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TRANSNEURONAL INDUCTION OF THE HIGHLY SIALYLATED ISOFORM OF THE NEURAL CELL ADHESION MOLECULE FOLLOWING NERVE INJURY*

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The polysialylated, embryonic form of the neuronal cell adhesion molecule (PSA-NCAM) is known to participate in a whole series of synaptic rearrangements even in adult animals. The possible role of this molecule in neuroplastic changes of the adult rat somatosensory cortex induced by unilateral transection of the infraorbital branch of the trigeminal nerve was studied with PSA-NCAM immunostaining at light microscopic level. Two- and three-month-old CFY albino rats were sacrificied on days 1, 4, 6, 14 and 21 following operation and PSA-NCAM immunoreaction was examined at three levels of the vibrissa-cortex neuraxis, namely, in the principal nucleus of the trigeminal nerve, in the ventral posteromedial nucleus of the thalamus and in the somatosensory cortex. The lower levels of the neuraxis remained free of PSA-NCAM labeling, similarly to control, intact animals. However, a large number of scattered small neurons became PSA-NCAM immunoreactive in layers IV–VI on both ipsi- and contralateral sides of the somatosensory cortex from day 6 onwards, suggesting a possible transynaptic regulation of NCAM sialylation state.

Keywords: PSA-NCAM – neural plasticity – immunohistochemistry – rat – cortex

INTRODUCTION

In the beginning of the last century Ramon y Cajal [18, 19] suggested that neurons are capable of morphological changes in response to their environment. Although his idea was later contested, the central nervous system has definitely overpassed by now the view of its static, hard wired nature. It is generally accepted that morphological rearrangements and modifications of neural activity do occur not only during development, but in the adult as well, under both normal and pathologic conditions. These ultrastructural alterations include changes in number of synaptic contacts, e.g. following long-term potentiation and epileptic seizures in the adult [20]. Functional alterations are reported in several areas of the nervous system, especially in connection with regenerative processes, like in the vestibular nucleus after unilateral deafferentation [10], or in the barrel field of the somatosensory cortex following infraorbital nerve transection [27].

*Dedicated to Professor József Hámori on the occasion of his 70th birthday.

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Such neuroplastic changes usually imply membrane-membrane interactions which suggest the involvement of cell adhesion molecules in the mechanism of synaptic restructuring. One of the cell surface molecules, which has been extensively studied in this respect, is the polysialylated form of the neural cell adhesion molecule (PSA-NCAM). It bears a long polysialic chain made up from α-2,8-linked sialic acid moieties that renders the molecule negatively charged, having a large hydrated volume. Consequently, homofilic binding established by PSA-NCAM expressing cells does not create stable cell-cell adhesion, rather, it provides an appropriate membrane environment for cellular mobility. PSA-NCAM is also called the embryonic form of the neural cell adhesion molecule, since it is expressed on all neural surfaces during embryonic development. After birth, however, the embryonic form of NCAM is progressively replaced by an isoform which lacks the long polysialic acid chain. As a result, the isoform characteristic of the adult brain is thought to be involved in stabilizing newly formed synaptic contacts by enhancing adhesivity between interacting cellular surfaces. Thus, the fundamental difference between the embryonic and adult isoforms resides in their PSA content. The polysialylation of the protein backbone of NCAM is carried out by at least two enzymes, the ST8SiaII (STX or sialyltransferase X) and ST8SiaIV (PST or polysialyltransferase 1). Both ST8SiaIV (PST) and ST8SiaII (STX) transcripts can barely be detected at embryonic day 8 (E8) but are increased after E9, and they are present in substantial quantity between E11 and E15, coinciding with the period when maximum synthesis of polysialic acid is required. Ten days after birth, the level of ST8SiaII (STX) transcript declines substantially, whereas the level of ST8SiaIV (PST) transcript persists in the adult brain [16]. An increasing number of evidence indicates that ST8SiaIV is the major regulator of NCAM polysialylation [23].

Since the expression of this enzyme is maintained in the adult, this allows the possibility for maintaining the polysialylated state of NCAM as well. However, under normal conditions, PSA-NCAM immunoreactivity (IR) in adult brain is restricted to those areas which can undergo morphological reorganization, e.g. the dentate gyrus of the hippocampus, the supraoptic, suprachiasmatic, paraventricular and arcuate nucleus of the hypothalamus, or the olfactory bulb in intact animals, and various regenerating brain regions following injury [2].

