Figure 17.

Illustration of Proposed Hypothesised Model of Systemic CNS Regeneration after a Pre-Conditioned Peripheral Lesion.

A) Normal CNS response following optic nerve crush (ONC, Control Group - ONC only); **B)** Hypothesised extent of CNS nerve regeneration following a pre-conditioned peripheral nerve injury 7 days prior to ONC (Test Group - sciatic lesion + ONC). Red fibres represent positive labelled neurons with anterograde tracer, dextran tetramethylrhodamine (Fluororuby). Illustrations modified from Filbin (2006, p715).

Figure 18.

Anatomical Representation of Injuries in Pre-Conditioned Lesion Model.

A) Location of optic nerve injury site ipsilateral to (**B**) sciatic nerve injury site. Note these structures reside in the CNS, are not anatomically connected and are not up to scale.

Figure 19.

FR^{+} Labelled Fibres Found Only Proximal to Optic Nerve Crush Site 4 Weeks Post CNS Lesion.

A) Cartoon illustration of the optic nerve showing FR injection site, crush site and 3-4mm distally from crush; **B)** No FR^{+} fibres found distally to crush site in pre-conditioned lesion test group (no different than control group), only FR^{+} granules from degenerated fibres were found (white arrows); **E)** Extent of optic nerve damage after FR tracer injection was visualised by colocalisation of CD68^{+} macrophage cells and FR tracer (green); **G)** FR^{+} fibres found at and nearby crush site (yellow arrows) in close proximity to macrophage cells (**H-I**) (ONC site determined by GFAP immunohistochemistry [**F**], white arrows).

Note illustration not up to scale. White asterisks, represents tracer injection epicentre.

Scale bars B-C 500um, enlarged views D-I 200um.

Figure 20.

No FR^{+} Fibres Found in Optic Tract or Superior Colliculus 6 Weeks Post CNS Lesion.

No FR^{+} fibres detected in superior colliculus or in optic tract (**B, F, J**) except for FR^{+} granules of degenerated fibres (yellow arrow), together with an increased expression of macrophage cells (CD68^{+}; **A, E, I**) and astrocytes (GFAP^{+}; **C-D, G-H, K-L**). White arrows indicate presence of autofluorescent red blood cell.

Scale bars (E-H) 500um, enlarged views (A-D) and (I-L) 100um.

Figure 21.

Complete Stereotaxic Coordinates of Superior Colliculus.

A) Coronal section illustration of the rat brain showing location of SC numbered 1-7 (black asterisk – small parasagittal insert); **B)** Complete stereotaxic perimeter coordinate values for the SC in female adult SD rats (250-300gm weight) obtained for retrograde labelling of RGCs; **C)** Specific stereotaxic coordinates of two FB tracer injections delivered bilaterally within the SC region (red asterisks). Coronal illustration of rat brain (A) was modified from Paxinos & Watson (1998, p.115).

Note (-) sign indicates positions posterior from bregma (0) in a caudal direction. Total bregma-labmda length 7mm. Illustrations not up to scale.

SC = superior colliculus, RGCs = retinal ganglion cells, FB = fast blue

Figure 22.

Effective FB Labelling of Cell Bodies in the Superior Colliculus.

A) Cross-section illustration of the rat brain showing SC location and area of FB tracer injection delivery (yellow coloured area); **B-I)** Successful labelling of cell bodies in the SC (**B, F**) resulting in minimal astrocyte ($GFAP^+$; **C, G**) and/or macrophage ($CD68^+$; **D, H**) activation or infiltration into the SC region, as compared to normal uninjured tissue (data not shown). Scale bars montage 500um, enlarged views 200um. Illustration (A) modified from Paxinos & Watson (1998, p44).

SC = superior colliculus, FB = fast blue

Figure 23.

Effective Retrograde Labelling of Retinal Ganglion Cells from the Superior Colliculus.

A) Parasagittal illustration of the rat brain showing location of SC and region of FB retrograde tracer injection; **B)** Cartoon illustration of RGC bodies (yellow) as confirmed by FB^+ retrograde labelling in contralateral eye (**C-D**, white arrows), while no RGCs were labelled ipsilateral to ONC (**E**). Scale bars C and D 500um, enlarged view 100um. Parasagittal illustration (A) was modified from Paxinos & Watson (1998, p.121), and illustration (B) was modified from Filbin (2006, p.715).

SC = superior colliculus, FB = fast blue, RGCs = retinal ganglion cells, ONC = optic nerve crush

Figure 24.

Retraction and Collapse of Optic Nerve Fibres Observed Within 2 Weeks Post CNS Lesion.

A-I) Neuronal immunostaining showing retraction, collapse (white arrows; NF-200^{+}; **A, D, G**) and scar formation (GFAP^{+}; **B, E, H**) at crush site 2 weeks post CNS lesion; **J-O**) At 14 weeks post CNS lesion, NF-200^{+} fibres had retracted distally (yellow arrows) and were only seen as far as the optic chiasm, along with a consistent astrocyte (GFAP^{+}) expression throughout (**K-L, N-O**).

Scale bars A-F and J-O 500um, enlarged views G-H 200um. Directional key in A indicates P = proximal, D = distal.

Figure 25.

Greater Macrophage Numbers at CRUSH SITE and 3-4mm DISTAL in Pre-Conditioned Lesion Animals.

A) Elevated macrophage numbers detected by CD68 immunostaining in both experimental groups at 2 weeks post CNS lesion are most likely due to optic nerve injury. This difference in macrophage numbers reached statistical significant at 4 weeks post lesion, with higher cellular numbers in the pre-conditioned lesion group (*P<0.05), as compared to controls; **B)** Macrophage numbers 3-4mm distal from crush site revealed a significant increase at 2 weeks post CNS lesion, with a greater number of macrophage cells in pre-conditioned lesion animals (*P<0.05) as compared to controls.

