Atomic Force Microscopy Reveals Fundamental Differences in Axonal Resistance to Injury

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Supporting Information

Figures

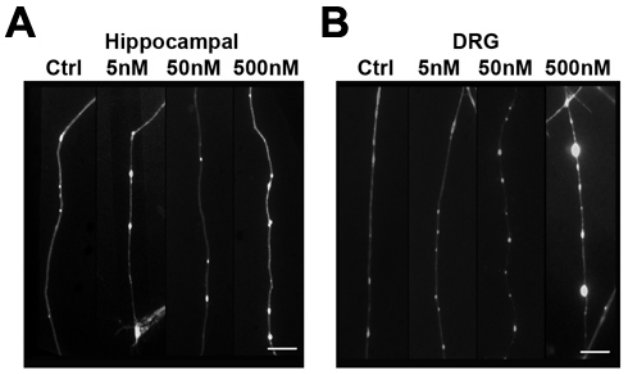

Figure S1. Vinblastine treatment impairs axonal transport and promotes changes in axonal morphology very similar to those observed in compressed axons. Hippocampal (**A**) and DRG (**B**) axons were treated with increasing concentrations of vinblastine for 1h then mitochondria were fluorescently labeled, revealing increasing FAS along the axons. Each panel represents one axon but at least 20 axons were tested in each condition with similar results. Scale bar, 10 μm.

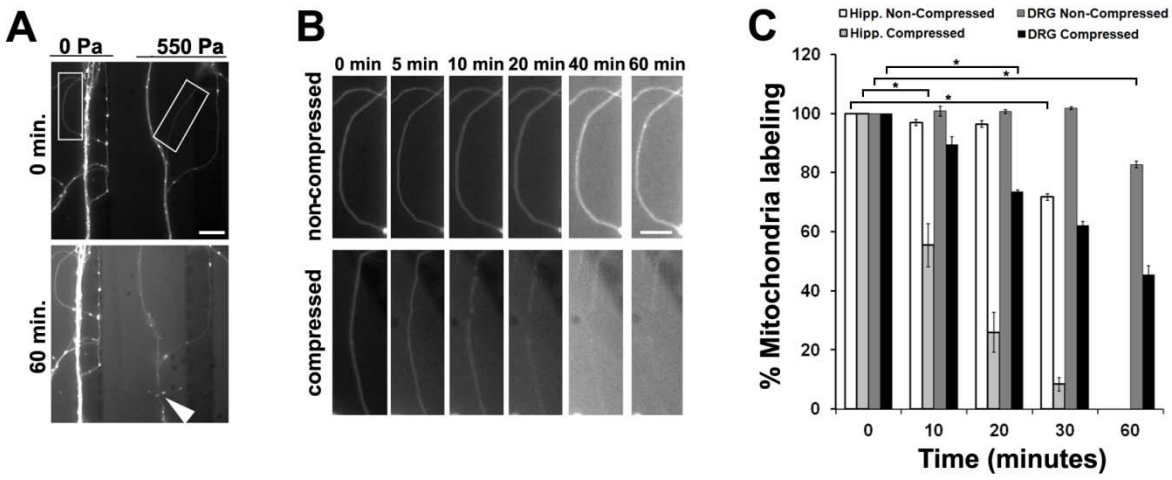

Figure S2. Mitochondrial loss during axonal compression. A Mitochondria were fluorescently labeled and compressed and non-compressed hippocampal axons were imaged for 60 minutes. Compression was applied for 60 minutes with a tipless AFM cantilever. Scale bar, 10 μm. The arrowhead points to the compression point and the image orientation is such that the soma lies below the axonal segment shown in the panels. Squares represent the regions analyzed in **B** before (0 min) and during compression. Brightness was increased in panels corresponding to 40 and 60 minutes to highlight the difference in the axonal labeling. Scale bar, 5 μm. **C**, Mitochondrial labeling was quantified using ImageJ (31) software in hippocampal and DRG axons compressed or not during 30 and 60 minutes respectively, revealing a significant loss of mitochondria labeling during compression. Each bar represents the average percentage of mitochondrial labeling remaining ± SE in at least 16 axons (*$p < 0.001$).

