**Figure 27.**

***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 FB) and anterograde (black asterisk – using BDA) 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.

BDA = biotinylated dextran amine, FB = fast blue

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

**Figure 28.**

***Liposome Structure and Mechanism of Action.***

**A)** Schematic representation of an armed liposome encapsulating clodronate molecules inside phospholipid bilayers with their hydrophilic head exposed on the outer liposomal surface; **B)** Liposome mechanism of action via apoptosis of macrophage cells: 1) internalisation of liposomes via endocytosis, 2) fusion with lysosomes containing phospholipases (arrowheads), 3-4) lysosomal degradation of liposome bilayers resulting in intracellular release of clodronate molecules. Images (A-B) were modified from van Rooijen & Sanders (1994, p.84-85).

L = lysosome, N = nucleus.

**Figure 29.**

***Timeline of Liposome Delivery via Intravenous (i.v.) Route to both Experimental Groups.***

All animals received three treatment injections containing either clodronate liposomes (Test group) or saline only (Control group), specifically delivered during the conditioning phase of adult DRG neurons (i.e. during peripheral nerve axotomy). The fact that depleted macrophage cells begin to repopulate 5-7 days after the last liposome treatment injection, we designed the following protocol to deplete these cells during: **A)** three days prior to sciatic nerve injury (SNI, -3d), **B)** on the same day as SNI (day 0), and **C)** three days prior to spinal cord injury (SCI, 7 days post SNI). Total length of animal survival period was 35 days.

**Figure 30.**

***Confirmation of Macrophage Cell Depletion Examined in the Spleens of Liposome-Treated Rats.***

**A-B)** During liposome treatment no macrophage cells were present in the spleens of treated animals, as confirmed by the absence in CD68- (macrophage marker) immunoreactivity; **C-D)** By 2 week post liposome treatment completion, macrophage cells ($CD68^{+}$) had already began to repopulate in the spleen of treated animals; **E-F)** At the end of experimental period (day 35) macrophage numbers in the spleen of treated animals had returned to normal levels, as compared to untreated spleens (**G-H**).

Scale bars A, C, E, G 500um, enlarged views B, D, F, H 200um.

**Figure 31.**

***No Regenerated FB$^+$ DRG Neurons Found in Liposome-Treated Animals.***

**A-B)** In the ipsilateral DRG, only saline-treated control animals showed FB$^+$ retrograde labelled neuronal cell bodies (white arrows), as compared to unlabelled cell bodies (red arrows) in liposome-treated animals (**C-D**); **E)** Quantification of FB$^+$ cell bodies in ipsilateral (black bar) and contralateral (white bar) DRGs revealed significantly more labelled neurons in saline-treated animals, as compared to unlabelled DRG neurons in liposome-treated animals, both ipsilateral and contralateral to sciatic nerve injury (****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.).

FB = fast blue tracer, DRG = dorsal root ganglia, CL = contralateral, IL = ipsilateral.

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

**Figure 32.**

***Anterograde BDA Labelled Fibres in the Spinal Cord of Liposome-Treated Animals.***

**A)** Montage of SCI epicentre (black asterisk), showing anterograde labelled fibres only in the distal segment (**C**), with no visible fibres present in the proximal segment (**B**). **D-F**) Closer observation in the distal segment revealed extensive neuronal collapse and retraction of BDA labelled fibres evident by their bulb-like structure (black arrows). **G**) Illustration of the region of interest (i.e. dorsal column cut).

BDA = biotinylated dextran amine, SCI = spinal cord injury. Directional key: D = dorsal, V = ventral, C = caudal, R = rostral.

Scale bars montage 800um, B-D 200um, E-F enlarged views 100um.

**Figure 33.**

***Anterograde BDA Labelled Fibres in the Spinal Cord of Saline-Treated Animals.***

**A)** Montage of SCI epicentre (black asterisk), showing extensive anterograde labelling of ascending fibres in both proximal and distal segments (black arrows) of the injured spinal cord; **B-G)** Closer observation at higher magnifications confirmed the presence of BDA labelled fibres extending in both segments (black arrows). This result is consistent with the retrograde labelling study previously discussed (Fig. 31).

BDA = biotinylated dextran amine, SCI = spinal cord injury. Directional key: D = dorsal, V = ventral, C = caudal, R = rostral.

Scale bars montage 800um, B-E 200um, F-G enlarged views 100um.

**Figure 34.**

***Macrophage and Astrocyte Quantification in the Injured Spinal Cord* of Treated Animals.**

**A)** Statistical analysis of macrophage numbers revealed that in saline-treated control animals, macrophage numbers were greater than those in liposome-treated test animals. In particular, in control animals these numbers were significantly higher 1-2mm both rostral and caudal from the SCI epicentre, as compared to test animals (*P<0.05, **P<0.01, respectively), while no differences were found at the SCI epicentre; **B)** Astrocyte quantification 3mm rostral from the SCI epicentre revealed a significant difference between groups, with a reduced astrocyte expression in control animals as compared to test animals. No differences were observed between groups caudally. A comparison of rostral and distal segments among groups revealed only a significant reduction in astrocyte expression in control animals rostrally, as compared to caudally (#P<0.05).