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

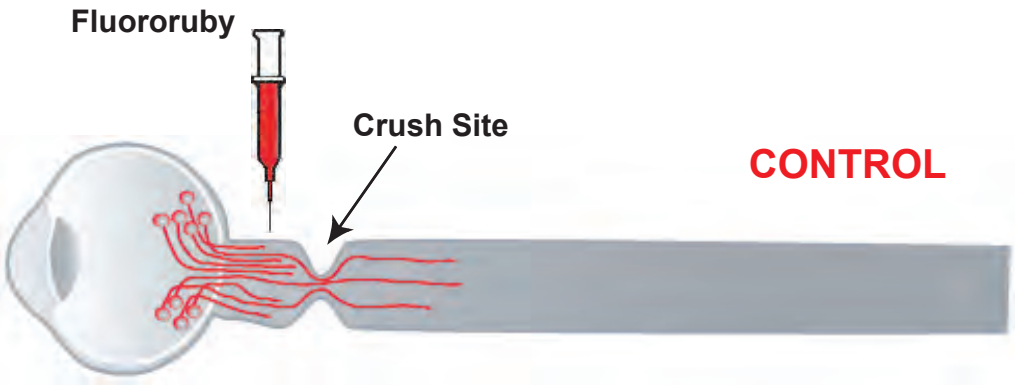
Figure 26.

Greater Macrophage Numbers in the OPTIC TRACT and SUPERIOR COLLICULUS in Pre-Conditioned Lesion Animals.

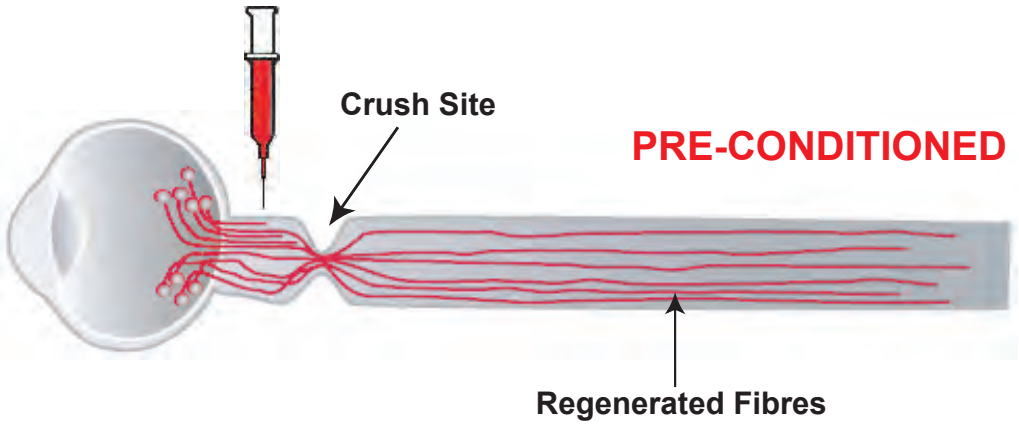
A) In the optic tract during the course of this study, macrophage numbers were significantly higher in pre-conditioned lesion animals at 7-14 weeks post CNS lesion (*P<0.05, **P<0.01), as compared to controls; **B)** A similar pattern was evident in the SC, with higher macrophages numbers found in the pre-conditioned lesion group at 7-14 weeks (*P<0.05) as compared to controls.

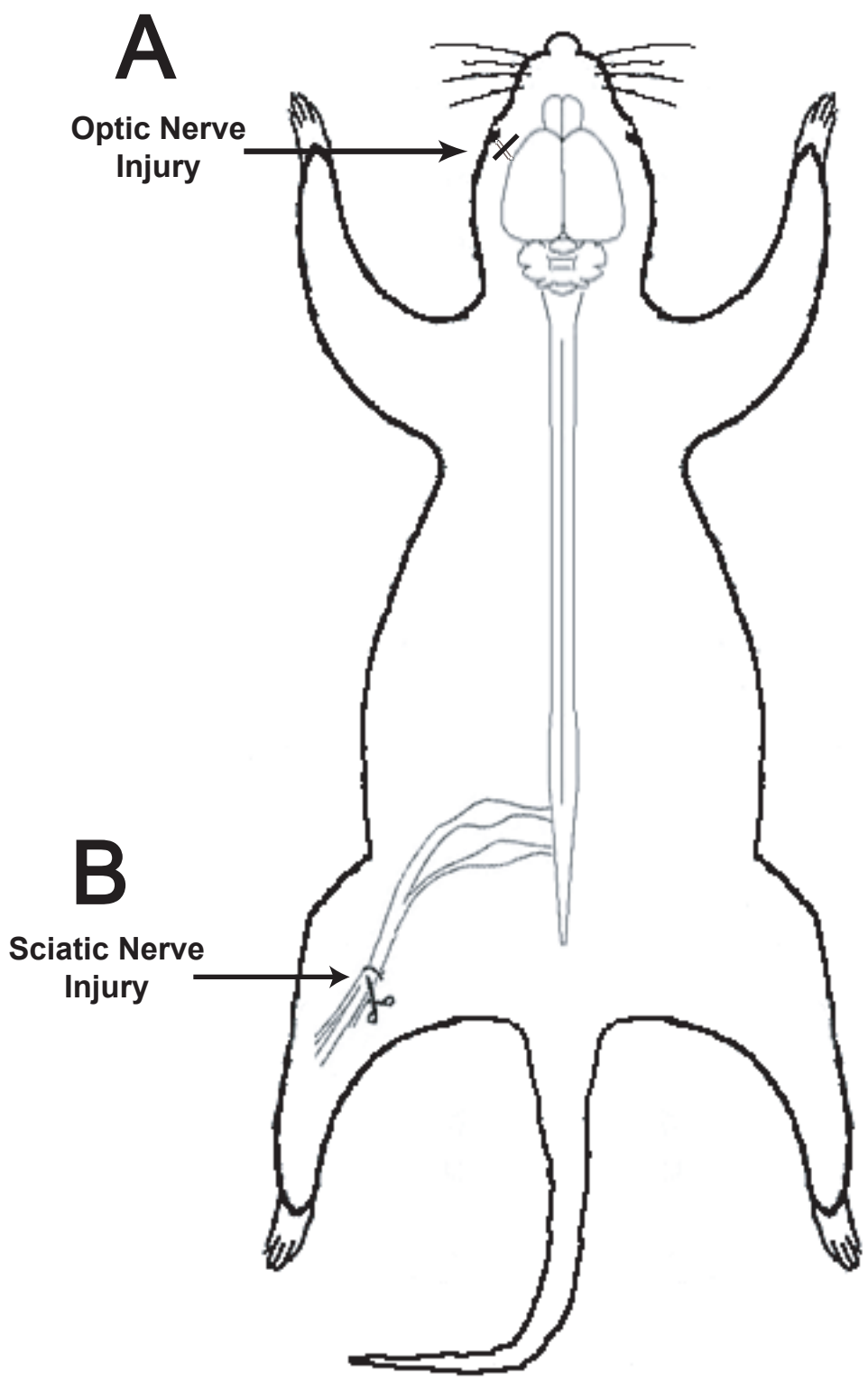
Columns represent an averaged mean (n=5) and error bars indicate error of mean (+/- S.E.).

