

Figure 3.

Schematic Representation of the Pre-Conditioned Model of Injury.

A) Shows the location of the peripheral (e.g. sciatic nerve) and central (e.g. spinal cord) nervous system injury site, as well as the location of tracer injection site for retrograde (red asterisk – using Fast Blue) and anterograde (black asterisk – using Fluororuby) tracing proximal or distal to spinal injury, respectively.

B) Cross-section anatomical representation of the spinal cord, showing area of injury (e.g. bilateral dorsal column cut - dashed line) and site of tracer injection in dorsal column ipsilateral to peripheral nerve injury. Note that this type of injury is specific for the corticospinal tract, which runs in the DC.

Note anatomical structures are not up to scale.

D, V, LC = dorsal, ventral, lateral column.

Figure 4.

Macrophage and Satellite Cell Immunoreactivity in the Ipsilateral DRG 7 Days Post Sciatic Nerve Lesion.

A-I) Immunostaining with a macrophage marker (CD68⁺) and satellite cell marker (GFAP⁺) revealed an upregulation of these cells in the ipsilateral DRG post peripheral nerve lesion. These CD68⁺ cells along with the formation of GFAP⁺ perineuronal rings (yellow arrows), were observed surrounding DRG cell bodies (white arrows).

Scale bars A-C 500um, D-F 200um, G-I 100um.

Figure 5.

Macrophage and Satellite Cell Immunoreactivity in the Contralateral DRG 7 Days Post Sciatic Nerve Lesion.

A-I) Immunostaining of macrophage cells (CD68⁺) and satellite cells (GFAP⁺) also revealed the extent of upregulation of these cells in the contralateral DRG after peripheral nerve lesion. These CD68⁺ cells (white arrows) although reduced in numbers, compared to those observed in the DRG ipsilateral to the peripheral injury, were consistently present surrounding some DRG cell bodies (white asterisks). Similar observations were found in GFAP⁺ cells with some upregulation in the contralateral DRG, forming perineuronal rings (yellow arrows) around DRG cell bodies.

Scale bars A-C 500um, D-F 200um, G-I 100um.

Figure 6.

Macrophage and Satellite Cell Quantification in Ipsilateral and Contralateral DRG 7 Days Post Sciatic Nerve Lesion.

A-B) Cellular quantification of macrophage cells (CD68⁺) in the ipsilateral DRG revealed a significant upregulation of these cells, as compared to lower cell numbers in the contralateral DRG (***P<0.0001). Similarly, satellite cells (GFAP⁺) were also significantly upregulated in the ipsilateral DRG and were observed forming perineuronal rings, as compared to lower numbers of these cells present in the contralateral DRG (**P<0.01).

Note that under normal conditions CD68⁺ and GFAP⁺ cells are present in the DRG, however, these cells do not surround neuronal cell bodies as compared to what is observed pathologically after injury and were therefore not counted.

Columns represent an averaged mean (n=5) and error bars indicate error of mean (+/- S.E.).

LS = left side, RS = right side, IL = ipsilateral, CL = contralateral, SNI = sciatic nerve injury.

Figure 7.

Greater BDNF Serum Concentration Level Found Post Peripheral Nerve Injury Only.

A) BDNF Concentration of serum samples revealed that only those samples collected 7 days post SNI only were significantly greater than normal BDNF concentration levels ($***P < 0.001$) and those from animals receiving a SCI 7D only ($***P < 0.001$). Note that 7 days post SCI only did not result in significant increases in BDNF serum concentration as compared to normal uninjured levels.

Columns represent an averaged mean ($n=5$) and error bars indicate error of mean (\pm S.E.).

BDNF = brain derived neurotrophic factor, SNI = sciatic nerve injury, SCI = spinal cord injury (i.e. dorsal column cut), 7D = seven days post injury.

Figure 8.

Greater Macrophage Numbers Found in the Spinal Cord of Pre-Conditioned Lesion Animals 7 Days After SCI.

A) Comparatively, the quantified macrophage (CD68⁺) numbers found in the SCI site 7 days after SCI only and/or in pre-conditioned lesion animals also 7 days post SCI, revealed significantly higher macrophage numbers rostral to (*P<0.05) and at the SCI epicentre (*P<0.05) in animals receiving a pre-conditioned lesion as compared to SCI only animals. While no differences between these two groups was found caudal to the injury.

B) Overall, pooled macrophage numbers from the examined area demonstrated greater macrophage numbers in pre-conditioned lesion animals, as compared to SCI only animals (*P<0.05).

Columns represent an averaged mean (n=5) and error bars correspond to error of mean (+/- S.E.).

SNI = sciatic nerve injury, SCI = spinal cord injury.

Figure 9.

High Astrocyte Expression in the Spinal Cord After SNI Only.

A) Astrocyte quantification in the lumbar region of the spinal cord revealed an increased expression of astrocytes (GFAP⁺) after SNI only, as compared to normal uninjured spinal cords (****P<0.0001).

B) Comparisons in astrocyte expression between animals that received a SCI only and/or a pre-conditioned lesion 7 days after SCI did not differ between these two groups. Note that GFAP⁺ expression was higher caudal to injury as opposed to rostral. Although not examined further, this most likely represents effects of Wallerian degeneration.

Columns represent an averaged mean (n=5) and error bars correspond to error of mean (+/- S.E.).

SNI = sciatic nerve injury, SCI = spinal cord injury.

Figure 10.

***Greater Number of Retrograde Labelled FB⁺ DRG Neurons in Animals
PNH Vaccinated as ADULTS.***

A-D) Qualitatively, PNH vaccination in adults resulted in more FB⁺ retrograde labelled DRG neurons (white arrows), as compared to control animals vaccinated with saline (red arrows).

E) Quantification of FB⁺ retrograde labelled DRG neurons confirmed qualitative observations, with a greater number of FB⁺ DRG neurons found in PNH vaccinated animals, as compared to controls (**P<0.01). Note that the contralateral DRG was used the uninjured side control.

Columns represent an averaged mean (n=5) and error bars indicate error of mean (+/- S.E.).

PNH = peripheral nerve homogenate, FB = fast blue tracer, DRG = dorsal root ganglia, IL = ipsilateral, CL = contralateral.

Scale bars A & C 500um, enlarged views 200um.

Figure 11.

***Greater Number of Retrograde Labelled FB⁺ DRG Neurons in Animals
PNH Vaccinated as NEONATES.***

A-D) Similar to PNH vaccinated adults, neonatal PNH vaccination also resulted in more FB⁺ retrograde labelled DRG neurons (white arrows), as compared to control animals vaccinated with saline (red arrows).

E) Quantification of retrograde labelled DRG neurons revealed a significantly higher number of FB⁺ DRG neurons found in PNH vaccinated animals, as compared to controls (**P<0.01). Note that the contralateral DRG was used the uninjured side control.

Columns represent an averaged mean (n=5) and error bars indicate error of mean (+/- S.E.).

PNH = peripheral nerve homogenate, FB = fast blue tracer, DRG = dorsal root ganglia, IL = ipsilateral, CL = contralateral.

Scale bars A & C 500um, enlarged views 200um.

Figure 12.

Reduced Number of Retrograde Labelled FB⁺ DRG Neurons in Animals PASSIVELY Exposed to PNH.

A-D) Maternal PNH vaccination and subsequent testing of respective offspring resulted in a reduced number of FB⁺ retrograde labelled DRG neurons in the ipsilateral DRG of those passively exposed to PNH (white arrows), as compared to control animals passively exposed to saline (red arrows).

E) Comparatively, this demonstrated a significantly higher number of FB⁺ DRG neurons in control animals, as compared to offspring born to PNH vaccinated animals (****P<0.0001). Note that the contralateral DRG was used the uninjured side control.

Columns represent an averaged mean (n=5) and error bars indicate error of mean (+/- S.E.).

PNH = peripheral nerve homogenate, FB = fast blue tracer, DRG = dorsal root ganglia, IL = ipsilateral, CL = contralateral.

Scale bars A & C 500um, enlarged views 200um.

