Transient co-localization of calretinin, parvalbumin, and calbindin-D28K in developing visual cortex of monkey

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Summary

This paper reports a double-labelling immunocytochemical study of the three calcium-binding proteins calretinin, parvalbumin, and calbindin-D28k in developing and adult Macaca primary visual cortex. In adult visual cortex, each protein marks a subset of GABAergic neurons with a characteristic laminar distribution and virtually no co-localization was found between these three proteins, suggesting that each calcium-binding protein may serve as a marker for one or more cortical subcircuits. The immature visual cortex, immunostained using identical techniques was then analysed to determine if each calcium-binding protein could serve as a developmental marker for these circuits. The Cajal-Retzius cells of layer 1 contained all three proteins during development. Calbindin-D28k and calretinin were co-localized starting at Fd (foetal day) 45 and after Fd 125, parvalbumin also was present in the same Cajal-Retzius cells. All three proteins continued to be expressed until the Cajal-Retzius disappeared postnatally. In layers 2–6 calbindin-D28k and calretinin were never co-localized. In contrast, parvalbumin and calretinin were found in neurons of deep layer 3 from Fd 155 to postnatal (P6) weeks with a few persisting even later. Before birth almost all PV+ neurons in layers 4–6 were CaB+, but by P3 weeks only a few PV+/CaB+ neurons remained in layer 4C and these completely disappeared by P6 weeks. Co-localization in layer 4 neurons overlaps the period of ocular dominance segregation, suggesting that the onset of cortical maturity coincides with segregation of calcium-binding proteins within the GABA interneurons.

Introduction

Previous studies have shown that three intracellular calcium-binding proteins of the EF-hand family, calbindin-D28k (CaB), parvalbumin (PV) and calretinin (CaR), are specifically expressed in neurons (Celio & Heizmann, 1981; Rogers, 1987; Winsky et al., 1989; Braun, 1990; Celio, 1990; Rogers et al., 1990). The function of these calcium-binding proteins is not known, but they have been useful neuronal markers. For instance, all three proteins are found in GABAergic cortical interneurons, but they are rarely co-localized in the same interneuron in adult cortex (Hendry et al., 1989; van Brederode et al., 1990; Demeulemeester et al., 1991; Rogers, 1992; Conde et al., 1994; Yan et al., 1995). PV+ neurons are most numerous (70% of all GABA neurons contain this protein), followed by CaR+ neurons (about 20% of all GABA+ cells), while CaB is found in about 10% of GABA+ neurons (van Brederode et al., 1990; Yan et al., 1995). Consistent with this observation, it has been found that each protein is found in morphologically and functionally different subpopulations of interneurons (van Brederode et al., 1990; Conde et al., 1994; Yan et al., 1995), suggesting that they can serve as markers for distinct inhibitory subcircuits within the adult visual cortex.

In the adult primate cortex, PV+ neurons are most numerous in the thalamo-recipient layers (layers 4A and 4C). Calretinin-positive and CaB+ neurons are most numerous in the supragranular layers and avoid layer 4C altogether (van Brederode et al., 1990; Conde et al., 1994; Yan et al., 1995). These patterns change dramatically over development (Hendrickson et al., 1991; Yan et al., 1995). For instance, CaB+ neurons are found in great numbers in layers 4A and 4C for a brief period around birth and CaR+ neurons are found in layer 4A. Both CaB and CaR+ immunostaining in layer 4 progressively decline after birth, while at the same time PV labelling in this layer rapidly increases. This change in protein distribution overlaps the period when cortical inputs normally are establishing ocular dominance columns and geniculo-cortical inputs become stabilized (Rakic, 1977; LeVay et al., 1980). The overlap in laminar location of immunostaining for CaR, CaB and PV raises the possibility that transient co-localization of these calcium-binding proteins occurs...
during the time when the visual cortex is most sensitive to functional alterations in its thalamic input, and that final separation of these proteins into distinct sub-populations of interneurons marks the end of plasticity of these inhibitory circuits. Furthermore, since changes in intracellular calcium-concentration might occur during the critical period (Bode-Greuel & Singer, 1991; Lin et al., 1994), it is possible that cortical neurons co-express more than one calcium-binding protein in response to an increased influx of calcium.

We carried out the present study in order to determine whether the three calcium-binding proteins are co-localized during development of monkey striate cortex, and if so, how the pattern of co-localization relates to the known pattern of maturation of the neuronal circuitry of the primary visual cortex and its afferent input.

Material and methods

Macaca nemestrina monkeys used in this study were timed conception at foetal day (Fd) 55, 65, 90, 125, 150, 155, 157, postnatal (P) 1 day, P3, 6 and 16 weeks and adult. Animals were obtained from the Regional Primate Center at the University of Washington or from the Primate Research Centre, Bogor, Indonesia. Birth occurs at Fd 165-170 in this colony. Foetal animals were delivered by aseptic cesarean postnatal (P) 1 day, P3, 6 and 16 weeks and adult. Animals were tranquilized with ketamine followed by deep intraperitoneal injection of barbiturate immediately after delivery to insure that they were anaesthetized. Postnatal animals were removed, and post-fixed overnight in the same fixative. Blocks of occipital cortex containing primary visual cortex were also found (Fig. 1C, D). An earlier study in the same area of the cortex showed that CaB and PV are rarely co-localized in the same neuron in the monkey (van Brederode et al., 1990). Combined with the results reported in this study, these data show that the three calcium-binding proteins occur in largely non-overlapping neuronal populations of neurons in the adult striate cortex.

Results

ADULT CORTEX

Double-label immunofluorescence for CaR and CaB (Fig. 1) detected virtually no overlap between these two proteins. Double-labeling for CaR and PV, however, showed that in a few cortical neurons these proteins are co-localized (Fig. 1C, D). These CaR+/PV- cells were located in layer 3 and were mainly multipolar neurons, although some bi-tufted cells were also found (Fig. 1C, D). An earlier study in the same area of the cortex showed that CaB and PV are rarely co-localized in the same neuron in the monkey (van Brederode et al., 1990). Combined with the results reported in this study, these data show that the three calcium-binding proteins occur in largely non-overlapping neuronal populations of neurons in the adult striate cortex.

DEVELOPING CORTEX

The pattern of expression of CaR, CaB and PV immunoreactivity in developing monkey visual cortex has been described in detail in previous studies (Hendrickson...