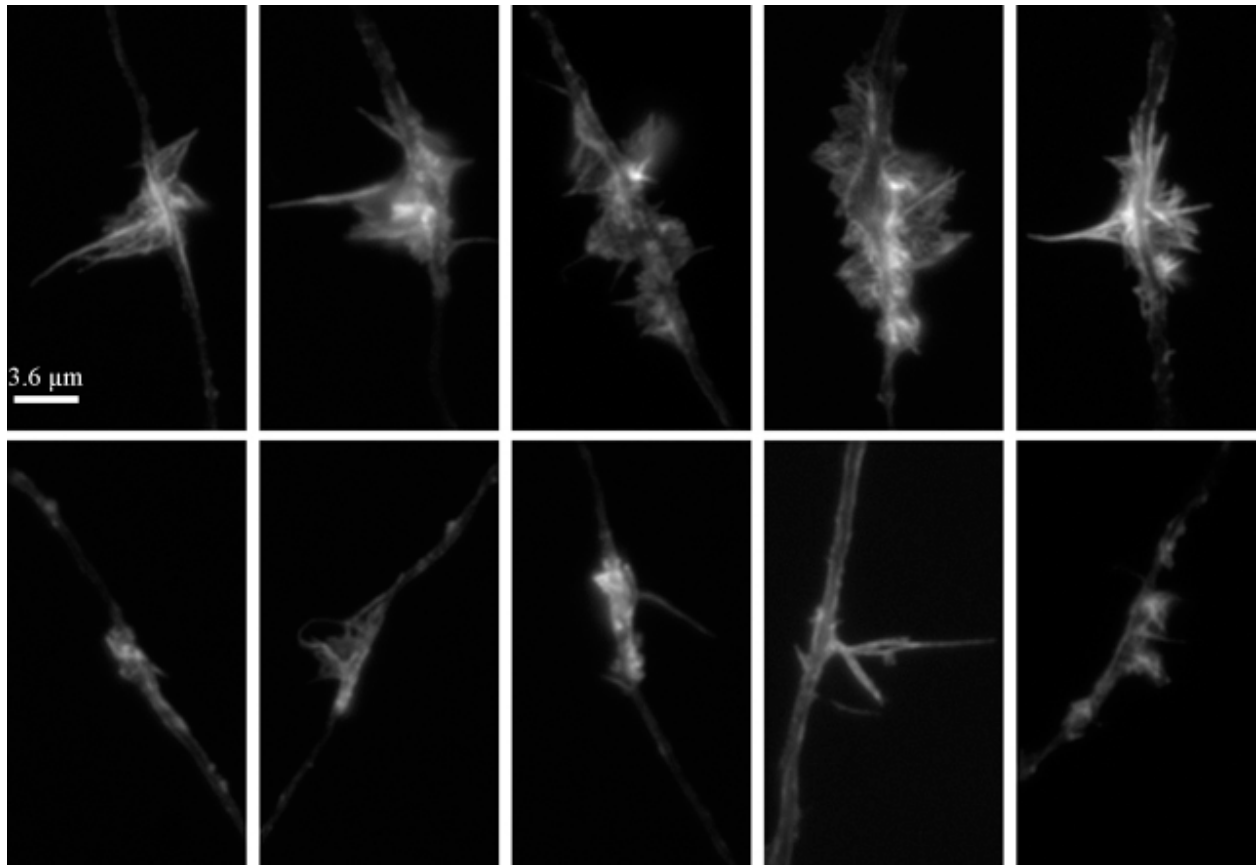


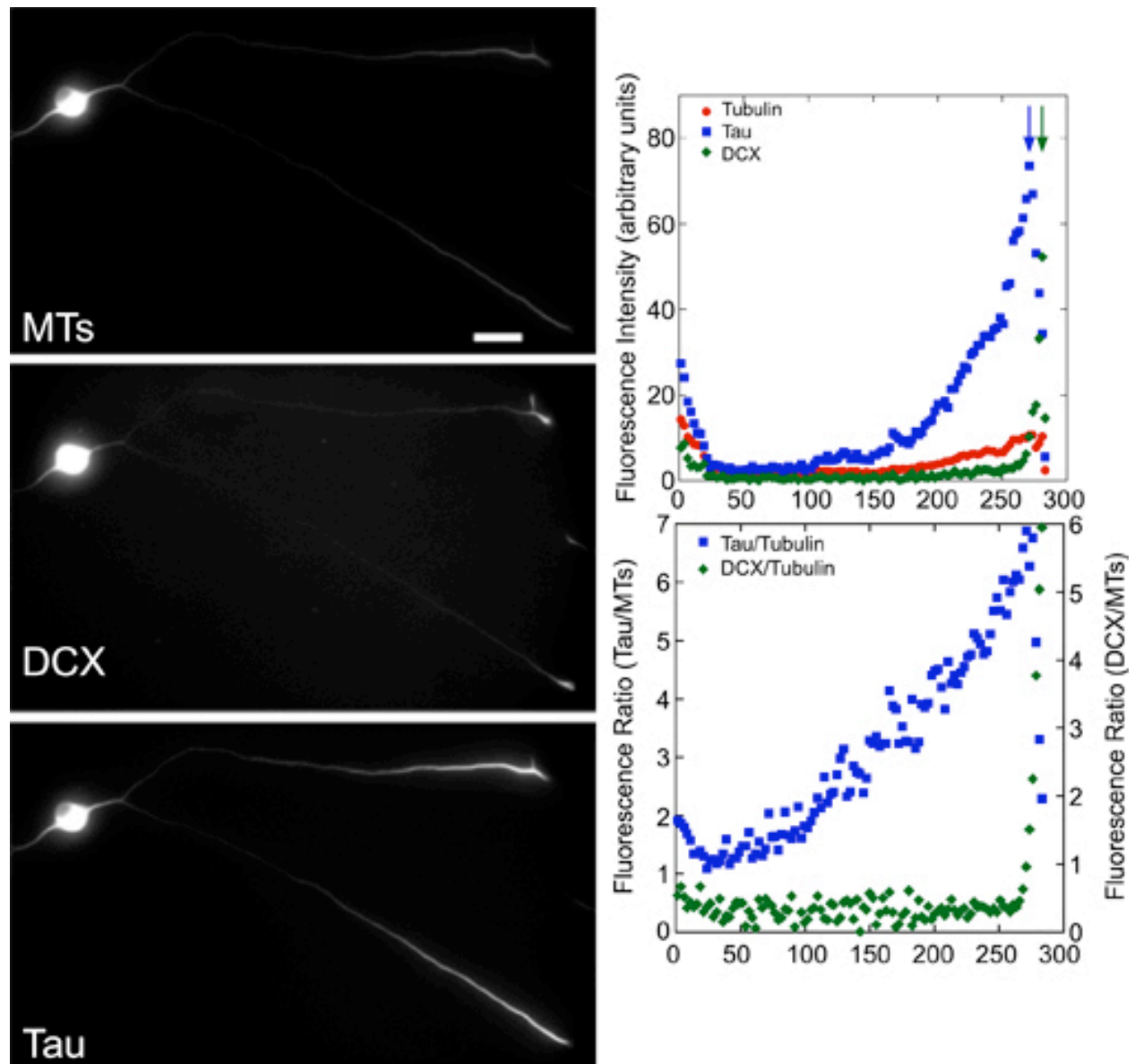
Supplementary materials

Supp. Figure 1.



Supp. Figure 1. Several examples of focal accumulations of actin along axons of cultured hippocampal neurons. The upper panels are all examples of accumulations with prominent lamellipodia, whereas the lower panels are examples without obvious lamellipodia. All of these images have been enhanced identically. Scale bar = 3.6 μm .