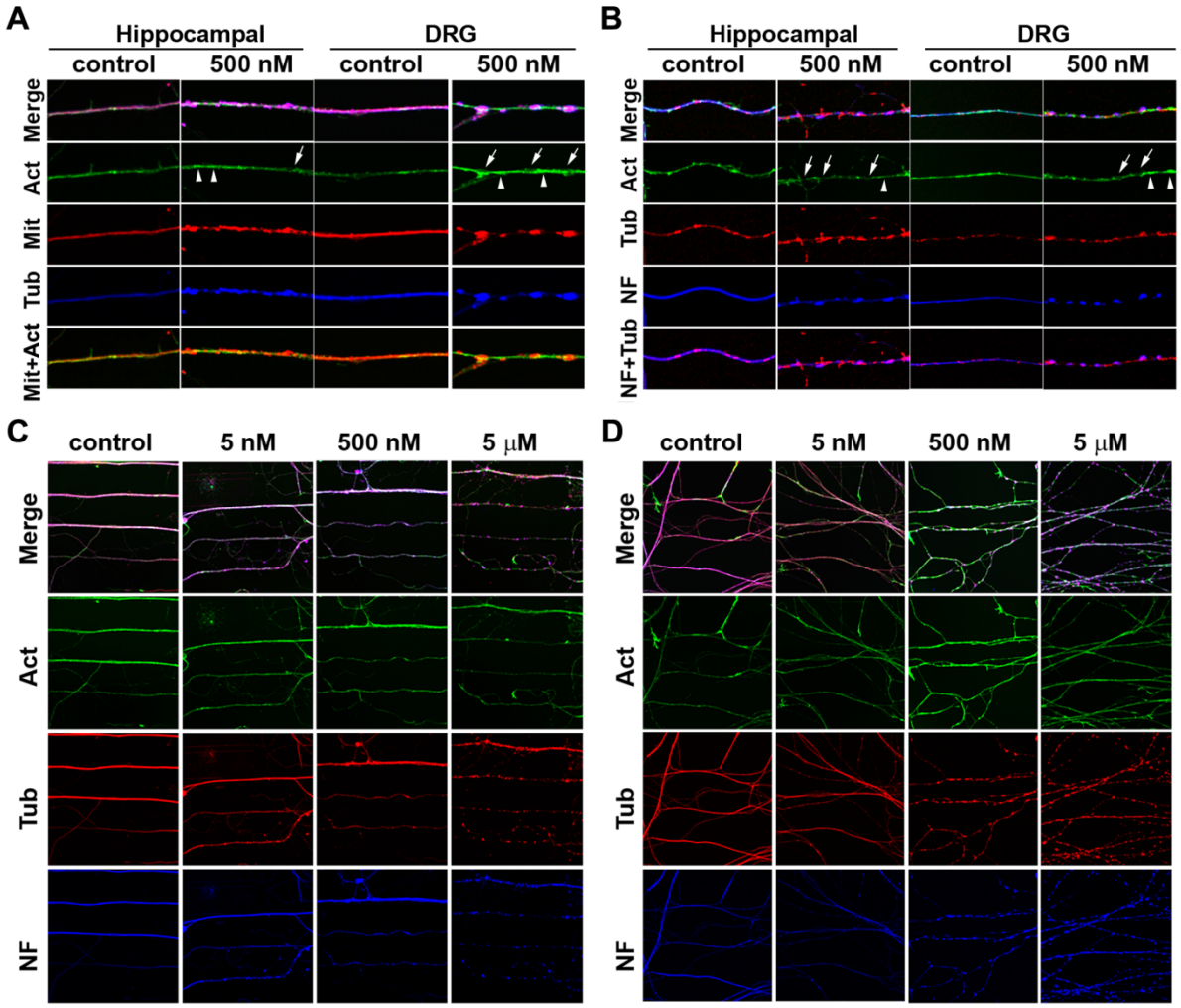

Fig. S3. Impairment of axonal transport promotes changes in the cytosolic organization of hippocampal and DRG axons. A, Confocal microscopy images of hippocampal and DRG axons treated or not with vinblastine for 1h, then fixed and immunostained fluorescently. Mitochondria were immunolabeled with antibodies against mitochondrial preprotein translocases of the outer membrane FL-145 (Mit), tubulin was labeled with anti-tubulin (Tub) antibodies and F-actin was revealed with fluorescently conjugated phalloidin (Act). In DRG and hippocampal axons, mitochondria co-localized with tubulin fragments (red mitochondria and blue tubulin co-localizing in purple in the "merge" panel) while actin accumulated between FAS (arrowheads) and deformed in regions in which actin co-localizes with FAS (arrows). Mitochondria do not co-localize with actin (red and green fluorescence in the "Mit+Act" panel). **B**, Same as in (**A**) but axons were immunostained fluorescently for neurofilament (NF) and tubulin (Tub) while F-actin was stained with fluorescently conjugated phalloidin (Act). Confocal images highlight the co-localization of microtubules and neurofilaments, while actin accumulated between FAS (arrowheads) and deformed in regions co-localizing with FAS (arrows). **C**, Hippocampal and **D,** DRG axons, treated with increasing concentrations of vinblastine for 1h, then fixed and labeled fluorescently as in (**B**), emphasizing the concomitant collapse of microtubules and neurofilaments with increasing concentrations of vinblastine. Scale bars, 10 μm (**A** and **B**) and 50 μm (**C** and **D**).