Figure 13.

FR⁺ Neurons in the Injured Spinal Cord of Adult PNH Vaccinated Animal.

A) Montage of regenerated ascending FR⁺ labelled fibres in the injured spinal cord of an adult PNH vaccinated animal. Note autofluorescent FR⁺ fragments found throughout the spinal cord segment (yellow arrows), are believed to be FR⁺ phagocytic cells.

B-I) Closer examination of the spinal cord revealed the presence of ascending FR⁺ fibres (white arrows) shown from a rostral (**B-G**) to caudal (**H-I**) direction through the injury epicentre (white asterisk).

Directional key: R = rostral, C = caudal, D = dorsal, V = ventral, PNH = peripheral nerve homogenate, FR = Fluororuby, dextran tetramethylrhodamine.

Scale bar of montage 500um, enlarged views 100um.

Figure 14.

***Greater Macrophage Presence in the Injured Spinal Cord of Animals
PNH Vaccinated as ADULTS.***

A) Quantified macrophage numbers (CD68⁺) were significantly higher in PNH vaccinated pre-conditioned lesion animals, as compared to saline vaccinated controls. Specifically, this difference was observed rostral to (*P<0.05), caudal to (**P<0.001), and at the SCI epicentre (**P<0.01), as compared to respective controls.

B) No differences were found in astrocyte expression (GFAP⁺) between PNH vaccinated and saline vaccinated control animals, with similar GFAP⁺ expression levels observed rostrally and caudally to SCI epicentre. Note that, GFAP immunoreactivity was higher caudal to the injury, as opposed to rostral.

Columns represent an averaged mean (n=5) and error bars indicate error of mean (+/- S.E.).

SCI = spinal cord injury, PNH = peripheral nerve homogenate, mm = millimetres.

Figure 15.

***Greater Macrophage Presence in the Injured Spinal Cord of Animals
PNH Vaccinated as NEONATES.***

A) Similar to adult PNH vaccinated animals, quantified macrophage numbers (CD68⁺) were highest in PNH vaccinated pre-conditioned lesion animals, as compared to saline vaccinated controls. This resulted in significant differences observed throughout the examined area, rostral to (**P<0.001), caudal to (*P<0.05) and at the SCI epicentre (*P<0.05).

B) Astrocyte expression as previously shown in adult PNH vaccinated animals, did not reach statistical significance between both of the neonatal vaccinated groups. Yet again, higher GFAP⁺ expression was observed caudal to injury.

Columns represent an averaged mean (n=5) and error bars indicate error of mean (+/- S.E.).

SCI = spinal cord injury, PNH = peripheral nerve homogenate, mm = millimetres.

Figure 16.

***Reduced Macrophage Presence in the Injured Spinal Cord of Animals
PASSIVELY Exposed to PNH.***

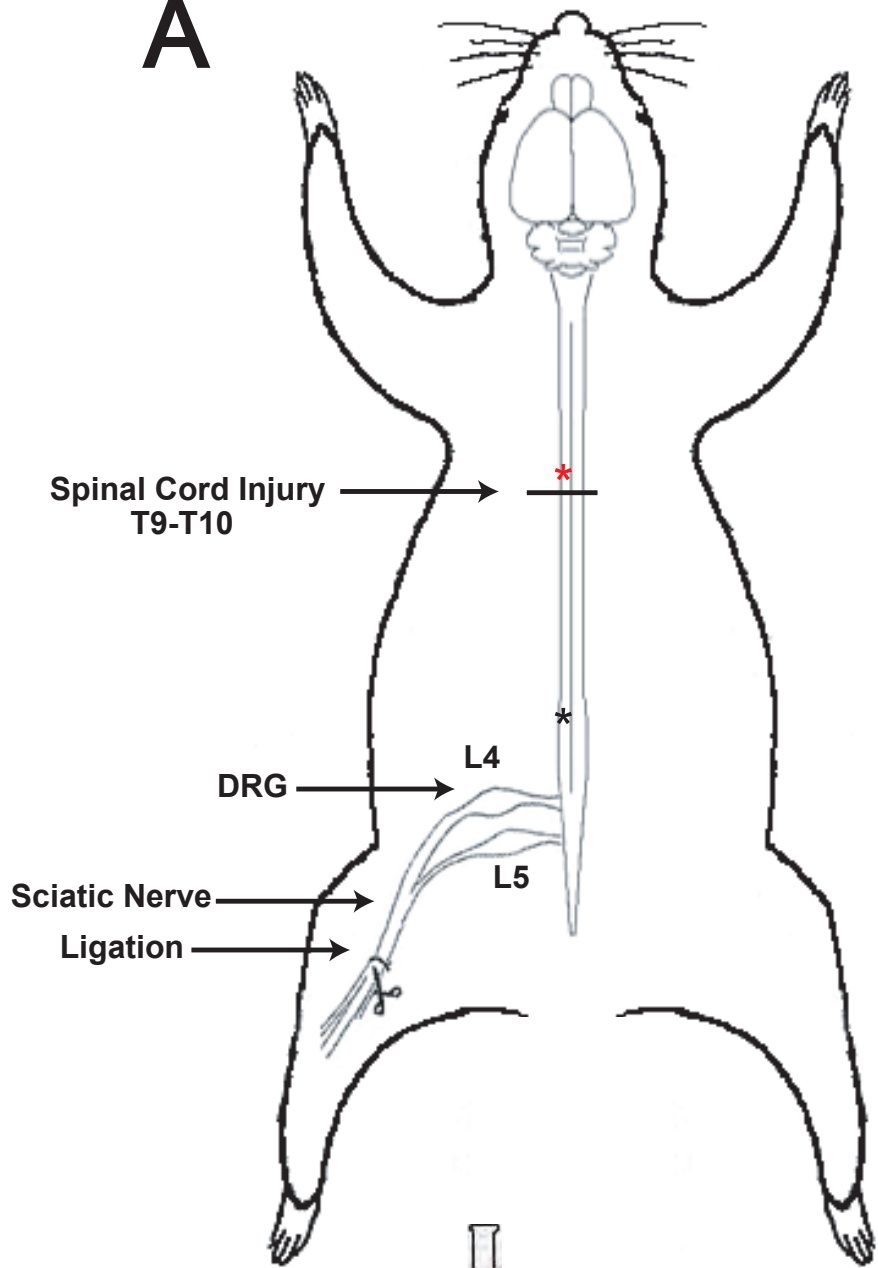
A) Quantified macrophage numbers between passively exposed animals to either PNH or saline through maternal vaccination, resulted in significantly reduced macrophage numbers (CD68⁺) in the former group born to PNH vaccinated animals, as compared to those born to saline vaccinated animals. Specifically, this difference was observed caudally (*P<0.05), with no other differences found rostral to or at the SCI epicentre.

B) Astrocyte quantification revealed a significantly higher GFAP⁺ expression caudal to the SCI epicentre in animals passively exposed to PNH, as compared to control animals passively exposed to saline (*P<0.05). No difference between treatments was found rostral to injury.

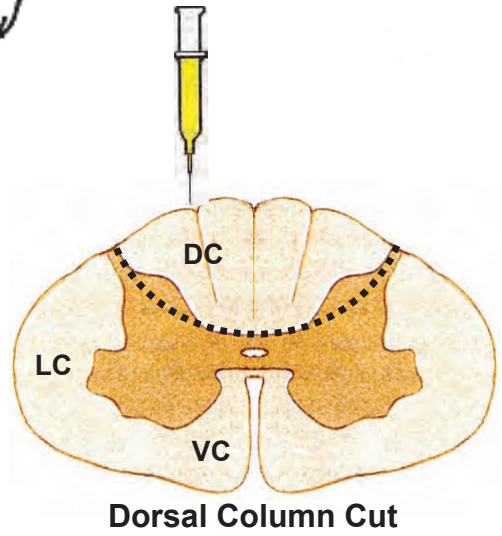
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SCI = spinal cord injury, PNH = peripheral nerve homogenate, mm = millimetres.

