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The Function of Spontaneous and BDNF-induced Repair of the Rat Olivocerebellar System

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TABLE OF CONTENTS

ABSTRACT

Research on improving recovery of neural function after adult brain injury has focused on axonal regeneration, i.e. the concept of recapitulating developmental axonal growth in the central nervous system (CNS). Although studies that increase axonal regeneration give rise to some functional improvement, it rarely ameliorates complex neural actions. Some attention has been directed to the type of *reinnervation* that takes place in the neonatal CNS, in which neurons surviving after injury *spontaneously* develop new axons to replace the damaged path and re-form its specific pattern with correct afferent-target connections. Such reinnervation is partly recreated in the maturing CNS by injection of growth factors e.g. brain-derived neurotrophic factor (BDNF). The aim of this project was to examine *in vivo* the structure and function of spontaneous and BDNF-associated alternate reinnervation.

We used the rat olivocerebellar projection to characterise the anatomy, physiology and complex (cognitive) neural function of spontaneous and BDNF-associated post-lesion olivocerebellar reinnervation. In the adult, the olivocerebellar path has a well-defined topography where axons enter the cerebellum via the contralateral inferior cerebellar peduncle and terminate as climbing fibres (CFs) onto Purkinje cells (PCs) to regulate motor and spatial functions. Our model involves unilateral axonal transection of this path (pedunculotomy; Px), either at postnatal day (P) 3 to induce spontaneous reinnervation, or P11 when reinnervation only occurs after injection of BDNF.

First, we examined whether spontaneous olivocerebellar reinnervation compensated complex functions such as spatial learning. As reinnervation is partial in the region which mediates spatial cognition, its capacity to mediate navigation was unknown. We tested rats with (Px3) and without (Px11) reinnervation in simple locomotion and spatial (water maze) tasks. Px3 animals performed the spatial task as well as controls despite learning more erratically while Px11 animals did not learn the task. The amount of reinnervation directly correlated with spatial ability, suggesting that even partial reinnervation was associated with functional benefit in a complex task.

Next, we assessed the effect of increasing olivocerebellar reinnervation by BDNF on its associated functions. BDNF/vehicle-treated animals were tested on simple/complex motor and spatial tasks and the amount and distribution of reinnervation were analysed. BDNF did not affect basic motor skills, however on the rotarod BDNF-treated Px11 animals were similar to normal and Px3 groups. They also exhibited better spatial abilities than vehicle-treated Px11 animals. BDNF treatment increased the amount and distribution of reinnervation in both Px3 and Px11 animals. This suggests that neurotrophin-induced reinnervation facilitated appropriate complex (i.e. cognitive) actions.

Finally, as reinnervating synapses do not always induce appropriate target responses, we examined the synaptic function of BDNF-associated olivocerebellar reinnervation to assess any correlation between CF reinnervation and improved behaviour. CF currents (amplitude, paired-pulse depression [PPD], fatigue) were recorded from adult PCs and the structural CF-PC interactions measured. In synapses forming ~6days post Px3, BDNF was associated with impaired CF-PC interaction (smaller synaptic amplitude, greater PPD smaller CFs). Whereas BDNF-induced reinnervation (i.e.Px11) had increased PPD without affecting anatomical attributes. As PPD aids CF-PC transmission at low *in vivo* frequencies, these synaptic changes are unlikely to affect cerebellar function. Therefore, spontaneous and BDNF-associated reinnervation form functional synapses with associated functional recovery.

RESUME

La recherche sur la réparation fonctionnelle après un traumatisme cérébral chez l'adulte s'est construite autour du concept de régénération i.e. la capacité de (re)croissance des axones dans le système nerveux central (SNC). Bien que la régénération entraîne une amélioration fonctionnelle, les fonctions complexes sont rarement améliorées. Peu de travaux sont consacrés aux processus de *réinnervation* chez le nouveau-né, qui permettent aux neurones immatures de survivre après une lésion et de reformer spontanément de nouvelles connexions. Cette forme de réinnervation est reproduite partiellement chez l'adulte dans le SNC par injection de facteurs trophiques comme le brain-derived neurotrophic factor (BDNF). L'objectif de ma thèse a été d'examiner la structure et la fonction des réinnervations spontanée et induite par le BDNF *in vivo.*

J'ai utilisé la voie olivocérébelleuse chez le rat comme modèle expérimental et étudié l'anatomie, la physiologie du circuit néo-formé et le comportement des animaux après réinnervation post-lésionnelle spontanée et associée au BDNF. Pour cela, les cellules de Purkinje (CPs) ont été désafférentées en sectionnant l'un des pédoncules cérébelleux (Px) soit à 3 jours postnatal (P3) afin d'induire la réinnervation spontanée des CPs par des fibres grimpantes (FGs), soit à P11, la réinnervation étant induite par le BDNF.