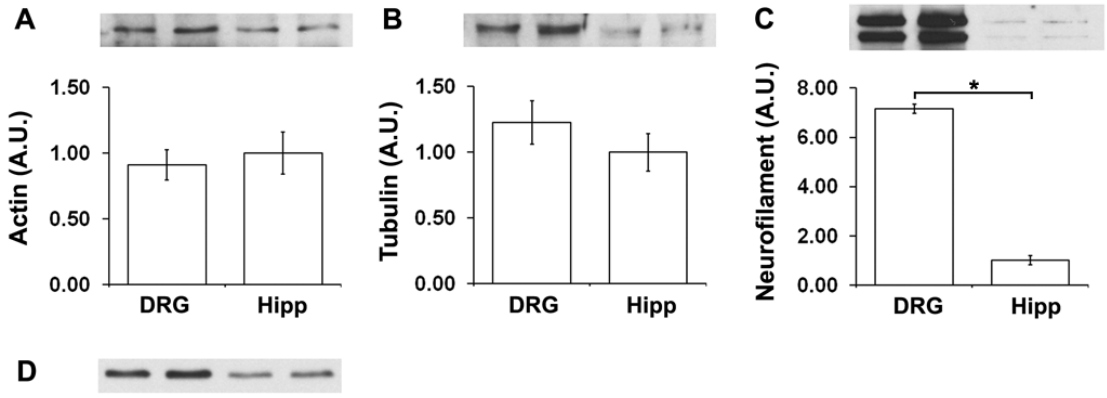

Figure S4. DRG axons contain more neurofilament than hippocampal axons. A, DRG and hippocampal axons were allowed to extend in microfluidic chambers for 7 and 14 days, respectively, then the soma were removed by suction and the axons were solubilized in RIPA buffer containing protease inhibitors. After protein quantification, 10 μmol of protein of each preparation was loaded in 10 % SDS-PAGE gels and transferred to nitrocellulose membranes. Each panel shows 2 lanes with material from DRG axons and 2 lanes from hippocampal axons tested with the respective antibodies: **A,** anti-actin; **B,** anti-tubulin; **C,** anti-neurofilament; **D,** anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The intensity of each band was quantified using ImageJ (31) and divided by the intensity of the bands in the lanes probed with antibodies against the housekeeping gene GAPDH. Each bar represents the intensity ± SE of at least 6 lanes resulting from 3 independent experiments (*$p < 0.001$).

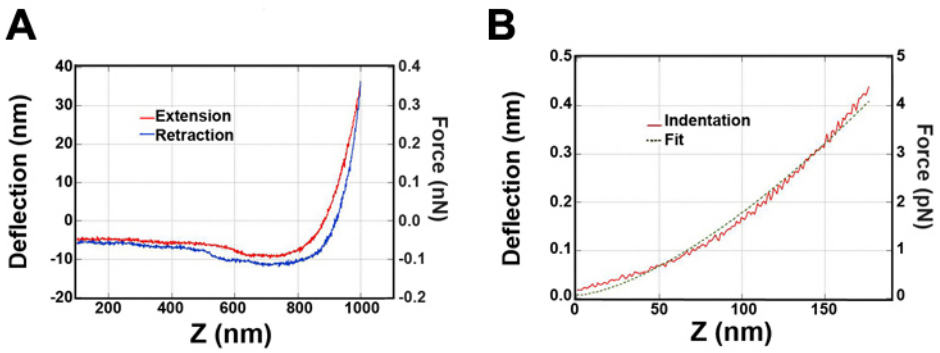

Figure S5. Elastic Modulus of DRG and hippocampal axons. A, Typical extension (red) and retraction (blue) force curve of an axon. **B**, The extending portion of the curve (red) was fitted (dashed line) using the Hertz contact model with modifications described in the Materials and Methods section.

Movies S1 and S2. DRG axons are more resistant to compression than hippocampal axons. Mitochondria were fluorescently labeled and hippocampal (**Movie S1**) and DRG (**Movie S2**) axons were compressed with a bead attached to the AFM cantilever with 550 ± 220 Pa for 30 minutes then imaged for another 15 minutes after compression release. Movie S1 shows growth cone retraction, reduction of mitochondria labeling, formation of FAS containing mitochondria close and distal from the compression site and final rupture of the hippocampal axon. When compressed under the same conditions, Movie S2 shows that DRG axons present mitochondria accumulation and deformation under the compression point without significant reduction of mitochondria labeling and no axonal rupture. Instead, DRG axons recover their shape after compression release at this pressure setting. The movies are orientated so that the soma lie below the axonal segments shown in the movies.