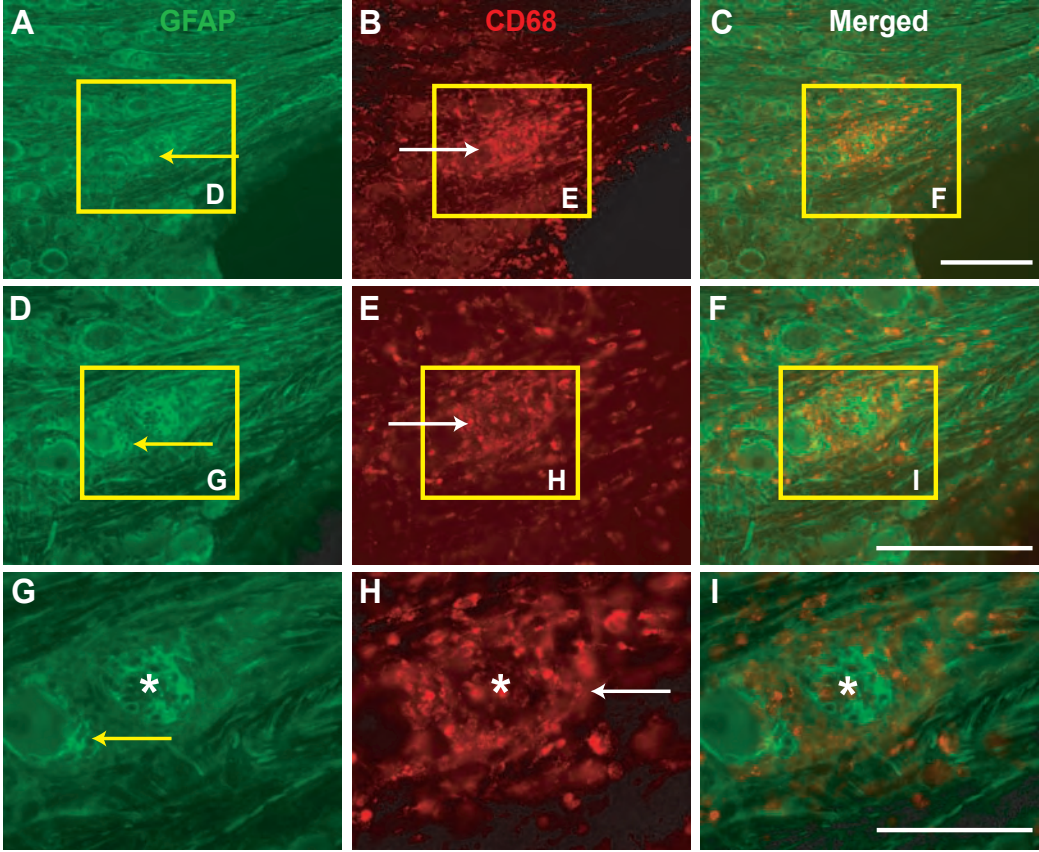
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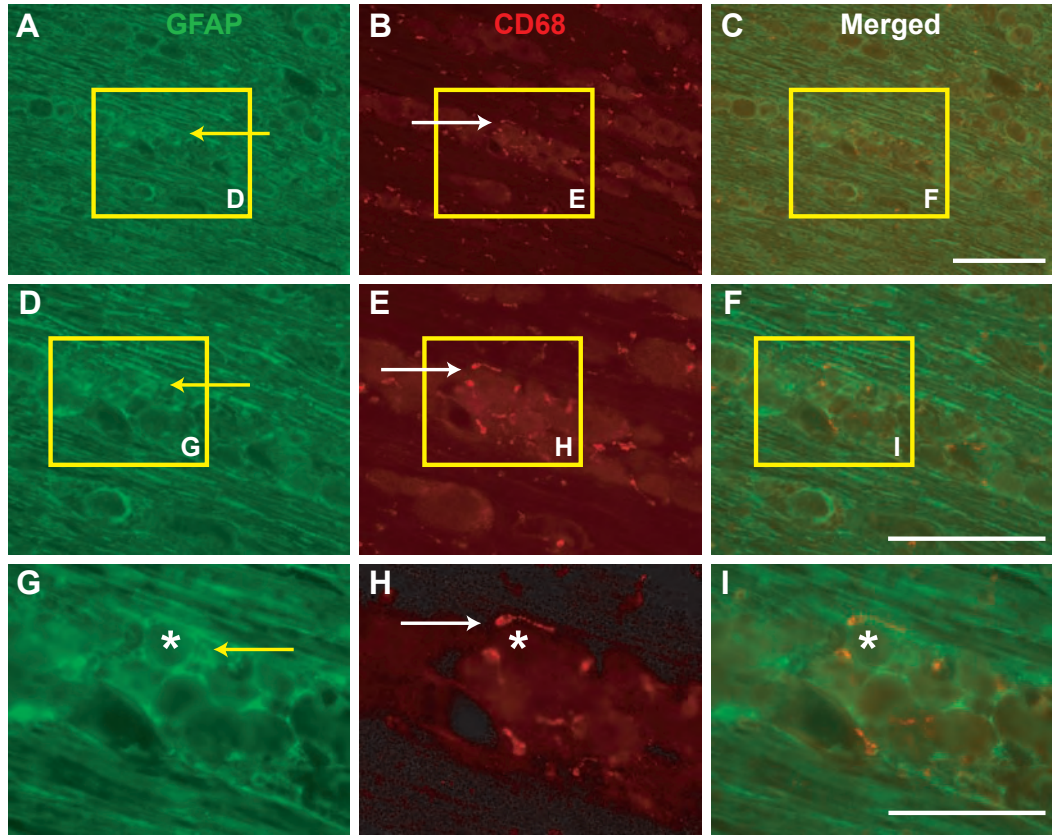
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Immunoreactivity in Ipsilateral DRG

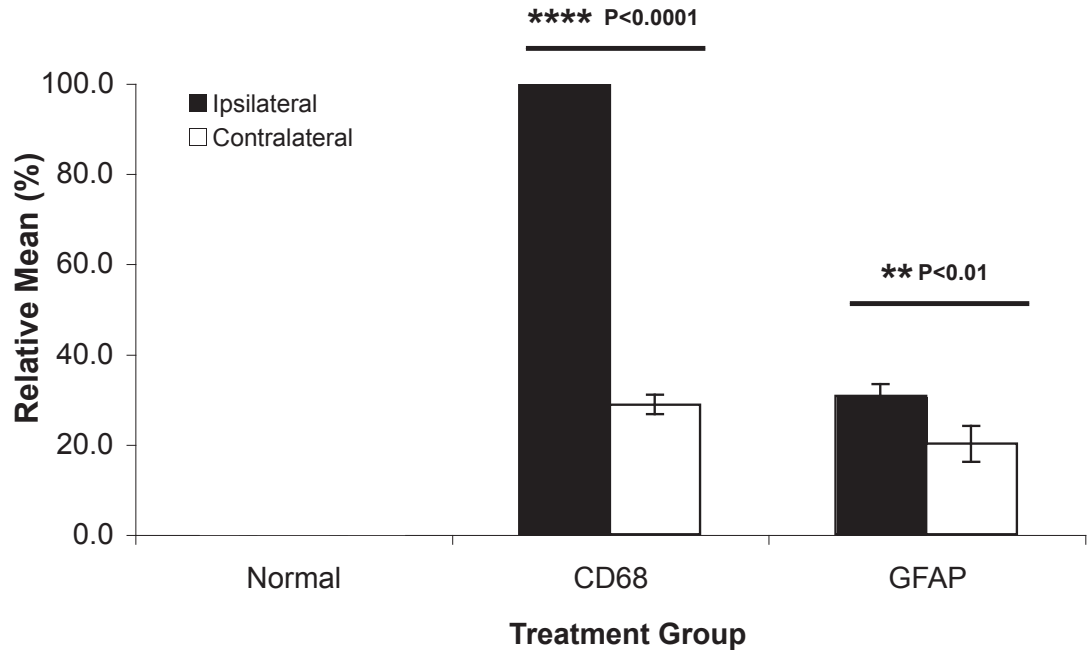


Immunoreactivity in Contralateral DRG



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Cellular Quantification in DRGs 7D Post SNI