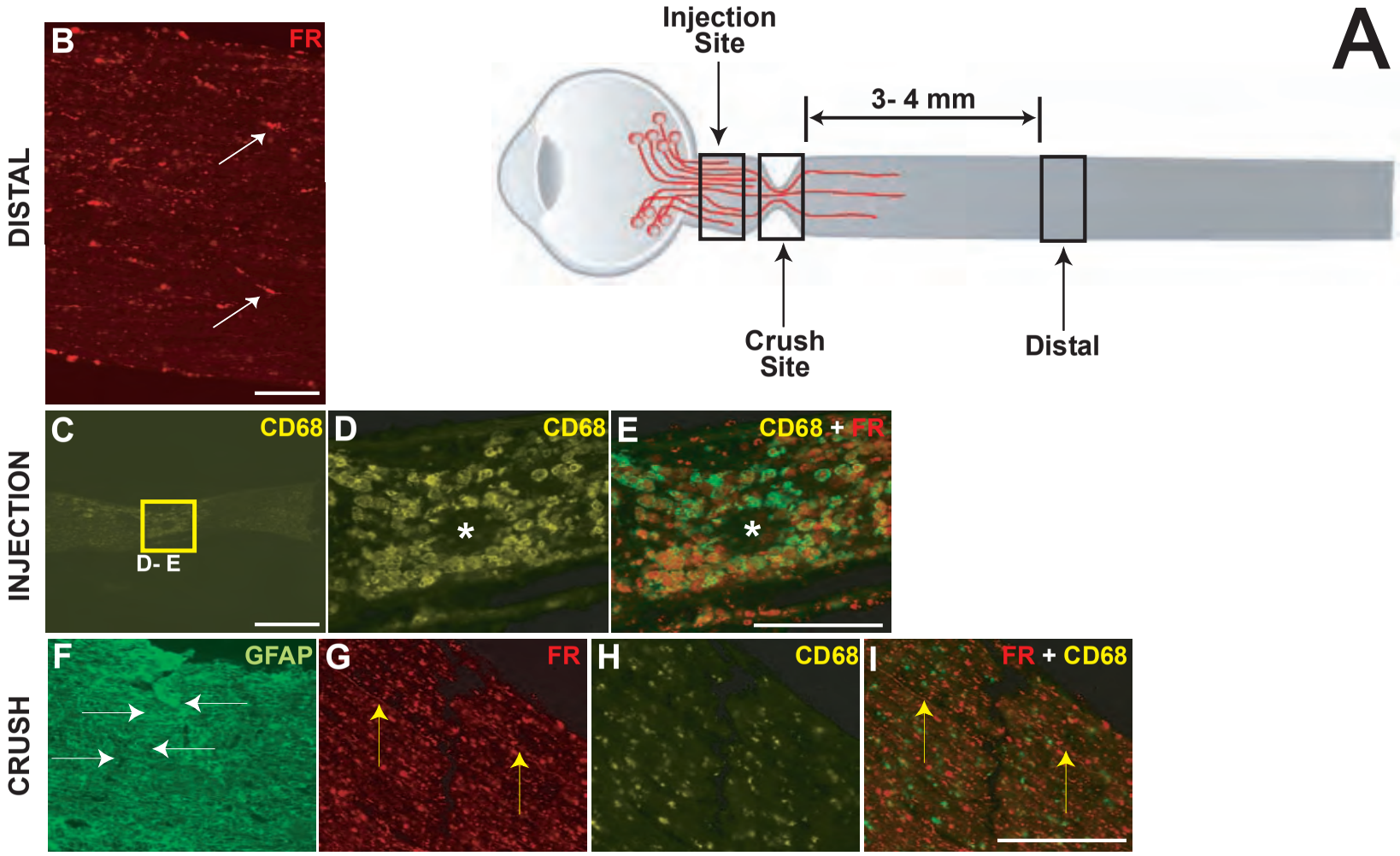
A

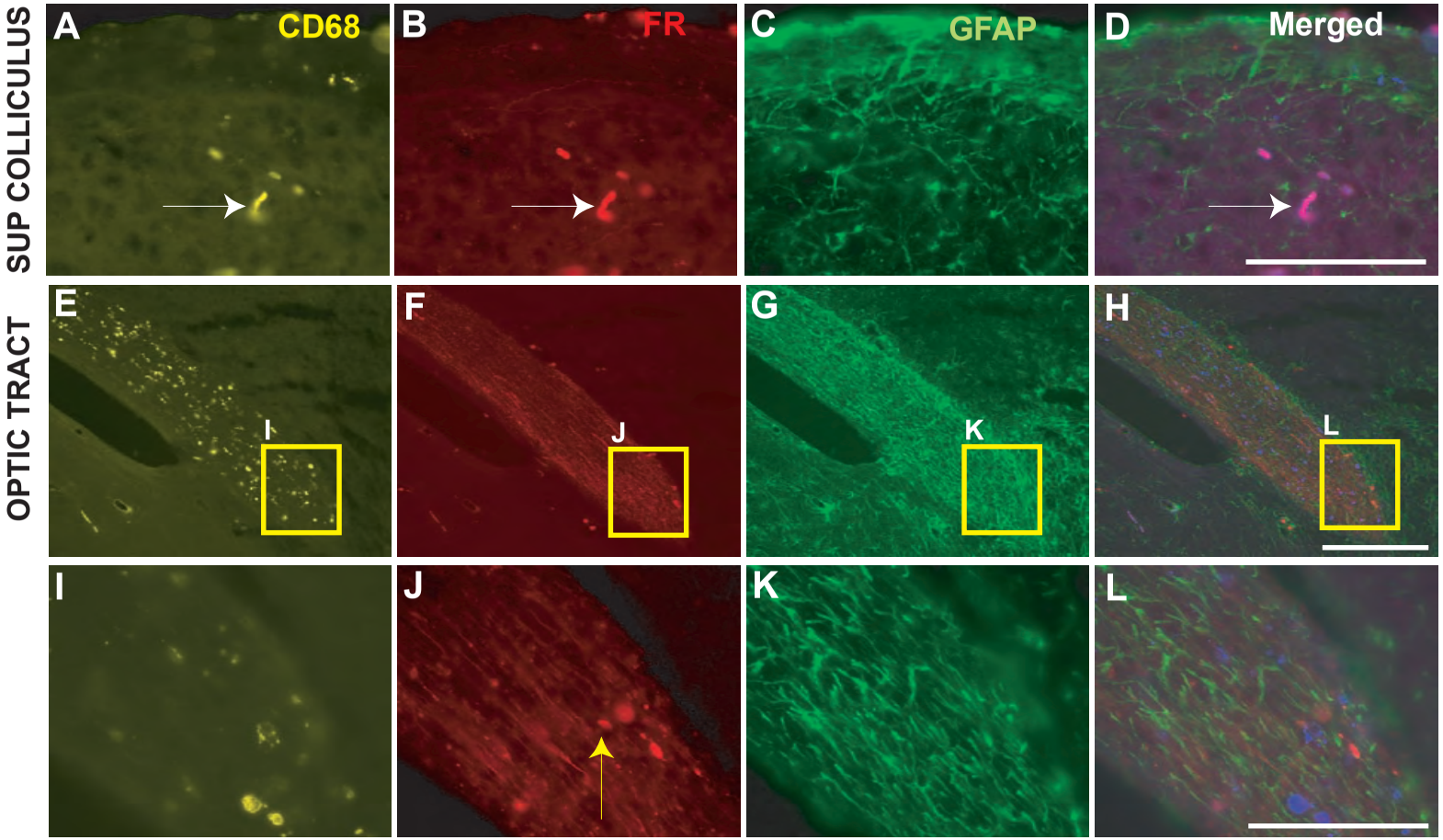

Fluororuby
Crush Site
CONTROL

B

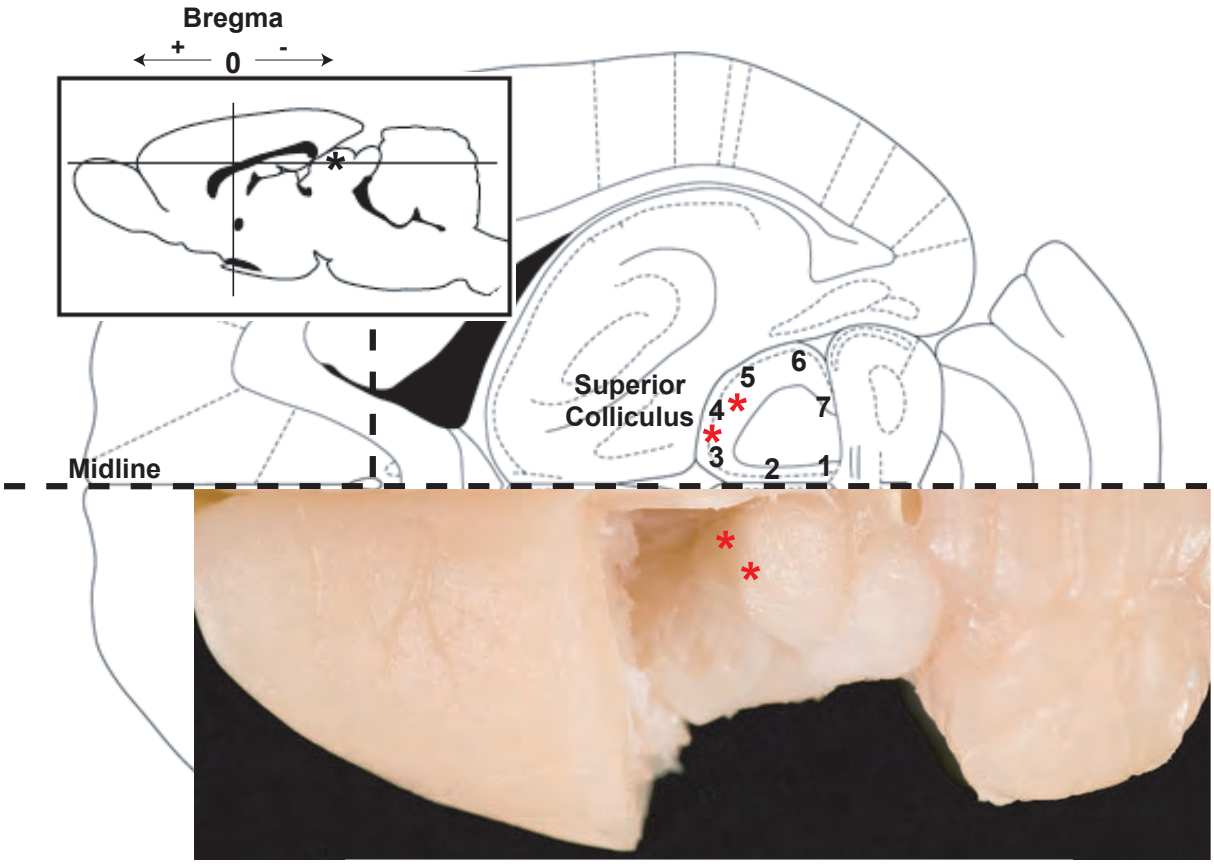

Crush Site
PRE-CONDITIONED
Regenerated Fibres


A
Optic Nerve
Injury
B
Sciatic Nerve
Injury


A
Injection Site
3- 4 mm
Crush Site
Distal
DISTAL
B
FR
INJECTION
C
CD68
D- E
D
CD68
E
CD68 + FR
CRUSH
F
GFAP
G
FR
H
CD68
I
FR + CD68


