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Deliverable Nature	
R = Report, P = Prototype, D = Demonstrator, O = Other	R
Dissemination Level	
PU = Public PP = Restricted to other programme participants (incl. the Commission Services) RE = Restricted to a group specified by the consortium (incl. the Commission Services) CO = Confidential, only for members of the consortium (incl. the Commission Services)	PU

A. Abstract / Executive Summary

Here at LENS, in collaboration with the EPFL institute in Lausanne, we combined in vivo two-photon microscopy with multi-photon nanosurgery and electron microscopy to characterize the subcellular details of the structural remodeling triggered by a lesion confined to a single-branch. Single axonal branches were ablated by laser axotomy. This very small denervation induced a consistent reshape in the connectivity of the injured axons with surrounding dendrites, both at the level of synaptic contact turnover and by triggering the sprouting of new branches towards the surrounding dendrites. We explored the hypothesis that newly formed branches were more than just exploratory sprouts with correlative light and electron microscopy. This combination of techniques reveals that the sprouted branch contains large numbers of vesicles accumulated most in varicosities in the close vicinity of Purkinje dendrites.

B. Deliverable Report

1 Introduction

The ability of adult neurons of the CNS to regenerate their axons in response to injury is limited in many neuronal types depending on both intrinsic and extrinsic factors [1-6]. Since axons in the CNS represent a challenging site for targeted manipulation and *in vivo* imaging, little is still known about their post-lesional reactive plasticity and how this is regulated by molecular mediators.

We investigated the reactive plasticity of an axonal terminal arbor by using the climbing fibers (CFs) as a model. Modern optical techniques like two-photon microscopy allows disclosing the real-time structural dynamics and synaptic reorganization of severed axons in the cortex of adult mice, defining the timescale and extent of degeneration and remodeling *in vivo*. Multi-photon laser nanodissection of a single axonal branch triggers axonal sprouting while eliciting synaptic remodeling in the surviving portion of the axon. Correlative light and electron microscopy allows a very subtle characterization of the subcellular details of remodeling events previously monitored in vivo. This combination of techniques revealed that the new varicosities formed on the sprouted branch lie next to Purkinje dendrites, and contain a large numbers of vesicles.

2 Results

We disrupted single axonal branches in live brain with multi-photon laser axotomy. This highly localized damage was performed by irradiating a distal branch of a labeled CF with a high energy dose of Ti:Sapphire laser. Injured CFs compensated for the synaptic loss by modifying their connectivity with surrounding neurons. The injury-induced structural rearrangement involved not only the increase in the turnover of presynaptic boutons but also of transverse branches.

