

ASTROCYTES ARE REMARKABLY VULNERABLE TO ISCHEMIC/ANOXIC INJURY

Viktória Éva Varga¹, Ádám Nyúl-Tóth², Réka Tóth¹, Ferenc Bari¹, István Krizbai², Eszter Farkas¹, Ákos Menyhárt¹

¹University of Szeged, Faculty of Medicine, Department of Medical Physics and Informatics, Szeged, Hungary
²Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

INTRUDUCTION AND RESEARCH GOAL

Glutamate excitotoxicity is responsible for cell death and lesion progression in multiple cerebrovascular disorders, such as ischemic stroke. Anoxic depolarization leads to extensive glutamate-release but its contribution to excitotoxicity remains unclear. Further, while the impact of glutamate excitotoxicity on neurons has been much investigated, less is known about the vulnerability of astrocytes.

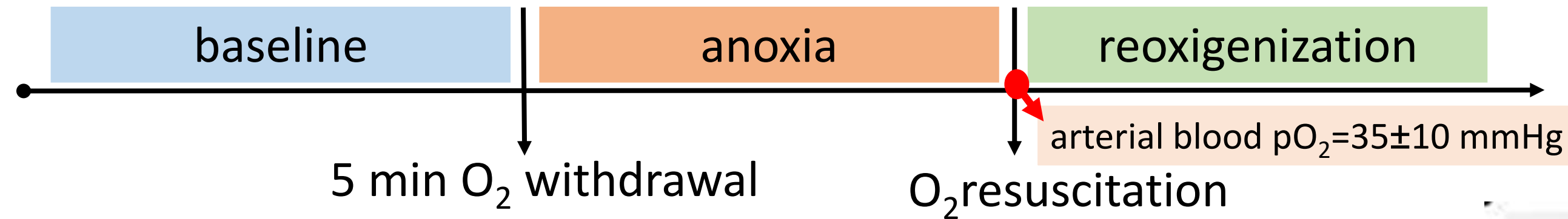
We aimed to compare the susceptibility of astrocytes and neurons to ischemia/anoxia regarding apoptotic cell death in the most sensitive brain regions.

MATERIALS AND METHODS

Animals: old (18 months) male Sprague-Dawley rats (n=5)

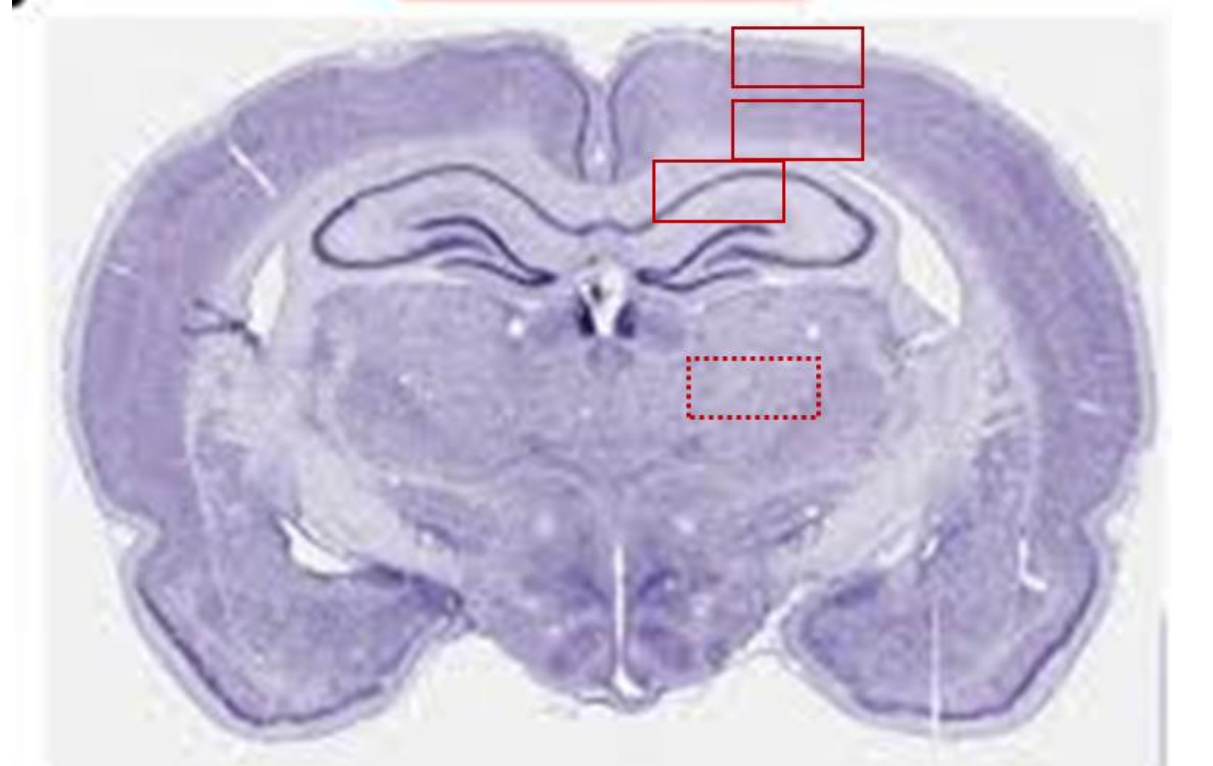
Anesthesia: 1.5-2 % isoflurane in N₂O:O₂= 2:1

