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Revisiting the Function of PSA-NCAM in the Nervous System

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Abstract

Since its first description the polysialylated form of NCAM (PSA-NCAM) is thought to be a major regulator of cell–cell interactions in the nervous system. Over the past few years many crucial questions have been answered concerning PSA biosynthesis and function. Among these are the identification and cloning of the key enzymes that are responsible for its synthesis and the fact that expression of PSA is not restricted to developmental stages but maintained in the adult nervous system. In the adult, PSA has been shown to be not only a marker of structural plasticity but seems to be a major player in these processes. Originally suggested to be a purely anti-adhesive factor, modulating cell-cell interactions in general and by this allowing plasticity, there is now increasing evidence that this might not be the whole story. Instead, it appears possible that PSA-NCAM interacts with secreted signaling molecules and by this fulfills a more instructive function in brain plasticity.

Index Entries: Axon growth; cell migration; plasticity; cell adhesion.

Introduction

Polysialylated NCAM has been shown to be a key player in the regulation of molecular interactions that participate in the formation and the activity-dependent remodeling of neuronal circuits (for review, *see* refs. *1–4*). Polysialic acid (PSA) is a long linear homopolymer of α2,8 sialic acid. In vertebrates, the major, if not the exclusive, carrier of PSA has been shown to be the neural cell adhesion molecule (NCAM) *(1,5–7).* PSA on NCAM is synthesized by two related transferases, namely ST8SiaII (STX *[8,9]*) and ST8SiaIV (PST or PST-1 *[10,11]).* Although their biochemical activities appear to be comparable, their temporal and spatial expression patterns differ significantly. The available data suggests that strong expression

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of ST8SiaII is responsible for the large amounts of PSA present during embryogenesis, but that its expression decreases shortly after birth. In contrast, expression of ST8SiaIV appears low during development, but persists in the adult nervous system, where PSA is restricted to brain regions showing ongoing plasticity, as for example the hippocampal formation, the olfactory system, and the hypophysis *(12–15).* However, the observation that some regions in the adult brain show PSA-expression in ST8SiaIV-knockout-mice *(16)* suggests that the situation is more complicated.

Here we will review recent data concerning the expression and function of PSA-NCAM in the aforementioned, but also other systems, and discuss the possible molecular mechanism(s) that could underly these functions.

PSA-NCAM in Cell Migration

The function of PSA-NCAM in the regulation of cell migration in the nervous system has been best studied in three different models. First, the dispersion of Oligodendrocyte-type 2 Astrocyte (O-2A) progenitors from neurohypophyseal explants, second, the migration of neural precursors from the subventricular zone of the forebrain to the olfactory bulb and third, the migration of lutheinizing hormone releasing hormone (LHRH) neurons from the olfactory placodes into the developing forebrain.

O-2A progenitors were first described in the optic nerve *(17).* However, explants from the newborn rat neurohypophysis generate a highly migratory cell population that shows molecular and morphological characteristics of such O-2A progenitors *(18).* Although both populations of O-2A cells express PSA-NCAM, axonophilic migration along the optic nerve seems to be independent of PSA-NCAM *(19).* In contrast, Wang and colleagues *(18)* demonstrated that treatment of the O-2A cell containing explant with the PSA-specific endosialidase Endo-N induced a complete blockade of the dispersal of the O-2A cell population. Interestingly, pharmacological blockage of *N*-methyl-D-aspartate (NMDA) receptors inhibited PSA-NCAM biosynthesis and concomitantly diminished O-2A cell migration *(20),* suggesting that glutamate-mediated neuronal activity is a regulating factor for PSA-NCAM-expression. Altogether, these findings provided the first direct evidence that PSA expression is essential for cell migration of a neural precursor cell population.