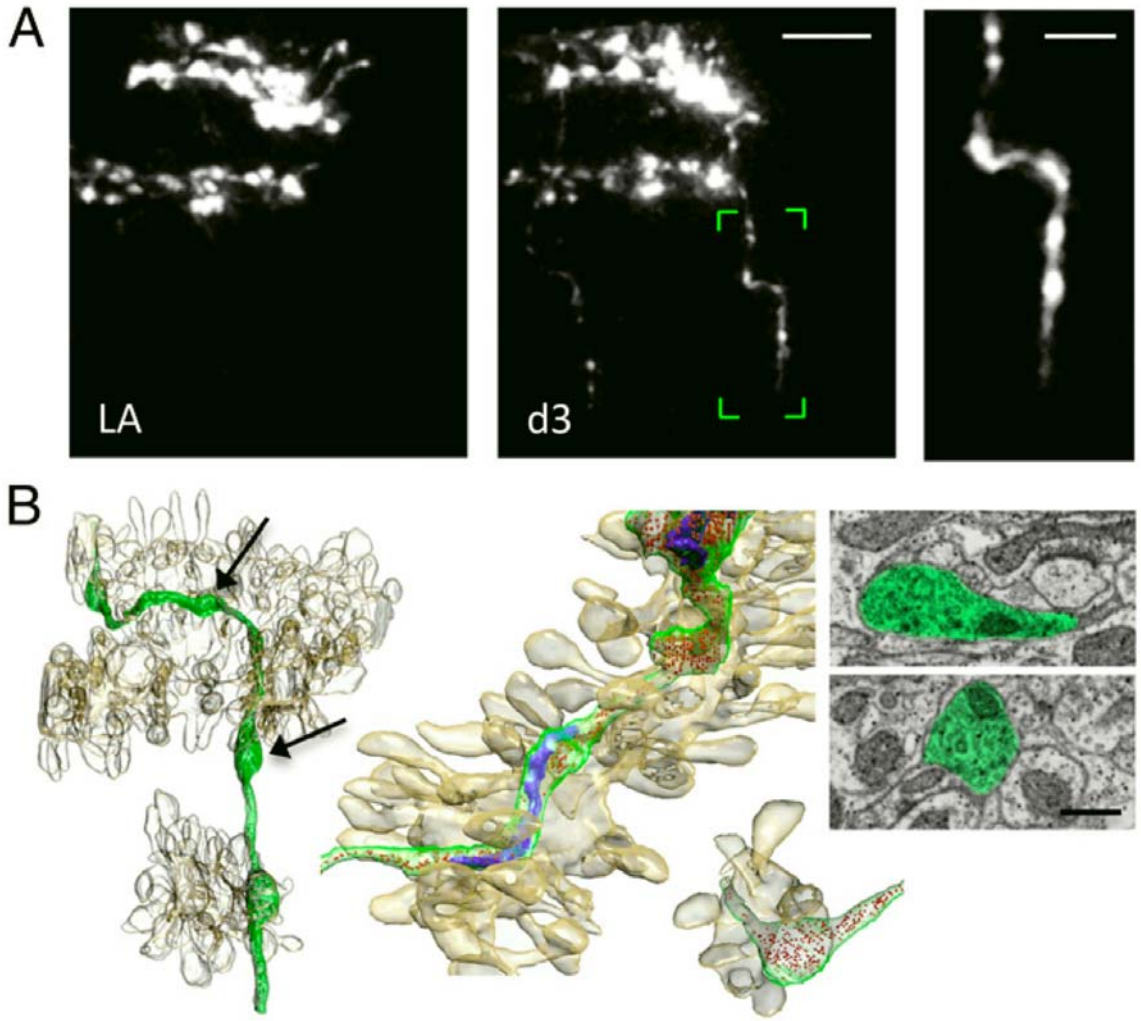

Figure 1: **Correlative light and electron microscopy of the sprouted axon.** (A) Time course of a CF showing the formation of a new branch 3 days after laser axotomy in vivo. Right hand image shows the sprouted axon boxed (in green) in the middle panel with clearly visible varicosities along its length. Scale bar, 10 µm. (B) Block face scanning electron microscopy, using FIBSEM, of the same sprouted axon, enables its reconstruction in 3D (shown in green) along with its mitochondria in blue and the vesicles colored in red. Also reconstructed were the surrounding dendritic segments and their spines (show in light brown). Lower right image shows another view of a single varicosity on this axon, showing its close proximity to two dendritic spines. Right, electrons micrographs from the series showing, pseudo-colored in green, two varicosities containing vesicles and mitochondria, but no indications of synaptic contacts. The electrons micrographs were collected at the level of two varicosities highlighted by the black arrows. Scale bar, 0.5 µm.

CFs transverse branches (TBs) are thin filaments emerging perpendicularly from the main plane of the CF whose functional role is still elusive. Although the mean length and motility of the TBs protruding from the injured fiber are not affected by laser axotomy, CFs reacted to injury by sprouting new transverse axonal branches a few days after laser axotomy (Fig. 1A). The sprouted axon does not protrude towards the site left vacant by the injured CF branch, but in nearby regions where the PC should be normally innervated. Newly formed branches presented varicosities suggesting

that new axons may have some components of the neurotrasmitter release machinery. Using focused ion beam scanning electron microscopy (FIBSEM) we imaged a portion of a sprouted branch previously imaged *in vivo*, and three days after its appearance. We found the new axon contained mitochondria, endoplasmic reticulum and large numbers of vesicles. These accumulated most in varicosities which were found in the close vicinity of Purkinje dendrites (Fig. 1B) suggestive of possible sites for synapse formation.

3 Conclusions

Previous works have shown that CFs are highly plastic and may expand or retract depending respectively on available denervated target or on target removal[7]. In this respect, we do not observe significant variation in the average length and motility of TBs in the injured axon, and at any time TBs turned out to be buds of sprouted branches. Nevertheless, the surviving portion of the axotomized CF reacts by protruding new branches in the same direction of TBs. Indeed, the sprouted axon elongate in a region where PCs should be regularly innervated. We reconstructed the sprouted branch previously imaged in vivo with focused-ion beam/scanning electron microscopy. Three days after its appearance, the new axon contained some components of the synaptic machinery. The newly formed varicosities lay next to Purkinje dendrites and gather a high density of vesicles, resembling developing sites for synapse formation.

A reshape in axonal connectivity is revealed by an increase in the turnover of varicosities on the portion of the injured CF not involved in remodeling events such as degeneration or sprouting. The focal lesion promotes synaptic reorganization on the entire CF, which possibly plays a compensatory role after damage.

4 References/Publications

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