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Engineering cultured neuronal networks with nanomaterials: carbon nanotubes and neuronal signaling

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NANOTECHNOLOGY
for BIOLOGICAL and BIOMEDICAL APPLICATIONS
ICTP October 10-14, 2011, Trieste - Italy

Engineering cultured neuronal networks with nanomaterials: carbon nanotubes and neuronal signaling

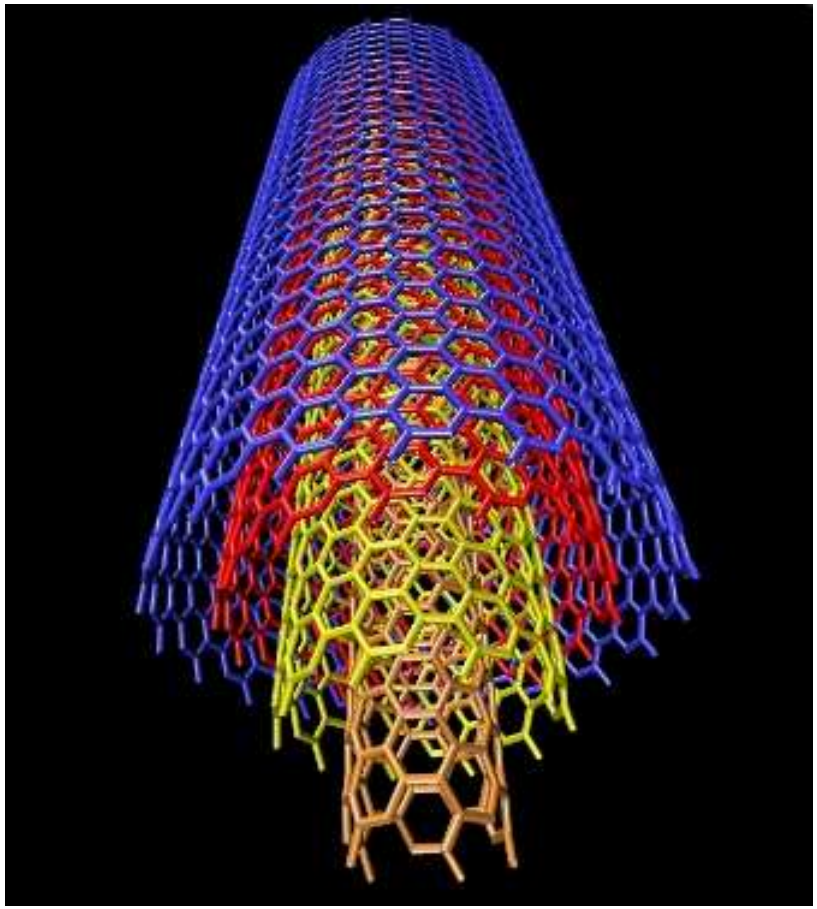
Laura Ballerini, MD, University of Trieste, Italy

- In the field of nanotechnology, carbon nanotubes (CNT) have received extensive attention due to their unique physical and chemical features that allowed the development of devices with outstanding electrical properties

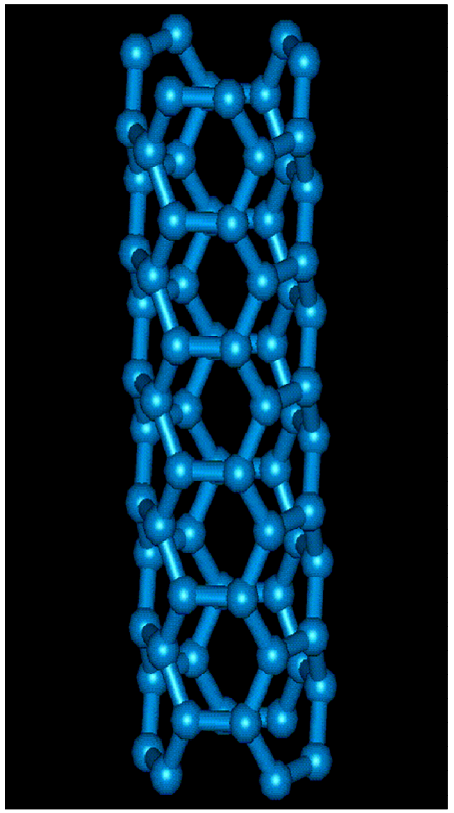
- CNT are one of the more promising nanomaterials for electronic, computer and aerospace industry

