Intrathecal injection of autologous macrophages genetically modified to secrete BDNF by ex vivo electroporation improves hind limb motor function after thoracic spinal cord injury in rats.

Tadanori Ogata[1], Hideki Horiuchi[1], Tadao Morino[1], Sintaro Yamaoka[1], Hiromasa Miura[2].

[1]Spine Center, Ehime University Hospital, Tohon-city, Ehime 791-0295, Japan

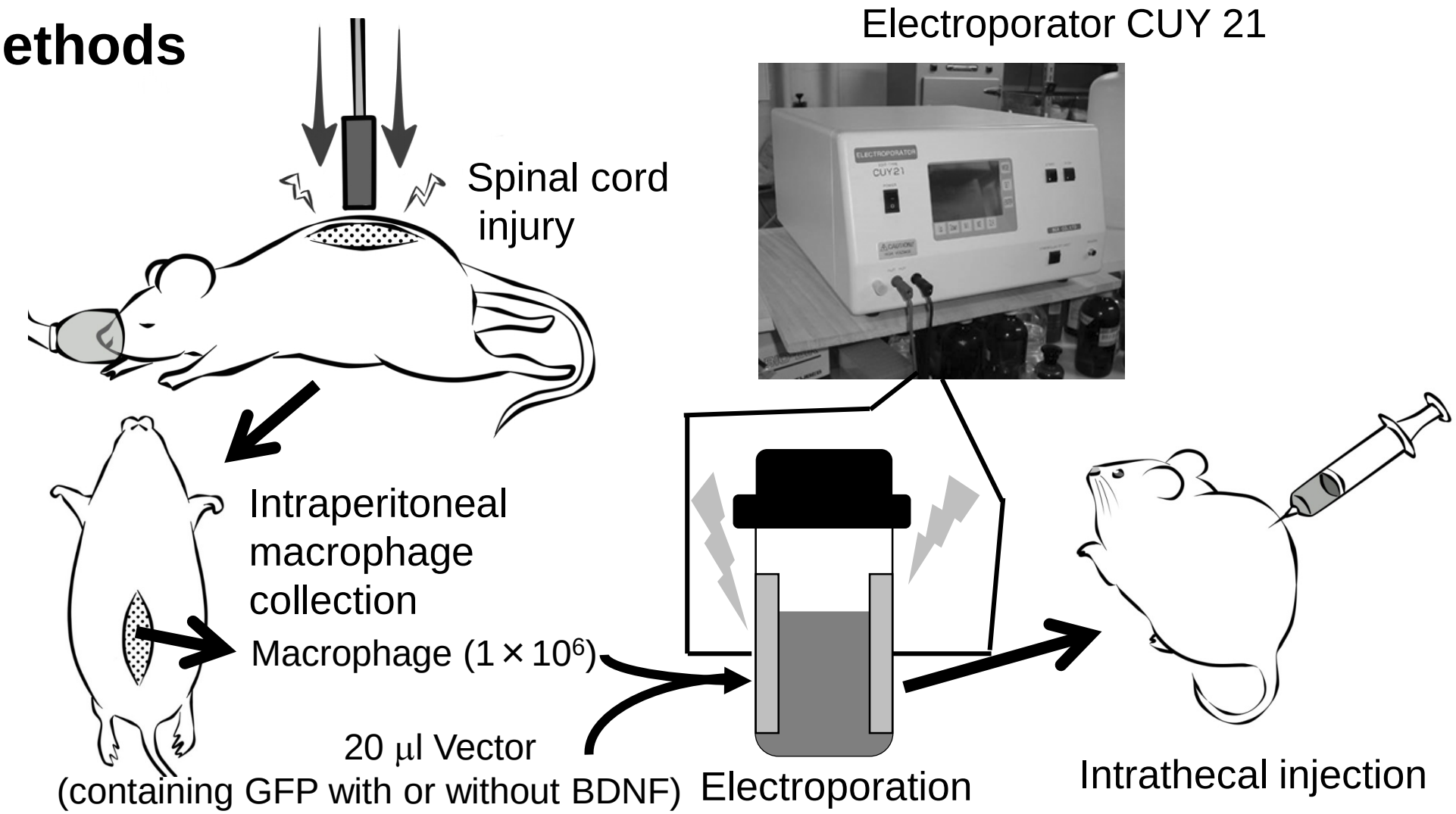
[2]Department of Bone and Joint Surgery, Ehime University School of Medicine, Tohon-city, Ehime 791-0295, Japan

Objectives:

Due to poor circulation in damaged spinal cord tissue, effective application of neuroprotective substances for the treatment of spinal cord injury (SCI) is difficult to achieve by intravenous infusion.

