

Supplementary material

Supplementary material 1, together with Supp. Figure 1:

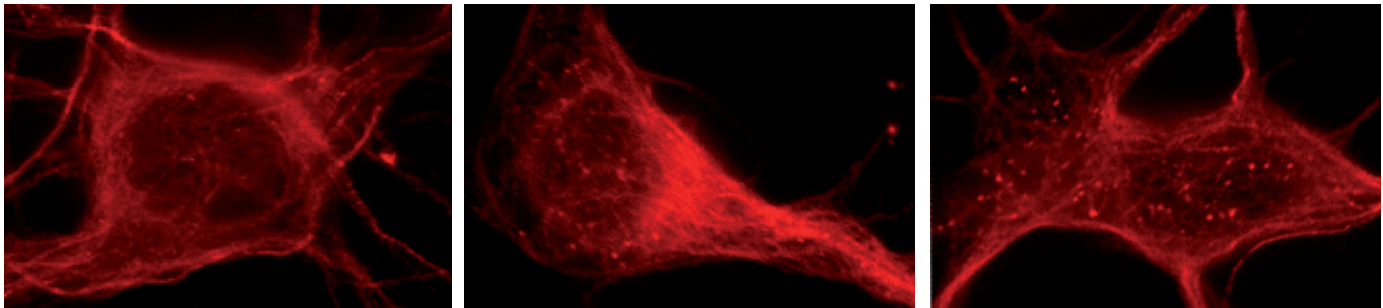
Hippocampal neuronal cultures were prepared from newborn rat or mouse brains according to Ray et al. 1993 and Kaech and Banker 2006. Briefly, the hippocampi of animals less than 2 days old were recovered, minced and digested in 0.01% trypsin for one hour at 37°C. Dissociated cells were plated on coverslips precoated with 5µg/ml poly-l-lysine and 7µg/ml collagen at densities of 2×10^5 /ml and incubated at 37°C with a 5% CO₂ atmosphere. Twenty-four hours later, the plating solution was replaced by B-27 neurobasal medium, and the second day cytosine arabinoside (20 µM) was added to eliminate nonneural cells.

On day 7 cultures were incubated for 6 hours with the biotinylated NFL-TBS.40-63 (10µM). As described for T98G cells, MT were detected using mouse anti-βIII-tubulin and an Alexa 568 nm labeled anti-mouse antibody (red). NFL-TBS.40-63 was detected using Alexa 488 nm labelled-avidin (green). Neurons that did not incorporate detectable levels of peptide demonstrated a clear MT network within their cell bodies (Supp. Figure 1A). In contrast, 18% of the total number of cells had incorporated detectable levels of peptide and approximately half of these (9% of the total number of cells) demonstrated a damaged MT network (Supp. Figure 1B). Similar results were obtained with rat and mouse neurons.

Supp. Figure 1

A

β III-tubulin

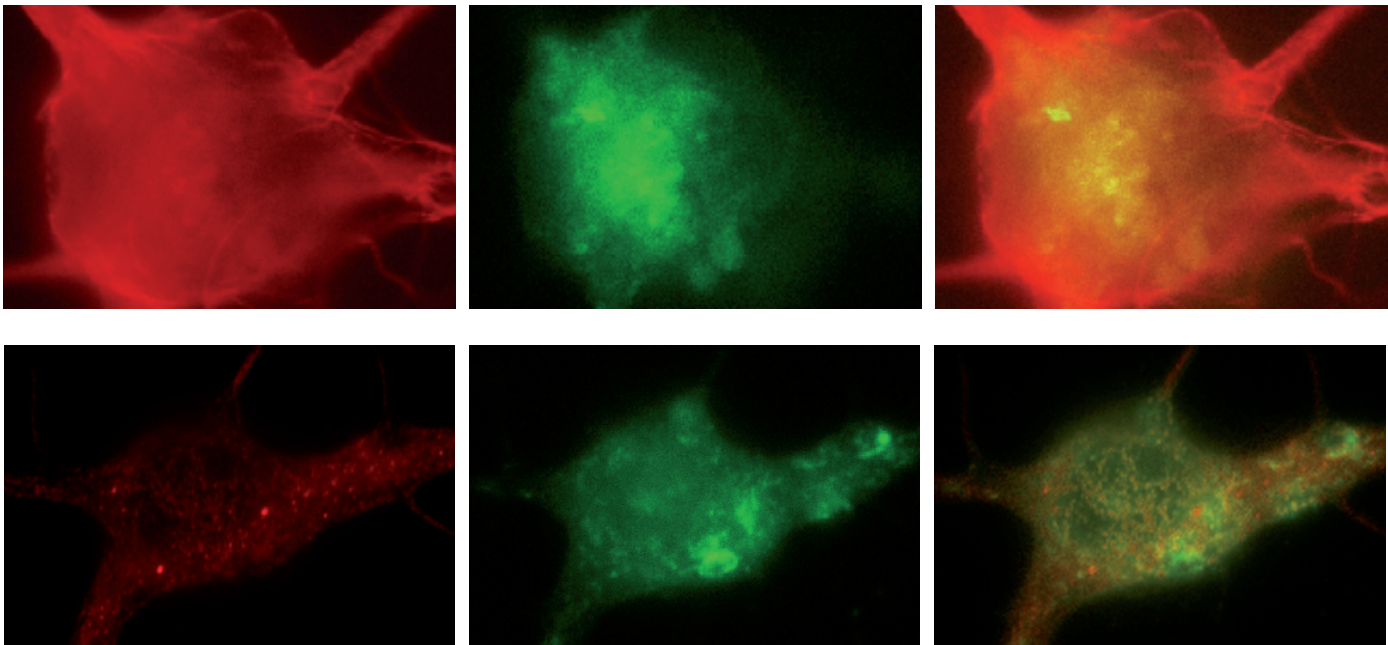


B

β III-tubulin

NFL-TBS (40-63)

Merged



Supplementary material 2:

Based on the current understanding of NF and tubulin structure, the maximum number of tubulin monomers potentially sequestered by a unit length (e.g., 1 μ m) of an axonal NF can be estimated. Tubulin is a 4nm globular protein, so a protofilament of 1 μ m contains approximately 250 tubulin monomers. As MT are composed of 13 protofilaments, a 1 μ m long MT contains $250 \times 13 = 3250$ tubulin monomers. In a normal axon with a diameter of 10 μ m and a cross sectional area of 78.5 μ m², the density of MT is $9.44 \pm 1.4/\mu$ m². Thus, it contains approximately 741 MT (78.5×9.44) or 2,408,250 tubulin monomers (741×3250) per μ m of axonal length. As NF-deficient axons assemble 2.5 times more MT, they contain 6,020,625 tubulin monomers per μ m of axonal length ($2,408,250 \times 2.5$). As average axonal NF density is 150/ μ m² (Waxman et al, 1995), an axon with a diameter of 10 μ m would contain 11775 NF (150×78.5). Based upon the supernumerary MT that form in the absence of NF, these must sequester 3,612,375 tubulin monomers ($6,020,625 - 2,408,250$). Thus, each 1 μ m of NF has the apparent capacity to bind 307 tubulin monomers ($3,612,375 / 11775$). Viewed in a different manner, this model predicts that tubulin sufficient to form one mature MT could be bound by approximately 11 NF of equivalent length ($3250 / 307$). Of course, different physiological states may lead to NF modifications such as phosphorylation with currently unknown affects on predicted tubulin binding capacity.