Microglia markers: Distinguishing Surveillance and Reactive States with Immunohistochemistry

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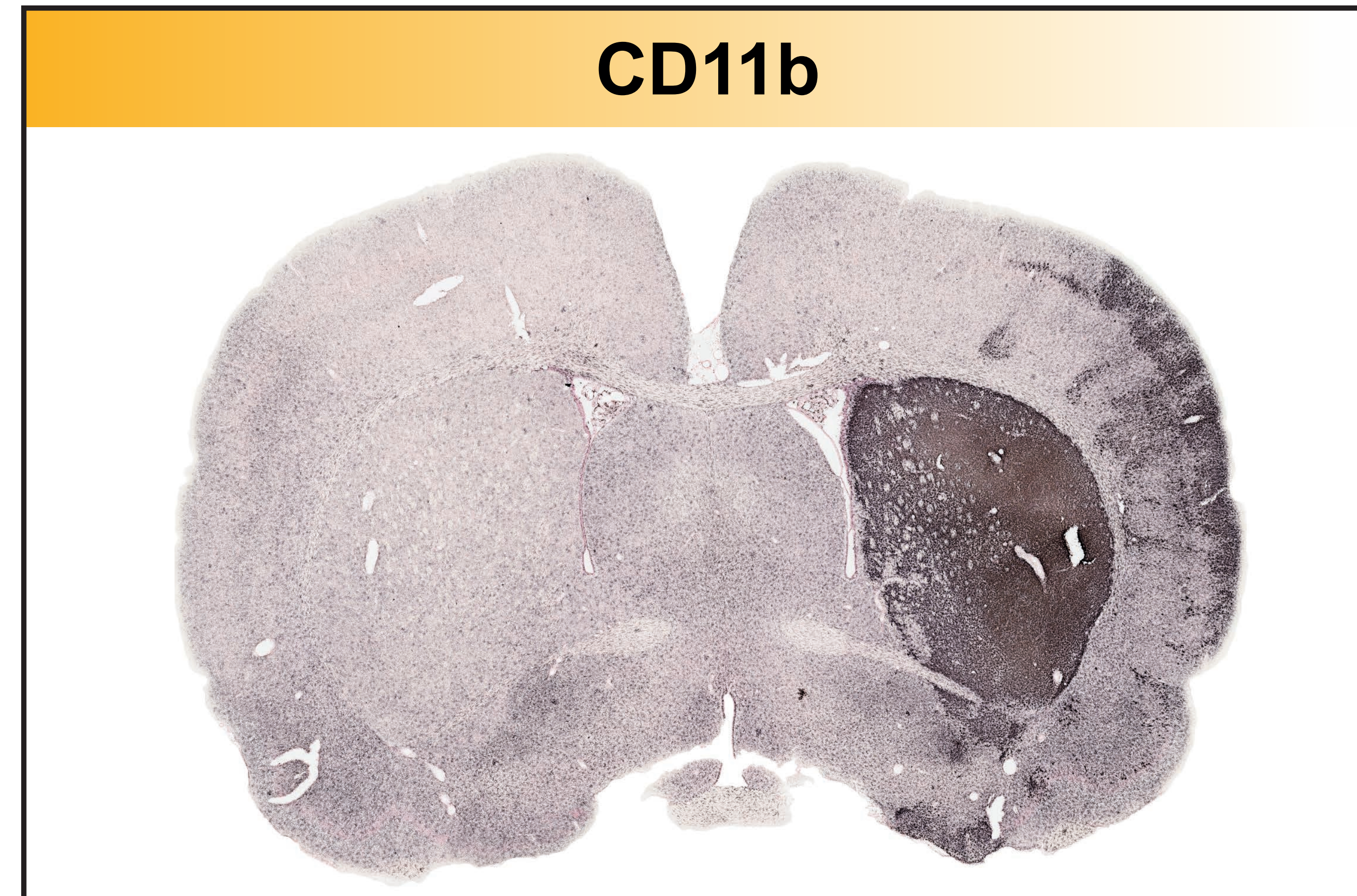[2]Louisiana State University, School of Medicine, New Orleans, LA 70112