SUP COLLICULUS
OPTIC TRACT
A
CD68
B
FR
C
GFAP
D
Merged
E
F
G
H
I
J
K
L

A

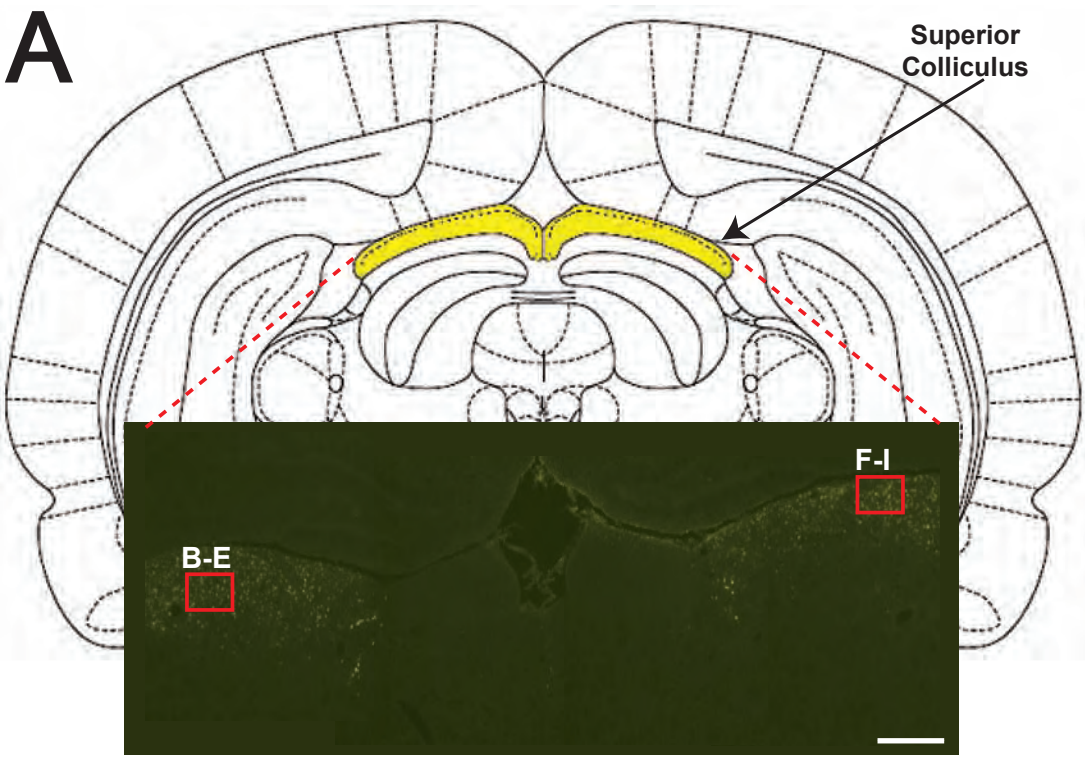


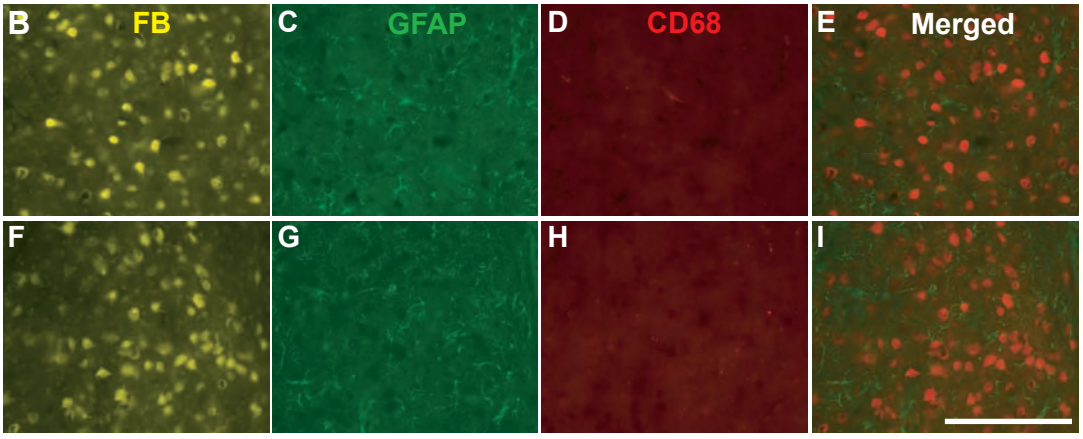
B

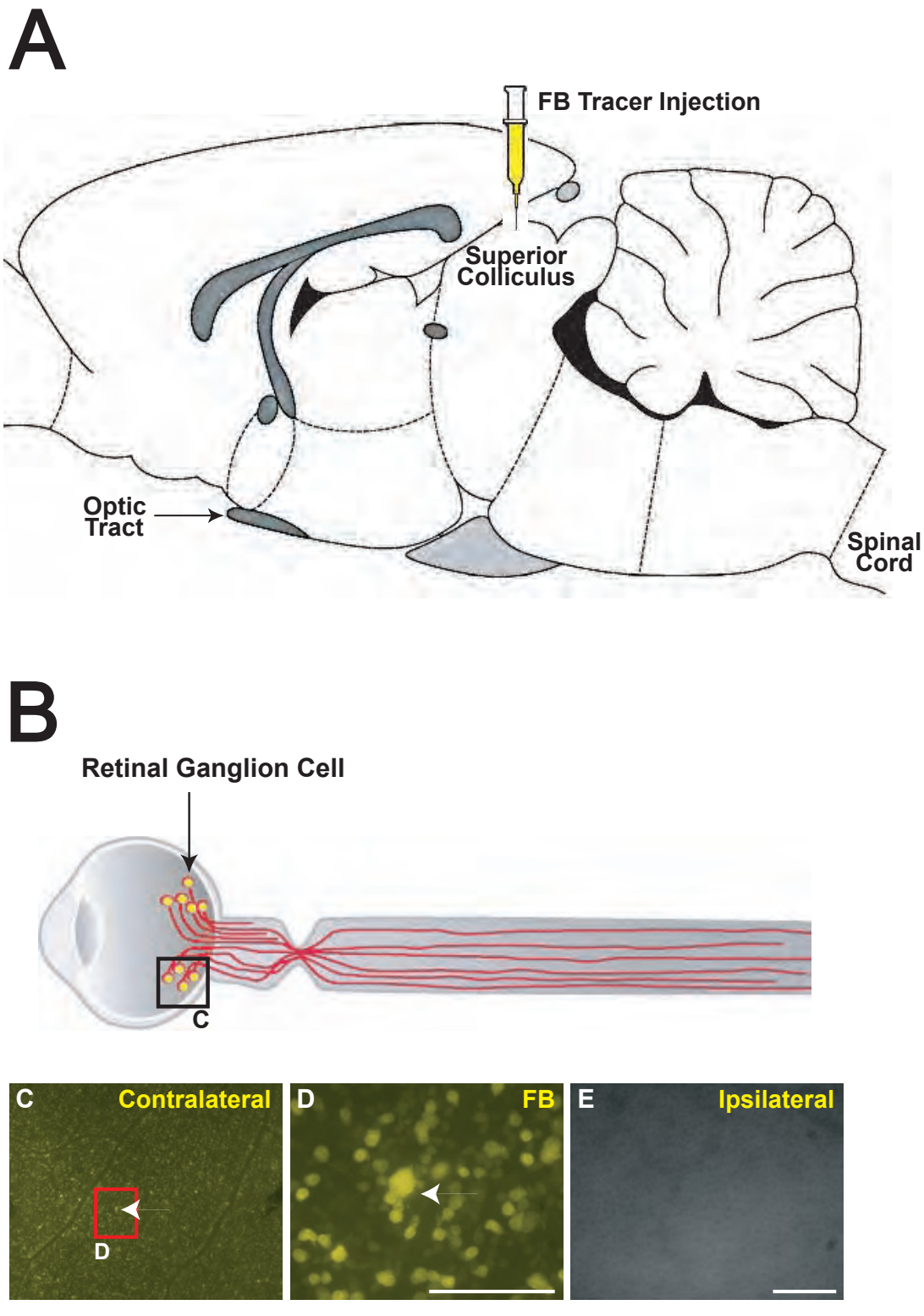
Location	Bregma (0mm)	Midline (mm)	Depth (mm)
1	- 8.50	0.80	3.00