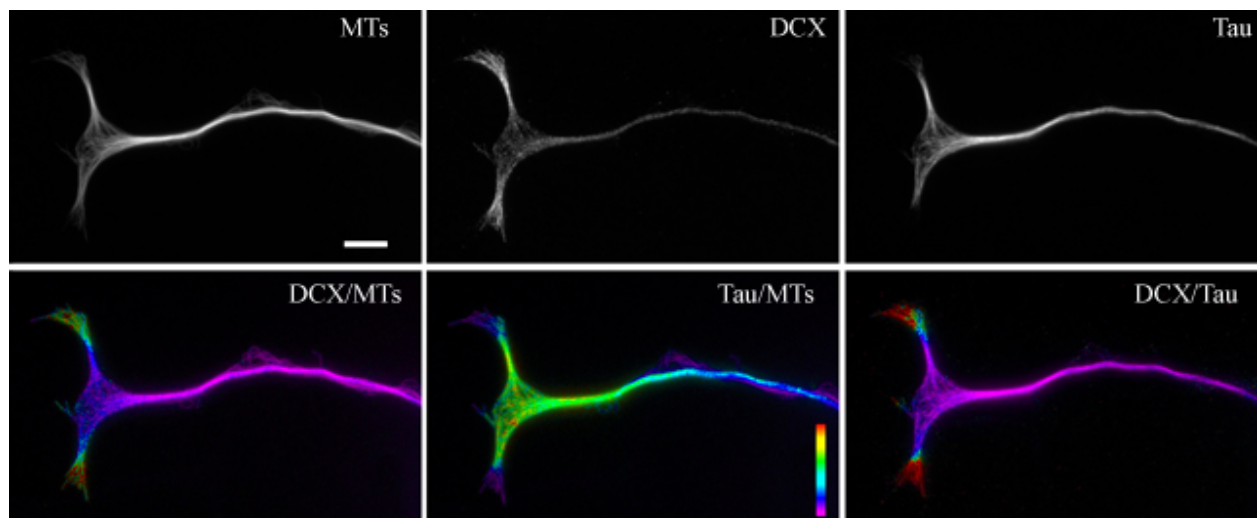
Supp. Figure 2.



Supp. Figure 2. Differential distribution of tau and DCX in axons and growth cones of cultured sympathetic neurons. The left-hand panels show a neuron triple-stained to reveal microtubules (MTs), DCX and tau, as indicated; scale bar = 29 μ m. While the fluorescence images show that both DCX and tau are present in the growth cone, tau clearly has a much broader distribution along the axon compared to DCX. This is shown quantitatively in the upper graph on the right, which shows fluorescence intensity plotted against distance from the cell body. Tau staining

increases progressively over the distal $\approx 200 \mu\text{m}$ of the axon, reaching a peak $\approx 15\text{-}20 \mu\text{m}$ from the axonal tip (blue arrow, see also Black et al, 1996). By contrast, DCX levels are negligible within the axon until its distal $\approx 20 \mu\text{m}$, where the levels rise sharply to a peak (green arrow) within the growth cone. The lower graph shows the ratio of tau and DCX to microtubules as a function of distance along the axon. These analyses show that within the growth cone, tau levels on microtubules decline in a proximal-to-distal direction whereas DCX levels on microtubules increase. This has been confirmed using higher resolution analyses (see Supp. Figure 3).