Abstract

Assessing neuroinflammation based on immunohistochemical (IHC) displays of microglia and astrocytes is a key dimension in characterizing different disease states and the results of physical or chemical perturbations to the brain and spinal cord. The antibody Iba1 (ionized calcium-binding adapter molecule) has proven to be a real workhorse for assessing the state of microglia in that it is not species restricted and the antigen is robust and not susceptible to long term exposure to formaldehyde fixative. Iba1 allows visualization of the full range of morphologic changes: surveillance states, 'reactive' hypertrophy and amoeboid. The reactive morphologic states span a wide range of extremes: barely different than surveillance state to gnarly processes. To quantify degrees of reactivity across such a gradient of morphologic changes is a considerable challenge. Some image analysis systems can characterize the range by examining individual microglia. To make such assessments across a whole cross section of the brain becomes cumbersome. Other histologic IHC markers for microglia preceded the discovery of Iba1 and others have emerged since. In the course of applying some of these other antibodies to tissues from a rat MCAO stroke model we found that the OX42* clone of CD11b antibody robustly stained reactive microglia in and around the affected ischemic zone in rats but microglia on the contralateral side were visible to varying degrees suggesting different degrees of expression of the antigen detected by OX42. This 'built in' attribute of improved signal to noise allows image analysis methods to parse out different degrees of reactivity and provide quantitive data. We strove to find a clone of CD11b that would perform similarly in mice. Only one of those that we tried (Life Sciences LSC #294688) came close to matching the performance of the OX42.

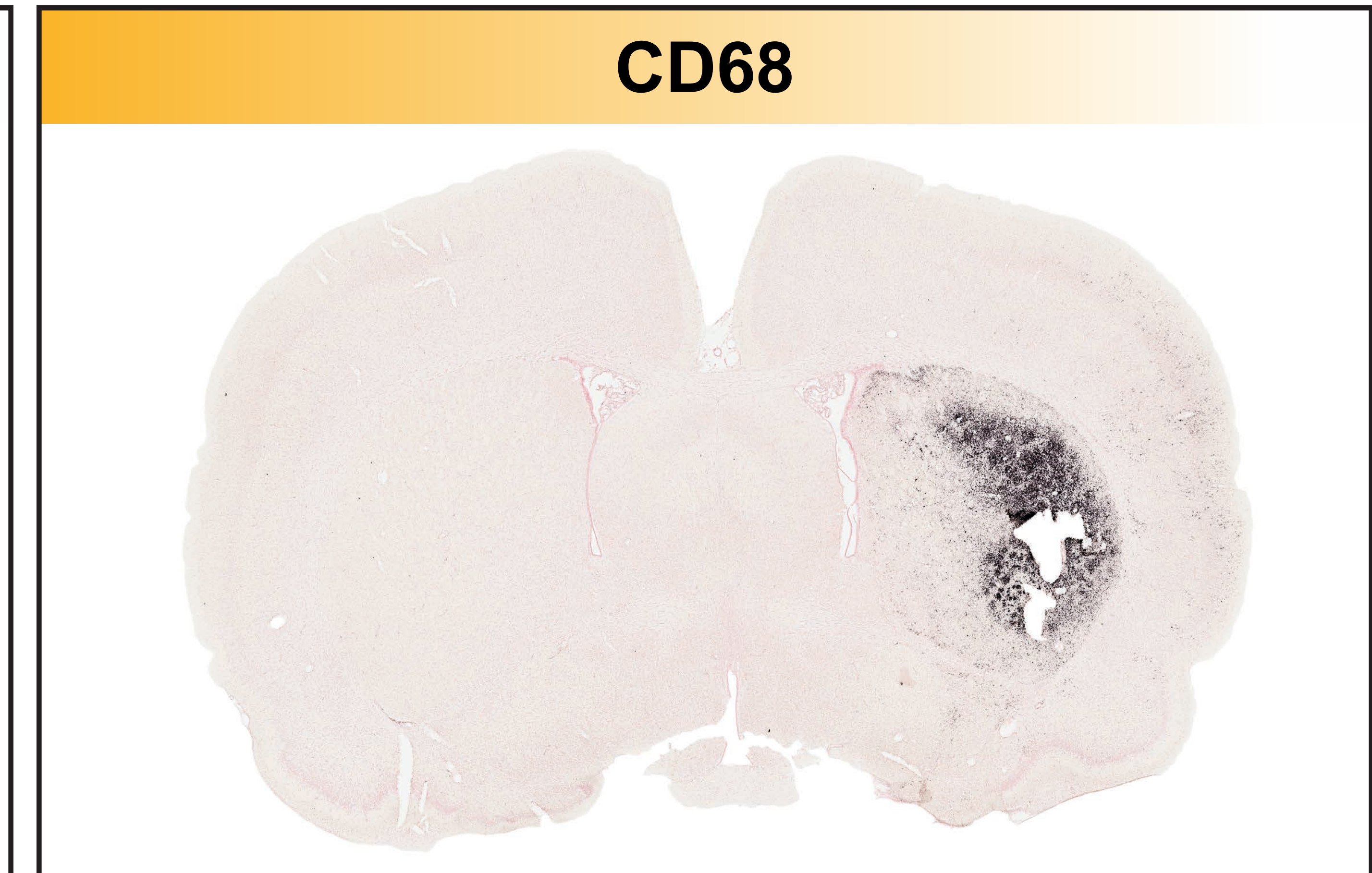* OX42 tends to be sensitive to time in formaldehyde. Perfusion and overnight fixation has proven sufficient.