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

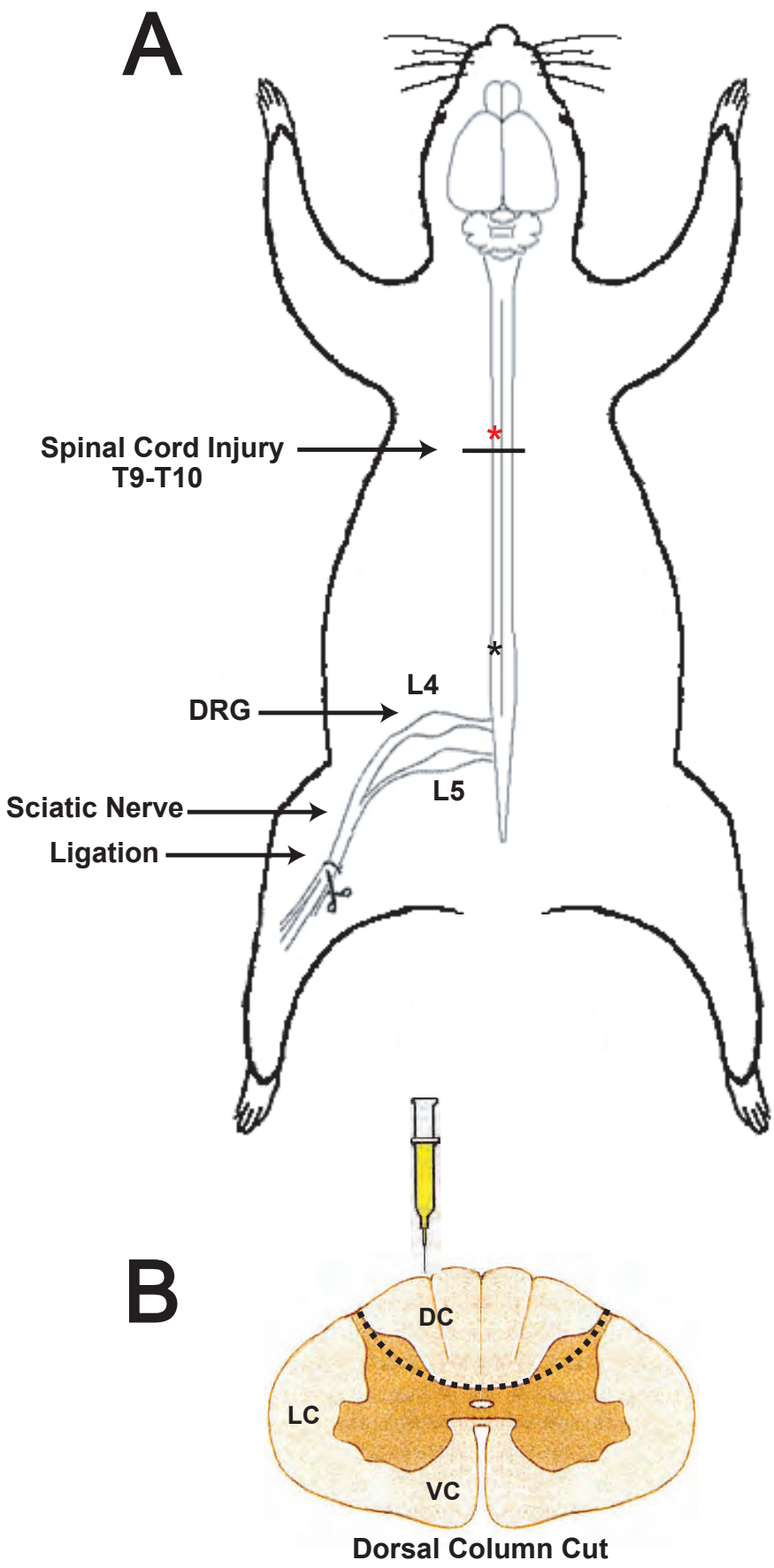
**Figure 35.**

***Higher BDNF Concentration Serum Levels in Saline-Treated Controls.***

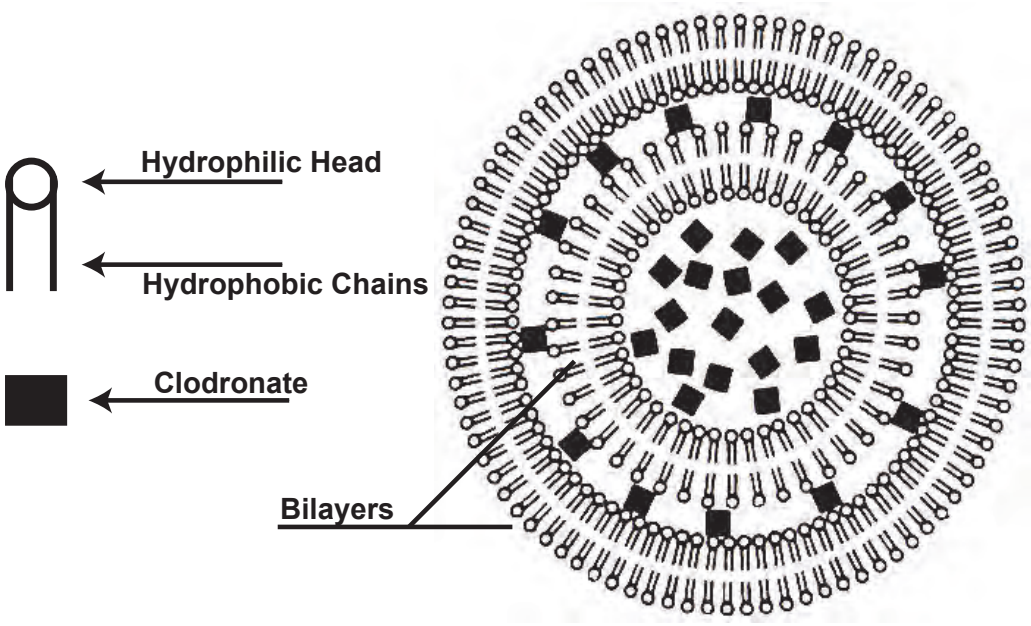
Analysis of serum trophic levels at the end of the experimental period revealed an increased BDNF serum concentration in saline-treated controls, as compared to liposome-treated animals (*P<0.05).

