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Expression of polysialyltransferases (STX and PST) in adult rat olfactory bulb after an olfactory associative discrimination task

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ABSTRACT

Neuronal plasticity and neurogenesis occur in the adult hippocampus and in other brain structures such as the olfactory bulb and often involve the neural cell adhesion molecule NCAM. During an olfactory associative discrimination learning task, NCAM polysialylation triggers neuronal plasticity in the adult hippocampus. The PST enzyme likely modulates this polysialylation, but not STX, a second sialyltransferase. How the two polysialyltransferases are involved in the adult olfactory bulb remains unknown. We addressed this question by investigating the effect of olfactory associative learning on plasticity and neurogenesis. After a hippocampo-dependent olfactory associative task learning, we measured the expression of both PST and STX polysialyltransferases in the olfactory bulbs of adult rats using quantitative PCR. In parallel, immunohistochemistry was used to evaluate both NCAM polysialylation level and newly-born cells, with or without learning. After learning, no changes were observed neither in the expression level of PST and NCAM polysialylation, nor in STX gene expression level and newly-born cells number in the olfactory bulb.

1. Introduction

Post-translational protein modification contributes to cell plasticity, especially the addition of membrane glycans, such as glycoproteins, which interact with other cells and extracellular components. In particular, polysialic acid (PSA) attachment to neural cell adhesion molecule (NCAM) plays a major role in neuronal plasticity.

Many studies show NCAM involvement in homotypic (neuron/neuron) and heterotypic (neuron/glia) interactions (Drazba & Lemmon, 1990), synaptic changes during learning and memorizing (Cremer et al., 1994; Cremer et al., 2000) and late stages of memory consolidation (Arami, Jucker, Schachner, & Welzl, 1996). Data suggest a relation between NCAM-mediated interactions and cognitive functions. Indeed, intraventricular infusions of anti-NCAM inside cerebral ventricles disturbs the consolidation of a passive avoidance response (Doyle, Nolan, Bell, & Regan, 1992). Moreover, the injection of anti-NCAM antibodies into hippocampus CA1 region inhibits long-term potentiation (LTP) (Ronn, Bock, Linnemann, & Jahnsen, 1995) and NCAM-deficient mice show impairments in spatial learning (Cremer et al., 1994; Kraev et al., 2011). The degree of NCAM sialylation directly regulates its ability to promote cell adhesion. Indeed, the attachment of large homopolymers of PSA down-regulates cell interactions, attributing anti-adhesive properties to PSA–NCAM (Bonfanti, 2006; Johnson, Fujimoto, Rutishauser, & Leckband, 2005), whereas loss of PSA increases the relative adhesivity of cells (Bonfanti, Olive, Poulain, & Theodosis, 1992; Rutishauser, Acheson, Hall, Mann, & Sunshine, 1988).

PSA–NCAM is largely expressed in the developing brain, where it mediates cell migration, neurite outgrowth, and synaptogenesis (Ono, Tomasiewicz, Magnuson, & Rutishauser, 1994). In the adult nervous system, PSA–NCAM mediates structural plasticity (Bonfanti, 2006; Gascon, Vutskits, & Kiss, 2007; Rutishauser, 2008) and is involved in activity-dependent synaptic remodeling (Hoyk, Parducz, & Theodosis, 2001; Stoenica et al., 2006). PSA–NCAM involvement in learning, memory and consolidation has been extensively reported in the rodent hippocampus. During learning processes, synaptic remodeling is associated with an increase of polysialylated NCAM (Fox, O'Connell, Murphy, & Regan, 1995; Murphy, O'Connell, & Regan, 1996; Sandi et al., 2003). PSA removal (e.g. through microinfusion of endoneuraminidase-N in the hippocampus) decreases rat performances in the Morris water maze (Becker et al., 1996) and reduces freezing responses to a conditioned context (Lopez-Fernandez et al., 2007). Similarly, adult mice deficient in PSA–NCAM exhibit spatial learning deficit (Markram, Gerardy-Schahn, & Sandi, 2007).

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The direct link between PSA expression in the adult olfactory bulb, olfactory learning and the memorization process is still unclear. The olfactory system includes the nose with the olfactory mucosae, the olfactory bulb and the piriform and entorhinal cortices (Shipley & Ennis, 1996), leading to the hippocampus. These neuronal circuits represent the pathway involved in olfactory learning and memory storage (Wilson, Best, & Sullivan, 2004). The olfactory system is a unique example for structural plasticity, as PSA–NCAM is found to be expressed in the olfactory bulb (Bonfanti, 2006; Bonfanti et al., 1992; Miragall, Kadmon, Husmann, & Schachner, 1988; Rockle & Hildebrandt, 2015). The loss of PSA leads to and coincides with the differentiation of newly-born interneurons, suggesting that the expression of PSA prevents the differentiation of the neuronal precursors migrating from the subventricular zone (SVZ) (Petridis, El-Maarouf, & Rutishauser, 2004). Moreover, PSA may promote the survival of the newly-born neurons *in vitro* (Vutskits, Gascon, Zgraggen, & Kiss, 2006). More recently, the generation of PSA-deficient mice impairs the neuroblasts migration in the rostral migratory stream and the interneuron composition in the olfactory bulb (Rockle & Hildebrandt, 2015). The main olfactory bulb, the first site for processing of odor discrimination, is involved in learning and memory tasks (Brennan, Kaba, & Keverne, 1990; Brennan & Keverne, 1997; Wilson & Stevenson, 2003). The link between learning, memory and NCAM polysialylation in the olfactory bulb is not clearly established because the addition of PSA in this structure is associated with neurogenesis, a process also involved in mnesic processes (Bonfanti, 2006). Nevertheless, olfactory enrichment enhances the survival of newborn cells and improves olfactory memory (Rochefort, Gheusi, Vincent, & Lledo, 2002). In addition, NCAM-deficient mice exhibit a smaller olfactory bulb associated with impaired odor discrimination (Gheusi et al., 2000; Lledo & Gheusi, 2003). Moreover, PST-deficient mice display olfactory deficits, associated with a decrease of PSA–NCAM expression in the brain, that lead to impaired social behaviors (Calandreau, Marquez, Bisaz, Fantin, & Sandi, 2010). Nevertheless, other studies revealed that changes in neurogenesis and/or NCAM are not related with concomitant changes in cognitive function (Lledo, Alonso, & Grubb, 2006). Since then, a study suggested that learning modulates survival of the adult-born neurons in the olfactory bulb and those adult-born neurons might participate in the long-term retention of olfactory information through unknown mechanisms (Sultan et al., 2010). Another study determined that the survival of the adult-born neuron during olfactory learning is governed by consolidation in the olfactory bulb (Kermen, Sultan, Sacquet, Mandairon, & Didier, 2010).

