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# Punctate Staining as Indirect Evidence for Microglial Ramification

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Microglia are finely ramified cells, uniformly distributed in the tissue of brain and spinal cord. Upon activation, they migrate towards the activating stimulus, dramatically changing their morphology. Their fine processes disappear, and the cells become ameboid. In the present study we demonstrate that in an immunohistochemical staining for microglia, the areas between activated cells become lighter, compared to the noticeable presence of immunoreactive puncta around quiescent cells. This can be interpreted as an evidence for the presence of extremely fine microglial processes around resting cells, which are lost following activation.

*Key words*: microglia, cell processes, activation, immunohistochemistry

## Introduction

Microglia are a population of cells of mesodermal origin, distributed all over the central nervous system. They constitute the intrinsic defense system of the nervous tissue and can act as macrophages and antigen-presenting cells [1]. Through intricate ramifications, each of the microglial cells occupies its own territory [2]. In this territory, microglial cells actively interact with synapses depending on their activity [5].

Following trauma of the nervous tissue, microglial cells are among the first cells to react. They direct their processes towards the site of the lesion, and can actively move their cell body through the tissue. When microglial cells are phagocytic, they alter their morphology by becoming ameboid, rather than ramified [2].

## Aim

The present study aims to illustrate the morphological transformation of activated microglia by demonstrating changes in the immediate vicinity of the cells. These changes can be indirectly related to the arborization of their processes.

## Materials and Methods

We used male adult Sprague-Dawley rats, which were kept in cages under standard conditions. The animals were inhalatory anaesthetized with isoflurane and fixed to a stereotactic frame. Following a punctiform craniotomy using a high speed drill, a 26G steel cannula was lowered into the parietal cortex at the following coordinates, relative to bregma: AP: +0.2, L:-3.5 [3], retained for 5 minutes, and retracted. Extracranial soft tissues were sutured and the animals were left to recover. 7 days after surgery, following terminal anesthesia with ketamine and xylazine, the animals were perfused transcardially with 300 ml ice-cold 4% solution of paraformaldehyde in phosphate-buffered saline. Following perfusion, brains were removed and postfixed in the same fixative overnight. They were transferred to a 20% sucrose solution for cryoprotection and sectioned in 40 µm sections on a freezing microtome. The resulting free-floating sections were processed for immunohistochemistry following a standard protocol. Anti-Iba1 (rabbit; Wako, 019-19741) was used as a primary antibody in a 1:700 dilution. After overnight incubation and washing, the sections were incubated with the secondary antibody (anti-rabbit biotinilated IgG; Dako, E0432). Vectastain ABC HRP Kit was used per manufacturer's directions and 3,3-diaminobenzidyne was employed as a chromogen. The sections were then mounted on glass slides, air-dried, dehydrated and coverslipped.

For evaluation of the specificity of the immunohistochemical reaction, sections were processed in control experiments, in which the anti Iba1-antibody was omitted, the secondary antibody was omitted, the ABC reagent was omitted, or non-immune serum was used instead of anti-Iba1 antiserum.

## Results

Immunoreactive microgliocytes with radiating processes were seen in all brain structures. Intermingled with them, immunoreactive puncta could also be observed, without being properly associated with individual cell bodies or processes (**Fig. 1**).

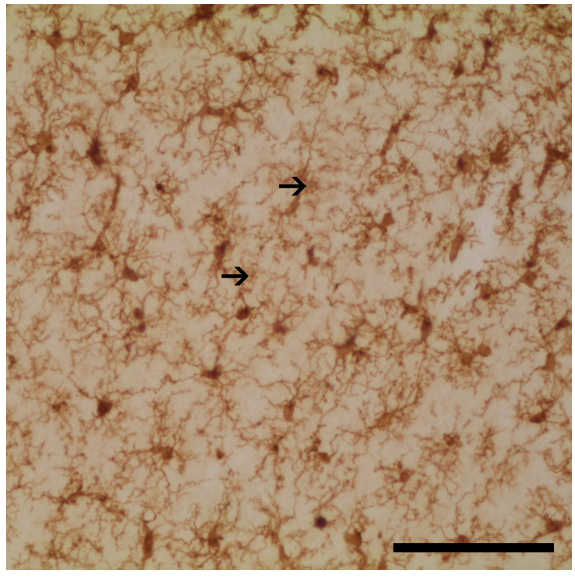

**Fig. 1.** Normal cortical Iba1+ microglia. Microgliocytes with typical radiating processes are evident. Arrows point to some of the existing immunoreactive puncta. Scale bar = 50 µm

