Cytomechanics
of Neuronal Growth
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A major aspect of the development of the nervous system is the outgrowth of axons, sometimes over considerable distances, from the cell bodies of neurons. At the very earliest times of development, axonal elongation occurs as a result of the motility of a specialized leading tip of the axon, the growth cone.   This arrangement has been likened to a dog on a leash that gets longer as the dog pulls on it.   In addition, axons elongate substantially after they have contacted their target cell, i.e. after synaptogenesis, as a result of tension exerted by growth of the skeleton.   These two types of axonal elongation suggest that mechanical tension is a stimulus causing growth, not just stretching, of neural axons.

Over the last 15 years, the research in our lab has shown that mechanical tension is a robust regulator of axonal development in several types of cultured embryonic neurons. Specifically, we have obtained direct evidence that mechanical tension can stimulate four phases of axonal development: 1) initiation of process outgrowth from the cell body; 2) growth cone-mediated elongation of the axon, i.e. the growth cone is acting as a 'tractor;' 3) elongation of the axon after synaptogenesis as mentioned above; and 4) axonal elimination by retraction. The axons initiated and/or elongated by experimental tension, applied by glass microneedles, appear normal by all available assays (Fig. 1) . Particularly significant, the relationship between the force and the growth response is surprisingly simple: the neurite elongates like a Newtonian fluid mechanical element, a dashpot (e.g. the leaky piston on the screen door that prevents slamming). That is, elongation rate is directly proportional to tension (above the threshold), and this simple linear relationship obtains both within the physiological range of growth rates and far-above-physiological rates (Fig. 2). Thus, tension apparently integrates the complex biochemistry of axonal elongation, including cytoskeletal and membrane dynamics, to produce a simple fluid-like relationship between the force input and growth output. Current efforts are devoted to determining whether tension can be used as a practical, clinical stimulator of axonal outgrowth as an aid to neural regeneration.   We are examining whether adult neurons retain the high capacity of embryonic neurons for tension-induced axonal elongation.   We also are developing a method of pulling on axons more suitable than glass microneedles to pull on injured nerves using iron beads and magnets.
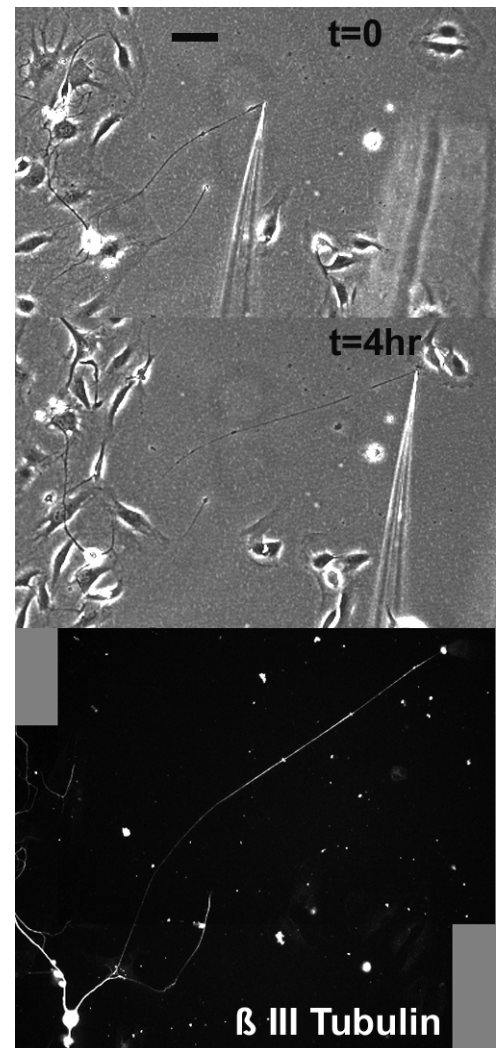

Fig. 1- Adult rat sensory neurons were cultured for 3 days before applying experimental tension by glass microneedles.   Immediately prior to needle attachment (0 time) the axon had extended some 300 µm via growth cone activity. The distal, growth cone end of the axon was pulled by a calibrated glass needle at a force of approx. 100 nanograms. After 4 hours of towing in this manner, the neuron added an additional 260 µm of axonal length.   After towing for 4 hours, the cell was fixed and visualized by a fluorescent, neuron-specific marker (note the satellite cells seen in the towing images are not stained).

Fig. 2 - Relationship between axonal elongation rate and magnitude of experimentally applied tension for two embryonic rat brain (hippocampal) neurons at different stages of development. Each data point represents a period of 60-90 minutes of 'towing' of an axon at a constant force by a glass microneedle. The growth rate is simply the difference in the length of the axon at the beginning and end of the towing bout divided by the elapsed time of towing. The average growth-cone-mediated elongation rate for this cell type is 10 um/hr so these data also show that experimental tension can greatly speed axonal outgrowth. We find that all cultured embryonic neurons tested to date show both the simple linear relationship between growth rate and tension and the capacity to grow at higher than normal rates in response to applied tension.