The polysialylation of NCAM is mediated and regulated by two enzymes, sialyltransferase-X (STX/ST8SiaII) (Kojima, Tachida, Yoshida, & Tsuji, 1996; Scheidegger, Sternberg, Roth, & Lowe, 1995) and polysialyltransferase (PST/ST8SiaIV) (Eckhardt et al., 1995; Nakayama, Fukuda, Fredette, Ranscht, & Fukuda, 1995). In the brain, STX expression predominates during the embryonic period and declines after birth, while PST expression peaks during adulthood (Hildebrandt, Becker, Murau, Gerardy-Schahn, & Rahmann, 1998; Ong et al., 1998).

Cognitive processes, such as learning and memorizing, require structural changes in brain, including especially new synapses and synaptic modification efficacy (Breen, Coughlan, & Hayes, 1998). Adult structural remodeling of the neuronal network involved two different mechanisms. First, synaptic plasticity of the mature neuronal network, in which PSA–NCAM is involved during learning through its adhesive properties (Guiraudie-Capraz et al., 2011). In this case of plasticity, the addition of PSA on NCAM seems to result from PST over-expression, at least in the dentate gyrus of the hippocampus (Manrique et al., 2014). Secondly, neurogenic processes are thought to be involved in learning and memorizing (Bonfanti, 2006). Cell proliferation and newborn neuron survival also require the expression of PSA–NCAM on the newly born cell surfaces. Previous works suggest that STX is responsible of the addition of PSA on NCAM on these immature cells, at least in the dentate gyrus of the hippocampus (Manrique et al., 2014; Nacher et al., 2010).

The mammalian olfactory system have general relevance for understanding the neurobiology of memory. Then, to further study the involvement of the two polysialyltransferases, STX and PST, in olfactory mnesic processes, we evaluated if learning an olfactory associative discrimination task modulates their expression in the olfactory bulb. Adult rats were submitted to an olfactory associative task. STX and PST mRNA expression levels were first measured in the olfactory bulb of "Learner" rats using quantitative RT-PCR (qPCR). Secondly, PSA–NCAM protein expression was investigated using immunohistochemistry. Finally, we investigated neural cell proliferation by determining BrdU immunoreactivity in the olfactory bulb.

2. Material and methods

2.1. Animals

Ten week-old male Sprague–Dawley rats (Charles River Laboratories, France), weighing 350 g, were housed in individual cages and supplied with food and restricted access to water. The housing temperature was constant (22 °C) under a 12-h/12-h light–dark cycle (light on at 6:30 am) during 10 days before the beginning of behavioral experiments. All the behavioral experiments were realized in accordance with the European guidelines concerning the procedures for animal experimentation (official authorization number A4/12/12) following the European Community Council Directive (2010/63/UE). All efforts were made to minimize animal suffering and to reduce the number of animals used while complying with statistical constraints.

2.2. Apparatus and training procedure

Rats were trained using their sense of smell and their associative memory performance was tested using an olfactory associative discrimination task developed in our laboratory (Chaillan, Roman, & Soumireu-Mourat, 1998; Manrique et al., 2014). The olfactory training apparatus was described previously (Manrique et al., 2014). Briefly, the system consisted in a rectangular box made of wire mesh with a conical odor port, drilled horizontally through a triangular wedge of Plexiglas, mounted in one corner of the cage. A circular water port in the shape of a well was placed directly above the odor port. A photoelectric circuit was used to monitor the responses to the odor presentation. Two flashlight bulbs were placed outside the cage, one on each side of the odor and water ports. Odors were individually delivered by forcing clean air (0.7 bar) through one of two Erlenmeyer flasks containing 2‰ of chemical odorants (jasmine (S+)/strawberry (S−); Sigma) mixed in water. Between each odor presentation, non-odorized air was delivered by sending air through a flask that contained only water. Water (0.1 mL) was delivered using a gravity-fed system through a valve into the water port. All experiments were conducted simultaneously in four cages to ensure training under the same conditions. Animals were trained to make two odor-reward associations. Two odors were associated with a specific reward, one designated as positive (S^+) and the other as negative (S^-) (Go /No-Go paradigm). A daily session was made of 60 individual trials (S^+ or S^-) run in a quasi-random fashion. When S^+ was delivered into the cage, if the rat responded, a water reward was delivered. The same response to S^- resulted in no water delivery and activa-

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