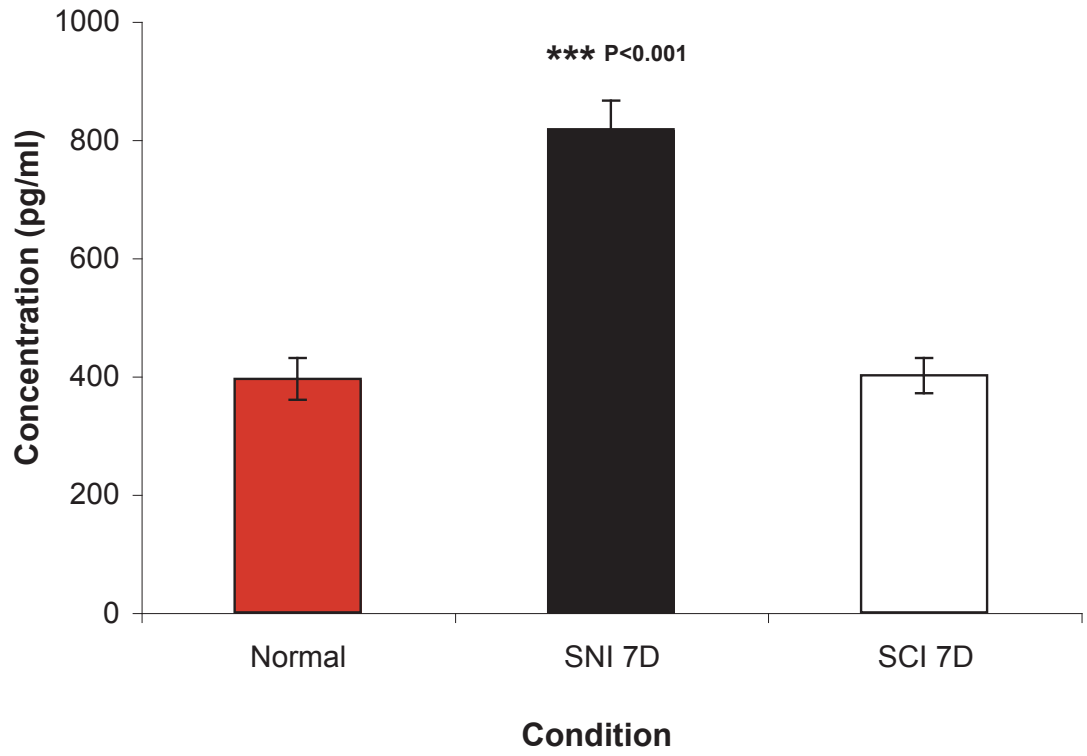
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Percentage of Cellular Presence in DRGs 7D Post SNI

	Normal		CD68 ⁺ Cells	CD68 ⁺ Cells	GFAP ⁺ Cells	GFAP ⁺ Cells
	LS	RS	IL	CL	IL	CL
Total	0.0 %		100.0 %	28.7 %	30.7 %	20.1 %

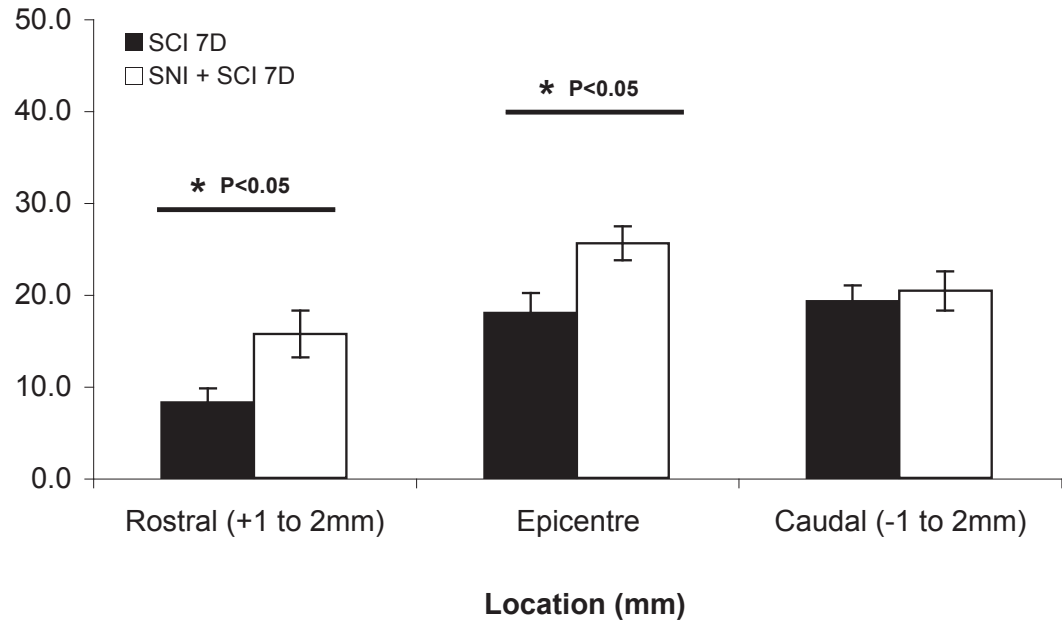
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Quantified BDNF Serum Concentration



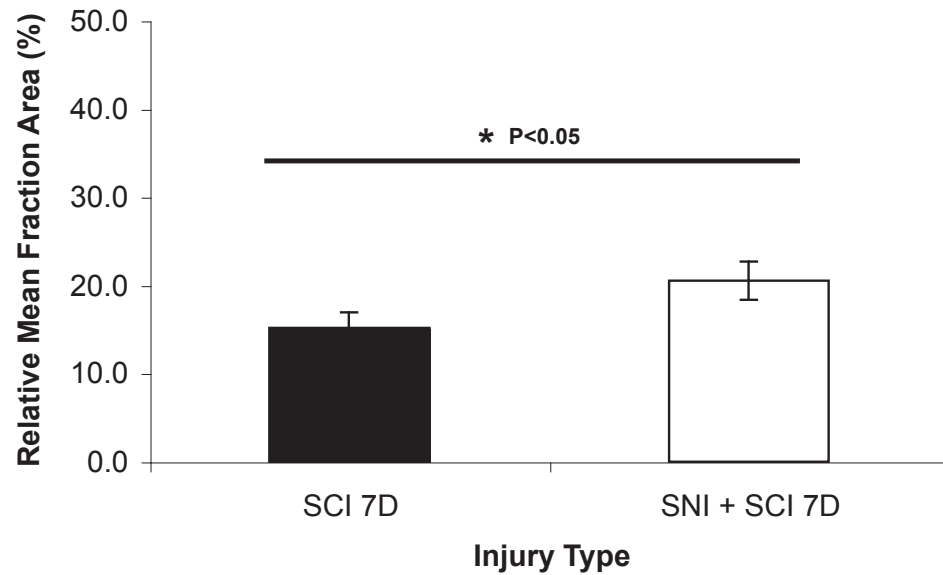
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Macrophage Cell Quantification