2	- 7.50	0.80	3.00
3	- 6.70	0.80	3.00
4	- 5.50	1.80	3.50
5	- 6.50	3.00	4.00
6	- 7.50	3.00	3.50
7	- 8.50	3.00	3.50

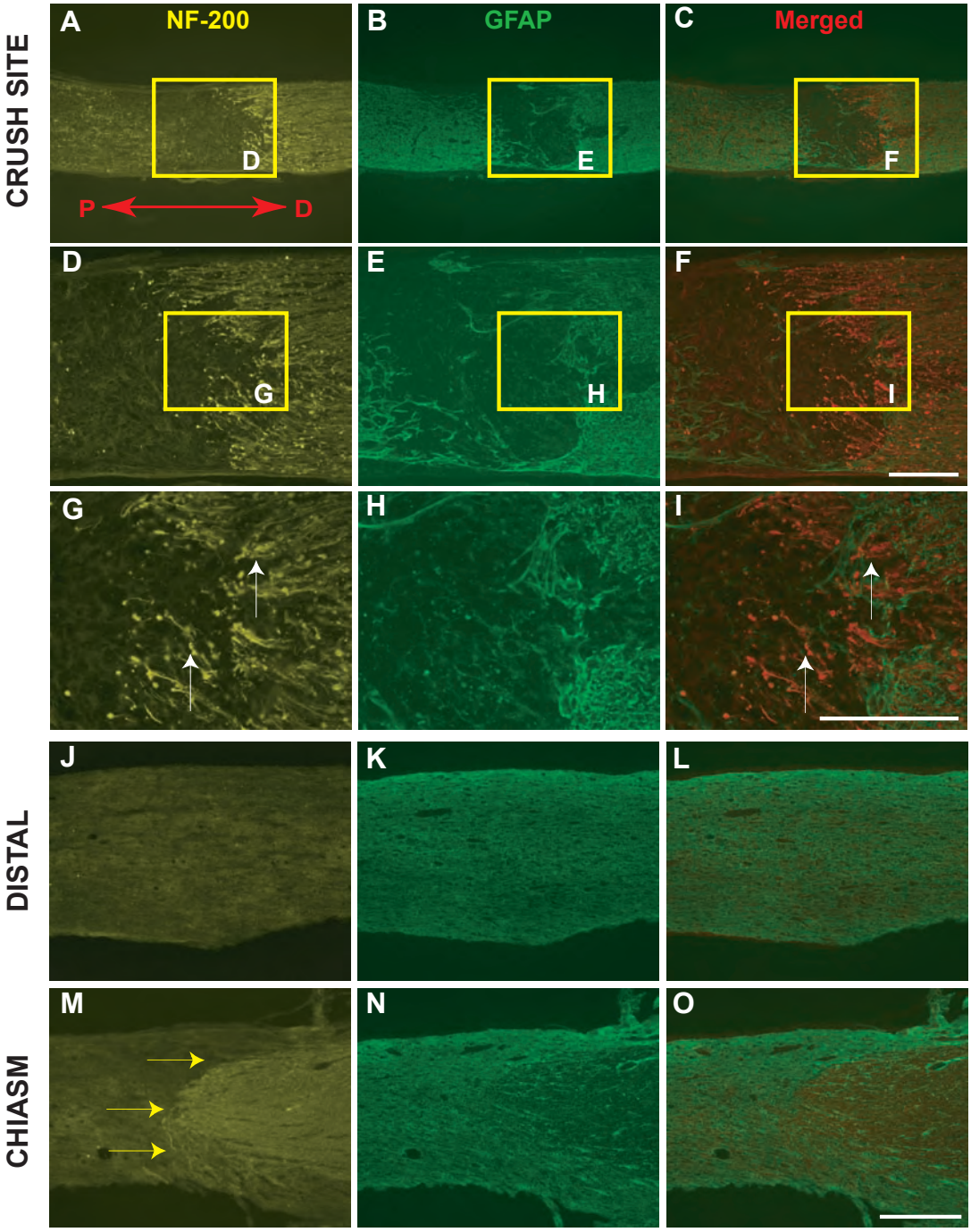
C

Location	Bregma (0mm)	Midline (mm)	Depth (mm)
A	-6.00	1.00	3.50/4.00
B	-6.50	2.50	4.00/4.50

