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Research Report

Postnatal development of GFAP, connexin43 and connexin30 in cat visual cortex

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Abstract

In cat visual cortex, neurons acquire progressively mature functional properties during the first postnatal months. The aim of this study was to analyze the development of astrocytes during this period. The patterns of expression of the glial fibrillary acidic protein (GFAP) as well as of two gap junction proteins expressed in astrocytes, connexin43 (Cx43) and connexin30 (Cx30), were investigated by immunohistochemistry and optical density measurements, in visual cortical areas 17 and 18 at four different ages: 2 weeks (postnatal days 12 to 15, P12–15), 1 month (P27–31), 2 months (P60–62) and beyond 1 year. Since visual experience is a key factor for neural development, the patterns of expression of these three proteins were studied both in normally-reared and monocularly deprived animals. Interestingly, the distribution of GFAP, Cx43 and Cx30 was found to change dramatically but independently of visual experience, during postnatal development, even beyond P60. During the first postnatal month, GFAP and Cx43 were mainly localized in the white matter underlying the visual cortical areas 17 and 18. Then, their distributions evolved similarly with a progressive decrease of their density in the white matter associated with an increase in the cortex. Connexin30 expression appeared only from the second postnatal month, strictly in the cortex and with a laminar distribution which was similar to that of Cx43 at the same age. In adults, a specific laminar distribution was observed, that was identical for GFAP, Cx43 and Cx30: their density was higher in layers II/III and V than in the other cortical layers.

Theme: Development and regeneration
Topic: Visual system

Keywords: Astrocyte; Glia; Gap junction; Area 17; Area 18

1. Introduction

The visual cortex of mammals is immature at birth and visual neurons develop progressively mature functional properties during a postnatal "critical period". In kittens, in visual cortical areas 17 and 18, this period lasts for 4 to 7 months: the first two postnatal months correspond to a period of high plasticity with a peak between the 4th and the 6th week. Then, plasticity decreases progressively with age [9,14,19,25,42].

While the maturation of neurons has been extensively studied in this brain area, only little information is available about the development of glial cells, in particular for astrocytes, although they are now considered as active partners of neurons. For example, astrocytes are involved in the regulation of both the electrical activity and the synaptic transmission of neurons [3,11,18,32]. In the cat visual cortex, one sole study reports the postnatal development (from birth to the 7th postnatal week) of two astrocytic markers, the glial fibrillary acidic protein (GFAP) and the S-100 protein [28]. The expression of these two proteins was found to increase in the visual cortex between the third and seventh postnatal week. This suggests a relation between the maturation of astrocytes and the development of the neurons

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in this area [28]. The first aim of the present study was to investigate the development of GFAP in the same cortical region at four postnatal ages including the period of high plasticity of the visual cortex: 2 weeks (postnatal days 12 to 15, P12–15), 1 month (P27–31), 2 months (P60–62) and after 1 year. Thus, we completed the previous sole study with observations of the development of GFAP beyond the age of P49, until adulthood.

Astrocytes can establish intercellular communication through gap junctions that contribute to the formation of a cellular network [16]. These intercellular junctions are composed of specific proteins, named connexins (Cxs) [6]. Connexin43 (Cx43) and connexin30 (Cx30) are the main connexins expressed in astrocytic gap junctions [37,40], where they are often co-localized [30,31]. Interestingly, mature neurons in cultures have been shown to control gap junctional communication between astrocytes and/or the expression of Cx43 and Cx30 [12,39]. This suggests that Cxs in astrocytes represent a target in neuron–glia interactions. Immunolabeling patterns of Cx30 and Cx43 have previously been studied in rodent in various cortical regions and in a lower extent in cat and human brain [30,31]. However, the expression of these connexins in the visual cortex has not been explored yet. The second goal of this study was thus to investigate the developmental patterns of Cx expression in the cat visual cortex and to compare them to the distribution of GFAP.

The postnatal visual experience in mammals has been shown to be critical for neuronal development in the visual cortex. For instance, a monocular visual deprivation strongly modifies the development of ocular dominance columns [5,19]. Several studies also investigated the influence of the postnatal visual experience on astrocytes in the visual cortex by analyzing the distribution of GFAP (or of S-100 protein) after different visual deprivations and in various species. But rather contradictory observations have been reported. Some studies indicated no effect of visual deprivation on the density of astrocytes [33] or on the expression of GFAP [8], while others pointed out an influence of visual deprivation on astrocytes development [4,15,17]. In the cat, dark-rearing induces a delay of the maturation of astrocytes [27]. Furthermore, several observations indicate that astrocyte maturation could influence the plasticity of visual neuron properties [21,29]. Altogether, these studies suggest that interactions between astrocytes and neurons may occur within cat visual cortex, during postnatal development. Accordingly, the third aim of this study was to compare the development of the astrocytes in normal and monocularly deprived animals by observing the expression of GFAP as well as the expression of connexins (Cx43 and Cx30).

We observed that an early monocular visual deprivation does not affect the postnatal development of GFAP, Cx43 and Cx30 expression patterns in visual cortical areas 17 and 18. However, we showed that the distributions of these three proteins change dramatically during postnatal development. In the adults, a specific laminar distribution was observed and was similar for these three proteins.

2. Results

2.1. Effect of visual deprivation on the postnatal development of GFAP, Cx43 and Cx30 expression

Comparing the distribution and the relative densities of GFAP, Cx43 and Cx30 at each age, we observed no significant difference ($P > 0.05$ for each cortical layer and for the white matter) between normally-reared (NR) and monocularly deprived (MD) animals. This indicates that an early monocular visual deprivation does not affect these parameters during development and in the adults. Observations from both groups (NR and MD) were thus combined and are presented together in the following sections.

2.2. Postnatal development of GFAP-positive astrocytes, connexin43 and connexin30

2.2.1. Two weeks (P15)

2.2.1.1. GFAP. The GFAP-positive astrocytes were mainly localized in the white matter where they appeared distributed homogeneously (Figs. 1A, 3B). In the cortex, only a few GFAP-positive astrocytes were observed and most of them had processes, usually termed as “end feet”, in contact with blood vessels (Fig. 3A). In all kittens, the optical density of GFAP labeling was significantly higher in the white matter than in the cortical layers ($P < 0.01$) (Fig. 6A).

2.2.1.2. Cx43. Connexin43 staining was also mainly localized in the white matter with a high density (Figs. 4A and 6E). In all kittens, the optical density of Cx43 labeling in the white matter was thus significantly higher than the one in the cortical layers ($P < 0.01$) (Fig. 6E). The highest intensity of Cx43 staining was observed around the blood vessels with a honeycomb pattern. In contrast to GFAP staining, Cx43 labeling was not distributed homogeneously in the white matter: a zone with a lower density of labeling was observed in the central part of the white matter (arrow in Fig. 4A). This zone was more clearly defined in NR kittens ($n = 3$) than in MD ones ($n = 2$). The mean optical density of Cx43 labeling was thus significantly lower inside this zone than in the rest of the white matter in NR kittens ($P < 0.01$), but this difference was not statistically significant in MD kittens. This was the only difference observed between NR and MD kittens at all ages. In the cortex, Cx43 labeling was mainly observed around blood vessels.

2.2.1.3. Cx30. No Cx30 staining was observed either in the cortex or in the white matter (data not shown).