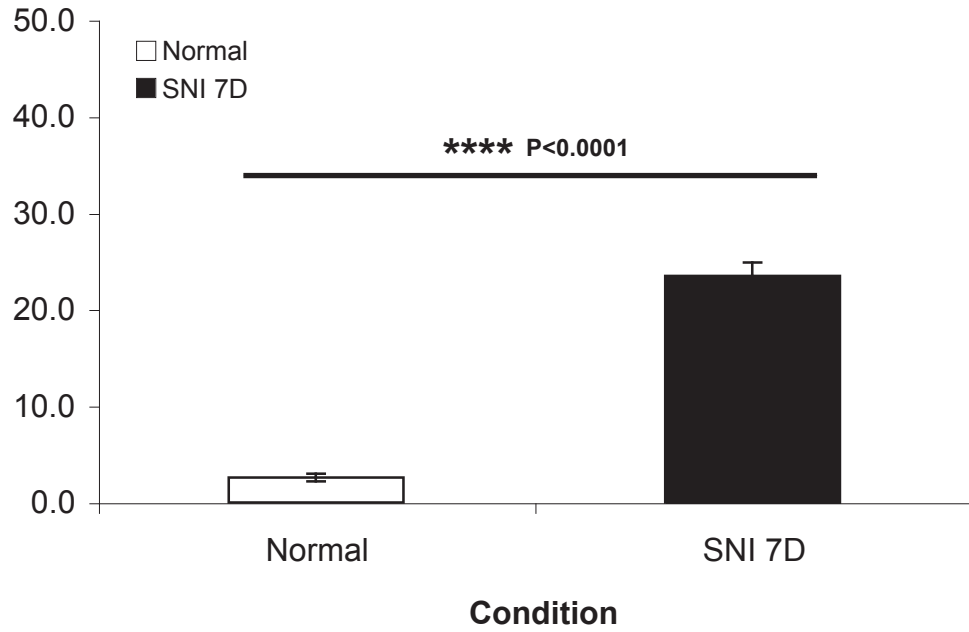
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Macrophage Quantification - Pooled Data



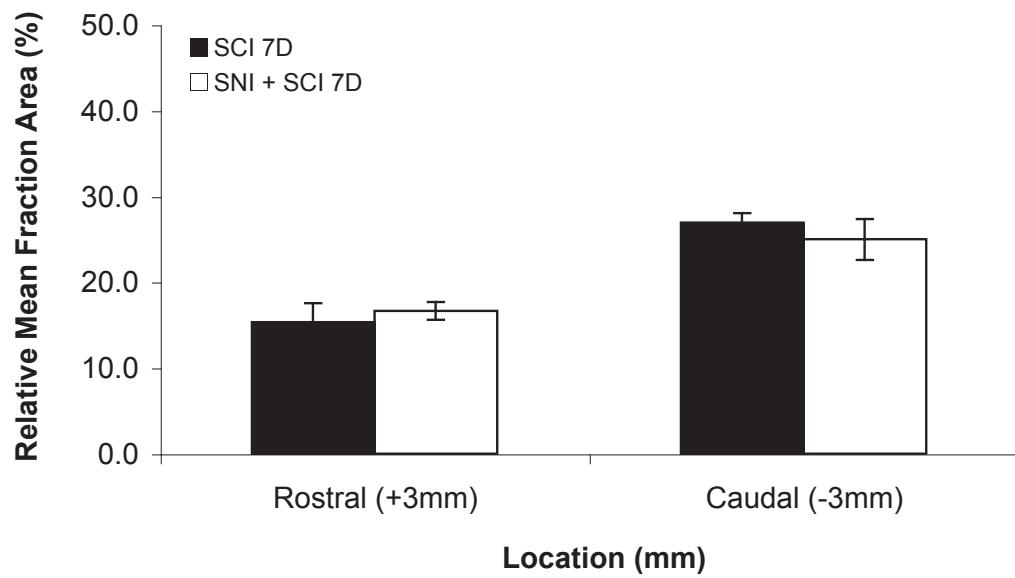
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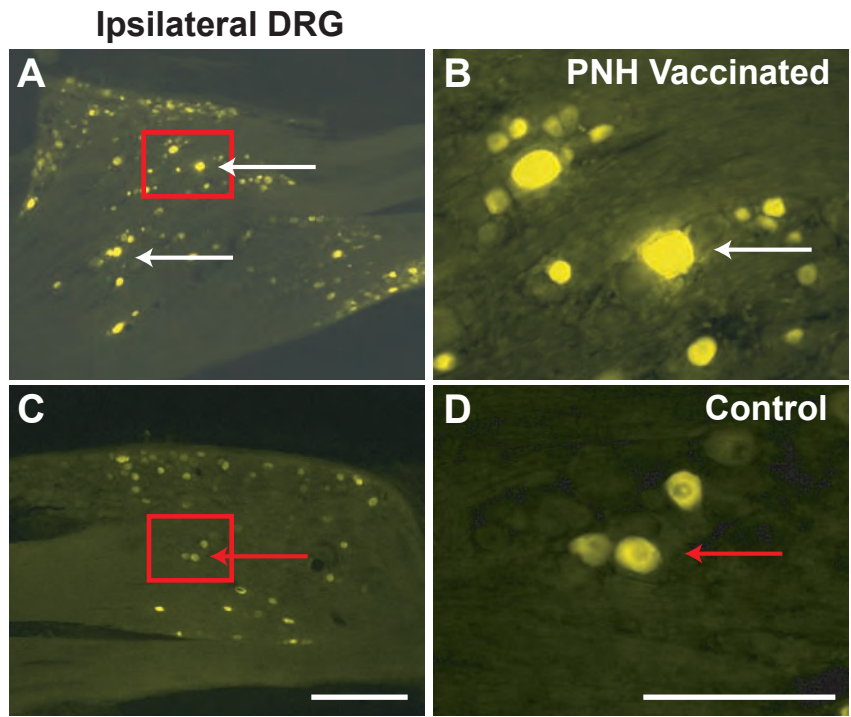
Astrocyte Quantification



B

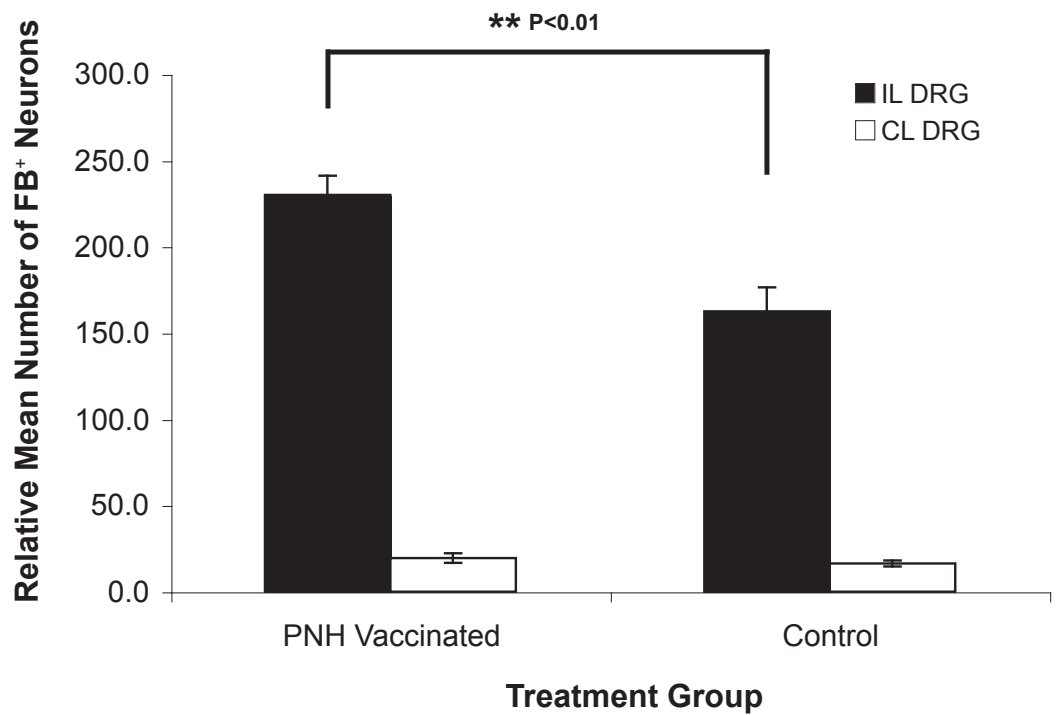
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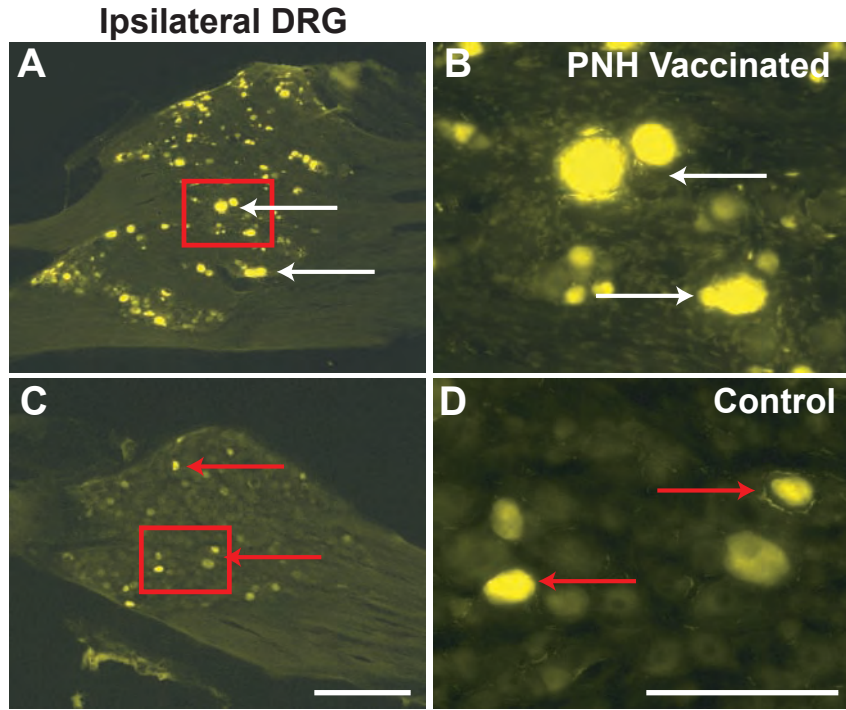




