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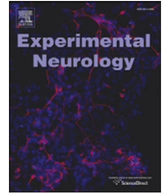

Increased synapsin expression and neurite sprouting in lamprey brain after spinal cord injury

Billy Y.B. Lau, Andrea E. Foldes [1], Naila O. Alieva [2], Paul A. Oliphint, David J. Busch, Jennifer R. Morgan *

Section of Molecular Cell and Developmental Biology, Institute for Cell and Molecular Biology, Institute for Neuroscience, University of Texas at Austin, Austin, TX 78712, USA

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ABSTRACT

Spinal cord injury induces structural plasticity throughout the mammalian nervous system, including distant locations in the brain. Several types of injury-induced plasticity have been identified, such as neurite sprouting, axon regeneration, and synaptic remodeling. However, the molecular mechanisms involved in injury-induced plasticity are unclear as is the extent to which injury-induced plasticity in brain is conserved across vertebrate lineages. Due to its robust roles in neurite outgrowth and synapse formation during developmental processes, we examined synapsin for its potential involvement in injury-induced plasticity. We used lamprey, a vertebrate that undergoes robust anatomical plasticity and functional recovery after spinal cord injury. At 3 and 11 weeks after spinal cord transection, synapsin I mRNA was upregulated >2-fold in lamprey brain, as assayed by semi-quantitative RT-PCR. Other synaptic vesicle-associated genes remained unchanged. *In situ* hybridization revealed that synapsin I mRNA was increased globally throughout the lamprey brain. Immunolabeling for synapsin I protein revealed a significant increase in both the intensity and density of synapsin I-positive structures in lamprey hindbrain at 11 weeks post-transection, relative to controls. Moreover, the number of structures immunolabeled for phospho-synapsin (serine 9) increased after injury, suggestive of neurite sprouting. Indeed, at the ultrastructural level, there was an increase in neurite density at 11 weeks post-transection. Taken together, these data show that neurite sprouting in the brain is an evolutionarily conserved response to a distant spinal cord injury and suggest that synapsin and its phosphorylation at serine 9 play key roles in the sprouting mechanism.

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Introduction

Spinal cord injury induces compensatory anatomical plasticity in both injured and spared neurons throughout the vertebrate nervous system (Blesch and Tuszynski, 2009; Bradbury and McMahon, 2006; Maier and Schwab, 2006). For example, spontaneous sprouting of both lesioned and unlesioned corticospinal axons has been observed after spinal cord injury in mammals, supporting recovery of movements via reconnections with propriospinal neurons (Bareyre et al., 2005; Courtine et al., 2008; Rosenzweig et al., 2010; Weidner et al., 2001). In addition, regeneration of injured spinal axons through the lesion site has been demonstrated for several ascending and descending tracts in vertebrates ranging from lampreys to mammals (Armstrong et al., 2003; Bradbury and McMahon, 2006; Mladinic et al., 2009; Oliphint et al., 2010; Rovainen, 1976; Yin and Selzer, 1983). Spinal cord injury also induces remodeling of injured neuronal connections at distant locations in the brain. For example, dorsal spinal cord lesions induce axon sprouting in the mammalian brainstem (Jain et al., 2000), which likely plays a role in functional reorganization of the somatotopic map in cortex (Jain et al., 1997; McKinley et al., 1987). Spinal cord injury also induces changes in the density, size, and length of dendritic spines in the motor cortex, indicating a remodeling of synaptic connections in the brain (Kim et al., 2006). Taken together, these findings indicate that spinal cord injury induces compensatory anatomical plasticity of neurites (i.e., axons and dendrites) throughout the vertebrate nervous system at locations both near to and far from the lesion site. Despite our growing understanding of the pathways that undergo anatomical plasticity after spinal cord injury, the molecular mechanisms underlying such plasticity remain poorly understood.

Synapsin has the potential to play an important role in injury-induced plasticity, due to its robust developmental roles in neurite outgrowth and synapse formation. Synapsin is an abundant neuronal phosphoprotein that is highly conserved across vertebrate and invertebrate species (De Camilli et al., 1983; Kao et al., 1999). The mammalian synapsin family consists of three genes (synapsin I, II, and III), while other organisms typically express synapsin I (and sometimes synapsin II) or an ancestral version of the gene (Cesca et al., 2010; Kao et al., 1999). During development of the mammalian

* Corresponding author at: Section of Molecular Cell and Developmental Biology, University of Texas at Austin, 2401 Speedway, Austin, TX 78712, USA. Fax: +1 512 232 5699.

