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Structural variation and interactions among astrocytes of the rostral migratory stream and olfactory bulb: II. Golgi and electron microscopic study of the adult rat

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ABSTRACT

Cytological characteristics of a cell sharing structure of both an astrocyte and a neuron, previously termed amphomorphic cell (AC), were defined here in adult rat rostral migratory stream (RMS). The AC perikaryon corresponds to that of the B1 cell of the adult mouse subventricular zone (SVZ)-RMS. The AC and its processes are confined to the RMS. Each AC originates four sets of processes that overlap with those from its homologues and adjacent neural and stromal elements. ACs interact between them via reciprocal sets of processes: those directed caudally bear spheroidal vesicles (SVP) and form gap junctions with pleomorphic vesicles (PVs) associated with the anterior set from the adjacent AC. Large asymmetric synapses, a set of them arising from the anterior olfactory nucleus, converge on each SV. The interlacing processes of the AC, together with a set of perikaryal out-growths form the glial cuff surrounding migrating neuroblasts described earlier. Small asymmetrical and symmetrical synapses terminate in subsets of differentiated A-cells, termed here A1, in the bulbar part of the RMS. Both AC- and A1-cells form electrical synapses between them and with their homologues. The strategic, wide-spread distribution between the neuropil and blood vessels of the AC, its processes, and migrating neuroblasts, suggests that the AC might mediate between both endogenous inductors and neurotransmitters, influencing the adult-born neurons it had previously originated.

1. Introduction

Discrete territories of the mammalian nervous system undergo a process of neurogenesis during adulthood. Although several areas of the forebrain exhibit varying degrees of plasticity, it is now accepted that the dente gyrus and the cortex of the olfactory bulb incorporate adult-born neurons to their preexisting circuits. However, prospective neurons are generated in the subventricular zone (SVZ), from which neuroblast should migrate within the SVZ, and its anterior extension or rostral migratory stream (RMS) to reach their eventual positions. In the olfactory bulb adult neurogenesis culminates with the incorporation of several cell types (Merkle et al., 2013), primarily granule- and periglomerular-cells (see Whitman and Greer, 2007).

A cell comparable to an astrocyte or B-cell divides asymmetrically, originating a rapidly dividing cell (i.e., C-cell) that, in turn, gives rise to migratory neuroblasts or A-cells. While this basic genealogy has been confirmed by implementing viral or DNA precursor incorporation and immuno-electron microscopic techniques (Doetsch et al., 1997), the precise role for each cell type in the dynamics from the B-cell to the mature granule cell is still evolving. It is clear, though, that migration of prospective neurons is assisted by a distinct glial (Lois et al., 1996, 2003) and vascular (Shen et al., 2008) frame-work providing them with mechanical guidance and tropic factors. It has also been demonstrated that the glial cuff or tubule enmeshing rows of migrating neuroblasts results from the convergence of the B-cell processes (Lois et al., 1996). Furthermore, the neuron stem-cells share structural and immunohistochemical features with astroglia (i.e., B1-cell), for which these cells have also been termed “astrocyte-like” cells (Doetsch et al., 1999).

In attempting to expand our knowledge of the structure and interactions of cells associated with the RMS, we now characterize in the adult rat a cell that was termed amphomorphic cell (AC) due to its combined phenotype of neuron and astroglial cell (Larriva-Sahd, 2014). Subtle modifications of the four sets of

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processes that persists in every AC justified to divide them into six subclasses. Among other differences between ACs and protoplasmic astrocytes is that the former exhibit a rostro-caudal polarity and processes overlap to each another. This polarity involves two sets of processes and is asymmetrical, because those directed caudally bear spheroidal vesicles (SVP) whereas processes proceeding rostrally exhibit pleomorphic vesicles (PVP). Thus, SVP and PVP interlace in what it was referred to as reciprocal polarity. The marked polarity of the AC, the asymmetric distribution of its cell processes, coupled with the initial electron microscopic observation of synaptic terminals on them, led me to suggest that the AC may represent "different stages of differentiation of adult-born neuron" (Larriva-Sahd, 2014). However, through subsequent observations at the electron microscope (vide infra) it became obvious that the AC perikaryon matches the B1-cell in structure (Doetsch et al., 1997). The limited resolution of our former light microscopic study precluded identification of junctional complexes and other membrane specializations associated with the AC and justified the present ultrastructural study of the RMS. The adult rat was chosen for this part of the study for two reasons: to obtain reliable stereotaxic lessoning of the anterior commissure (vide infra) (Price and Powell, 1970a; Larriva-Sahd, 2008; Marcellino et al., 2012), and for the superior preservation of fine structure achieved in this species (Larriva-Sahd, 2006, 2008, 2012). Conservation of the AC sets of processes defined earlier in the adult rabbit was first assessed here in a supplemental group of adult rats with the Golgi technique. Given the availability of a detailed account of the AC in adult rabbit (Larriva-Sahd, 2014), and the confirmatory nature of the present study, the reader is referred to the former article. While most cytological characteristics of the rabbit AC appear to be conserved in the adult rat, my recent interpretation of the AC as "migrating neuroblast" (Larriva-Sahd, 2014) was wrong. Despite the fact that ACs, like a migrating neuroblast, exhibits marked polarity, field processes overlapping, and receives synaptic inputs (vide infra), the present cytological observations support that the AC soma corresponds to the cell previously described as "Class 2" (Jankovski and Sotelo, 1996) or B1 (Doetsch et al., 1997), or to the "structural astrocyte" (Sawamoto et al., 2011) described elsewhere.