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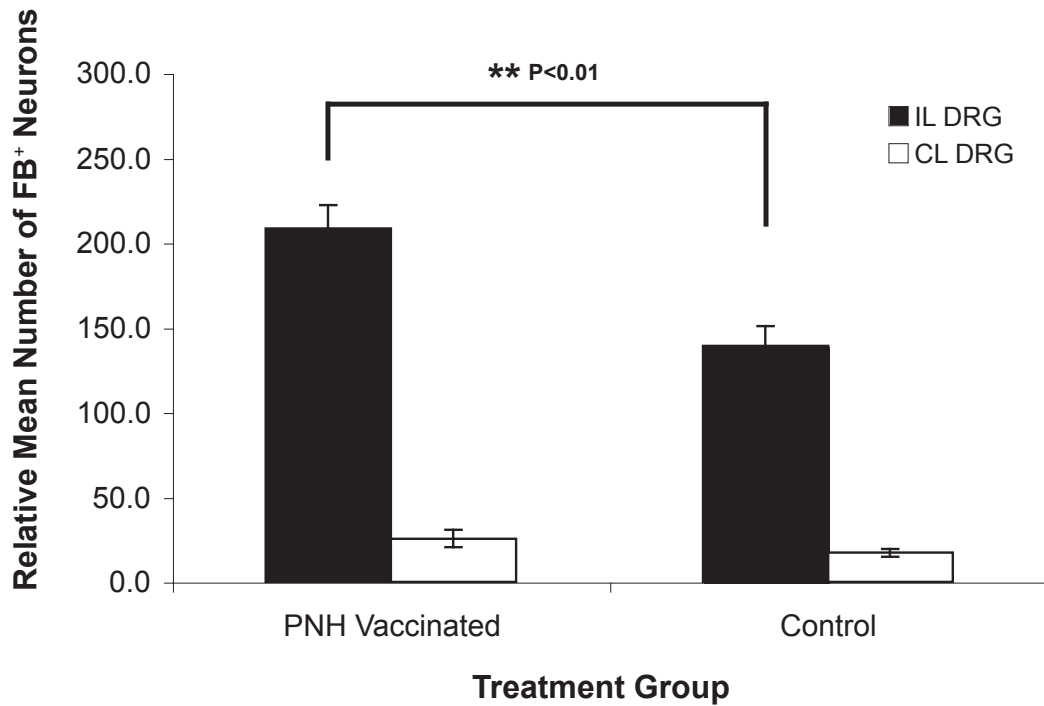
FB Quantification in DRGs of ADULT Vaccinated

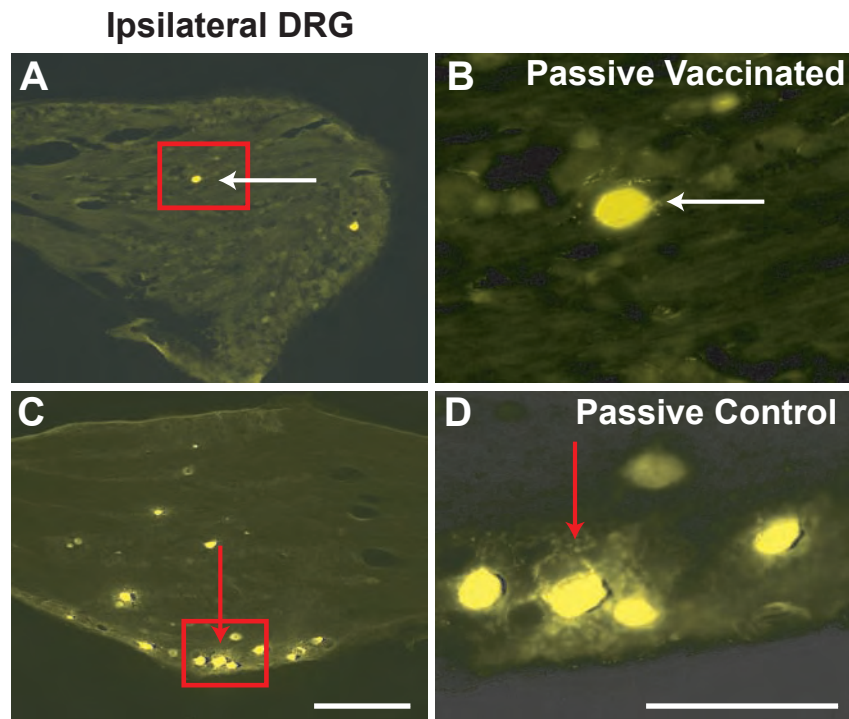




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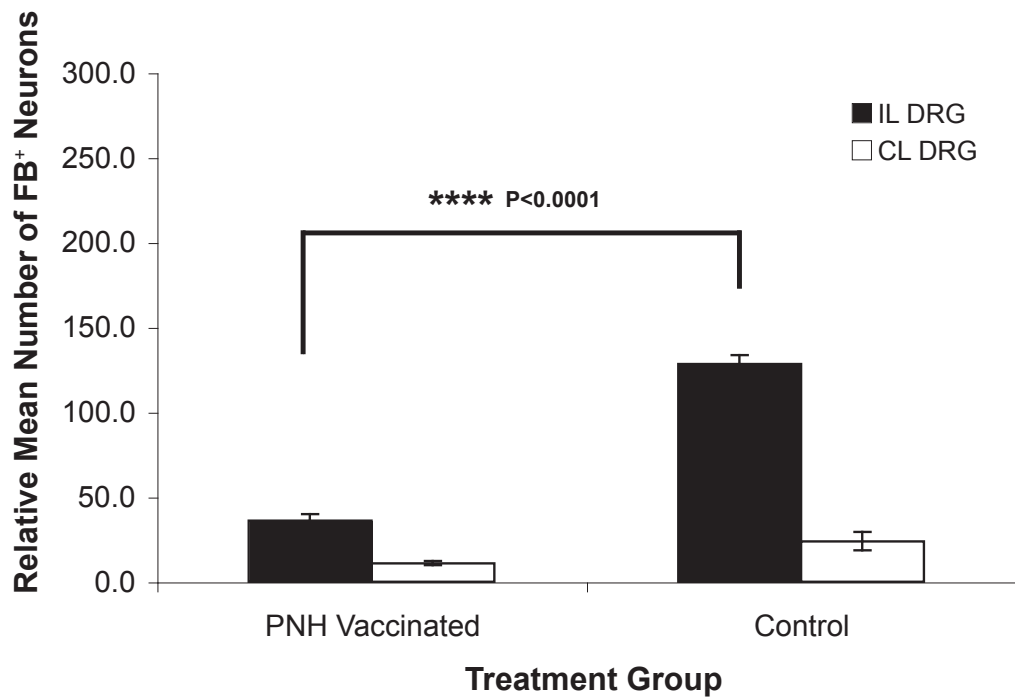
FB Quantification in DRGs of NEONATAL Vaccinated