E-mail address: jmorgan@mail.utexas.edu (J.R. Morgan).

[1] Current address: University of Texas Health Sciences Center in San Antonio, San Antonio, TX 78229, USA.

[2] Current address: Mechanobiology Institute, National University of Singapore, Singapore 117411, SIngapore.

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nervous system, synapsins I and II regulate initial neurite formation, axon specification and growth, and synapse formation (Chin et al., 1995; Ferreira et al., 1998, 1995; Han et al., 1991; Lu et al., 1992; Schaeffer et al., 1994). Synapsin III plays a role in axon growth, but has little effect on synapse formation (Ferreira et al., 2000). Overexpression of synapsin I or II induces non-neuronal cells to sprout thin, elongated, branched processes resembling neurites, suggesting that synapsins are sufficient for neurite sprouting (Han and Greengard, 1994). The extent of synapsin-mediated neurite outgrowth is positively regulated by cAMP-dependent protein kinase A (PKA)-mediated phosphorylation at serine 9, as shown in developing *Xenopus* neurons (Kao et al., 2002). In mature neurons, synapsin is highly expressed in synaptic boutons where it regulates the size of the vesicle pool (Gitler et al., 2008; Pieribone et al., 1995), exocytosis kinetics (Hilfiker et al., 1998), vesicle trafficking (Bloom et al., 2003; Ryan et al., 1996), and synaptic plasticity (Rosahl et al., 1993). Thus, the multi-faceted roles for synapsins in neurite outgrowth, as well as synapse structure and function, in both developing and mature neurons make it an excellent candidate for mediating some aspects of injury-induced structural plasticity. However, this has not been tested in any model to date.

Here, we examined synapsin expression before and after spinal cord injury as a first step toward gaining a better understanding of its potential role in injury-induced plasticity. We utilized the nervous system of lampreys for several reasons. First, lamprey nervous system undergoes an exceptional degree of anatomical plasticity after spinal cord injury, including spontaneous regeneration of descending and ascending axons (Armstrong et al., 2003; Davis and McClellan, 1994; Oliphint et al., 2010; Rovainen, 1976; Selzer, 1978; Yin and Selzer, 1983), synapse regeneration (Oliphint et al., 2010; Wood and Cohen, 1979), and synapse remodeling (Cooke and Parker, 2009). The injury-induced anatomical plasticity temporally coincides with robust behavioral recovery (Cohen et al., 1986; Davis et al., 1993; Oliphint et al., 2010). Second, the lamprey synapsins have already been sequenced and are well characterized in the uninjured nervous system (Bloom et al., 2003; Kao et al., 1999; Pieribone et al., 1995). We report that spinal cord injury induced a robust upregulation of synapsin I mRNA and protein, as well as increased levels of phospho-synapsin (serine 9), in the lamprey brain. Injury-induced synapsin upregulation coincided with a significant increase in neurite density in the lamprey hindbrain. Taken together, these data indicate the existence of spinal cord injury-induced neurite remodeling in lamprey brain, as occurs in other vertebrates. The data are also consistent with a role for synapsin and phospho-synapsin (serine 9) in the injury-induced neurite sprouting mechanism, suggesting that at least some developmental processes are recapitulated after injury.