PSA-NCAM expression can be induced both on neurons and astrocytes that are normally immunonegative for PSA-NCAM during regeneration following injury [3]. Moreover, NCAM polysialylation state is known to be altered in some pathologic conditions, such as following transient global ischemia [6] and middle cerebral artery occlusion [8], and in some neurodegenerative diseases like Alzheimer's disease [15] and amyotrophic lateral sclerosis [30]. The induction of PSA-NCAM expression following injury is further supported by our present work suggesting a transynaptic regulation of PSA-NCAM polysialylation state in both the ipsi- and contralateral barrel fields of the adult rat somatosensory cortex after transection of the infraorbital branch of the trigeminal nerve.

MATERIALS AND METHODS

The experiments were carried out in accordance with the European Communities Council Directives (86/609/EEC). In the present study CFY albino rats, raised and maintained in a 12 h light : 12 h dark cycle, were used. Two- and three-month-old albino rats were anaesthetized with a mixture of Ketavet (10 mg/100 g) : Rompun (xilasine, 0.8 mg/100 g) at a ratio of 2 : 5. The infraorbital nerve was cut and a 2-mm-long section was removed in the area between the vibrissa pad and the eye.

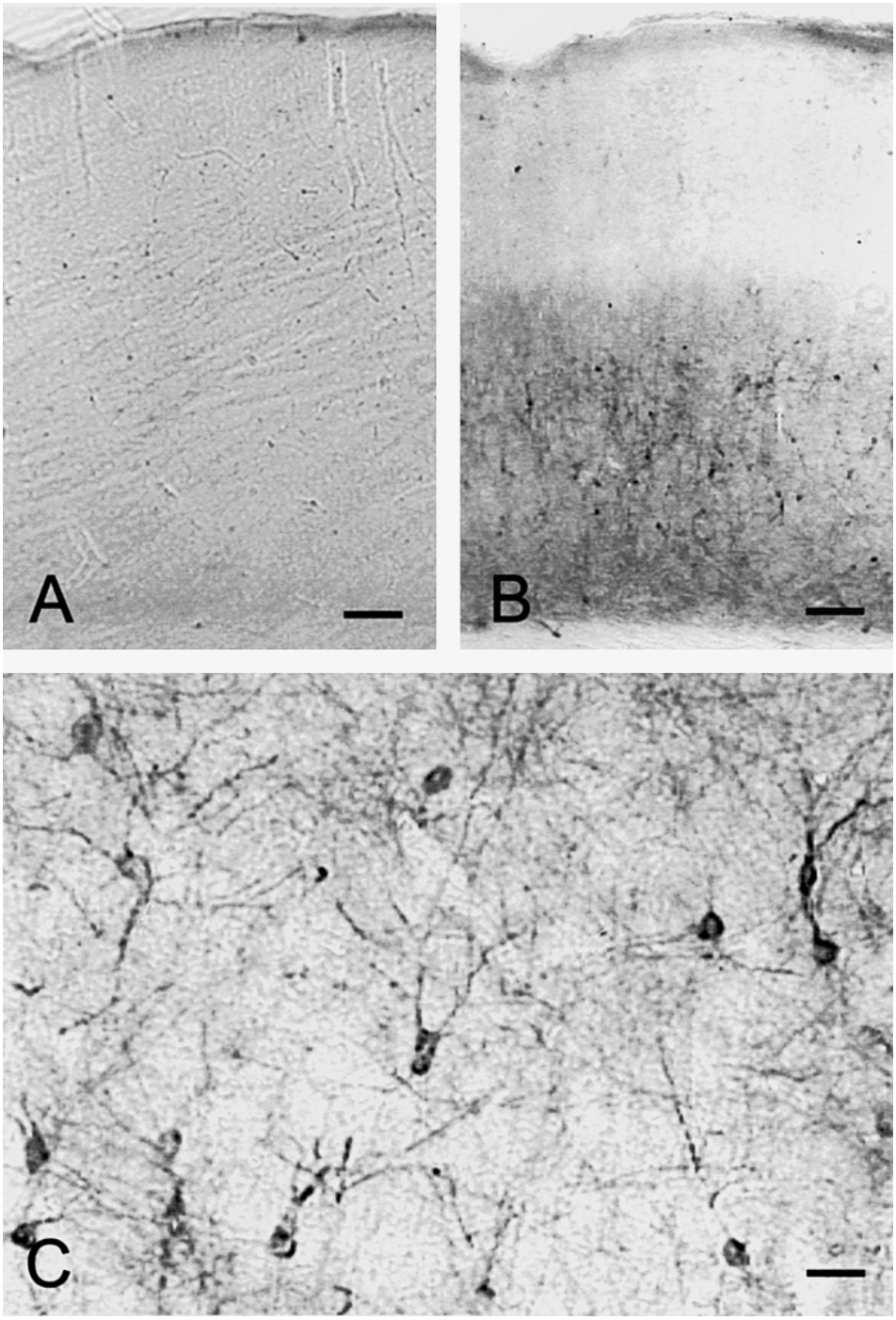
Animals were perfused on days 1, 4, 6, 14 and 21 following nerve transection through the left cardiac ventricule with 50 ml of 0.9% NaCl then with 250 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After perfusion, the brains were removed and placed in the same fixative at 4 °C overnight.