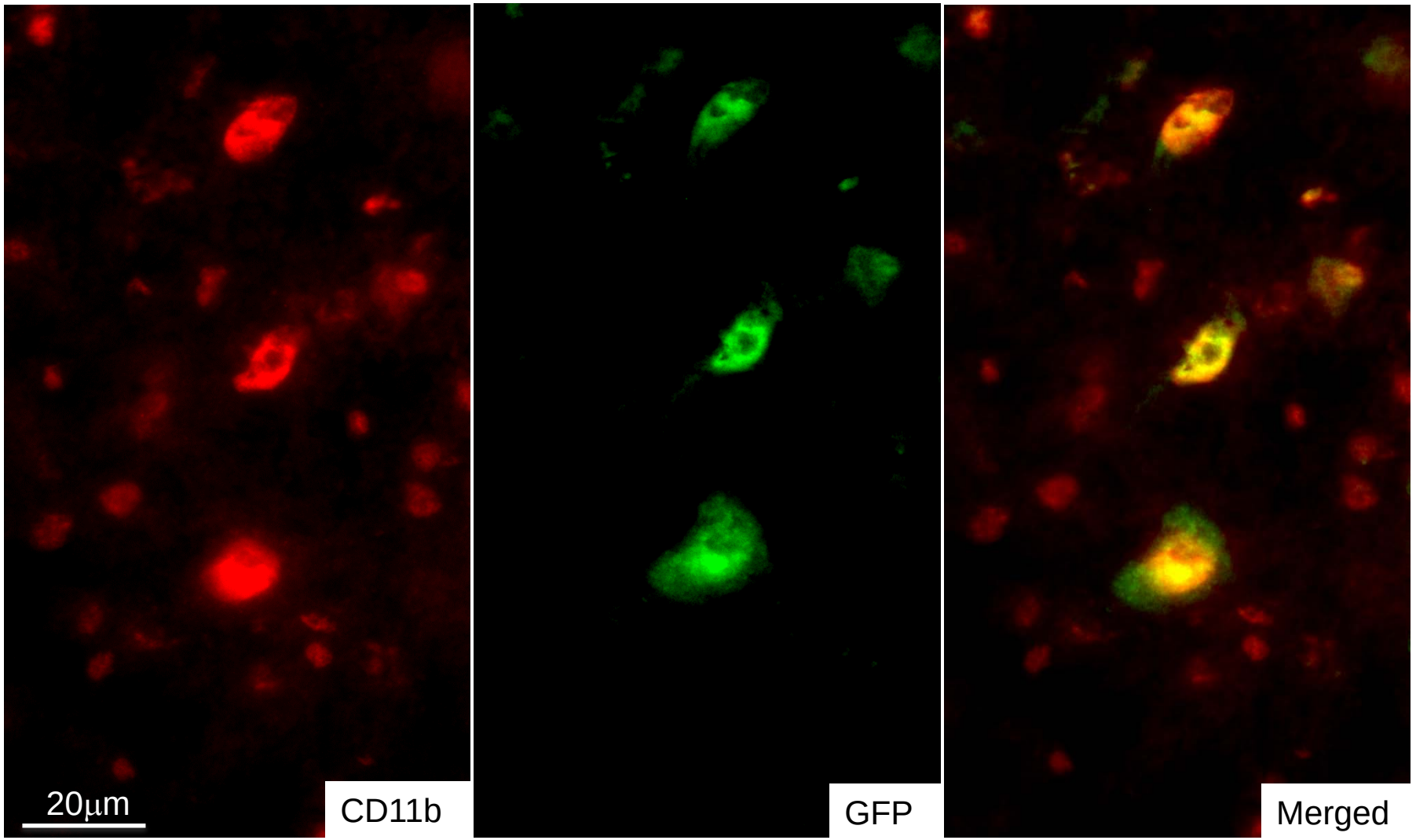
To develop a novel system for substance delivery to damaged ischemic tissue, we focused on the tissue-migration ability of macrophages. In the present study, to develop a new gene therapy for the treatment of SCI, we performed BDNF gene transfer by *ex vivo* electropolation into the autologous intraperitoneal macrophages, and injected them into the subarachnoid space of the experimental rat SCI model.