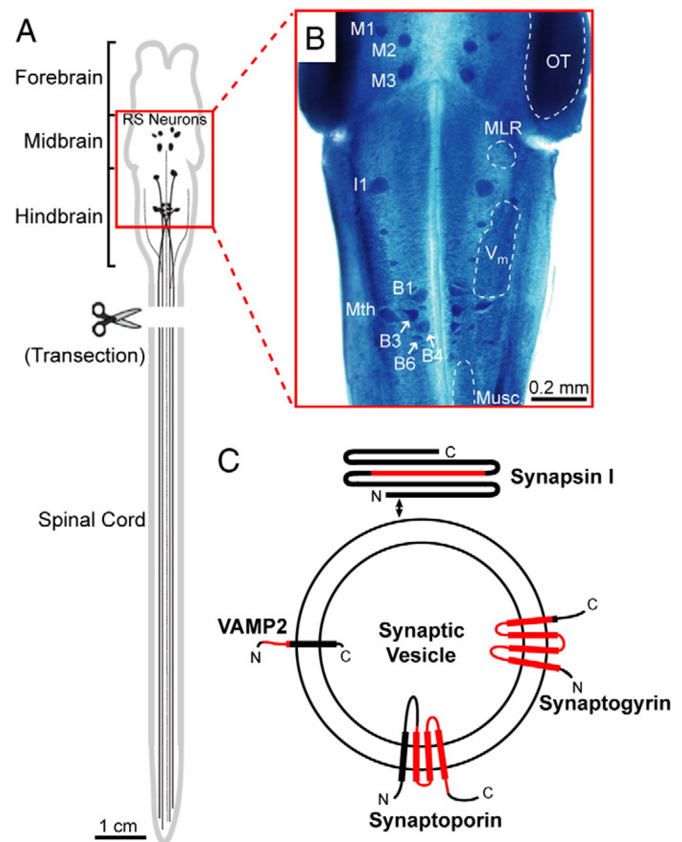

Fig. 1. Lamprey nervous system and genes examined in this study. (A) Diagram of the lamprey nervous system showing the general architecture of the brain, the locations of giant reticulospinal (RS) neurons, and the site of spinal cord transection. Red box highlights the brain regions from which cDNA libraries were generated. (B) Nissl stained brain from an uninjured control lamprey. The neurons and brain regions shown were predicted to undergo changes in gene expression in response to spinal cord injury. (Left) Several of the identified giant RS neurons are labeled, including mesencephalic neurons (M1–3), isthmic neuron (I1), bulbar neurons (B1, B3, B4, B6), and Mauthner neuron (Mth), all of which are bilaterally localized in the same position across individual lamprey brains. These neurons are severed upon spinal cord transection. (Right) Also shown are the optic tectum (OT), mesencephalic locomotor region (MLR), trigeminal motor nucleus (V_m), and muscarinoceptive neurons (Musc.), which project to RS neurons. (C) Diagram of a synaptic vesicle and the associated proteins whose gene expression levels were examined in this study. Red region on the protein corresponds to the gene fragment that was amplified by semi-quantitative RT-PCR.

Methods

Spinal cord transections and brain dissections

Late larval stage sea lampreys (*Petromyzon marinus*; 11–14 cm) housed at room temperature (RT; 25 °C) were used for all experiments. For spinal transections and sham operations, lampreys were anesthetized using MS-222 (0.1 g/l tank water; Argent Labs, Redmond, WA). Complete spinal cord transections were made at the level of the 5th gill, as previously described (Fig. 1A) (Jacobs et al., 1997; Oliphint et al., 2010). Alternatively, sham operated lampreys underwent the same anesthesia, skin and muscle incision, and suturing, but the spinal cord was not severed. Both spinal transected and sham operated lampreys were allowed to recover at RT for 1, 3, or 10–12 weeks (collectively called "11 weeks"). Then, the lampreys were re-anesthetized, and the brains were removed by dissection for further experimentation. For this study, we used a total of 23 uninjured control lampreys, 42 spinal transected lampreys, and 21 sham operated lampreys. All procedures were approved by the Institutional Animal Care and Use Committee at The University of Texas at Austin in accordance with standards set by the NIH.

Nissl staining

Nissl staining was performed as previously described with a few minor modifications (Selzer, 1979; Shifman et al., 2008). Briefly, lamprey brains were fixed in 4% paraformaldehyde (PFA) in 0.1 M PBS (pH 7.4). Then, the brains were stained with toluidine blue O (EM Sciences, Hatfield, PA) containing 1% Borax and no boric acid, followed by differentiation in Bodian's fixative for 20 min, dehydration in 95% and 100% ethanol for 5 min each, clearing in cedarwood oil, and mounting with Permount (EM Sciences, Hatfield, PA). Imaging was performed using a Leica DFC420C camera attached to a Leica MZ10F stereoscope (Leica Microsystems Inc., Bannockburn, IL).

Semi-quantitative reverse transcriptase PCR (RT-PCR)

Using an RNAqueous Micro Kit (Applied Biosystems, Ambion Inc., Austin, TX), total RNA was isolated from lamprey midbrain and hindbrain regions containing descending giant reticulospinal (RS) neurons

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