After several washes in tris-buffered saline (TBS, pH 7.4) 40 μm-thick coronal slices were sectioned with a vibratome (TPI Inc., St. Louis, USA) and processed for light microscopic PSA-NCAM immunostaining. The nonspecific sites were blocked with 20% normal goat serum (Sigma) diluted in TBS, then sections were incubated in anti-PSA-NCAM antibodies (gift of G. Rougon, specificity described in Rougon et al. [21], diluted 1: 6000 (48 h at 4 °C). Anti-mouse IgM immunoglobulins conjugated to horseradish peroxidase (HRP) raised in goat were used as immunolabels to reveal PSA-NCAM immunoreactivity (Sigma, dilution 1: 50, 2 h, room temperature). The HRP reaction product was visualized with 3',3'-diaminobenzidine and $NiCl_2$.

The sections were dehydrated in an ascending series of alcohol, coverslipped with Entellan (Merck) and examined with an Olympus Vanox T light microscope.

RESULTS

PSA-NCAM immunoreactivity was examined at three levels of the vibrissa-cortex neuraxis: in the principal nucleus of the trigeminal nerve, in the ventral posteromedial (VPM) nucleus of the thalamus and in the primary somatosensory cortex both on the ipsi- and contralateral sides after unilateral transection of the infraorbital nerve at different survival times ranging from 1 to 21 days. In control conditions these areas are free of PSA-NCAM labeling in the adult, as it was observed in control, intact animals in accordance with earlier results reported by others. In animals that have suffered nerve transection, the lower levels of the neuraxis, more precisely, the principal nucleus and the VPM remained PSA-NCAM immunonegative at both sides following injury. In contrast, the somatosensory cortex, representing the highest level of the neuraxis, showed a well-defined change in PSA-NCAM-IR. In agreement with literature data in control animals the cortex is devoid of immunoreaction (Fig. 1A), but PSA-NCAM immunoreactive scattered cell bodies and processes became visible in layers IV–VI on day 6 after transection of the infraorbital nerve (Fig.1B). These cells resembled small interneurons based on their morphology and localization (Fig. 1C). A punctate membrane labeling was apparent both on somata and processes,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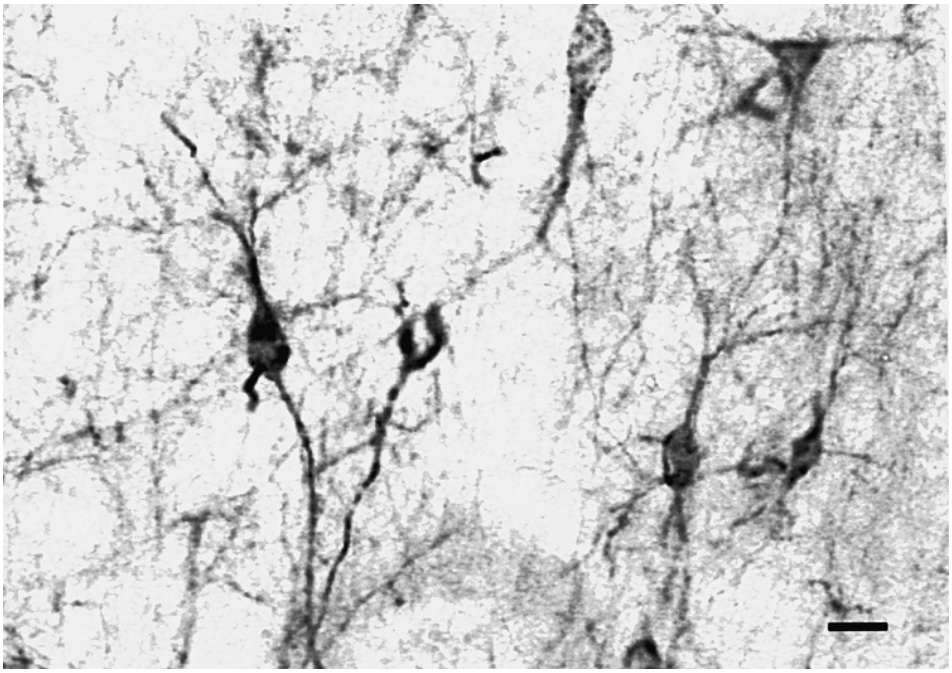