2. Material and methods

2.1. Animals

Sprague-Dawley male rats killed at 10 weeks of age were used. The animals were kept at constant temperature (24 °C) and photoperiods (14 h light/10 h dark) with free access to food and water. All experimental manipulations and sacrifices were made according to the ethical policies of animal care and handling of our institute, aimed at minimizing animal pain and suffering.

2.2. Rapid Golgi technique

The olfactory bulbs from twenty animals were utilized for this part of the study. All animals were killed by decapitation under deep barbiturate anesthesia, and the brains were removed and processed as detailed earlier (Larriva-Sahd, 2008). Indian-ink drawings were performed with a camera lucida adapted to a Zeiss-Axioplan 2 (Zeiss) microscope, utilizing 40- and 100× oil-immersion objectives. Images were replicated as accurately as possible, with the exception of that in Fig. 3C whose cell bodies and processes were exaggerated to allow size reduction to the journal standards.

2.3. Electron microscopy

The 14 adult rats used for this part of the study were sacrificed, and brain samples processed as previously described (Larriva-Sahd, 2006). Briefly, rats were deeply anesthetized and perfused through the left ventricle with 250 ml of Karnovsky's fixative (4% paraformaldehyde–2.5% glutaraldehyde in 0.15 M phosphate buffer, pH 7.3) cooled at 4 °C. Each brain was processed following the same procedure cited above up to flat embedding the tissue samples for inclusion in Epon. One micrometer-thin sections were obtained from the tissue blocks in a Leica ultramicrotome equipped with glass knives. The sections were stained with toluidine blue and coverslipped. In every block of tissue the bulbar cortex was identified and trimmed-out from the block so that only the RMS was further studied at the electron microscope. From the surface of these trimmed blocks, silver to gold ultrathin sections were obtained with a diamond knife and mounted on 200 mesh copper grids. Then, sections were sequentially stained with uranium and lead salts and observed in a JOEL 1010 electron microscope.

2.4. Transection of commissural axons

Under deep barbiturate anesthesia, the anterior commissure (AC) of six supplemental animals was cut with a modified Halaz knife utilizing a stereotaxic apparatus. All experimental procedures and sacrifices were performed as described elsewhere (Marcellino et al., 2012). To detect degenerative changes following axotomy, animals were left alive for a 48-h period. This allows to define both the synaptic terminals undergoing degenerative changes following axotomy as well as its postsynaptic element(s) to be defined (Price and Powell, 1970a; Marcellino et al., 2012).

2.5. Ultrastructural reconstructs

On the basis of the superior preservation, two to three blocks from each of five different animals were utilized to study serial sections. Appropriate areas were selected from the neuropil of the external layer of the RMS or stratum lucidum (SL) (Larriva-Sahd, 2014). Then, 45 series of 35–95 sections were obtained with a diamond knife, distributed into one to three, one-slot copper grids that had previously been covered with formvar film, and then sequentially stained (see above). Digital micrographs from equivalent areas were successively photographed at 5–30,000× magnification. Representative cells, synapses, and interactions between glial processes from these series were aligned, outlined, and reconstructed with a freely available Reconstruct program (http://synapses.clm.utexas.edu/tools/reconstruct/reconstruct.stm.). Prior to performing three-dimensional reconstructs of the interactions between ACs, each prospective AC was identified through the series according to the following criteria: first, the ovoid shape of the cell sending one or paired stem processes (Fig. 9); second, the presence of thick cytoplasmic fascicles of intermediate filaments (Figs. 9A,D and 10A,D); third, SVs in apposition to or embedded in the AC's cytoplasm (Figs. 5E and 10C–E); and fourth, identification of one or more synaptic boutons terminating on their SVs (Figs. 11, 12G–J). Following this approach the AC source of each type of vesicle shown in our reconstructs was previously defined throughout series itself.

3. Results

3.1. Light microscopy

3.1.1. Aniline stains

The rat olfactory bulb medulla hosts the rostral migratory stream (RMS) that extends from the anterior part of the lateral ventricle to resolve in the core of the olfactory bulb (Fig. 1). The previous parceling of the RMS into three successive territories described for the adult rabbit (Larriva-Sahd, 2014) will be used here: that is, a