Experimental protocol:



- Preparation of 20 µm coronal brain sections with a freezing microtome
- Immunofluorescent staining:
 - Cleaved caspase-3 (CC3) → apoptosis
 - GFAP → astrocytes
 - NeuN → neurons
- Cell counting (ImageJ)

transcardial perfusion with physiological saline and 4% paraformaldehyde



RESULTS

Our results show that astrocytes are more susceptible to anoxia-induced apoptotic cell death, than neurons. In the topical and deep cortical layers, as well as in the hippocampal CA1 region and the striatum less than 10% of the NeuN positive neurons were engaged in apoptosis, meanwhile more than half of the GFAP positive astrocytes expressed CC3 in every observed region. In addition, the presence of CC3 near the nucleus of astrocytes was clearly associated with GFAP-degradation.

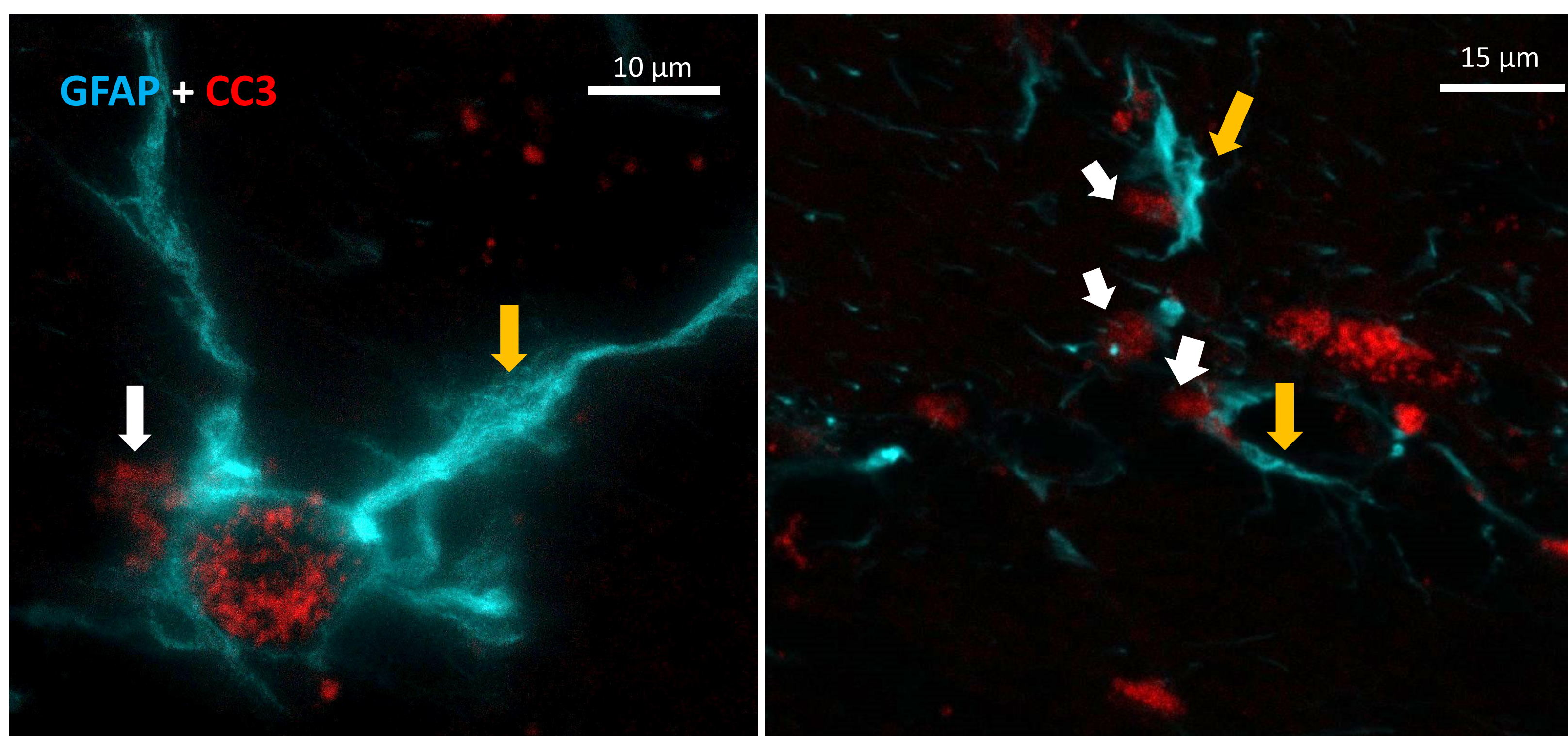