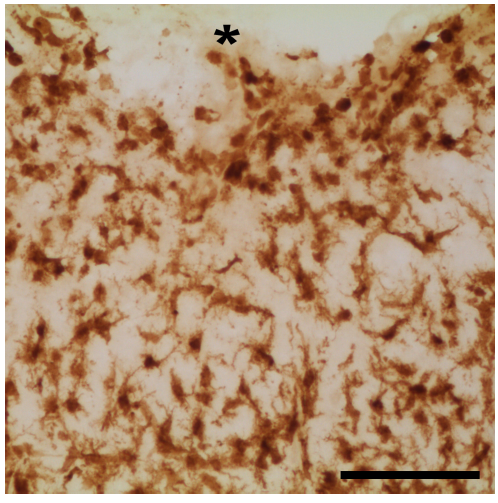

**Fig. 2.** Ameboid Iba1+ microglia adjacent to the cortical impact site (asterisk). Scale bar = 50 µm

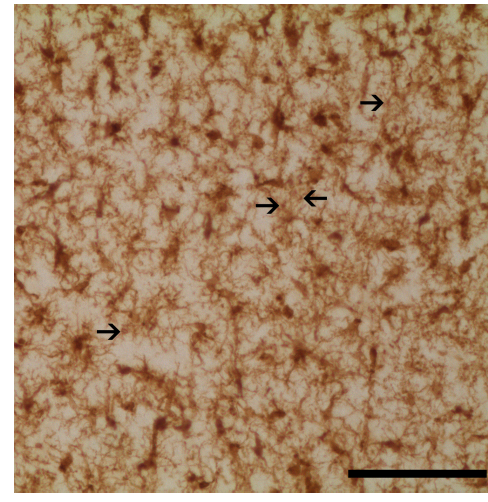

**Fig. 3.** Ramified Iba1+ microglia close to the cortical impact site. Note the intensive punctate background staining (arrows). Scale bar = 50 µm

The cortical impact site of the cannula on the brain surface was seen as a concavity, lined with microglia. The cannula tract itself was visualized as an intensively immunopositive band, crossing dorso-ventrally through the cortex at the coordinates used. The cannula tract was infiltrated with round and ameboid microglia, having a considerable density. In the proximity of the tissue defect ameboid microglial cells were almost exclusively present. Moving away from the site of mechanical influence, ameboid cells were gradually replaced with ramified ones.

Interestingly, the immediate vicinity of the ameboid cells adjacent to the cortical impact site, was almost not stained (**Fig. 2**). The cells were separated by zones without the characteristic immunoreactive puncta, seen between cells close to the impact site (**Fig. 3**) or in the normal cortical tissue, further away (**Fig. 1**).

## Discussion

The present study is based on observations of immunoreactivity of microglia and their alterations following stimulation. Here we attempt for the first time to analyze the nature of the immunoreactive puncta, visible around microgliocytes. Furthermore, we demonstrate these details of the immunohistochemical staining for the first time using Iba1 as a microglial marker.

We interpret our observation of loss of immunoreactive puncta as an evidence for the existence of very fine, delicate ramifications of microglial cells. Despite the considerable thickness of the sections and the good quality of staining, those ramifications cannot be visualized, even under a high magnification. Those extremely thin ramifications can be considered the morphological basis of the extreme sensitivity of microglial cells in their territorial domains [2]. Even though a similar loss of immunoreactivity has been previously reported, a morphological explanation has not yet been provided [4]. Therefore, the present study seems to give new knowledge regarding visual interpretation of microglial immunoreactivity on histological slides.

## Conclusion

Microglial cells, in their quiescent state, are extremely well ramified. Light microscopy, however, cannot demonstrate the finest arborizations of their processes. Despite this limitation, the loss of immunoreactive puncta following activation is an indirect sign for the presence of such arborizations.

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