Methods



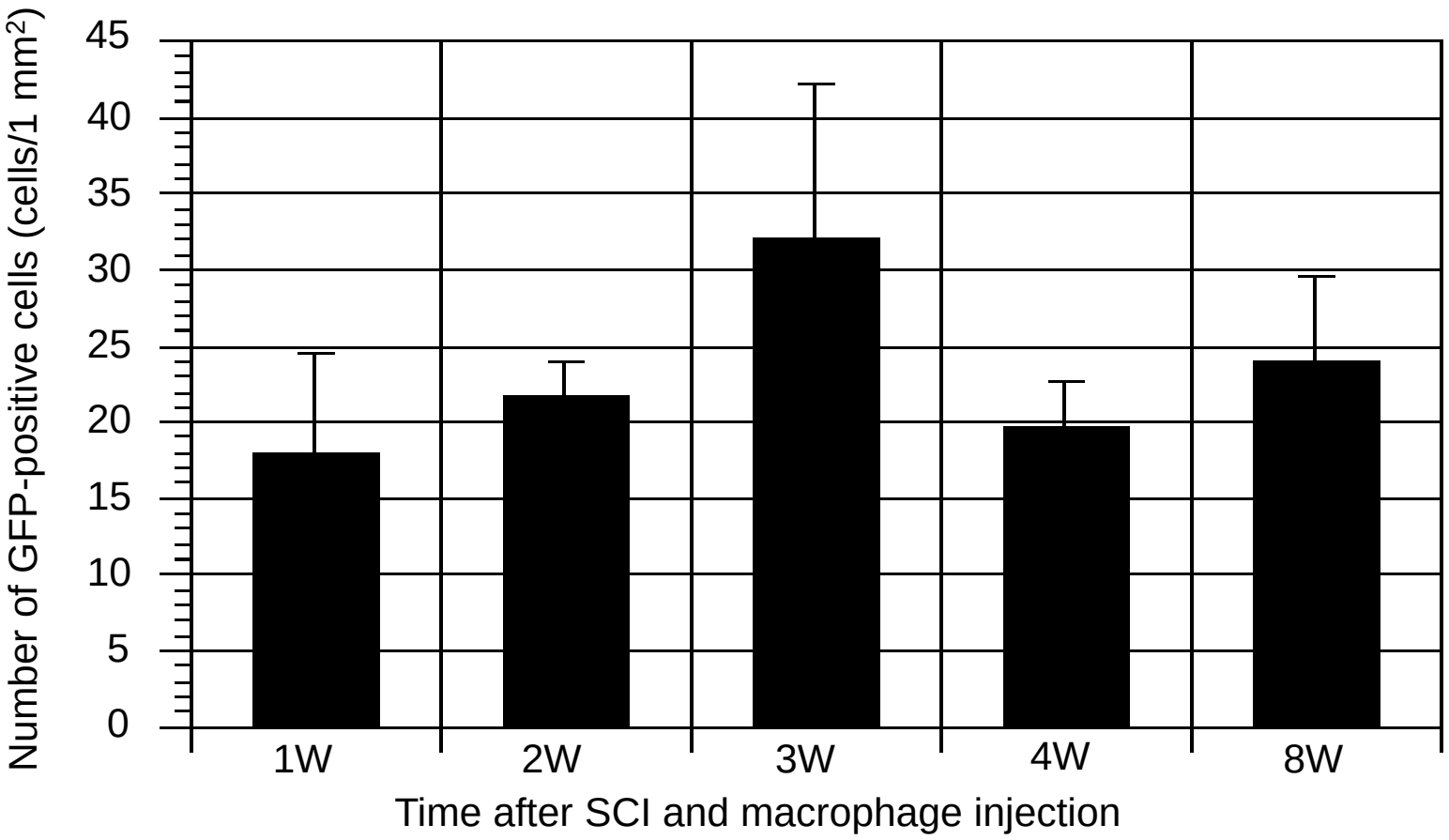
Intra-peritoneal autologous macrophages were harvested from Wistar rats, and then a vector encoding green fluorescent protein (GFP) gene with (BDNF animals) or without (vehicle animals) BDNF gene was transferred into the cells by electroporation. Rat SCI was performed at the 11th thoracic vertebral level using a MASCIS impactor. The gene-transfected autologous macrophages were injected into the subarachnoid space at the 4-5th lumbar intervertebral level.