Fig. 2. PSA-NCAM immunoreactive cells from the contralateral somatosensory cortex 6 days after unilateral transection of the trigeminal nerve. Note the typical membrane staining with punctate character. Bar: 10 μm

demonstrating the characteristic pattern of neural PSA-NCAM immunostaining (Fig. 2). No cells with stellate morphology and strong PSA-NCAM immunolabeling filling the whole cytoplasm were detected at either brain region examined, thus the possible presence of PSA-NCAM positive reactive astrocytes was excluded. The presumed small interneurons remained PSA-NCAM immunoreactive throughout the examination period. It is interesting to note that the neuronal PSA-NCAM expression in the somatosensory cortex is extended to both ipsi- and contralateral sides and seems to be transynaptic, since no change in PSA-NCAM-IR is detectable in the lower relay stations of the neuraxis.

←————

Fig. 1. PSA-NCAM immunoreactivity of the somatosensory cortex from control rats (A) and from animals fixed 6 days after unilateral transection of the infraorbital branch of the trigeminal nerve (B, C). Bar: 100 μm (A and B), 20 μm (C)

DISCUSSION

Previous works indicated that unilateral transection of the infraorbital nerve induces changes in physiological parameters of neurons in the somatosensory barrel cortex of the adult rat [27]. Moreover, vibrissectomy results in an enlarged representation of the spared vibrissa barrel in the contralateral cortex. It is suggested that removal of vibrissal input produces transynaptic induction of axonal sprouting within the barrel cortex [5]. As far as the morphological substrate of these functional alterations is concerned, thalamocortical arborization patterns were analyzed and two different types, a direct-projecting and a bifurcating one was identified. Direct-projecting axons arborize within a single cortical barrel and extend their fibers into adjacent barrels as well, but always have greater than 50% of their fiber length within layer IV. Bifurcating-type axons, in contrast, bifurcate in the subcortical white matter or in layer VI and only after that project to multiple barrel columns, where they arborize in layer IV, always having less than 50% of their total column fiber length in layer IV. This divergent projection pattern may provide an anatomical substrate for the observed functional plasticity in the somatosensory cortex [1].

Restructuring of the barrel field following altered afferent input is accompanied by complex neurochemical changes. The role of some neurotransmitters and growth factors has been extensively studied in this respect. It was shown that GABA-immunoreactive neurons undergo highly selective redistribution following neonatal sensory deprivation [14]. A possible participation of dopamine was also suggested, since a significant increase in the release of dopamine metabolites was found in the deafferented cortex [12]. Acetylcholine seems to be also involved in somatosensory cortical plasticity, because the basal forebrain cholinergic input from the nucleus basalis is an important facilitator of these ultrastructural reorganizations [22]. Norepinephrine is thought to play a similar role, given the fact that cortical norepinephrine depletion prevents changes in the representation of the spared vibrissa in the barrel field, although it has no effect on vibrissectomy-induced decrease in GAP-43-IR observed within the barrels that have lost their afferent input [5]. Techniques of molecular biology have also contributed to the better understanding of barrel field plasticity. RT-PCR studies revealed an increased BDNF mRNA content in the contralateral barrel region after cauterizing facial vibrissae [25].

