for each time point. (F-J) Boxes in A-E are shown in high power under respective low power cross-section image. (B, C) and (G,H) Acutely the lesion is characterized by myelin debris. (D,E) and (I,J) Myelin debris is cleared away by 7dpi. (K,L) Representative spinal cords photographed during tissue collection at 1 and 3dpi. There was no observable hemorrhage at later time points. Charcoal on cords (black) delineates level of microinjections. Scale bar (A-E)= 500µm, (F,J)= 20µm
Fig. 2: Longitudinal lesion spread of intraspinal hemorrhage and unilateral contusion model shows differences in % lesion area. (A-E) Representative mean lesions (* and within dotted line) from 7dpi rats injured via ISH showing rostral-caudal lesion extent after ISH. (F-J) Rostral-caudal lesion extent after hemi-contusion, which produces larger lesion at the epicenter compared to ISH, but does not extend as far rostral and caudal. Sections are labeled for neurofilament (axons) and eriochrome cyanine (myelin). (K) Quantitation of lesion area after ISH and hemi-contusion injuries reveals the larger but shorter lesion shape after contusion. The hemicontusion lesions at ~1mm to the epicenter have a lesion area more comparable to the epicenter lesions of the ISH model. Scale bar = 500 µm
Fig. 3: Astrocyte activation and macrophage accumulation occurs after both intraspinal hemorrhage and hemicontusion. (A-E) Distribution of GFAP+ astrocytes are depicted within low powered cross-sections. (D) By 7dpi astrocytes clearly define the lesion. Inserts (A-E) High powered views from boxes on lower power images show astrocytes at the white matter and gray matter lesion border. Inserts (D,E) By 7dpi astrocytes at the lesion border form a glial scar. (G) Microglial reactivity is slightly increased 1 day after ISH, (L) but there is no evidence of iron deposition. (H,I) At 3 and 7dpi, areas of macrophage activation in the lesion coincide with (M,N) increased iron staining. Both increased iron immunoreactivity and macrophage activation/accumulation is more pronounced in the gray matter areas of the lesion. Inserts (H-J) High powered views from boxes on lower power images show round, activated macrophages and Inserts (M,O) and morphologically similar cells stained for iron. (J) Macrophage activation is more pronounced in hemicontusion epicenters compared to ISH and (O) Iron staining is observed at 7dpi hemicontusion. Lesion (* and dotted line), Scale bar (A-O) = 200µm , Scale bar (Inserts A-E and F,K)= 50µm, Scale bar (Inserts G-J and L-O)= 20µm
Fig. 4: NG2+ Cells proliferate in response to intraspinal hemorrhage. (A-E) Cross-sections labeled for NG2/BrdU. Arrows denote examples of NG2+/BrdU+ cells. Boxed areas are shown in high powered views under respective images (F-J). (K) NG2 proliferation was significantly increased compared to vehicle controls and naïves (dotted line) at all time points. One-Way ANOVA, Dunnett’s Multiple Comparison Test. *p<0.05, **p<0.01, ***p<0.001) Lesion (* and dotted line), Scale bar (A-E) = 500µm , (F-J)= 50µm

Fig. 5 Oligodendrocytes lost after hemorrhagic injury are replaced by 7dpi. (A,B) At 7dpi activated GFAP+ astrocytes form a scar at the lesion border, and CC1+ oligodendrocytes are abundant therein. (C) Oligodendrocytes significantly decreased compared to vehicle and naïve (dotted line) at 1 and 3dpi return to vehicle and naïve levels by 7dpi. Hemicontusion cords have significantly fewer oligodendrocytes at the lesion epicenter at 7dpi compared to ISH (2-tailed t-test). One-Way ANOVA, Bonferroni’s Multiple Comparison Test. *p<0.05, **p<0.01, ***p<0.001), Lesion (* and dotted line), Scale bar (A) = 50µm , (B)= 20µm
REFERENCES