These first observations linking cell migration to PSA-NCAM expression on the migrating cells were consistent with data acquired in other processes, as for example the migration of interneuron precursors in the forebrain. In rodents, interneurons of the olfactory bulb (OB) are generated postnatally and throughout adult life at the subventricular zone (SVZ) of the lateral ventricles *(21).* Using a particular mode of migration in a chain-like organization, they translocate tangentially through the forebrain using a well-defined pathway, the rostral migratory stream (RMS) *(22–24;* for review, see refs. *25,26).* A variety of immunohistochemical studies using anti-PSA antibodies showed that the migrating cells express large amounts of PSA-NCAM *(15,27,28).* The functional importance of this expression has been first shown with the availability of mice in which NCAM, and consequently PSA-NCAM-expression, has been genetically altered. Such animals, either entirely devoid of NCAM *(29)* or exclusively NCAM-180 deficient *(30)* show a drastically reduced OB and an accumulation of neural precursors along the RMS, owing to a deficit in their migratory behavior. The observation that this effect is phenocopied by enzymatic removal of PSA in vivo *(31)* as well as in vitro *(29,32)* indicates the importance of the PSA moiety in this process.

The combination of in vivo and in vitro analysis allowed deeper insight into the possible function of PSA-NCAM in chain migration. Transplantation studies demonstrated that NCAM-180 mutant cells grafted into a wild-type SVZ migrated into the OB *(33).* The authors suggested that in such a situation the mutant cells would integrate into existing PSA-positive chains and were towed along. This is in agreement with results obtained in mouse-

chicken chimeras (in which mouse SVZ-derived precursors were grafted into the neural-crest pathway of chick hosts) showing that chain migration is a community effect independent of the environment *(34).* The reverse experiment in the mouse, grafting of wild-type cells into a mutant environment produced somehow contradictory results. While one group *(33)* found only very little migration, another *(29)* demonstrated less efficient but still considerable migration in such a heterotypic situation. However, both studies used animals of different age as host and donor as well as different survival times after grafting, which might account for the miscellaneous outcome. These studies were compatible with two models for PSA-function in this system. Either a shielding of the migrating cells from interactions with their environment, or a direct role in the maintenance of chains. However, the development of culture systems that allowed the in vitro analysis of chain migration *(35)* in association with knockout mice and Endo-N treatment *(29,32),* as well as the use of mouse-chicken chimeras *(34)* allowed to further characterize the function of PSA-NCAM in precursor migration. A surprising conclusion from these studies is that PSA-NCAM appears to be a positive regulator for chain formation, either by the promotion of adhesion or by an involvement in recognition between the migrating cells. This is in contrast to the general view of the molecule as a general inhibitor of cell-cell interactions.

The third model in which the function of PSA-NCAM in neural cell migration has been investigated is the migration of LHRH neurons. These cells move from their place of birth, the olfactory placode, via the OB into the developing forebrain *(36–38).*

Enzymatic removal of PSA from the developing brain significantly inhibited the migration of the LHRH neuron population both in mouse *(39)* and chicken *(40).* Surprisingly, the absence of PSA-NCAM in NCAM-knockout mice did not produce this effect *(39),* demonstrating that this type of migration is sensitive to acute (enzymatic), but not chronic (genetic), removal of PSA-NCAM.

The use of NCAM-180 mutant mice allowed the investigation of the behavior of LHRH neurons in a choice situation. Normally, LHRH neurons use two PSA-positive pathways for their migration: one leading to the accessory OB, expressing the NCAM-140 isoform and thus retaining PSA-expression in NCAM-180 mutants, the other leading to the forebrain, expressing NCAM-180 and thus specifically affected in these mutants. A shift in the choice of the migration route, resulting in a higher proportion of LHRH cells in the accessory OB, suggested a preference of a subset of migratory LHRH cells for a PSA-positive axon branch over a PSA-negative one. These data would be in agreement with the notion that PSA-NCAM acts as an inhibitor of cell-cell interactions, thereby creating a permissive environment that would favor migration into the accessory olfactory bulb. However, an alternative explanation would be that PSA facilitates recognition of the correct migratory route in a way comparable to the recognition of the migratory chains by SVZ-precursors. In this case PSA would play a direct role in the guidance process.