Rat (Stroke)
Acutely Induced

Mouse (AD)
Chronic State

Iba1
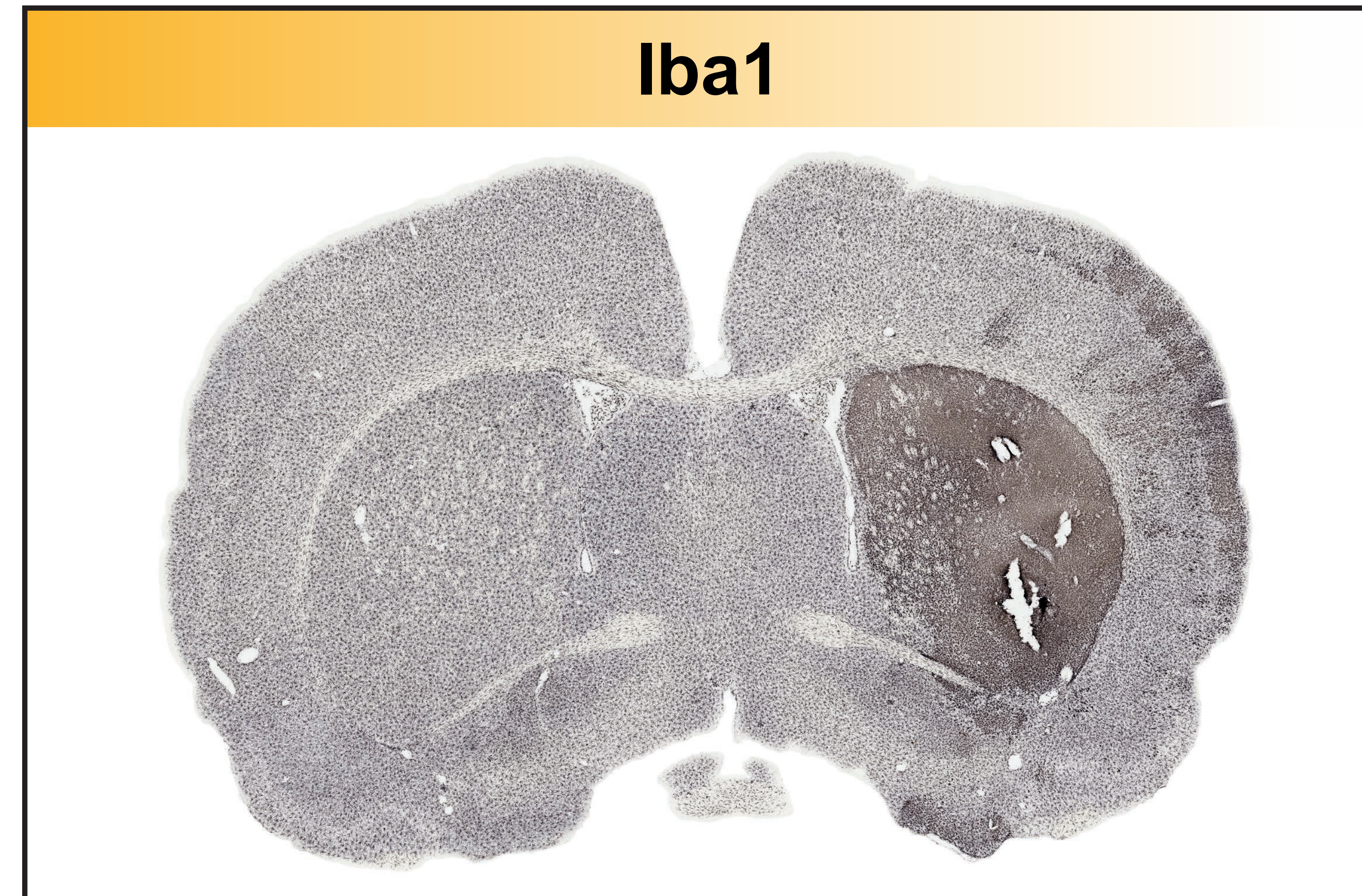
An MCAO induced stroke in rat induces widespread hypertrophy of microglia in the right hemisphere (72 hr. survival). This reactivity of microglia is clearly massive in striatum of this coronal section with lesser range of effect elsewhere in the right hemisphere-e.g. cortex. In the left hemisphere there is a range of reactivity as judged by morphology. Quantifying this by image analysis across any region has proven difficult.