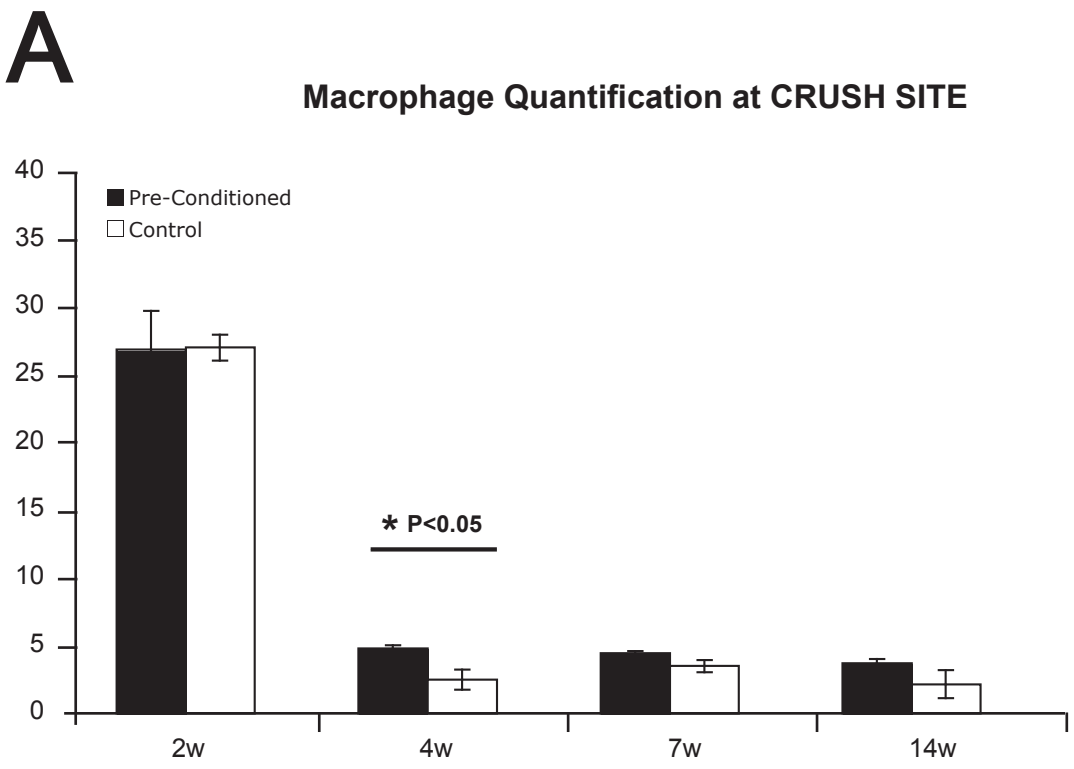

A
Superior
Colliculus
F-I
B-E


B
FB
C
GFAP
D
CD68
E
Merged
F
G
H
I

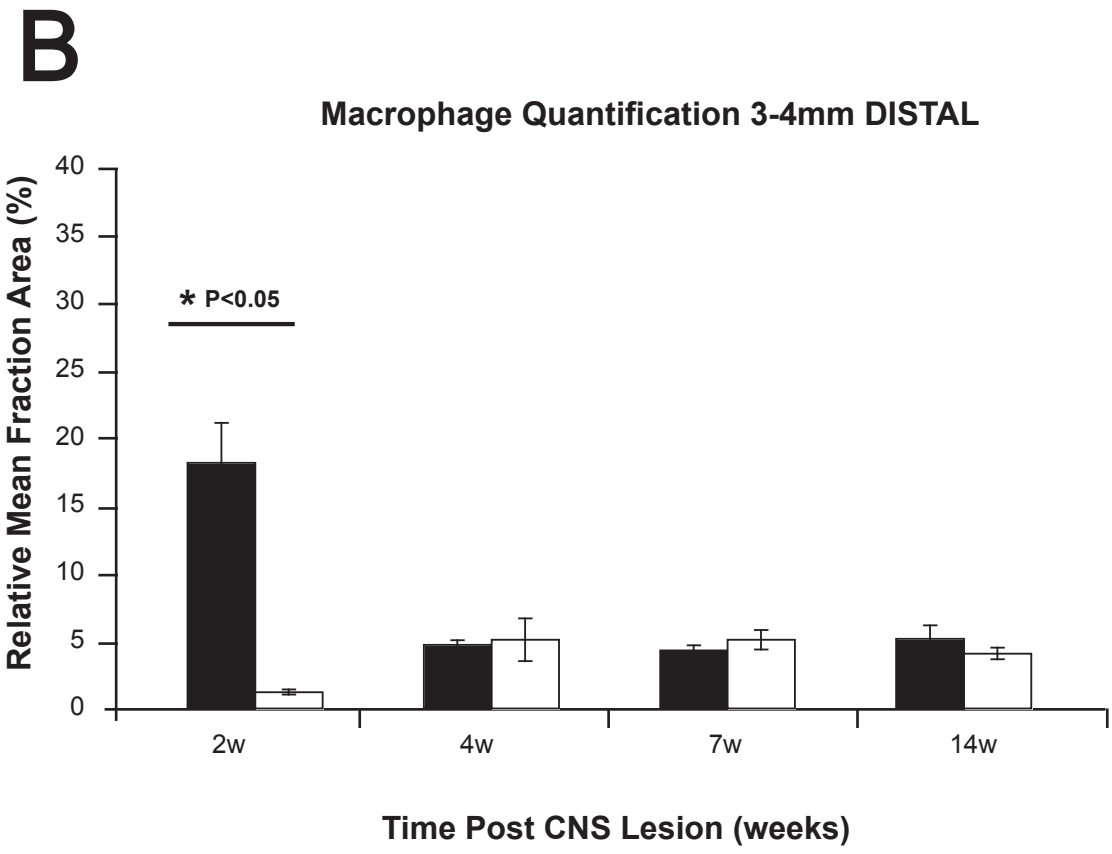

A
FB Tracer Injection
Superior
Colliculus
Optic
Tract
Spinal
Cord
B
Retinal Ganglion Cell
C
C
Contralateral
D
D
FB
E
Ipsilateral