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

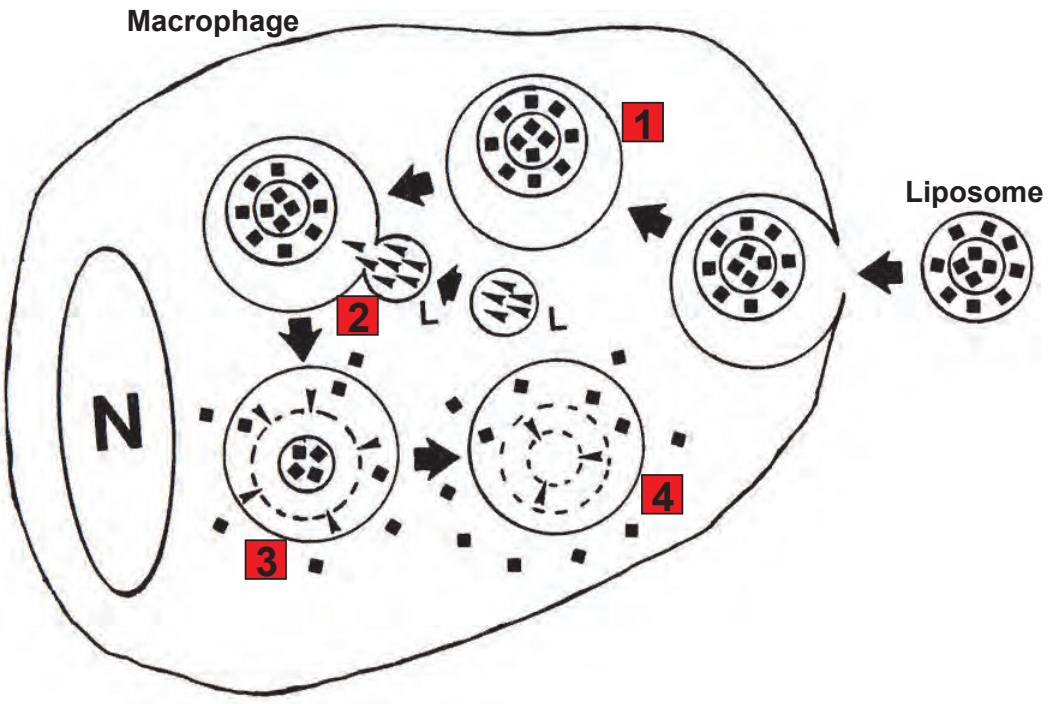
BDNF = brain derived neurotrophic factor.

