



Title	The Mechanisms of Chondroitin Sulphate Lyases Treatment in Promotion of Axonal Growth
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symptoms and nonmotor symptoms, which are mostly resulted from progressive death of dopaminergic neurons in the midbrain. The pathophysiological mechanisms are not thoroughly understood. A recent study reports that spines are selectively eliminated in striatal D2 receptor-expressing medium spiny neurons (MSNs) but not in D1 MSNs in the parkinsonian states. We wonder whether the corticostriatal transmission is affected in relation to different neuronal types. By taking advantage of bacterial artificial chromosome D2 transgenic mice, in which enhanced green fluorescent protein is expressed under the control of D2 receptor promoter, we compared paired-pulse ratios (PPRs) of cortically evoked excitatory postsynaptic currents in D1 MSNs and D2 MSNs in slice preparations from naïve and mouse models of PD using whole-cell patch-clamp recordings. Our results showed that corticostriatal AMPA PPRs and NMDA PPRs were specifically decreased in D2 MSNs, but not in D1 MSNs, following dopamine depletion. In the presence of cyclothiazide to block AMPA receptor desensitization, bath application of γ -DGG detected higher glutamate content in the corticostriatal synaptic cleft associated with D2 MSNs from dopamine-depleted mice, which was not caused by malfunction of glutamate transporters. All these observations suggested an increase in the corticostriatal glutamate release onto D2 MSNs in the parkinsonian states. This enhanced glutamate release further led to an increase in the AMPA receptor occupancy level and subsequently in the AMPA receptor desensitization in D2 MSNs. In conclusion, the corticostriatal glutamate release onto indirect-pathway striatal projection neurons was selectively increased in the parkinsonian states, which may underlie the motor deficits in PD.

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DERIVATION OF OLIGODENDROCYTES PRECURSORS FROM BONE MARROW STROMAL CELLS FOR MYLINATION THERAPY

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Myelin damage and disorders caused by physical damage or diseases like leukodystrophies result in severe loss of function. With an aim towards remyelination therapy, we attempted to direct differentiation of bone marrow stromal cells (BMSCs, adult rats) along the oligodendroglial lineage *in vitro*. BMSCs were first cultured as non-adherent spheres until they expressed markers of neural/glial progenitors. The neural/glial progenitors were then maintained in adherent culture supplemented with β -heregulin, PDGF-AA and bFGF. Oligodendrocyte precursors expressing the markers - NG2, Olig2, PDGFRA and Sox10, were detectable within two weeks and can be expanded in culture for up to 3 months with no observable decline in marker expression. To test for myelination capability, BMSC-derived oligodendrocyte precursor cells (OPCs) in a 2-week co-culture with dorsal root ganglion neurons extended myelin basic protein-positive processes along neurites, suggesting maturation into myelinating oligodendrocytes. *In vivo* myelination by BM-OPCs was tested by exploitation of unmyelinated axons of retinal ganglion cells of adult rats. Myelin basic protein-positive processes were also observable along retinal axons by 8 weeks post-injection. Our findings indicate BMSCs as a possible source of OPCs for remyelination therapy.

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THE MECHANISMS OF CHONDROITIN SULPHATE LYASES TREATMENT IN PROMOTION OF AXONAL GROWTH.

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In Injured nerves, chondroitin sulphate (CS) is upregulated forming barriers with astrocytes/fibroblasts and other extracellular matrix molecules, and thereby hampering nerve regeneration. Cleavage of CS using chondroitin sulphate lyases (*Proteus vulgaris*) promises axon regrowth through the barrier but the enzymatic efficacy remains to be improved. Two subtypes, endolyase and exolyase, have been found coexisting in the original host but only the former has been exploited for treatment of injured nerve tracts.

We hypothesise that the two subtypes are necessary for enzymatic efficacy on CSs. We therefore prepared recombinant enzymes of both subtypes. Enzyme kinetics study revealed feedback inhibition by limit digestion products: that of the endolyase by tetrasaccharides and that of the exolyase by the disaccharides. When the two subtypes were used in combination, the digestion efficiency increased. We then used TGF beta-1 to induce CS production by astrocytes in culture, mimicking reactive glia in injured nerves. In co-cultures of such astrocytes with cortical neurons, treatment with combinations of the two subtypes resulted in increased neurite lengths as compared to co-cultures treated with one of the subtypes. The limit digestion products of CS were further tested for their effects on neurite extension on astrocytes that had been treated with TGF beta-1. The CS disaccharides, both 4- and 6-sulphated but not the tetrasaccharides, promoted neurite extension significantly. Taken together, the combinatorial use of the ChABC subtypes not only improved efficacy of enzyme activity on the axon-restrictive CS moiety, but also increased the yield of CS disaccharides which contributed to axonal growth.

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ROLES OF PERINEURONAL MATRIX IN DEVELOPMENTAL PLASTICITY OF THE CENTRAL VESTIBULAR SYSTEM

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Perineuronal nets (PN), as detectable by *Wisteria floribunda* agglutinin (WFA) staining, are extracellular matrix molecules surrounding GABAergic neurons. We hypothesize that PN regulate developmental plasticity of central vestibular circuitry containing GABAergic neurons. To address this, we looked for changes in PN during postnatal development of the central vestibular system. WFA-stained PN in the vestibular nucleus (VN) changed from diffuse organization in neonates to consolidated network in adults. Previous studies suggested that PN formation is activity-dependent. Therefore, the effect of vestibular deprivation on PN development in the VN was also investigated. Following bilateral labyrinthectomy (BL) at P3, the number of VN neurons with PN at adult stage was less than in normal controls. However, following BL at P7 or later, the number of PN-bound VN neurons were similar to normal controls. Our findings indicate that the first postnatal week is a critical period during which inputs from the peripheral afferents provide the necessary trigger for PN formation. The relationship between PN formation and maturation of vestibular functions was further studied with a behavioral test for negative geotaxis (a vestibular reflex). Normal rats acquired the mature response at P9, the time when PN consolidation was first observed. However, the acquisition was delayed to P13 in rats pretreated with chondroitinase ABC injection into the VN at P6 to prevent PN formation. Therefore, PN consolidation in the VN is critical to timely acquisition of the vestibular reflex. Taken together, our results show that PN undergo activity-dependent reorganization that corresponds to the maturation of vestibular behavior, indicating that PN play important roles in plasticity of the developing central vestibular system. [Supported by HKRGC]

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REGULATORY ROLE OF PROHEPARANASE WITH PERI-SYNAPTIC HEPARAN SULFATE PROTEOGLYCAN AND AMPA-TYPE GLUTAMATE RECEPTOR IN SYNAPTIC PLASTICITY

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AMPA-type glutamate receptors (AMPA) govern excitatory synaptic transmission. Perineuronal heparan sulfates (HS) have been implicated in controlling the open-state of AMPAR. Our finding of neuronal heparanase expression in adult rats led us to test (1) if neuronal heparanase is secreted and (2) if the secreted form acts on perineuronal HS to modulate synaptic plasticity.

Neuronal secretion of heparanase was triggered by phorbol ester of rat hippocampal neurons in culture. Western blot analysis of the secreted product revealed enzymatically inactive proheparanase, but not the enzymatically active heparanase. Synaptosomes prepared from phorbol ester-treated rat cortex