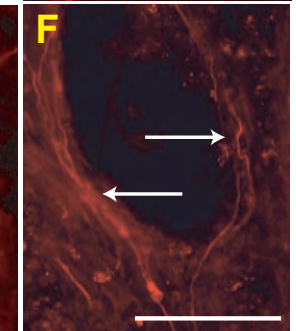
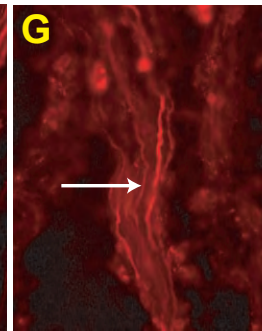
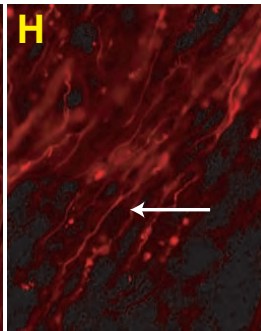
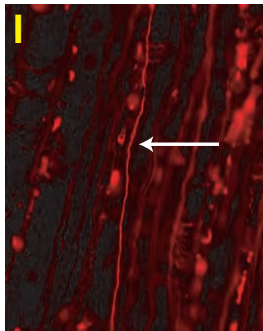
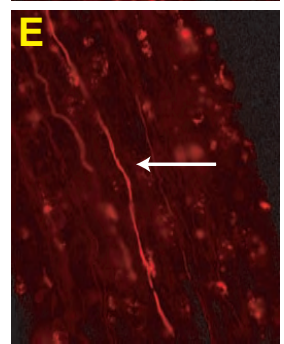
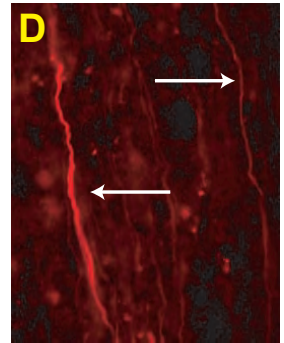
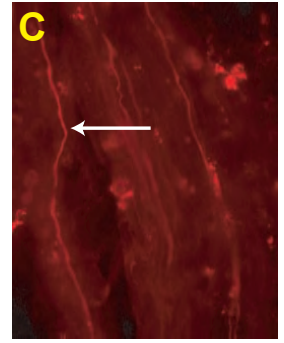
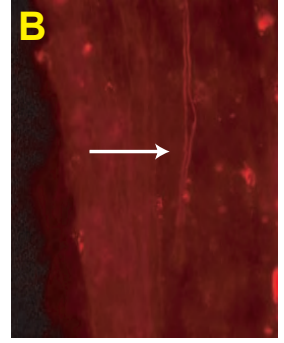
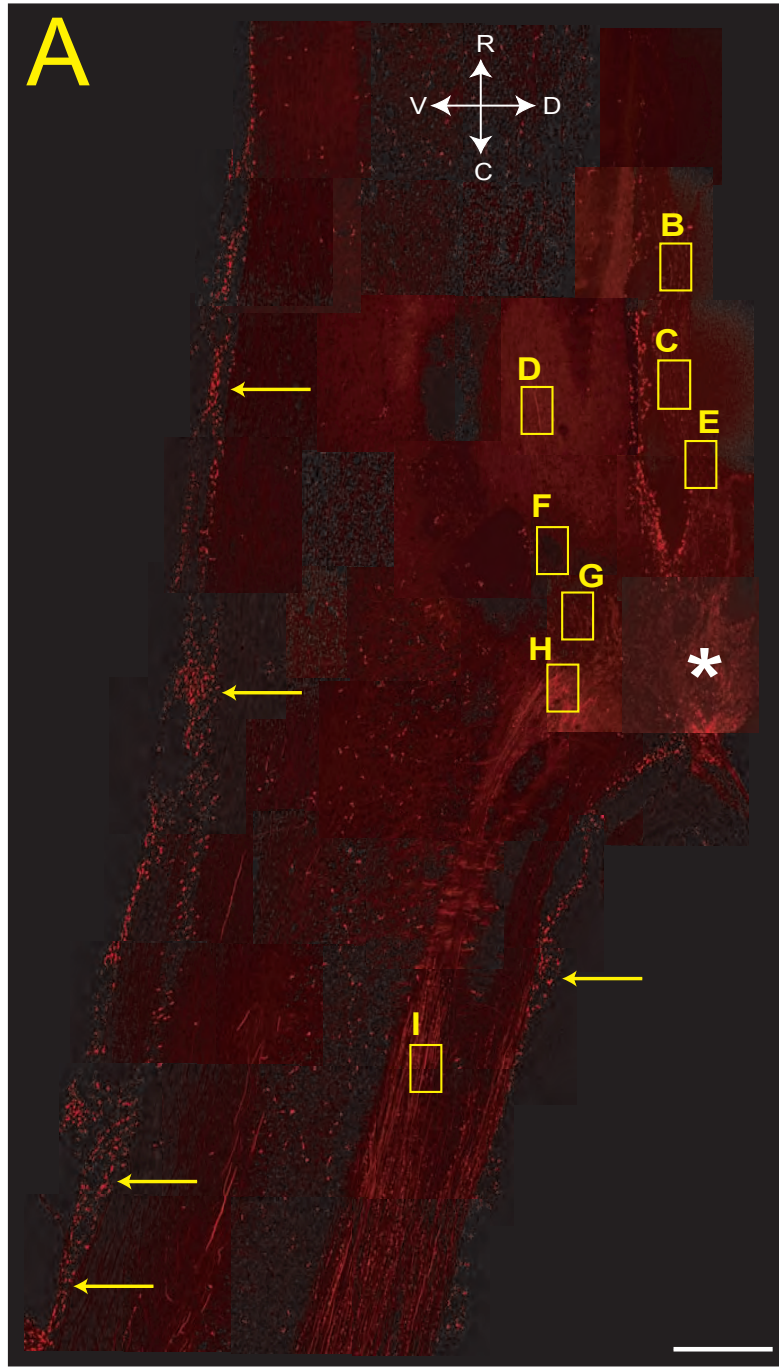




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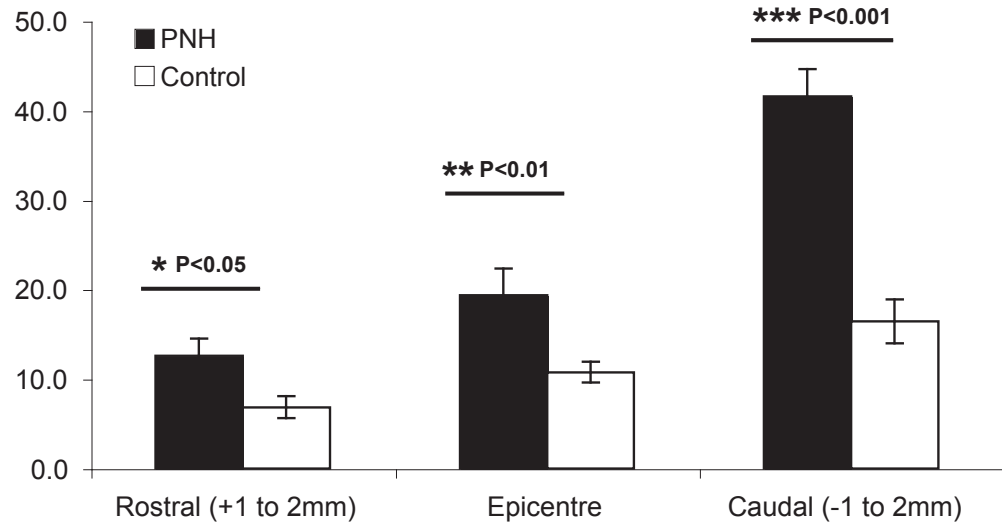
FB Quantification in DRGs of PASSIVE Vaccinated