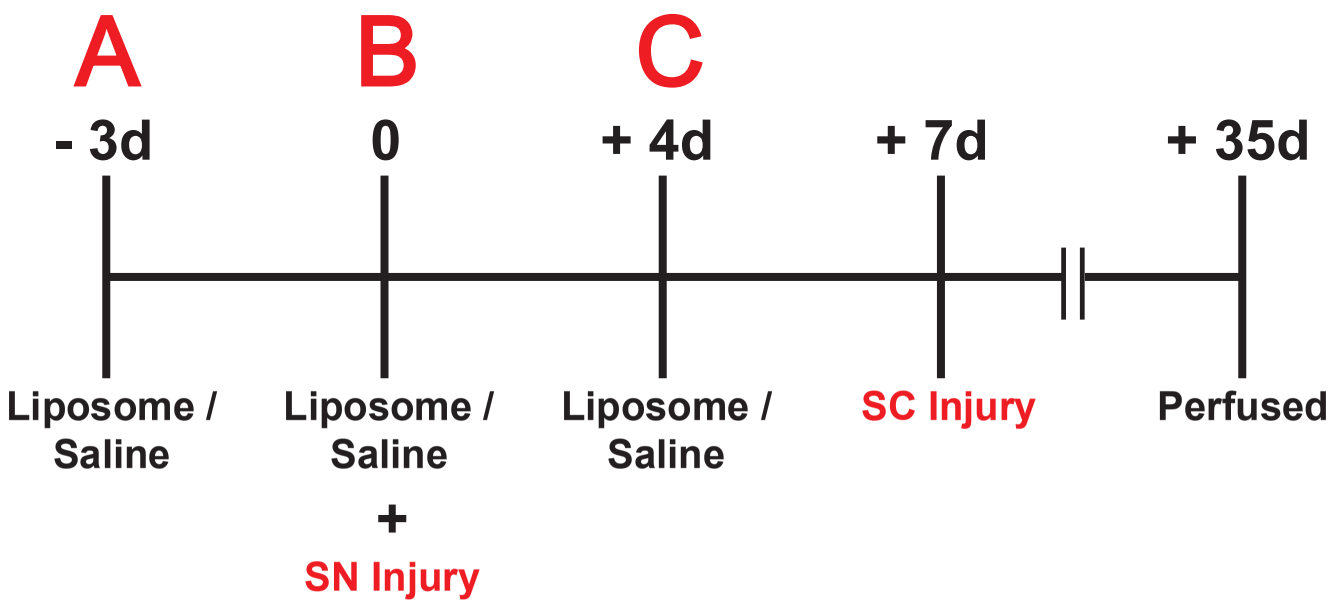

A
Spinal Cord Injury
T9-T10
L4
DRG
L5
Sciatic Nerve
Ligation
B
DC
LC
VC
Dorsal Column Cut

A

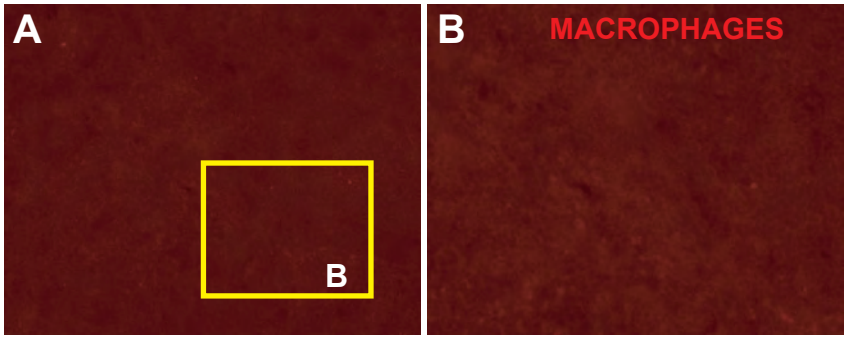

Hydrophilic Head
Hydrophobic Chains
Clodronate
Bilayers

B

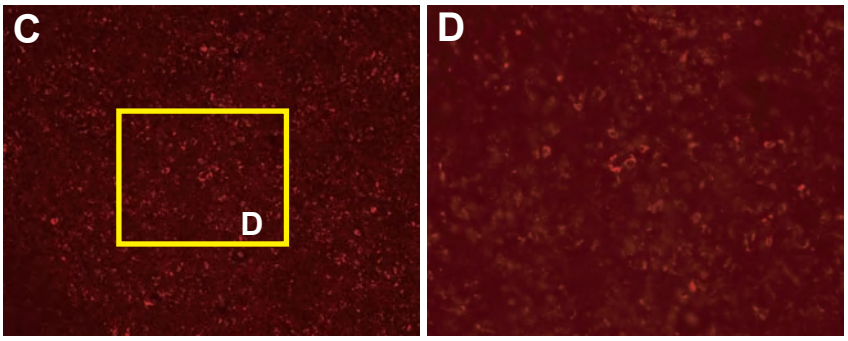

Macrophage
Liposome
1
2
3
4
L
L
N


A
B
C
- 3d
0
+ 4d
+ 7d
+ 35d
Liposome / Saline
Liposome / Saline
+
SN Injury
Liposome / Saline
SC Injury
Perfused