PSA-NCAM in Axonal Growth and Fasciculation

Since their identification, PSA and NCAM have been implicated in the regulation of axon growth and guidance *(41).* Using primary neurons growing on monolayers that expressed different NCAM-isoforms, Doherty and colleagues *(42)* demonstrated that NCAM-dependent neurite outgrowth of chicken retinal ganglion cells in vitro could be inhibited by removal of PSA from neuronal NCAM with EndoN. They suggested that changes in PSA expression specifically modulate NCAM-dependent axonal growth. In another paradigm, Zhang and colleagues *(43)* showed that loss of PSA from cultured retinal ganglion cells reduced neurite outgrowth on NCAM-coated membrane vesicles.

In vivo evidence for a role of PSA-NCAM in axonal growth has originally mainly relied on

injection of EndoN into the developing embryo. Using the chicken system, Tang and colleagues *(44,45)* demonstrated that application into the developing limb induced changes in the branching patterns of the arriving motor axons. Furthermore, injection of antibodies against the adhesion molecule L1, which is a major mediator of axon-axon fasciculation in this system, reversed the effects of PSA-removal. Based on these observations the authors suggested that the deficient branching was due to enhanced fasciculation. In this scenario, PSA might play a permissive role by weakening axon-axon interactions in the plexus and allowing the reorganization that is essential for correct motor axon targeting to muscle.

Interestingly, the use of EndoN in other systems led to somewhat contradictory results. PSA removal from cultured neural cells *(46)*, the developing optic *(47)* and cultured spinal cord neurons *(48)* induced a striking defasciculation. This has been attributed to a shift from axon-axon interactions to axon-substratum interactions in a situation where the "shielding" component PSA is absent, thus allowing for the premature response to guidance cues provided by the environment *(47).*

However, comparable results have been interpreted differently by others. For example, Monnier et al. *(49)* used intravitreal injection of anti-PSA antibodies and EndoN to approach PSA-function on outgrowth of retinal ganglion cell axons at the optic fissure. The observation that both treatments resulted in pathfinding errors of retinal ganglion cell-axons led the authors to suggest that PSA is involved in the recognition of orientation cues and that it might act via the sensitization of growth cones to environmental cues.

Further evidence for a role of PSA-NCAM in the control of axon growth has been acquired by a detailed analysis of the dentate gyrus in the hippocampal formation. This structure, like the SVZ, shows ongoing neurogenesis *(50)* and expression of PSA-NCAM in the adult *(15,51–53).*

Granule cells residing in the dentate gyrus send their axonal projection, the so-called mossy fibers, to innervate pyramidal cells in the CA3 area. In the normal situation PSA-NCAM positive mossy fibers are highly fasciculated and form *en passant* synapses in a strictly laminated fashion on the proximal region of the apical dendrite of their target neurons.

First indications for a function of PSA-NCAM in mossy-fiber outgrowth and organization came from the analysis of NCAM deficient mice. Using Timm's, Golgi, and immunohistochemical staining, a general disorganization and delamination of the mossy-fiber termination field was identified *(54).* Electron microscopic analysis and DiI injection provided evidence that the defect was associated with deficient fasciculation of the mossy-fiber axons *(54,55).* Furthermore, analysis of total NCAM or NCAM180-deficient mice and EndoN-treated mouse brains showed ectopic formation of mossy-fiber terminals within the CA3-subfield in the absence of morphological changes of the terminals themselves *(55,56).*

Based on these observations two models for PSA-NCAM function in the mossy-fiber system have been suggested. First, PSA-NCAM could play an active role in the fasciculation process, leading to altered synapse formation as a secondary consequence *(54).* Alternatively, it has been proposed that lack of PSA-NCAM either inhibits the withdrawal of or stabilizes the extension of the transitory axonal processes that mossy-fibers form in the pyramidal cell layer, inducing the formation of ectopic mossy-fiber boutons *(55).*

The last step in the maturation of many axons is the generation of an insulating myelin sheath, which allows for the high-speed transmission of action potentials. The first indication for a role of PSA-NCAM in the control of myelination in the central nervous system (CNS) was the finding that expression of the glycoprotein on the developing optic nerve decreased, and finally disappeared, with a time course paralleling myelination *(57).* A recent study addressed the functional consequences of this phenomenon. Charles et al. *(58)* demonstrated that the premature removal of

PSA from cultured mouse forebrain neurons, induced either by the use of EndoN or by antibody-induced internalization, increased myelinization four- to five-fold. Two possible mechanisms for PSA action in this system could be proposed: 1) PSA-NCAM could act by preventing the establishment of a close contact between axons and oligodendrocytes via steric inhibition; 2) PSA-NCAM could act by triggering a negative signal regulating oligodendrocyte maturation.