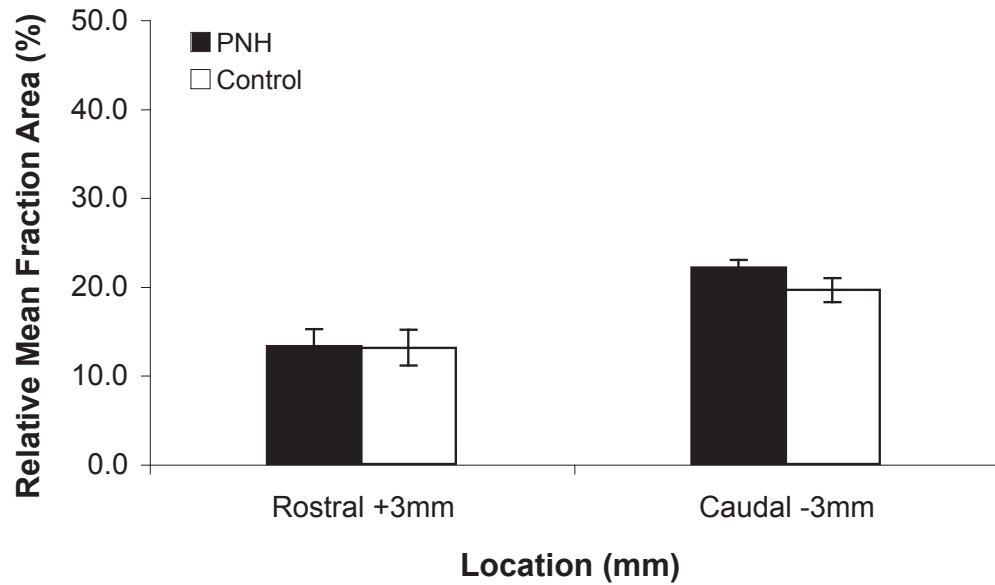
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Macrophage Quantification in ADULT Vaccinated



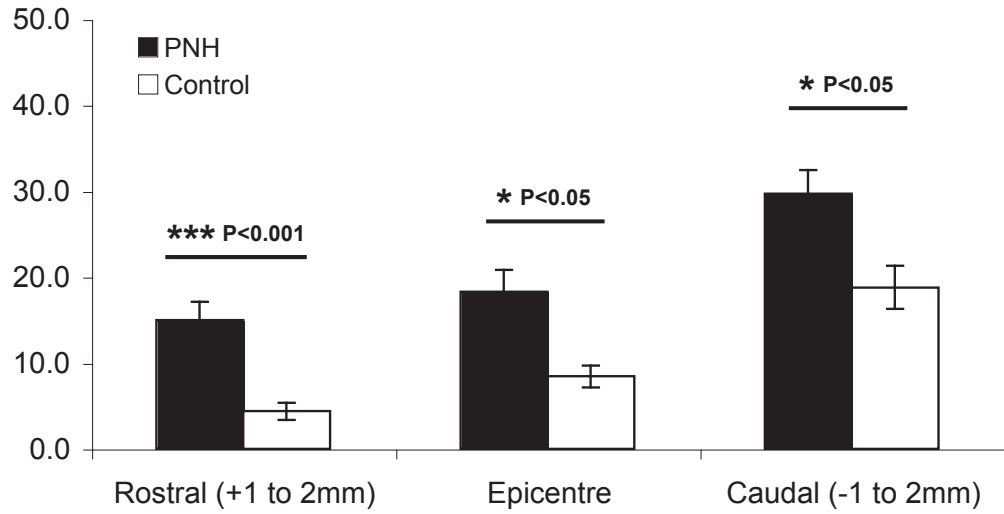
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Astrocyte Quantification in ADULT Vaccinated



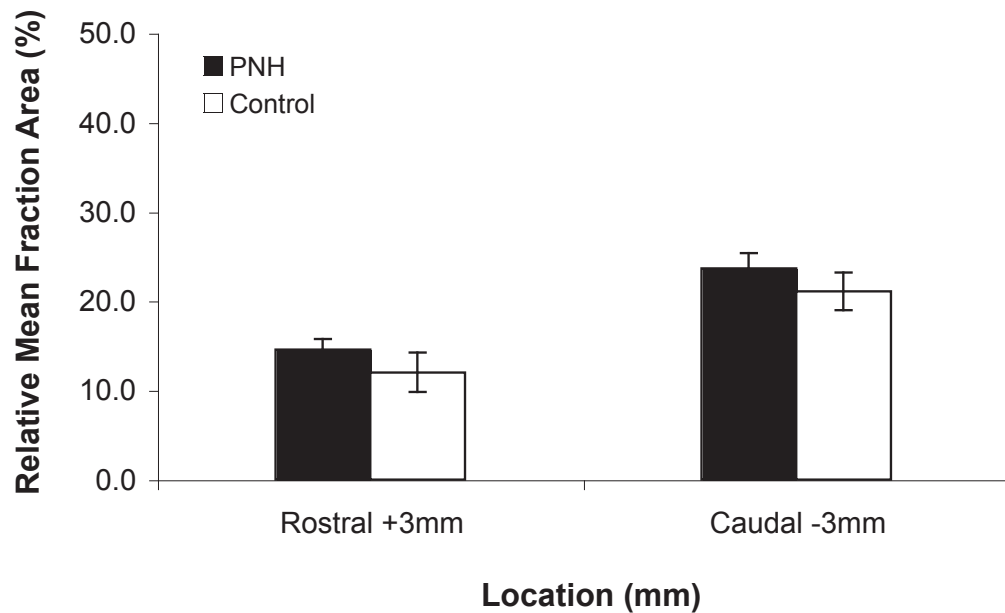
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Macrophage Quantification in NEONATAL Vaccinated



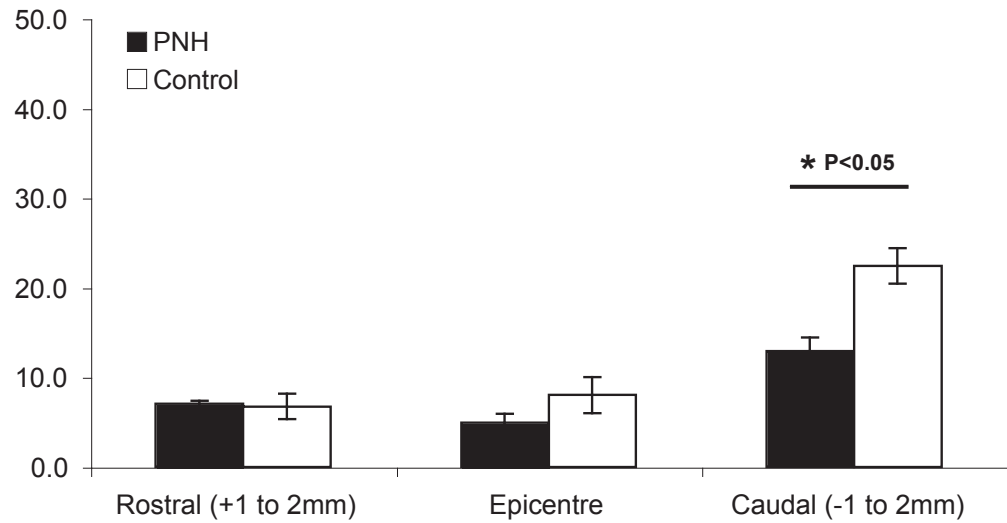
B

Astrocyte Quantification in NEONATAL Vaccinated



A

Macrophage Quantification in PASSIVE Vaccinated



B

Astrocyte Quantification in PASSIVE Vaccinated