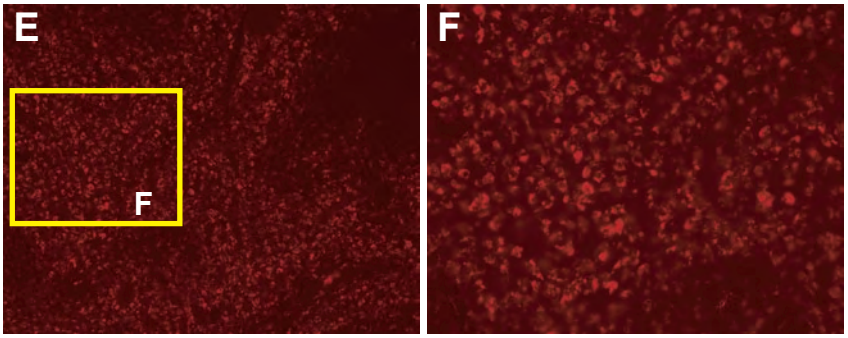
During Liposome Treatment



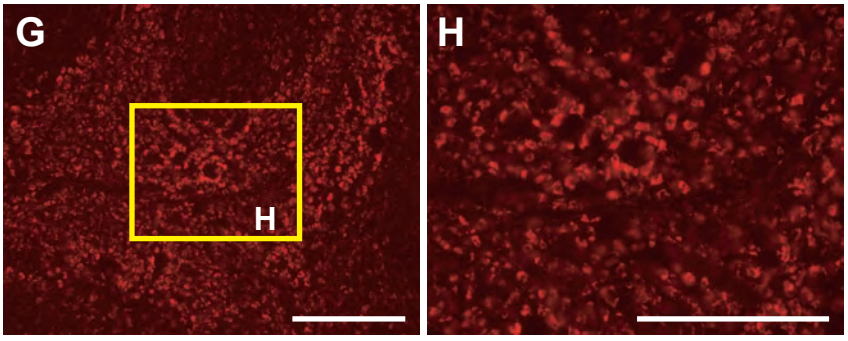

2w Post LiposomeTreatment Completion

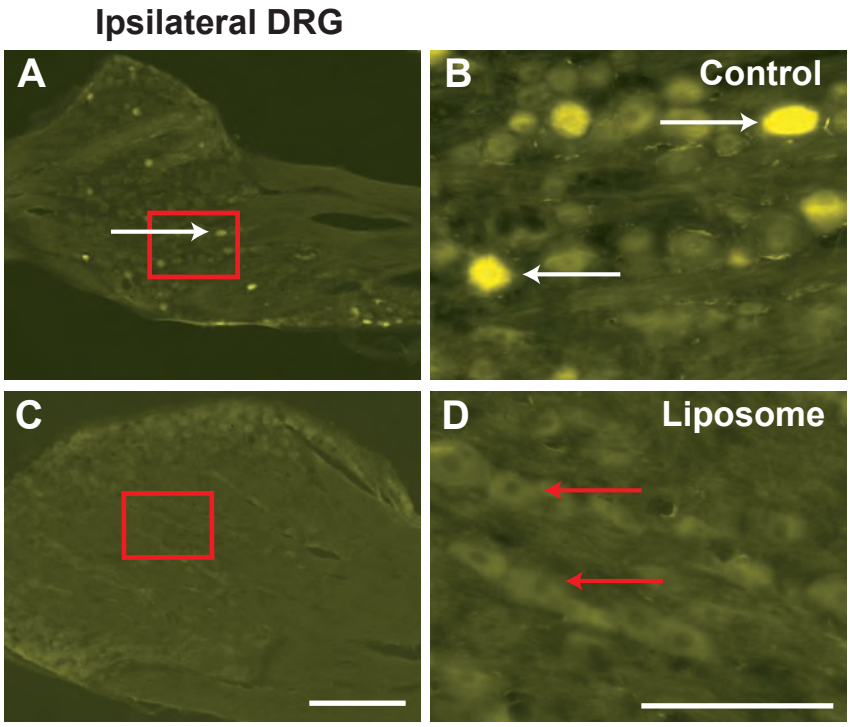



Day 35 (Study Completion)