In this Alzheimer disease mouse model, the location of amyloid plaques is revealed by microglia staining with Iba1. Hypertrophied microglia are also distributed throughout the brain. This mouse had also been intracerebrally injected with a test article in the cortex of the right hemisphere a seen by the line of hypertrophied microglia. In the region adjacent to the injection site increased staining reflecting microglia hypertrophy is also visible. Staining of 'normal' microglia intereferes with distinguishing minimally affected zones from those unaffected using image analysis.
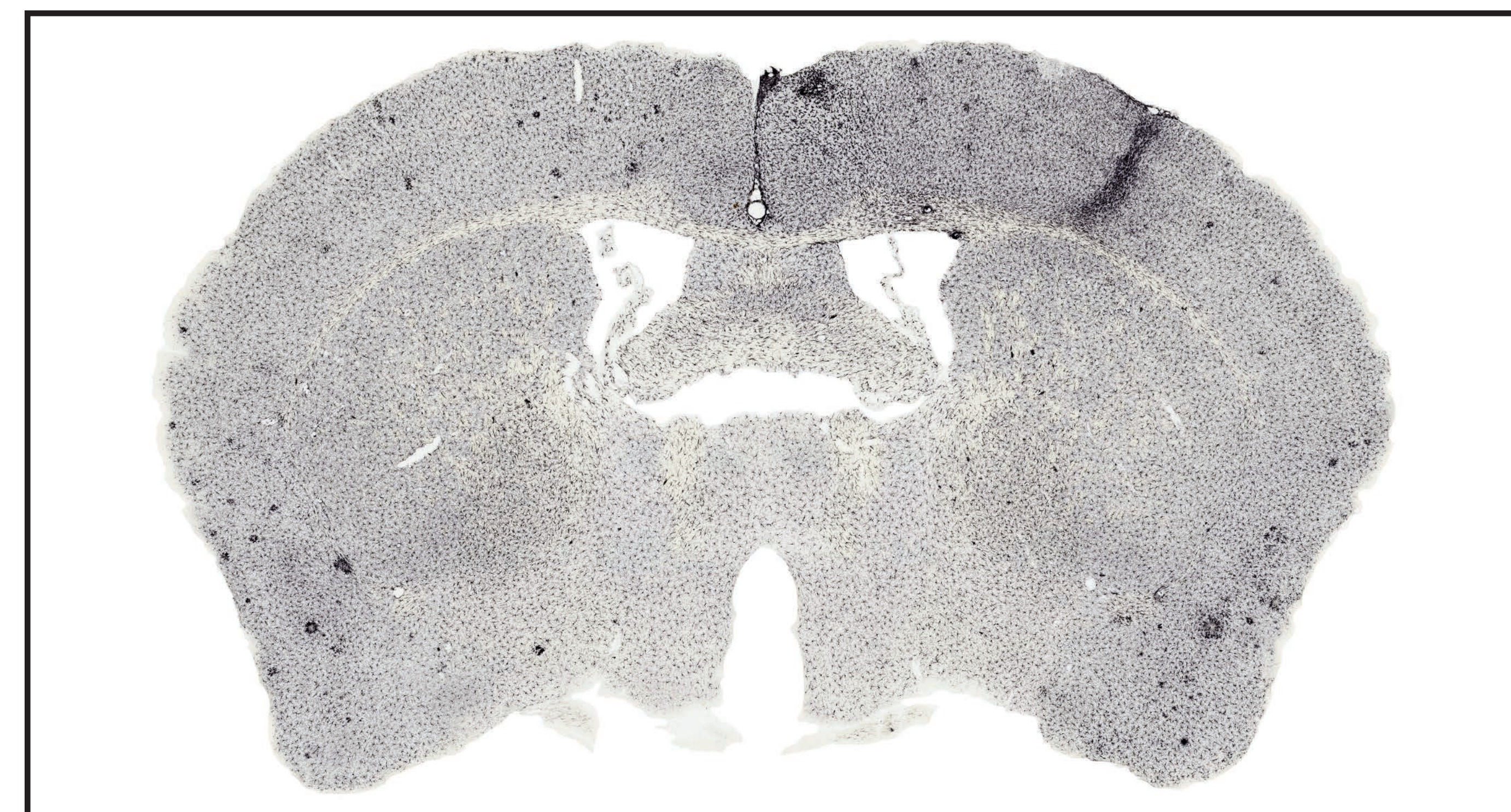
CD11b