Fig. 1. Subcellular distribution of GFAP and CC3 in astrocytes visualized with STED microscopy (20 µm Z-projection; 100x objective). As the active form of caspase-3 manifests in the perinuclear region, the GFAP immunopositivity dissipates (white arrows), whereas in the absence of CC3 colocalization, GFAP fibers remain intact (yellow arrows).

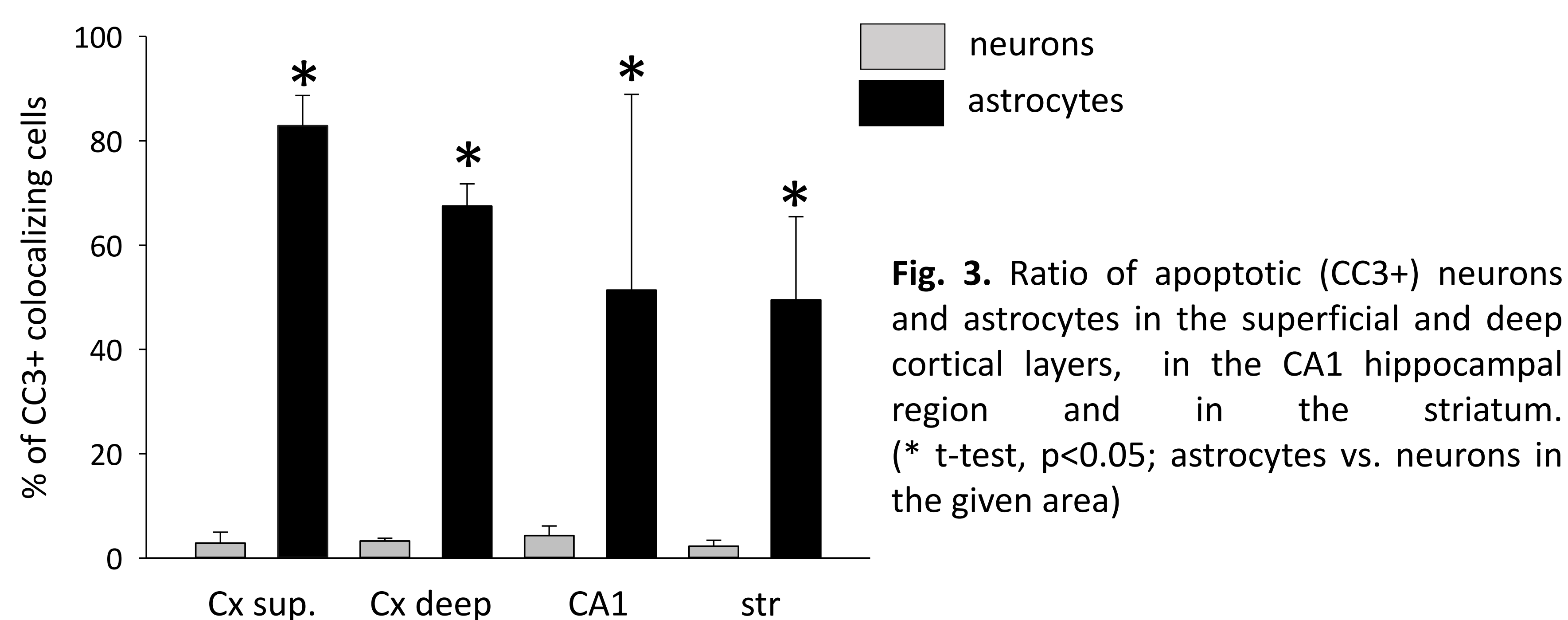


Fig. 3. Ratio of apoptotic (CC3+) neurons and astrocytes in the superficial and deep cortical layers, in the CA1 hippocampal region and in the striatum. (* t-test, p<0.05; astrocytes vs. neurons in the given area)

DISCUSSION AND CONCLUSION

While astrocytes are thought to be resistant to ischemic stress more than neurons, our data suggest that ischemia in combination with severe anoxia induces apoptosis predominantly in astrocytes rather than neurons in the old rodent brain. Meanwhile our data do not exclude the possibility of a more extensive necrotic loss of neurons.