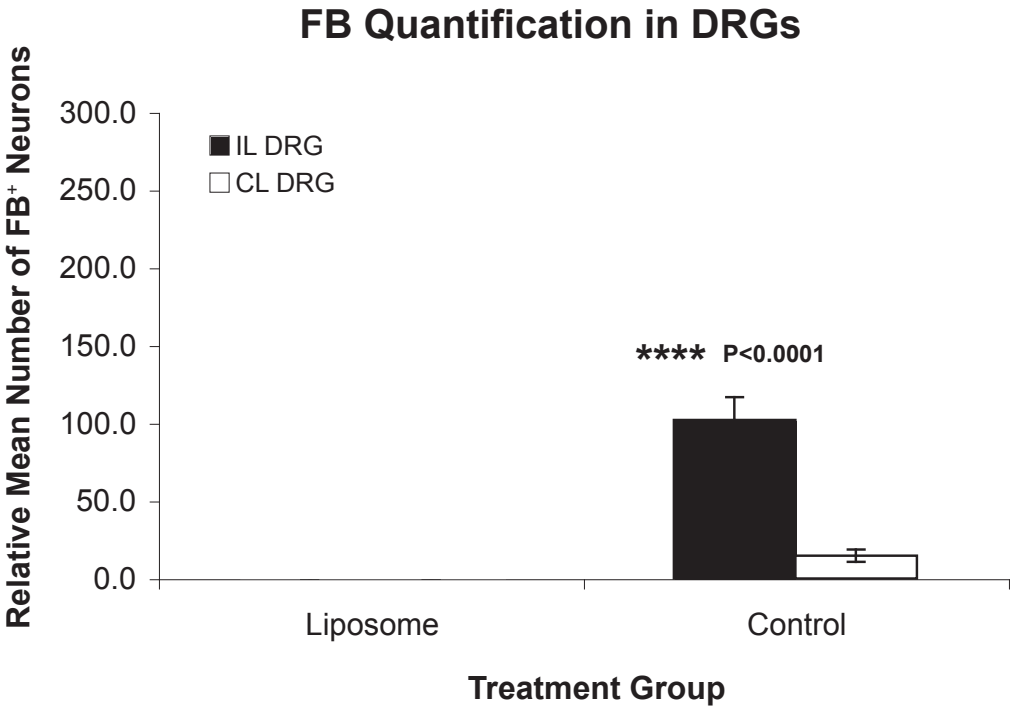


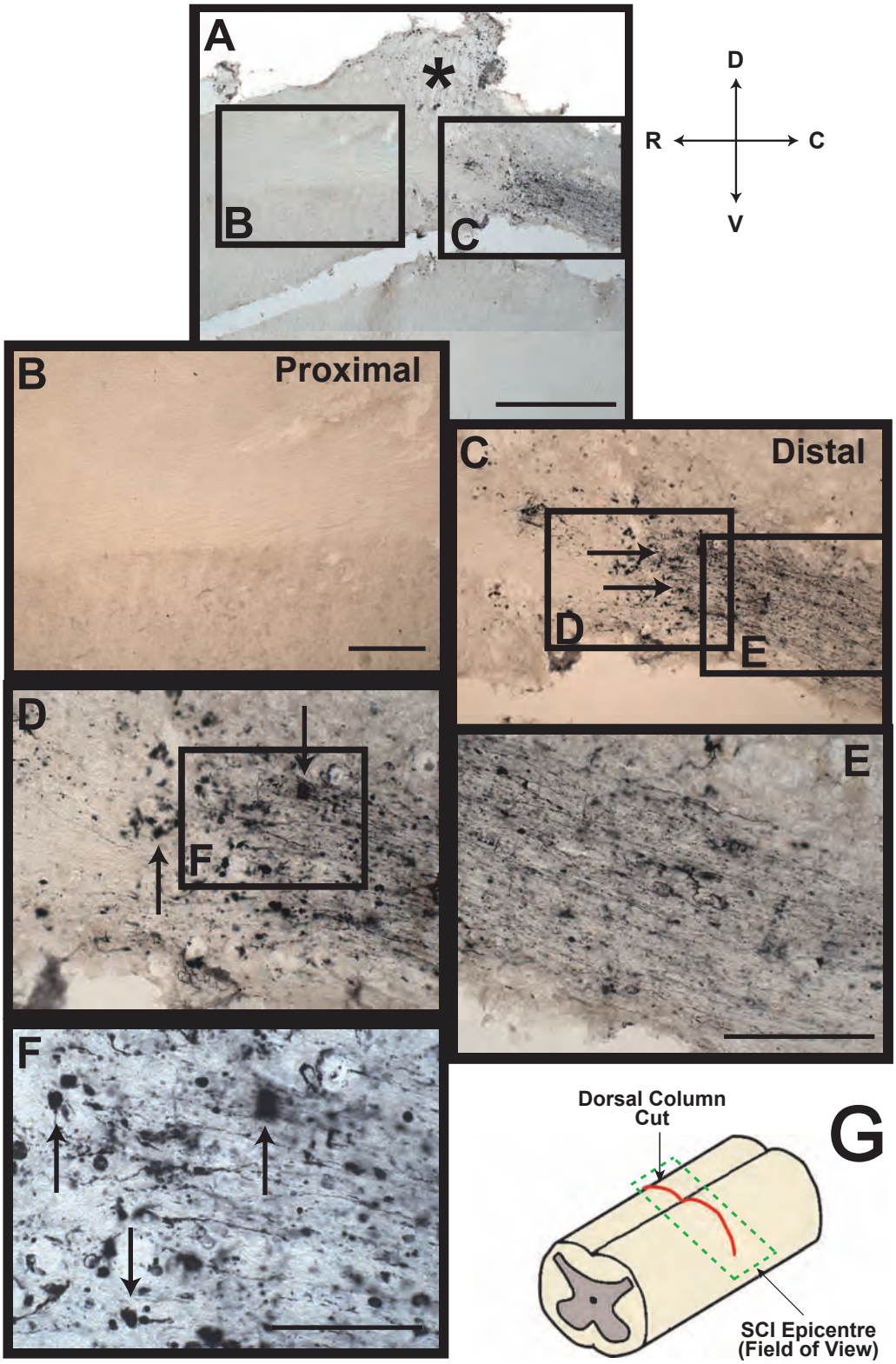

Normal Spleen (untreated)