In deploying different antibodies to distinguish levels of reactivity we found that one of the clones for CD11b, OX42, appeared to distinguish highly hypertrophied microglia from those less reactive and normal.
Take note of the cortex left and right. Intense staining marks the right side, and on the left the corresponding area of cortex is mildly stained, reflecting symmetry of intrahemispheric connections. These features offer a better opportunity for quantitative image analysis than afforded by Iba1.

We searched for an antibody against a clone of CD11b that would perform as OX42 does in rat and found the antibody #LSC 294688 from Life Sciences to be promising. Microglia not involved with plaques or the injection site stained weakly, if at all.
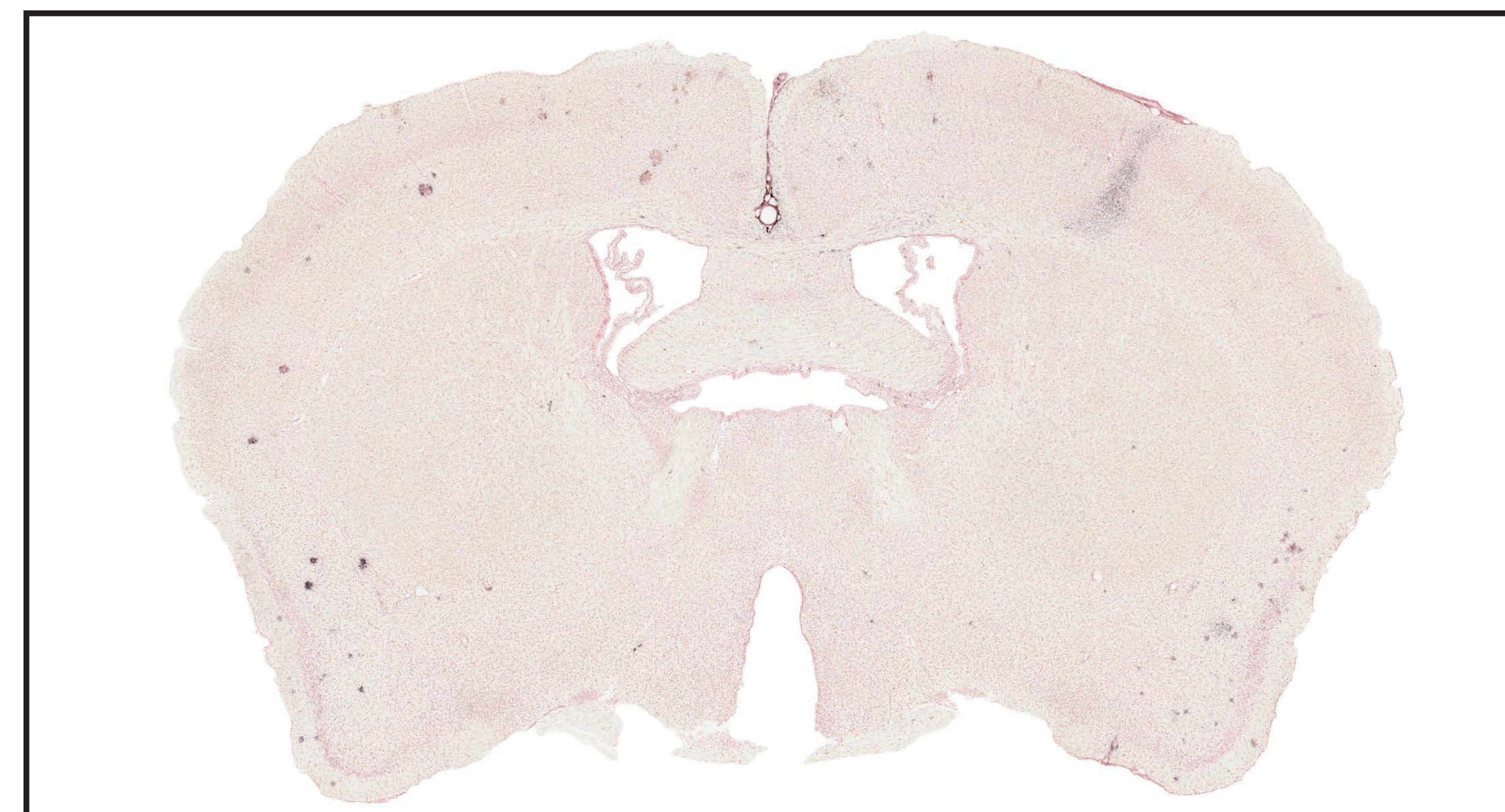
CD68