PSA-NCAM in Synaptic Plasticity

Long-term potentiation (LTP) i.e., long-lasting changes of synaptic transmission, is one of the leading models to be the basis for learning and memory (for review, *see* ref. *59*). First evidence for a specific function of NCAM in synaptic plasticity arose from the observation that antibodies or interfering peptides inhibited the induction of LTP in the hippocampal CA1 area *(60).* However, the analysis of two different mouse lines that carry targeted mutations of the NCAM-gene produced contradictory results. Muller and colleagues *(61)* described an inhibition of LTP in the CA1 area of NCAM-deficient mice that is in accordance with the finding that these animals show deficits in spatial learning *(7).* In a second strain of NCAM mutants, such a deficit in LTP has not been confirmed *(62).* Whether these differences are due to genetic background or to other changes in experimental parameters is not clear at the moment.

The analysis of the role of PSA-NCAM in synaptic plasticity in the hippocampus has been extended to the CA3 area, in which the synapses of mossy fibers terminate on the pyramidal neurons of this region. In contrast to CA1, where LTP induction is a postsynaptic event, LTP is expressed presynaptically in this pathway *(63).* A detailed analysis of the plastic properties of this synapse in NCAM-mutant mice revealed again a specific defect in LTP in the absence of alterations in basic synaptic transmission or short-term plastic properties *(56).*

However, the analysis of NCAM-deficient mice did not allow discrimination between the absence of the NCAM-protein or the lack of the PSA-modification. Furthermore, the discrimination of a developmental phenotype due to the absence of PSA-NCAM from its acute lack in synaptic plasticity was not possible in NCAM-mutant mice.

Evidence for a specific implication of PSA was obtained through the demonstration that removal of PSA by EndoN could mimic the defect seen in mutants *(61,64).* A recently presented mouse model further confirmed this finding *(16).* In these animals the gene coding for ST8SiaIV/PST, one of the two α2,8 transferases that link PSA to the NCAM protein (*see above)* has been inactivated. These mice express normal amounts of the NCAM protein and show normal histology, as for example correct lamination of mossy-fiber terminals in the CA3 region. Loss of PSA in the presence of NCAM and in the absence of striking morphological changes allowed studying the function of PSA in synaptic plasticity in a direct fashion. In these animals LTP in CA1 was affected in the same way as in NCAM-mutants. In contrast, mossy-fiber LTP in CA3 was normal *(16).* In accordance with the functional data, PSA-NCAM is expressed in synapses in CA1 *(61)* while mature mossy-fiber boutons expressed only the NCAM-protein *(65).* Altogether, these observations show that PSA is involved in LTP at the Schaffer collateral synapse, whereas NCAM is important for LTP in CA3 *(16).* This represents the only demonstration of a function for NCAM independent of PSA in the different mouse models so far.

Another electrophysiological study shed light on the possible mechanism of PSA action in synaptic plasticity *(66).* The authors demonstrated that the deficit in LTP in hippocampal slices prepared from NCAM-knockout mice or treated with EndoN *(see* above) can be rescued by the application of recombinant brain-derived neurotropic factor (BDNF), a secreted peptide growth factor that has been shown to have positive effects on LTP in the CA1 region *(67).* Excess soluble PSA or recombinant PSA-

NCAM-Fc chimera also prevented LTP. While it is possible that PSA and BDNF affect LTP by independent pathways, the fact that phosphorylation of the BDNF receptor trkB is decreased both in NCAM-deficient and EndoN treated slices suggest a functional interaction. The authors propose that PSA-NCAM expression could sensitize CA1 pyramidal neurons to BDNF action and thereby enhance the action of the factor in a selective manner.

