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Research Report

# The fate of "dark" neurons produced by transient focal cerebral ischemia in a non-necrotic and non-excitotoxic environment: Neurobiological aspects

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ABSTRACT

*Background information*: We recently proposed novel neurobiological ideas for discussion regarding the common nature (malfunction of a physicochemical phenomenon genetically programmed for the morphological execution of ontogenetic apoptosis), mechanism of formation (phase transition in an intraneuronal gel structure) and mode of death (neither necrosis nor apoptosis) of "dark" neurons. These ideas were deduced from morphological changes in neurons found in a visually undamaged environment after in vivo or postmortem mechanical or electric injuries and after hypoglycemia. *Objective*: In search of further support, this paper revisits these ideas in the case of transient focal cerebral ischemia by investigating the light- and electron-microscopic changes produced in neurons by a 1-h occlusion of the rat middle cerebral artery in non-necrotic and non-excitotoxic tissue areas, where extraneuronal pathological processes may not influence the intraneuronal events. *Results*: In the first hour after restoration of circulation, the soma-dendrite domains of "dark" neurons displayed hyperbasophilia, hyperargyrophilia, hyper-electron density and a dramatic compaction of ultrastructural elements. Between 1 h and 1 day of the restored circulation, the degree of ultrastructural compaction decreased and mitochondrion-derived membranous whorls appeared in several "dark" neurons indicating recovery. Further, the cytoplasm of scattered neurons manifesting the apoptotic condensation pattern of the nuclear chromatin displayed the same morphological features as those of the freshly produced "dark" neurons. After 1 day of restored circulation, both the non-recovering "dark" neurons and the apoptotic neurons fell into membrane-bound, compact and electron-dense fragments, which were subsequently engulfed by phagocytotic cells. *Conclusion*: These observations support each of the ideas mentioned above.



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## 1. Introduction

More than a century ago, Flesh (1887) drew attention to the fact that the brains of patients who had died of various neurological diseases contained different proportions of markedly shrunken and hyperchromatic neurons, which were scattered among normal-looking neurons of the same type. The pathogenesis of such neurons, traditionally called "dark", has been a controversial subject in neuropathology (Agardh et al., 1981). At least four morphological subtypes of "dark" neurons are currently accepted (Graeber et al., 2002): the Huntington type (observed in a mouse model of experimental Huntington disease), the artefactual type (produced by unintentional postmortem mechanical injuries of various kinds), the reversible type (early stages of hypoglycemic, epileptic or ischemic injury) and the irreversible type (late stages of hypoglycemic, epileptic or ischemic injury). The common features of the freshly produced "dark" neurons are hyperbasophilia, hyperargyrophilia, hyper-electron density and a dramatic compaction of the ultrastructural elements (Csordás et al., 2003; Zsombok et al., 2005).

During the past few years, we (Gallyas et al., 2002, 2004, 2005, 2006; Csordás et al., 2003; Zsombok et al., 2005; Kellermayer et al., 2006) have published novel ideas of general neurobiological character on the common nature, common mechanism of formation and common mode of death of the "dark" neurons produced in a visibly normal environment by a head injury, an electric shock or mild hypoglycemia.

In order to draw the attention of researchers other than neuropathologist to the above ideas, the present paper demonstrates their validity for the "dark" neurons produced by transient occlusion of the middle cerebral artery in the rat, an animal model of human cerebral stroke. Since the widely used adjective "ischemic" does not conform exactly to the facts in this case (Lipton, 1999), such "dark" neurons are

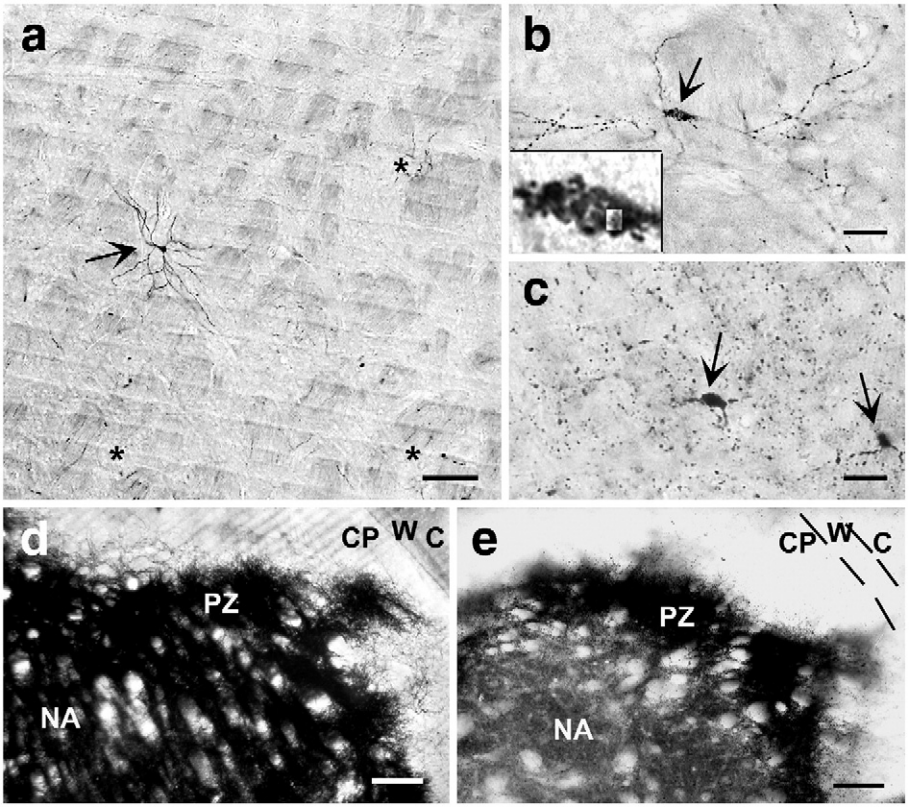


**Fig. 1 – Light-microscopic appearance of silver-stained "dark" stellate neurons in 150 μm vibratome sections of the caudate putamen in rats exposed to a 1-h occlusion of the middle cerebral artery. Rats were sacrificed immediately (a), 1 h (b and d) or 1 day (c and e) after the occlusion. Arrows in a–c point to freshly produced, regenerating or dying "dark" neurons, respectively, which are situated in a non-necrotic and non-excitotoxic environment. Around the asterisks in a, dendrites of "dark" neurons present in a neighboring section are stained. Note that the silver-stained dendritic fragments in c are somewhat larger than the silver-stained dendritic mitochondria in b. The inset in b, which is a 6× magnification of the soma indicated by the arrow, visualizes better the punctate nature of silver staining of the regenerating "dark" neurons. A square part in the nucleus is electronically lightened. In d and e, CP denotes caudate putamen, W subcortical white matter, C temporal cortex, NA necrotic area and PZ penumbra zone. The blurred black or gray staining represents the cumulative light absorption of a multitude of superposed, silver-stained neuronal elements. Round clear patches correspond to cross-sectioned axon bundles. In e, the white matter is contoured by dashed lines. Scale bars: a, ~200 μm; b and c, ~100 μm; d and e ~500 μm.**