Results



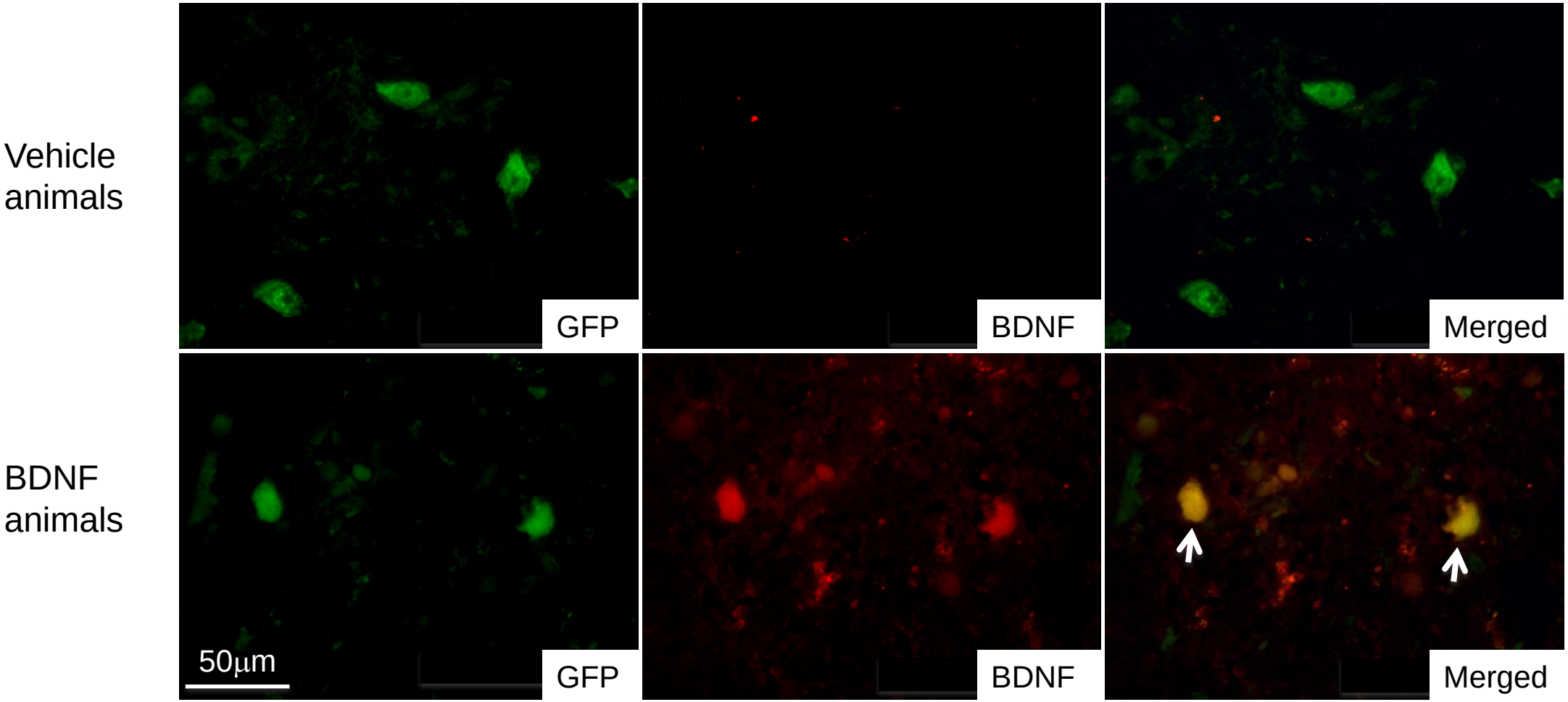
Anti-GFP and anti-CD11b staining of the spinal cord (axial sections in the thoracic spinal cord) two weeks after transplantation in BDNF animals.
The area peripheral to the center of the injury was filled with the cells positive to CD11b, a marker of microglia and macrophage. The cells positive to CD11b and GFP were migrated gene-modified macrophages.

Number of GFP-positive cells in the damaged spinal cord tissue of BDNF animals.