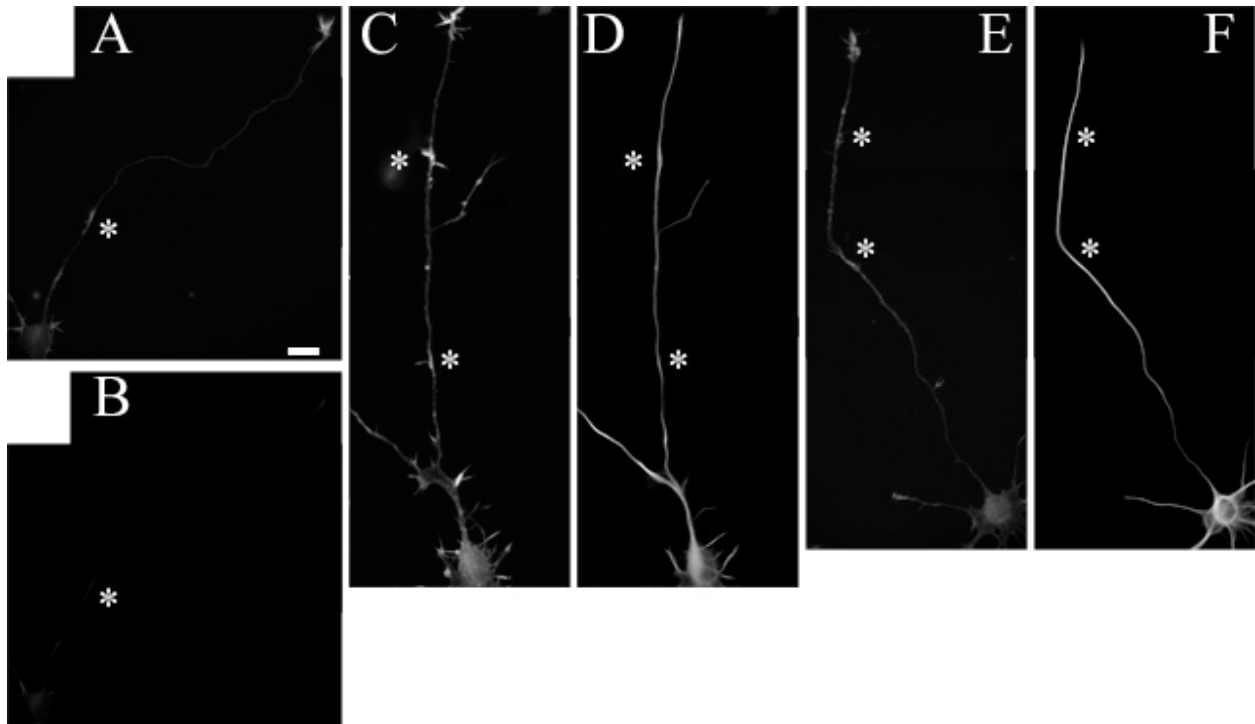
Supp. Figure 3



Supp. Figure 3. DCX and tau are differentially distributed on microtubules in growth cones of cultured sympathetic neurons. Higher magnification views of a growth cone fixed with glutaraldehyde + detergent and then stained to reveal microtubules (MTs), DCX and tau as indicated. DCX is especially intense on microtubules in the very distal parts of the growth cone, whereas tau is especially prominent in the neck and central regions of the growth cone. This can be seen especially clearly in the ratio-images depicting the abundance of DCX or tau relative to

microtubules. The ratio-image depicting DCX relative to tau also highlights the differential distribution of these proteins in the growth cone. Scale bar = 7.2 μm .

Supp. Figure 4.



Supp. Figure 4. Images of cells expressing low (A,B) or high (C-F) amounts of GFP-DCX. In preparing the figure, all of the actin images (A, C, E) were enhanced identically to permit visual comparison, and the same was also true for the GFP-stained images (B, D, F). The asterisks identify focal accumulations of actin. In the low expressing cell (A,B) the focal actin accumulation is also associated with a focal accumulation of DCX, although this is very dim. In the cells expressing high levels of GFP-DCX, GFP-DCX is present at relatively high levels throughout the axon. Many of these axons also have focal actin accumulations. In some axons, GFP-DCX staining is enriched in the regions of the focal accumulations (C, D), whereas in other axons, GFP-DCX is abundant in the region of the focal actin accumulation, but not obviously

enriched relative to adjacent axonal regions (E,F). The lengths of the axons of the three different neurons are 261 μm (A,B), 308 μm (C,D) and 293 μm (E,F). The scale bar = 18 μm .

Supp. Movie 1. Time-lapse phase movie of proximal-to-distal movement of focal axonal lamella containing a focal accumulation of GFP-DCX. This movie depicts the same cell shown in figure 10. Images were acquired every 2 sec for 10 min. Elapsed time in seconds is indicated in the lower left.