PSA-NCAM in Neuroglial Plasticity in the Hypophysis

The mammalian hypothalamo-neurohypophysial system (HHS), which secretes oxytocin and vasopressin, shows remarkable plasticity and strong expression of PSA-NCAM in the adult in response to physiological stimulation *(68,69).* This makes it an attractive model for studying the regulation and the role of PSA in activity dependent plasticity. The pituicytes are the principal cellular element of the neural lobe of the neurohypophysis (NH). They have the morphological characteristics of glial cells and are immunoreactive for markers such as glial fibrillary acid protein (GFAP) *(70),* vimentin *(71),* and S-100 *(71).* In the resting state, pituicytes surround the neurosecretory axons and terminals of the magnocellular neurons located in the hypothalamus, which suggests that they form a physical barrier between the blood vessels and the neurosecretory terminals.

Stimulation of neurohormone secretion such as parturition, lactation, or dehydration, results in the retraction of astrocytic processes accompanied by enlargement and multiplication of neurosecretory terminals. This is thought to facilitate the release of neuropeptides into the general circulation. All these changes are reversible with cessation of stimulation *(68,69,72).* PSA-NCAM is highly expressed in the mature HHS by both neurons and glial cells *(69).* On magnocellular neurons, PSA immunoreactivity is mainly associated with axons within the NH; expression on astrocytes is restricted to processes. The morphological modifications in the hypothalamus during lactation are accompanied by changes in the PSA-NCAM expression pattern. Both modulation of PSA-NCAM expression and regulation of the PST enzyme *(73)* over the time of stimulation offer strong correlative evidence that the molecule is an important player for the morphological plasticity of the system. Using enzymatic removal of PSA on NCAM, Theodosis and colleagues have recently obtained direct evidence of such a role *(74,75).* They showed that the ability of pituicytes to undergo morphological changes as well as synaptic remodeling in response to physiological stimuli are dependent on their expression of the carbohydrate *(74,75).* Surprisingly, the absence of PSA leading to the perturbation of morphological changes induces no measurable consequence to HNS function, suggesting that compensatory mechanisms do exist. These observations are in agreement with a mechanism whereby PSA could decrease adhesion via physical interference or change repulsion leading to structural remodeling.

Conclusions

Against all predictions based on expression patterns and in vitro analyses, PSA on NCAM appears not to be essential during embryogenesis, but rather acts as an important regulator of plasticity in the adult nervous system. Up to now the investigation of knockout mice lacking either the NCAM-protein (and consequently also lacking PSA) or the PSA-generating enzyme ST8SiaIV, show no striking defects during development *(7,16,30).* What could be the basis for this surprising observation? One obvious explanation might be that compensatory mechanisms exist during embryogenesis. Alternatively, even if at the end of development the whole system is set up correctly, errors or delays in development might exist, but so far escaped the phenotypic analysis. Such a scenario is suggested by the finding that the neuromuscular synapse in NCAM-

mutant mice is normal in the adult. However, in a well-defined time window at the early postnatal stage, maturation of the synapse is delayed *(76).* In the adult, PSA-NCAM is associated with regions that show ongoing morphofunctional plasticity. In these regions processes that normally occur during development, such as cell migration, axonal growth, and synaptogenesis, are not restricted to a certain time window but extended throughout life. Thus, in a situation where errors are continuously produced, the system may be unable to "catch-up" as during development.

Another question that arises is which molecular mechanism underlies the functions of PSA-NCAM. In general, PSA on NCAM was thought to attenuate adhesion forces mediated by NCAM and modulate overall cell-surface interactions, thereby regulating changes in the shape and movement of cells or cell processes *(48).* Thus, PSA was thought to act as a purely anti-adhesive factor regulating, in a nonspecific manner, cell-cell interactions in general (Fig 1A). Although this model is in agreement with many of the experimental findings some of the data discussed earlier, such as chain migration in the RMS or axon fasciculation in the optic nerve or the hippocampal formation, do not support such a mechanism. Here, the data suggests a more instructive role of the PSA-NCAM.