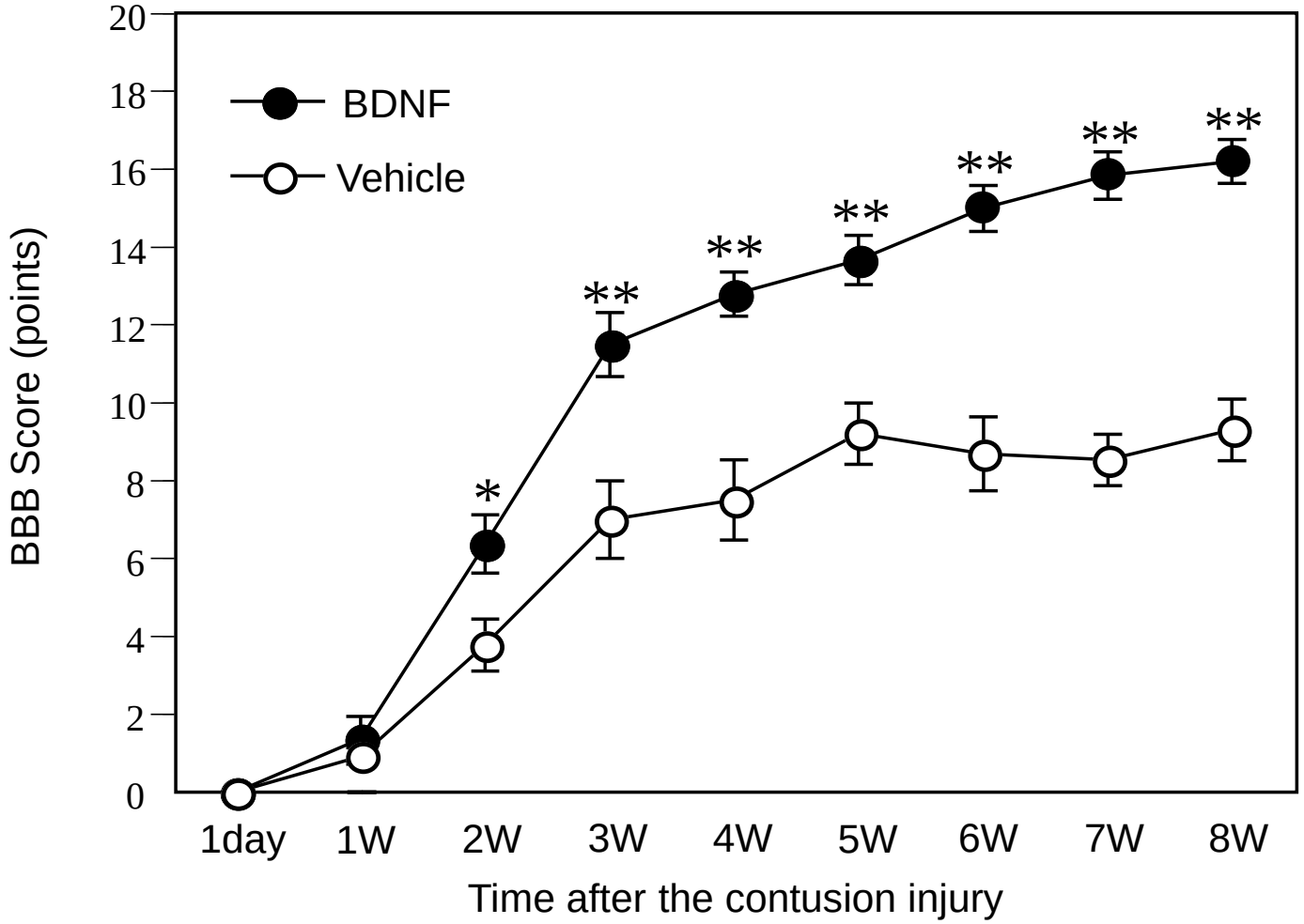
The cells, which revealed significant autofluorescence in the area peripheral to the center of impact injury, were counted. Data are shown in mean ± SEM (n = 6).
At 3 weeks after injury/injection, the number of GFP-positive cells was 32.0 ± 10.3 cells / mm^2 in 10 mm slice. Autofluorescence was detected even 2 months after the injury/injection. The gene-transfected macrophages successfully stayed in the injured area and survived for a long period of time.

Double immunostaining by anti-GFP and anti-BDNF antibodies in the damaged spinal cord two weeks after SCI/macrophage injection.



In the vehicle animal, the cells positive to anti-GFP antibody hardly stained by anti-BDNF antibody. On the other hand, cells positively stained by both anti-GFP antibody and anti-BDNF antibody were observed in the BDNF animals (arrows).

The hind-limb function remarkably improved by the transplantation of gene modified macrophages.

