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Neutrophils rarely invade dorsal root ganglia after peripheral nerve lesions

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Dear Dr. Raine,

We read with interest the recent article by Morin et al. (*J. Neuroimmunol.* 184, 164–171, 2007) which the authors had entitled “Neutrophils invade lumbar dorsal root ganglia after chronic constriction injury of the sciatic nerve”.

The title of this paper surprised us because we have frequently stained using immunohistochemistry for neutrophils in dorsal root ganglia (DRGs) of rats after a variety of sciatic lesions but we consistently fail to find evidence of other than the rarest instance of neutrophil invasion. It is certainly the case that neutrophils, demonstrated using a commercial antibody specific for rat neutrophils (Accurate Chemicals, USA; Perkins & Tracey, 2000) or an antibody to S100 A8 (produced by Carolyn Geczy), are present in increased numbers within the meninges and perineurial membranes around the DRGs and spinal roots. They occasionally appear close to blood vessels within the parenchyma, but it is very difficult to say that they have invaded the parenchyma. It is also notable that they often

appear in the membranes around the DRG (particularly at the sub-arachnoid angle) on the contralateral side and this may occur more frequently than in naïve animals.

We attach micrographs showing typical fields taken from two rats, each one week after a chronic constriction injury, one stained for neutrophils and the other S100A8. These animals showed pain behaviour (see Hu, Bembrick, Keay & McLachlan, 2006). We have confirmed by staining nuclei with DAPI that these cells are polymorphonuclear. We have seen similar images after sciatic transection (n=8) and spinal nerve transection (n=5), as well as chronic constriction injury (n=5).

Among the most abundant proteins expressed by myeloid cells are S100A8 and S100A9, which together form a heterodimer which constitute ~45% of neutrophil cytosolic protein (Hobbs et al., 2003). The antibody to S100 A8 does not cross-react with other S100 proteins and detects all neutrophils in murine bone marrow and blood (Iismaa et al., 1994).

We examined Figure 3 in the paper by Morin et al. particularly carefully and confirm that it shows a distribution of neutrophils similar to what we typically see. Only Figure 3c suggests that one cell lies close to a neurone soma and, in the example shown, it is not clear whether a capillary lies adjacent to the stained cell (to the right of the edge of the figure).

When we looked carefully at the paper, we were confused by the description of the immunohistochemistry procedure (p. 165, para 2.3, l. 13), where the primary antibody RP3 is described as a goat antibody, but we assume that this is a typographical error. Further, the esterase staining kit used, as stated by the supplier (Sigma), is considered specific for cells of the granulocyte lineage, which includes mast cells, which we have occasionally seen in similar locations on the outside of the DRG and spinal roots when staining with a more specific marker.

Our observations suggest that the results obtained by Morin and colleagues, using cells dissociated from DRGs, probably reflect the activity of neutrophils present within the membranous sheaths of the ganglion and associated nerve bundles or perivascularly. This emphasizes the importance of identifying the *in situ* location of cells in order to interpret correctly measurements made of the activity of cells isolated *in vitro*.

Yours sincerely,

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Figure.

Neutrophils lie outside L5 dorsal root ganglia after sciatic nerve lesions.

Section (14 μm thick) from female Wistar rats with chronic constriction injuries applied to the right sciatic nerve 7 days previously. A, B. Section double stained using antibodies to (A) rat neutrophils and (B) the α/β T-cell receptor (TCR) and fluorescent secondary antibodies, showing accumulations of T-lymphocytes in the sub-arachnoid angle (lower left corner) but neutrophil restricted to the meningeal membranes outside the ganglion. C, D. High and low power micrographs of a section from another showing immunofluorescent staining for the intracellular protein S100A8. Calibration in C is 200 μm and applies also to A,B.