A commonly used antibody to show 'reactive' microglia in rat is the ED1 clone of CD68. Here we used the polyclonal Rockland antibody for CD68 (#600-401-R-10), which stains positively in both mouse and rat tissue. As can be seen in this image, the staining is confined to the most heavily affected area in the right hemisphere.
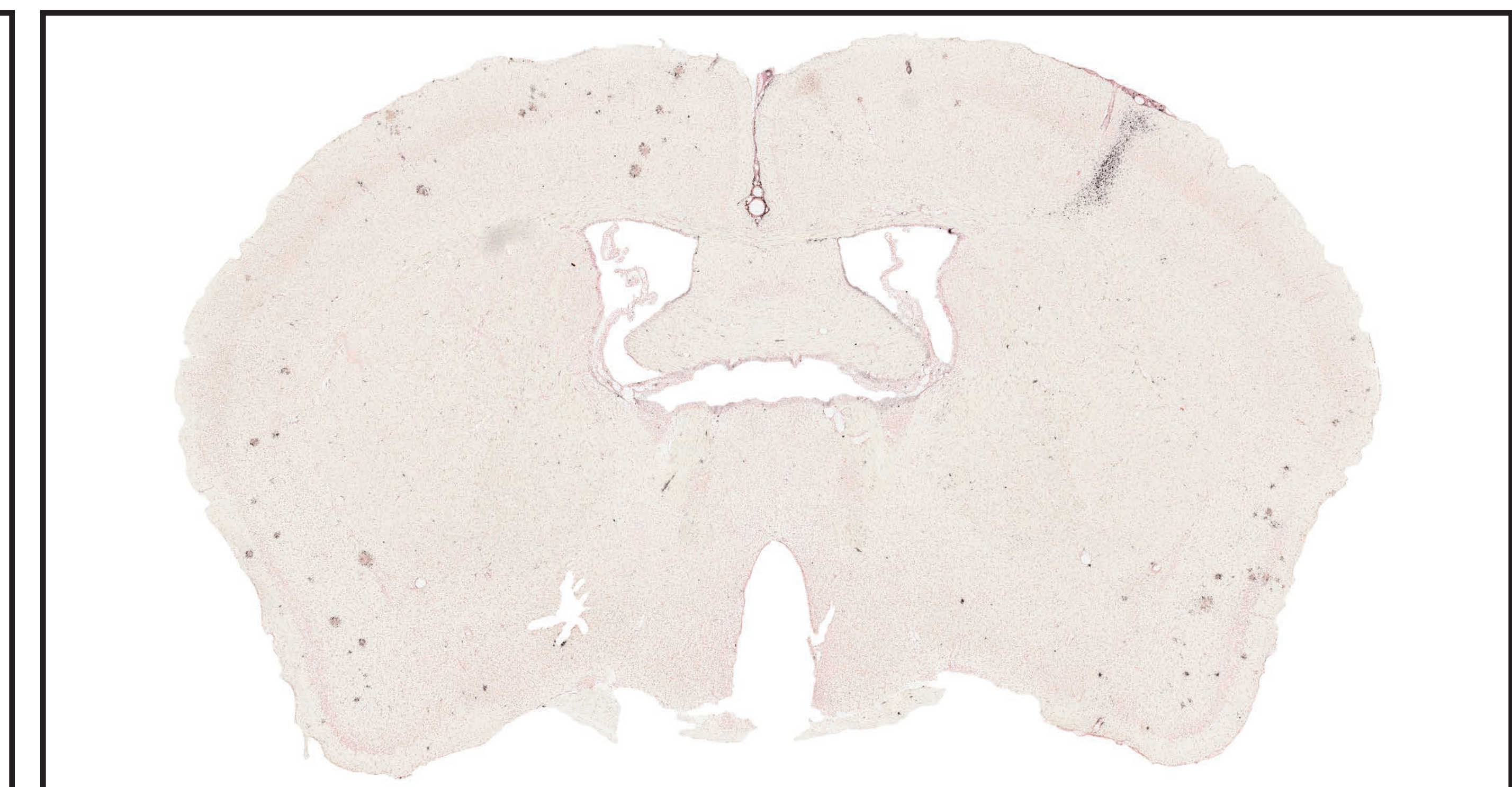

Instead of the usual FA11 clone of CD68 appropriate for mice we used the Rockland polyclonal (# 600-401-R10) and found a similar staining pattern as for the LSC antibody for CD11b.

Comments & Conclusions

Background
- The most general trait of reactive microglia is hypertrophy as shown with the Iba1 IHC in the Rat stroke model. Different degrees of hypertrophy are manifest as enlargement of cell bodies and processes.
- Quantifying different degrees of hypertrophy based on Iba1 staining is challenging due to a shallow gradient of staining intensity.
- Revealing a range of degrees of hypertrophy by IHC with different antibodies can provide a steeper gradient (greater signal-to-noise) of visible changes amenable to quantification.

Overview
- Two different types of induction of reactivity of microglia are presented. Acute or Chronic induction of microglia hypertrophy may reveal different characteristic profiles of reactivity.

Results
- In the MCAO acutely induced lesion there is a marked difference in the appearance of three different antibodies.
- For the chronic disease state of the AD mouse there is less of a difference between CD11b and CD68 and yet, the Iba1 displays widespread reactivity. Perhaps this reflects the mixed proinflammatory and anti-inflammatory states.

Outlook
- With the discovery of more IHC probes to reveal different types or degrees of microglia reactivity, it will be possible to quantify each and build characteristic profiles of reactivity.