CRUSH SITE
DISTAL
CHIASM
A
NF-200
B
GFAP
C
Merged
D
E
F
P
D
G
H
I
J
K
L
M
N
O

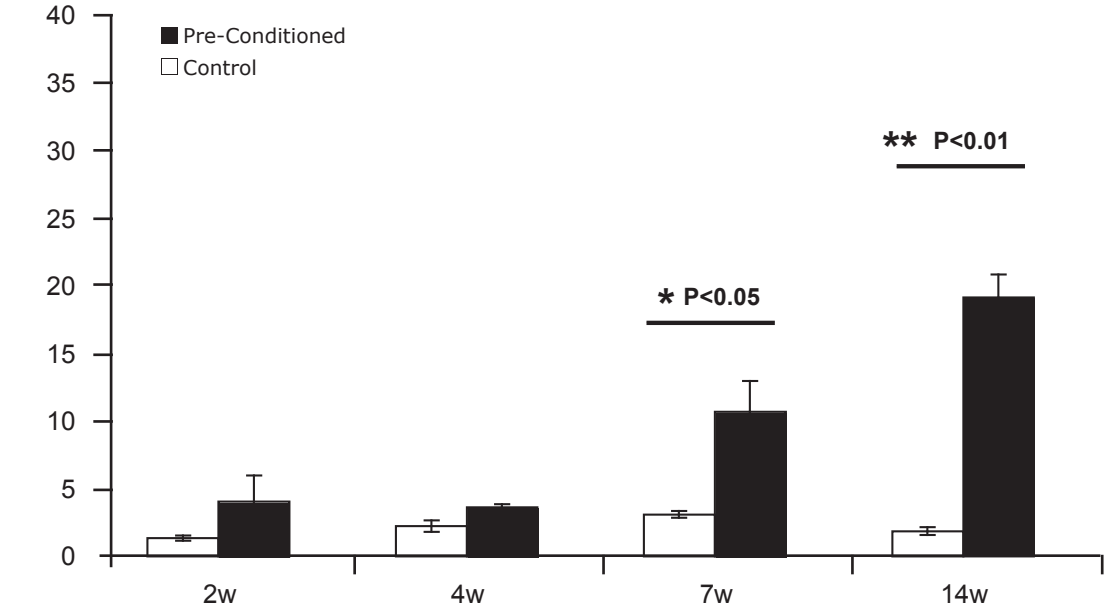
A


Macrophage Quantification at CRUSH SITE
Pre-Conditioned
Control
40
35
30
25
20
15
10
5
0
* P<0.05
2w
4w
7w
14w

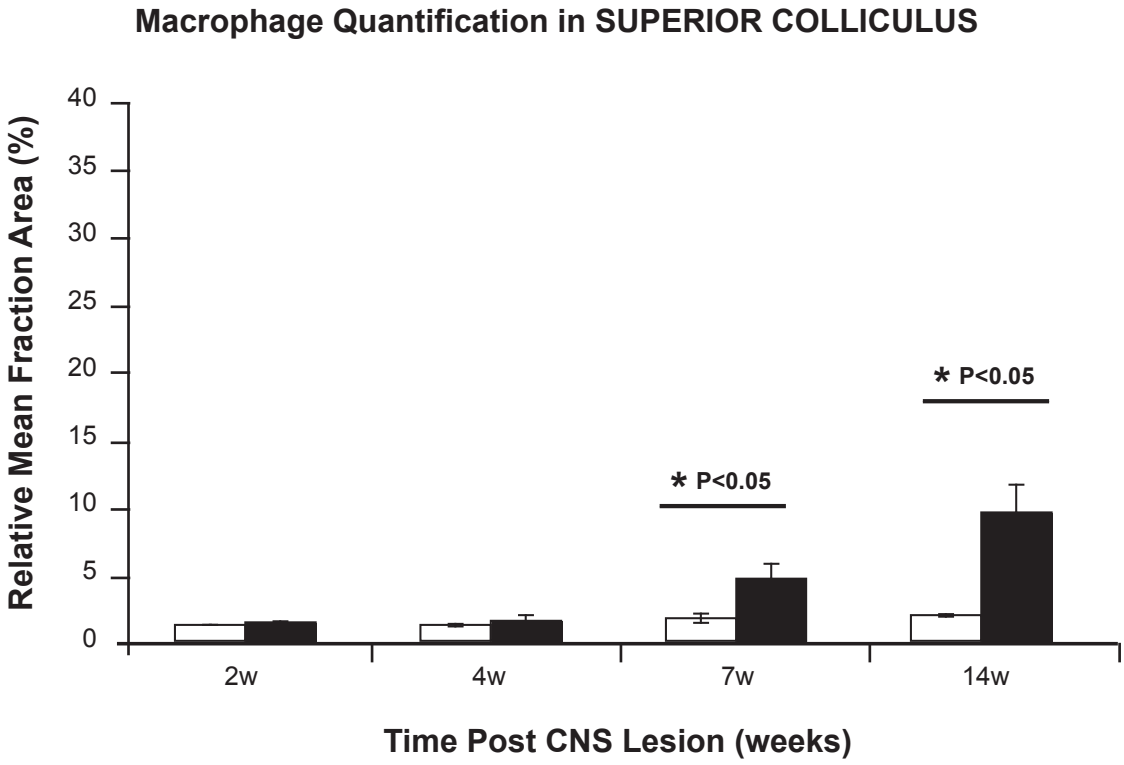
B


Macrophage Quantification 3-4mm DISTAL
Relative Mean Fraction Area (%)
40
35
30
25
20
15
10
5
0
* P<0.05
2w
4w
7w
14w
Time Post CNS Lesion (weeks)

A


Macrophage Quantification in OPTIC TRACT
Pre-Conditioned
Control
40
35
30
25
20
15
10
5
0
** P<0.01
* P<0.05
2w
4w
7w
14w

B


Macrophage Quantification in SUPERIOR COLLICULUS
Relative Mean Fraction Area (%)
40
35
30
25
20
15
10
5
0
* P<0.05
* P<0.05
2w
4w
7w
14w
Time Post CNS Lesion (weeks)