The recent work by Muller and colleagues *(66)* suggests another model for PSA-NCAM action. As described earlier, presence of PSA-NCAM sensitizes hippocampal pyramidal neurons to BDNF, thereby modulating activity dependent synaptic plasticity. In line with these findings, Vutskits and colleagues *(77)* demonstrated comparable synergistic effects of PSA-NCAM and BDNF on the survival of cultured cortical neurons. Three molecular mechanisms have been proposed that might explain these results.

First, a direct interaction between PSA-NCAM and BDNF due to the physico-chemical characteristics of the two molecules could lead to an increased concentration of BDNF in the vicinity of the PSA-positive cell (Fig. 1B). In this scenario PSA-NCAM would play the role of a permissive factor by facilitating the interaction of BDNF and the trkB receptor. However, it has been demonstrated that the survival effect of BDNF and PSA-NCAM is not reproduced by the application of NT3, a factor that shows comparable physico-chemical properties as BDNF, and thus should also interact with PSA. This suggests that the interaction between PSA and BDNF is not merely due to general charge interactions but might require a specific molecular conformation. Second, PSA could act specifically on the trkB receptor and thereby augment the efficiency of signaling (Fig. 1C). One way to achieve this could be via a *cis* interaction that induces a conformational change of trkB, which, in turn, would facilitate ligand binding. Third, PSA-mediated *cis* interactions at the cell surface could induce a reorganization of multifunctional signaling complexes that include for instance trkB (Fig. 1D). A comparable *cis* mechanism of PSA has already been proposed by Rutishauser and Landmesser *(2).* In the model presented here, the presence of PSA would reorganize clusters of membrane bound molecules, thereby un-masking the trkB receptor and allowing trkB-BDNF signaling.

Chain migration of neural precursors in the forebrain has been shown to be PSA-NCAM dependent. How does the existing data fit into the aforementioned models on the putative function of PSA? Chain formation depends on tight contacts and specific interactions between the migrating cells. As discussed earlier, genetic and enzymatic removal of PSA from the migrating cells in vivo as well as in vitro, induces a disturbance of chain integrity. This is not easily explainable with an anti-adhesive function of PSA-NCAM.

The concomitant expression of PSA-NCAM and BDNF in the forebrain *(28,78,79)* allows us to postulate that in this system, like in the hippocampus, PSA could regulate migration by modulating BDNF signaling. Intraventricular application of BDNF has been shown to increase the proliferation of newly generated neuronal precursors in the SVZ and the RMS,