J'ai tout d'abord déterminé si la réinnervation spontanée post-lésionnelle chez l'animal jeune (Px3) pouvait compenser une fonction complexe comme l'apprentissage spatial. Dans ces conditions, la réinnervation est partielle dans les régions latérales du cervelet qui sont impliquées dans cette fonction, et donc potentiellement insuffisante pour un comportement normal. J'ai comparé les performances d'animaux Px3 et Px11 non traités par le BDNF dans des tests de comportement moteur et spatial à l'âge adulte. Les animaux Px3 ont acquis la tâche spatiale aussi bien que les contrôles malgré des patrons d'apprentissage plus erratiques, contrairement aux Px11. L'ampleur de la réinnervation chez les Px3 était corrélée au comportement spatial, suggérant qu'une réinnervation même partielle peut améliorer la fonction.

J'ai ensuite évalué les conséquences fonctionnelles de la réinnervation par le BDNF. Les comportements moteur et spatial ont été comparés à la distribution et à l'importance de la réinnervation. Le BDNF n'a eu aucun effet sur le comportement moteur, mais a amélioré considérablement le comportement moteur complexe et spatial des animaux Px11 traités par le BDNF par rapport aux Px11 non traités. L'administration du BDNF chez les Px11 comme chez les Px3 a augmenté la l'importance et l'étendue de la réinnervation. Ces résultats suggèrent que la réinnervation associée au BDNF a permis la récupération des fonctions complexes.

J'ai enfin réalisé une étude électrophysiologique des synapses néoformées en présence de BDNF par la technique du patch-clamp. Le BDNF a entraîné des modifications de la réponse synaptique (amplitude et PPD) et des arborisations des FGs chez les Px3 alors que chez les Px11 seule la PPD était modifiée. La PPD favorise la transmission synaptique à basse fréquence qui est caractéristique du réseau olivocérébelleux *in vivo*; il est donc peu probable que les modifications observées altèrent le fonctionnement des réseaux cérébelleux. En conclusion, dans ce modèle de réparation post-lésionnelle, la réinnervation qu'elle soit spontanée ou associée au BDNF permet une récupération des fonctions comportementales.

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ABP	Actin-binding protein
AMPA	α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor
3-AP	3-acetylpyridine
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
CaM kinase	Ca^{2+}/calmodulin-dependent protein kinase
CAM	Cell adhesion molecule
CF	Climbing fibre
CNS	Central nervous system
CREB	Cyclic AMP response element binding
DAG	Diacylglycerol
DAO	Dorsal accessory olive
DCN	Deep cerebellar nuclei
DRG	Dorsal root ganglion
E	Embryonic ages
ECM	Extracellular matrix molecules
EGL	External germinal layer
EPSC	Excitatory post-synaptic current
ERK	Extracellular signal-regulated kinase
GABA	Gamma-aminobutyric acid
GAP-43	Growth associated protein-43
GC	Granule cell
Grb-2	Growth factor receptor bound protein-2
HCb	Hemicerebellectomy
IGL	Internal granule layer
IP3	Inositol trisphosphate
JNK	Jun N-terminal
LTD	Long-term depression
LTP	Long-term potentiation
MAG	Myelin-associated glycoprotein
MAO	Medial accessory olive
MAP	Microtubule-associated proteins
mEPSC	mini excitatory post-synaptic currents
mGluR	Metabotrophic glutamate receptor
MF	Mossy fibre
MWM	Morris water maze
NGF	Nerve growth factor
NGR	Nogo receptor
NMDA	N-methyl-D-aspartic acid
nr	nervous
NT-3→7	Neurotrophin – 3 to 7
OMgp	Oligodendrocyte myelin glycoprotein
p75	Pan-low affinity receptor
P	Postnatal ages
PC	Purkinje cell
pcd	Purkinje cell degeneration
PF	Parallel fibre
PKC	Protein kinase C
PI-3K	Phosphatidylinositol 3- kinase
PLC_{γ}-1	Phospholipase C

PNS	Peripheral nervous system
PO	Principal olive
PPD	Paired-pulse depression
Px	Pedunculotomy
RGC	Retinal ganglion cell
Trk	Tropomyosin-related kinase
TTX	Tetradotoxin
VOCC	Voltage-gated calcium channel