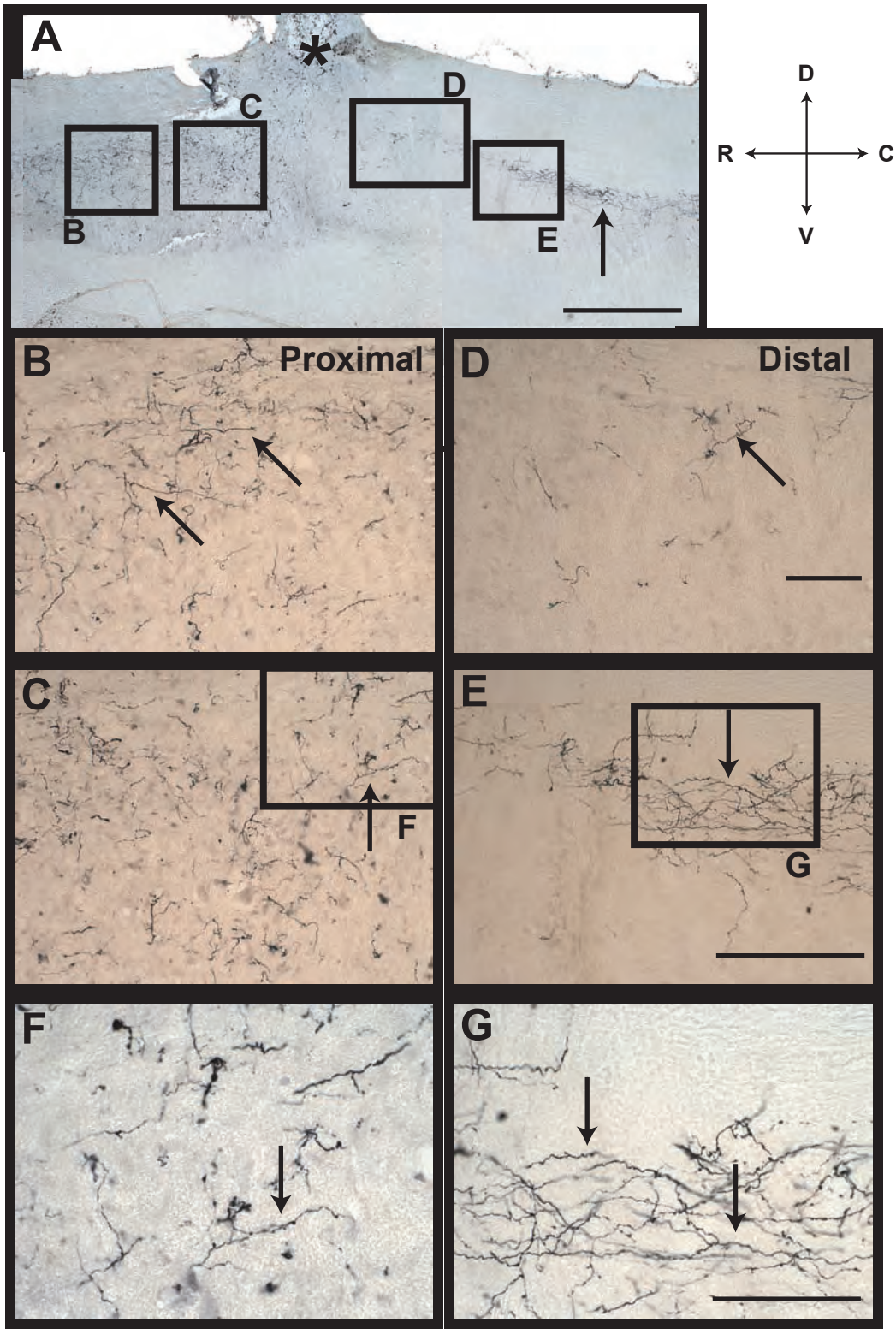



Ipsilateral DRG
A
B
Control
C
D
Liposome

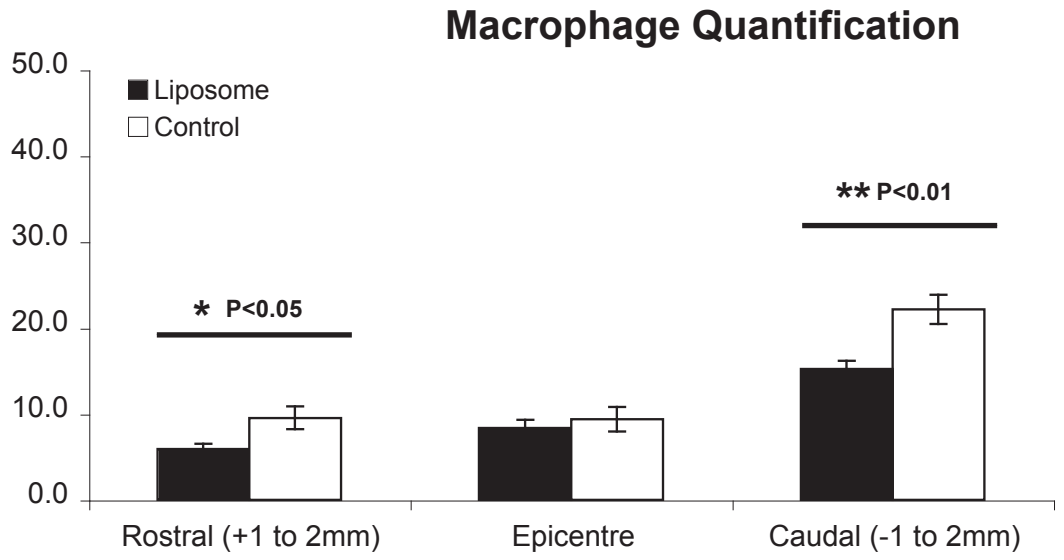
E


FB Quantification in DRGs
Relative Mean Number of FB+ Neurons
300.0
250.0
200.0
150.0
100.0
50.0
0.0
IL DRG
CL DRG
**** P<0.0001
Liposome
Control
Treatment Group