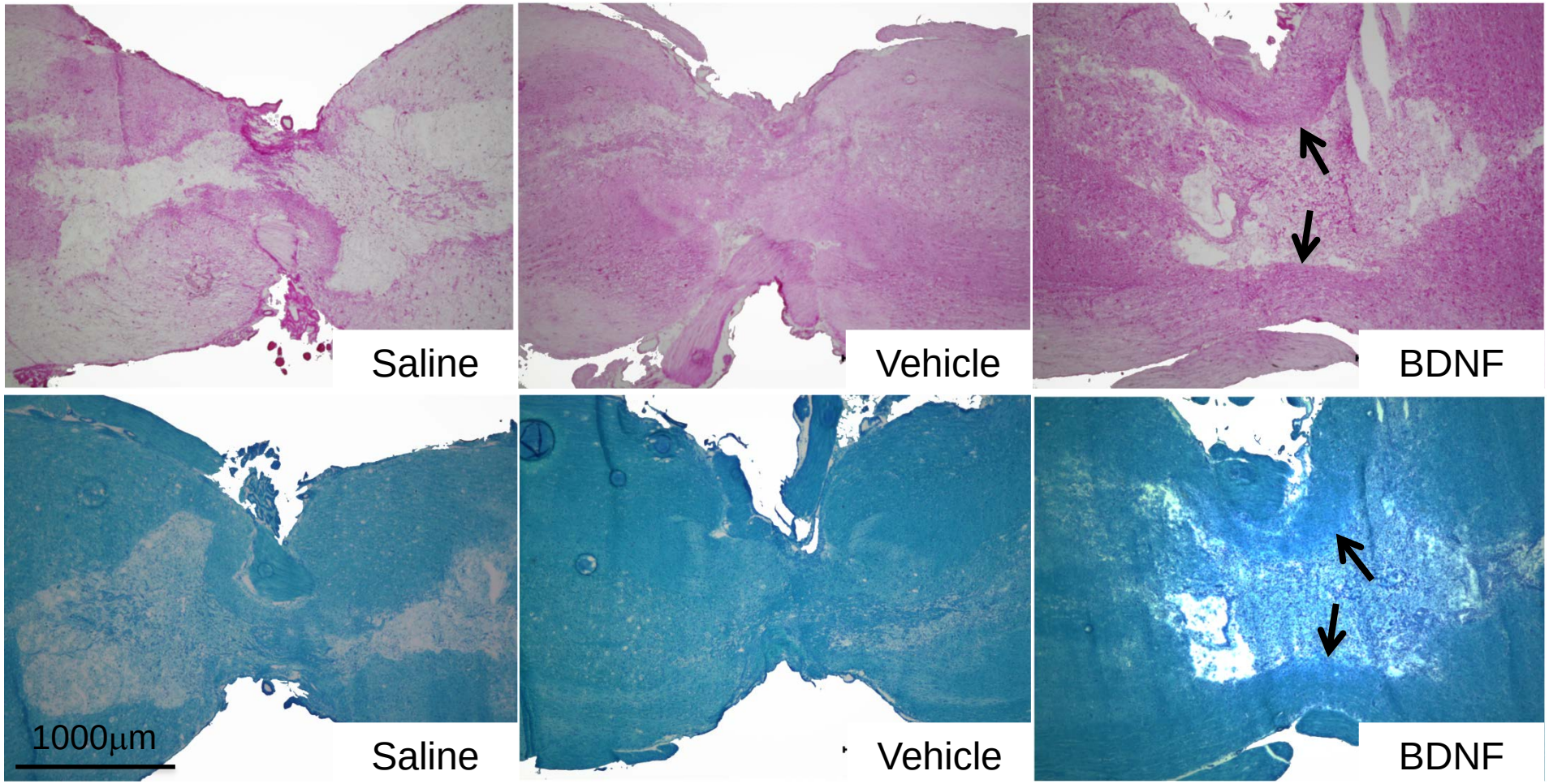


The hind-limb function, evaluated by the BBB scale, in the BDNF (n = 20), was significantly better than that in the vehicle animals (n = 14) from two weeks to eight weeks after the SCI. (*$p < 0.05$, **$p < 0.01$ by Mann-Whitney's U-test).

Preservation of myelin structure in the white matter by transplantation of macrophages genetically-modified to secrete BDNF six weeks after spinal cord injury.

H–E staining

Luxol fast blue (myeline staining)



Saline animals were injected 100 μl of saline instead of vector/cell containing solutions after SCI. Compared with saline animals and vehicle animals, myeline structure in the white matter was apparently preserved in the BDNF animals (arrows).

Conclusions:

Transplantation of gene-transfected autologous macrophages by lumbar puncture successfully improved hind-limb motor function via BDNF production after spinal cord injury.

This method may be a useful substance-delivery system for the treatment of spinal cord injury.

Conflict of interest

The authors declare no conflict of interest.