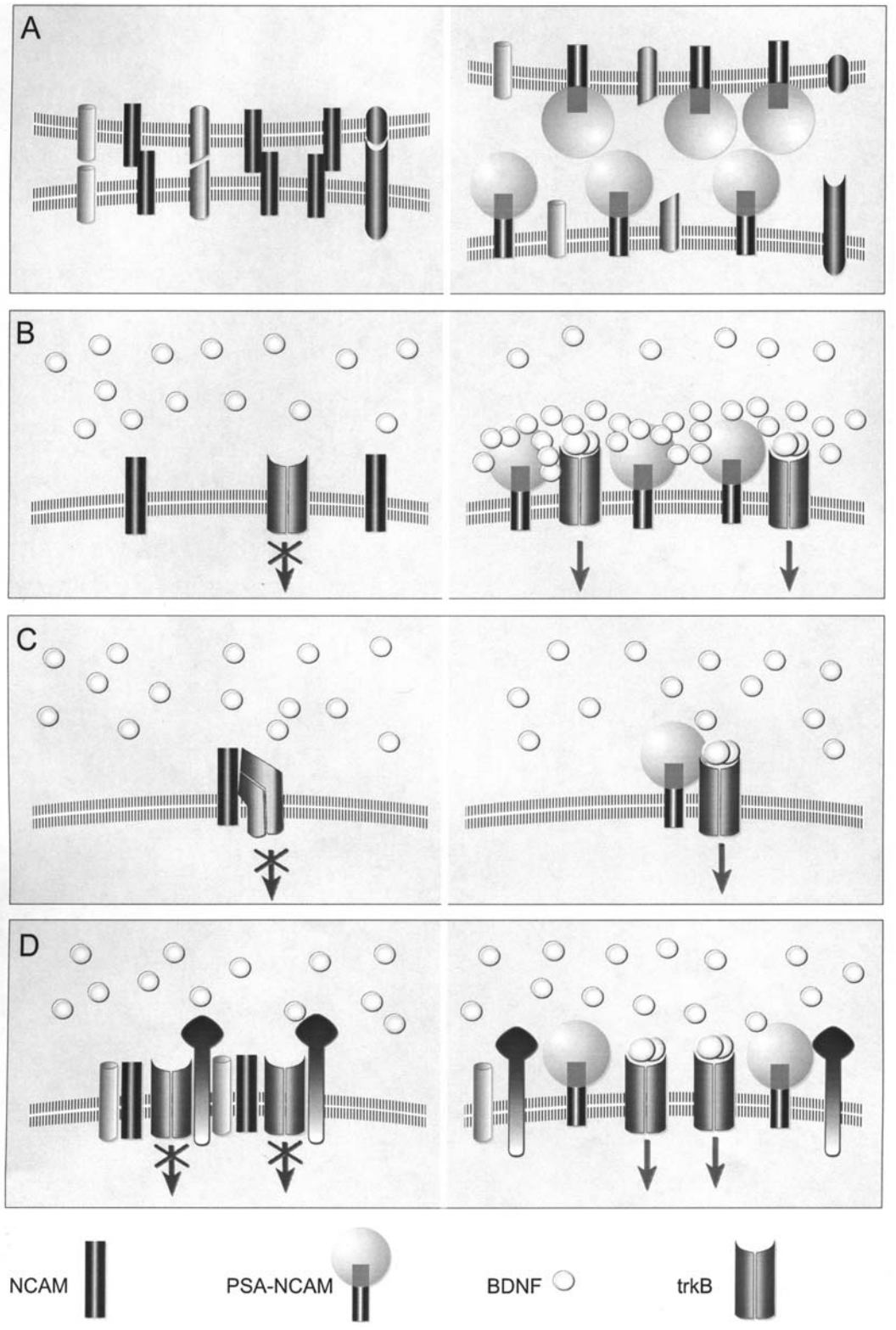
A
B
C
D
NCAM
PSA-NCAM
BDNF
trkB

Fig. 1. Possible molecular mechansims of PSA-NCAM action. **(A)** The classical model of PSA-NCAM as a general anti-adhesive factor regulating cell–cell interactions in a non-specific manner via steric or charge impedance. **(B)** PSA-NCAM at the cell surface induces an increase in in the concentration of soluble factors. The physico-chemical characteristics of PSA on NCAM could lead to an augmented concentration of soluble factors in the vicinity PSA-positive cells. In the case of BDNF such a mechanism would allow trkB-mediated signaling. **(C)** A conformational change due to a cis interaction with PSA-NCAM leads to an activation of surface receptors, as for example trkB. **(D)** Reorganization of multifunctional signaling complexes by PSA-NCAM. The presence of PSA on NCAM would modify clusters of membrane bound molecules, thereby unmasking surface receptors. For the trkB-example, this would allow BDNF-binding and subsequent signaling.

demonstrating that the trkB-BDNF signaling pathway is active in these cells *(79).* However, a direct function in the regulation of migration has to our knowledge not been demonstrated so far.

An attractive alternative possibility is that PSA on the migrating precursors is capable of modulating other ligand-receptor complexes in this system. The migration defect in NCAM-deficient mice could be due to a deficit in the recognition of guidance cues within the pathway.

The secreted factor *slit* is expressed by the septum and has been shown to be a repellent cue acting through its *robo* receptors expressed by migrating precursors in the RMS *(80).* It appears conceivable that this complex interacts with PSA-NCAM in a way comparable to the models presented earlier for BDNF and trkB.

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