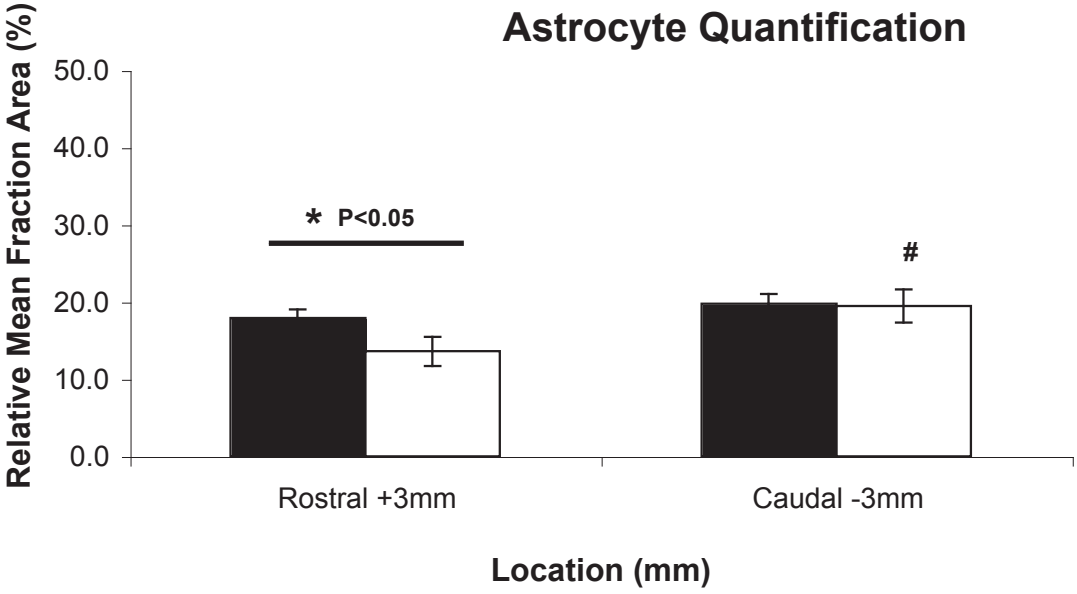

A
*
B
C
D
R
C
V
B
Proximal
C
Distal
D
E
D
F
E
F
Dorsal Column
Cut
G
SCI Epicentre
(Field of View)


A
*
B
C
D
E
D
R
C
V
B
Proximal
D
Distal
C
F
E
G
F
G

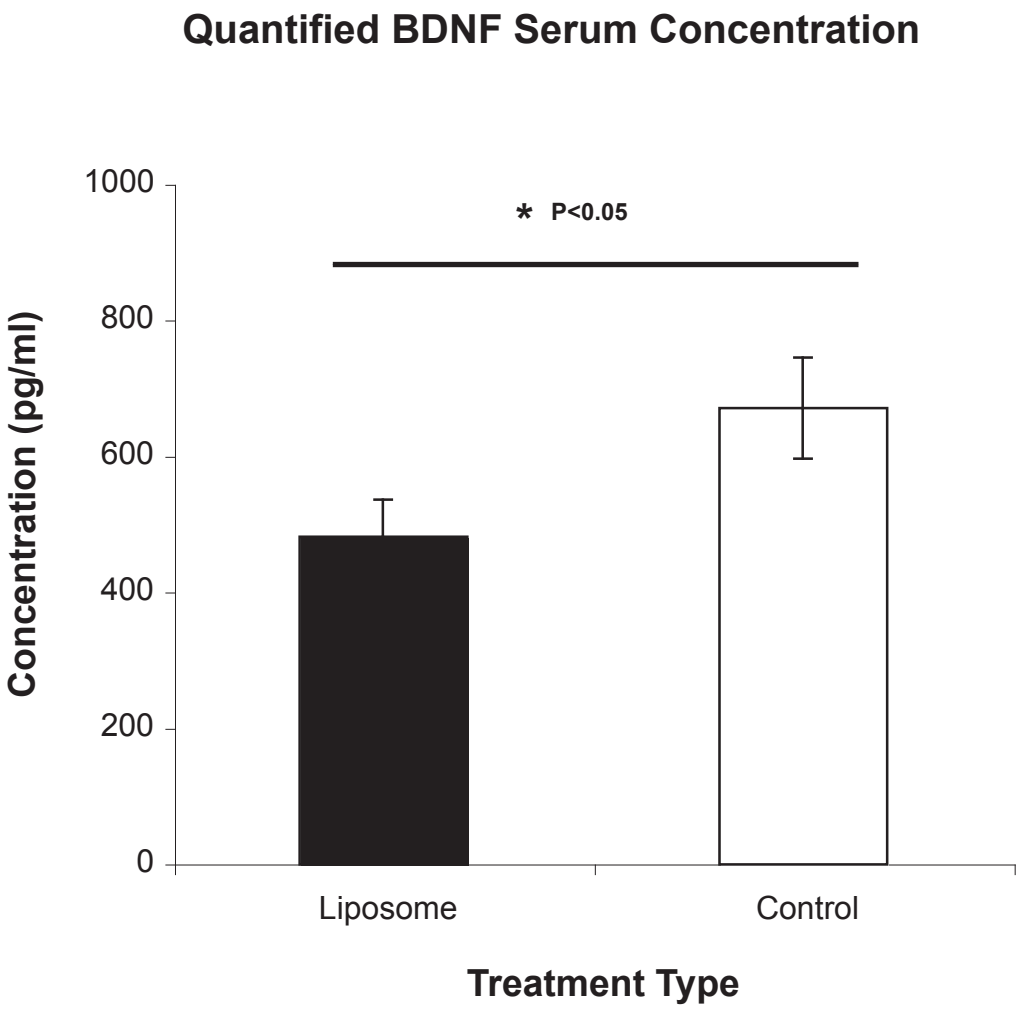
A


Macrophage Quantification
50.0
40.0
30.0
20.0
10.0
0.0
Liposome
Control
* P<0.05
** P<0.01
Rostral (+1 to 2mm)
Epicentre
Caudal (-1 to 2mm)

B


Astrocyte Quantification
Relative Mean Fraction Area (%)
50.0
40.0
30.0
20.0
10.0
0.0
* P<0.05
#
Rostral +3mm
Caudal -3mm
Location (mm)

# A


Quantified BDNF Serum Concentration
1000
800
600
400
200
0
* P<0.05
Concentration (pg/ml)
Liposome
Control
Treatment Type