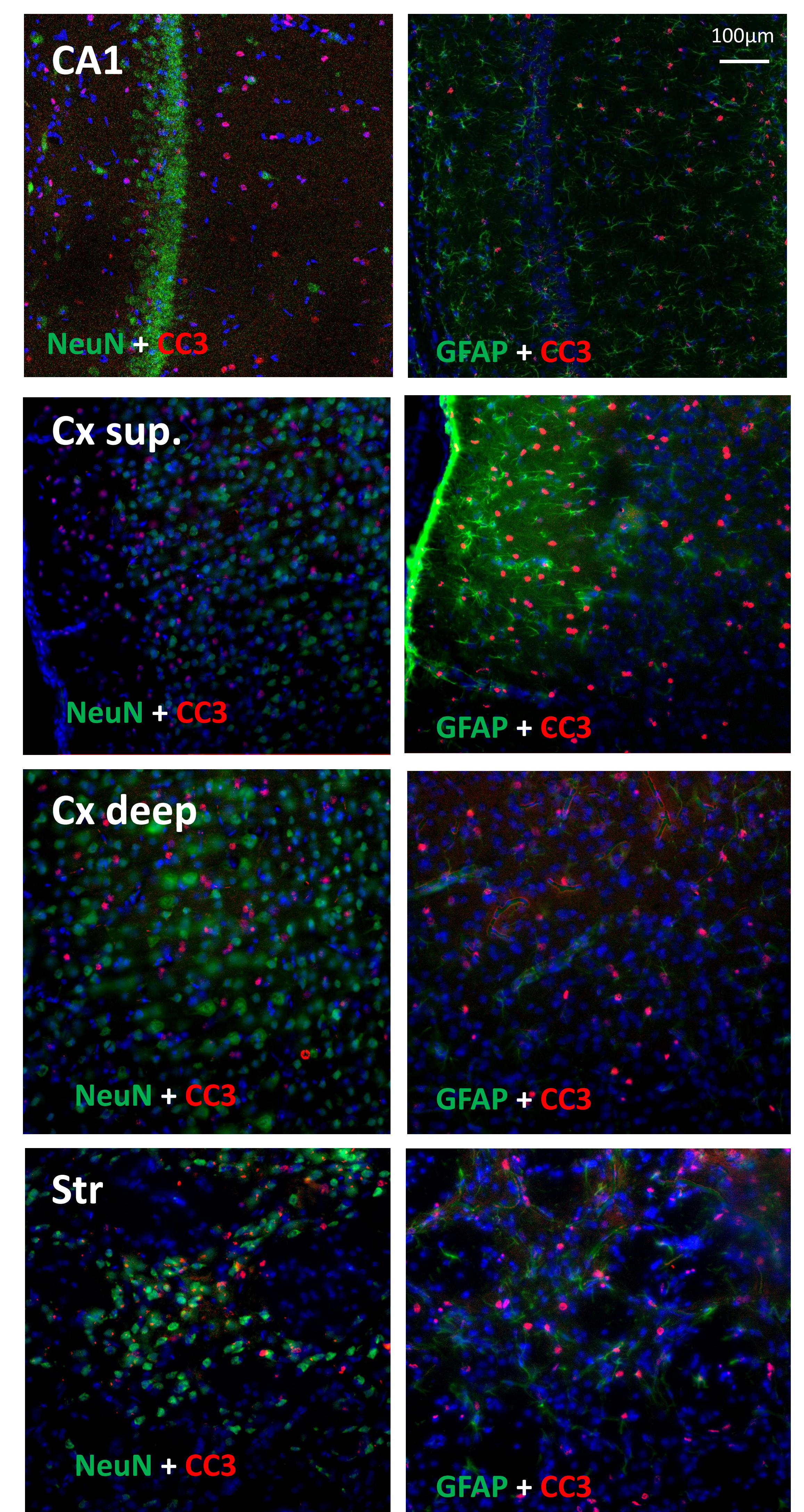


Fig.3. Representative photomicrographs of hippocampal, cortical and striatal areas of ischemic rat cortex, showing the colocalization of neuronal (NeuN) and astrocytal (GFAP) markers with the apoptosis marker cleaved caspase-3 (CC3) (20x magnification)

CA1 – hippocampus CA1, Cx sup. – superficial layers of cortex, Cx deep – deep layers of cortex, Str - striatum

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