Changes induced by peripheral nerve injury in the morphology and nanomechanics of sensory neurons.

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RESUME :
To our knowledge, our study is the first that investigated the effects of conditioning injury on the morphology and mechanical properties of live sensory neurons membrane. In this work, we use differential interference contrast microscopy (DIC), fluorescence microscopy and atomic force microscopy (AFM) to study the morphological and nanomechanical properties of mice dorsal root ganglia (DRG) sensory neurons in regenerative growth mode. DIC results show that conditioned axotomy, induced by sciatic nerve injury, did not increase somatic size of adult lumbar sensory neurons but promoted the appearance of longer and larger neurites and growth cones. Our AFM data indicate that conditioned neurons are characterized by softer growth cones and cell bodies, compared to control neurons. As cell elasticity is related mainly to the intrinsic properties of the cell membrane and cytoskeleton structures such as microtubules and actin fibers, the increase of the cell membrane elasticity suggests a modification in the ratio and the inner framework of the main structural proteins. Furthermore, in order to evidence structural differences between conditioned and control somas and growth cones, we use immunocytochemistry to localize actin and neuronal microtubules. These results show a slight actin spreading as observed in control in combination with a very thin tubulin prolongation at the conditioned growth cone tip, while actin is hardly visible in conditioned cell bodies as compared with a clear spreading of actin in control somas.

DESCRIPTION :

DRG contain a variety of sensory neurons that transduce somatic stimuli. Following peripheral nerve injury, sensory neurons have to adapt to a new environment in order to successfully promote their axonal elongation. Unsuccessful regeneration leads to post-traumatic neuropathies, such as ataxia and pain-related behavior, which are often chronic and mostly resistant to current treatments. Understanding the cellular and molecular mechanisms leading to improved neurite re-growth is a major step to propose a new therapy for nerve repair. It has been demonstrated that a prior in vivo nerve injury enhances peripheral axonal regeneration following a second injury. In vitro, the neurons conditioned by the first traumatism display a faster, elongated mode of neurite growth called regenerative growth. The neuronal growth cone is a highly motile mechanosensitive structure at the tip of the axon. Elasticity is a determining parameter of membrane mechanical properties and provides important information toward the health and function of the cell. We have investigated, using AFM, the morphology and the membrane mechanical properties of adult sensory neurons from mice DRG following left sciatic nerve injury.

Methods

1- Animal surgery
Adult Swiss mice were anesthetized by constant isoflurane inhalation. The left sciatic nerve was exposed at the mid-thigh level and sectioned and a 3-5 mm fragment of nerve was removed. Mice were kept alive for 5 to 7 days.

2- Neuronal culture
DRG from adult mice were dissociated and sensory neurons were plated on collagen/ laminin substrate.

3- Contrast microscopy
- For three dimensional live imaging, dissociated neurons were plated on glass coverslips and imaged via an optical differential interference DIC system mounted on a Nikon TE2000-E inverted microscope.

4- Fluorescence microscopy
- For cell size analysis, neuronal cultures were observed with an inverted Zeiss Axiosvert 200M equipped with a CCD camera and a motorized platine driven with Metamorph 7.0 software.

5- Atomic Force Microscopy AFM
The AFM experimental system used for both cell imaging and force mapping was the Asylum MFP-3D. Young’s modulus (E) was calculated for each force, according a modified Hertz model.

RESULTS

Fig.1 DIC microscopy images of mice dorsal root ganglion (DRG) sensory neurons: (A) DIC image of a neural cell following peripheral nerve injury, (B) DIC image of a control neuron. Scale bar: 25µm.

Fig.2 Rheology of somas: AFM force-volume results. Scale bar: 7µm.

Fig.3 Immunostaining of βIII-tubulin and actin in somas.

Fig.4 AFM (contact mode) topography images show areas with different heights corresponding to the P and C domains of the growth cone. Scale bar: 7µm.

Fig.5 Nanomechanics of growth cones AFM force-volume results. Scale bar: 7µm.

Fig.6 Immunostaining of βIII-tubulin and actin in growth cones.

Fig.7 Nanomechanics of growth cones AFM force-volume results. Scale bar: 7µm.