The purpose of the present study was to identify further molecular factors that may contribute to such neuroplastic changes. Since ultrastructural rearrangements require membrane-membrane interactions, cell adhesion molecules, among them especially PSA-NCAM, are likely to play a role in these processes. It was found that PSA-NCAM expression is induced on small interneurons of IV–VI layers of the somatosensory cortex by one week after unilateral transection of the infraorbital nerve. It is known from earlier data that expression of PSA-NCAM is needed for reversible synaptic reorganizations taking place e.g. in the hypothalamo-neurohypophyseal system and in the arcuate nucleus of the hypothalamus [7, 26]. In these areas the polysialylated state of NCAM is maintained throughout life and the presence of PSA provides a permissive cell surface environment for synaptic restructur-

ing whenever it is induced by a proper stimulus [11]. The exact regulation of NCAM polysialylation is not yet clearly revealed. The key issue in this respect is to find out how the expression of PSA on NCAM is regulated both during development and physiological processes in the adult. Moreover, in the adult there is some discrepancy in data on PSA-NCAM immunoreactivity and those of the expression of polysialyltransferase mRNA as shown by in situ hybridization studies. It is generally accepted that PSA-immunoreactive neuronal cell bodies in the adult brain are located only in the dentate gyrus of the hippocampus and in the olfactory system, namely in layer II of the piriform cortex, in the entorhinal cortex, in the olfactory bulb and in the rostral migratory pathway. In this latter system, however, the expression of ST8SiaIV polysialyltransferase mRNA, which is thought to be responsible for NCAM polysialylation in the adult, is rather weak, thus the existence of a yet unknown polysialyltransferase cannot be ruled out [9]. In the olfactory bulb no ST8SiaII signal was detected by Kurosawa et al. [13], which contradicts the findings of Phillips et al. [17] and makes uncertain which of the two known polysialyltransferases is responsible for NCAM polysialylation in this brain region. With regards to the neocortex, it is reported to be free of PSA-NCAM immunolabeling by Bonfanti et al. [2] while Seki and Arai [24], using another type of monoclonal antibody directed against the polysialic chain of NCAM found that PSA-NCAM expression was progressively attenuated from one week onwards after birth till it almost vanished. In situ hybridization experiments carried out by Hildebrandt et al. [9] showed a sustained expression of ST8SiaIV mRNA in widely dispersed cells of the adult neocortex, which might explain the slight controversy of immunohistochemical data.

A profound study by Brusés and Rutishauser [4] on the regulation of NCAM polysialylation put forward the possibility of a nontranscriptional control of polysialyltransferases. A strong dependence on intracellular Ca^{2+} was found, and, on the other hand, PSA synthetic activity itself was also Ca^{2+}-dependent. Namely, a decrease in intracellular Ca^{2+} concentration leads to decreased polysialylation. Thus, it is suggested that mobilization of Ca^{2+} from intracellular stores which are presumably connected with the Golgi apparatus can result in rapid and localized control of NCAM polysialylation state. Among the numerous factors that influence intracellular Ca^{2+} concentration, nerve-target interaction and neuronal activity seem to have major impacts. It is clearly documented that the electrical activity of neurons in the barrel field is altered due to changes in afferent inputs. More precisely, an enhanced Ca^{2+} permeability of non-NMDA glutamate receptors, a stronger spiking activity and an increased neuronal excitability is detected. These changes are more pronounced in the cortex than in the thalamus. Our results showing an induction of PSA-NCAM expression in layers IV–VI of the somatosensory cortex, but with no change in PSA-NCAM-IR in the thalamus are in accord with previous electrophysiological data reported by Waite [29] and Világi et al. [27]. Moreover, our recent findings support the view of Vutskits et al. [28] on the role of PSA-NCAM in enhancing the sensitivity of neurons to BDNF, which is expressed in increased quantities in the barrel region following cauterization of facial vibrissae as already mentioned above [25].

Taken together, our results clearly indicate that the embryonic form of the neural cell adhesion molecule, PSA-NCAM, participates at the plethora of events induced in the somatosensory cortex by loss of afferent inputs.

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