- CNT have outstanding physicochemical properties such as: ordered structure, ultralight weight, high mechanical strength, high electrical conductivity and high surface area/large aspect ratio

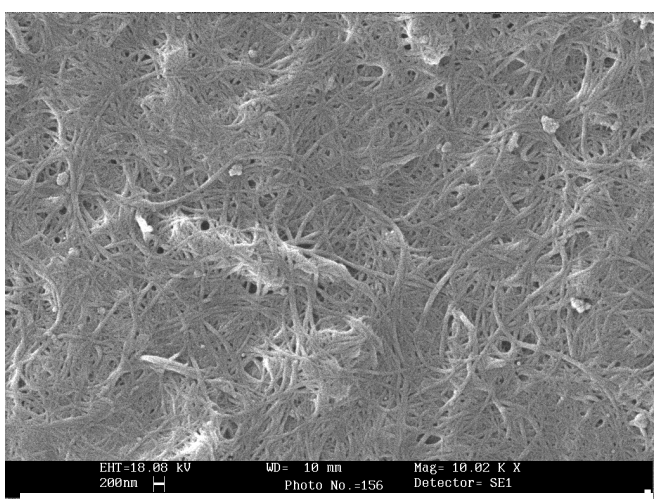
- Recently, these unique materials have been rapidly developing as a platform technology for biomedical applications

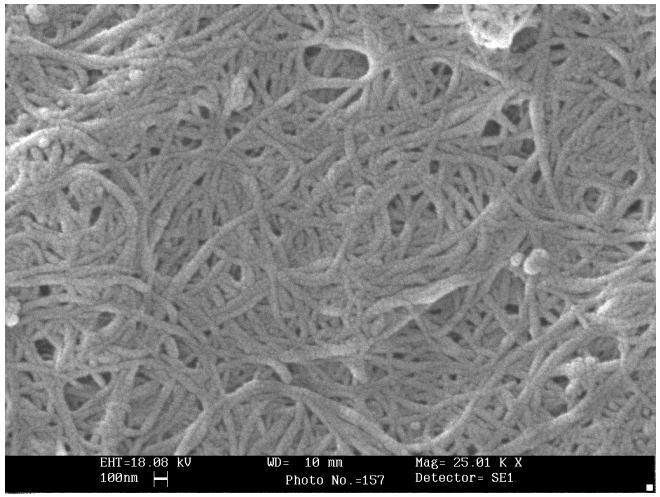


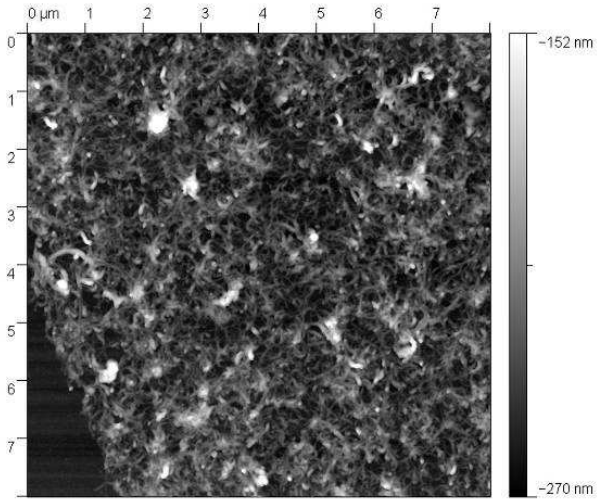
Length:
from few tens of nm to
several µm

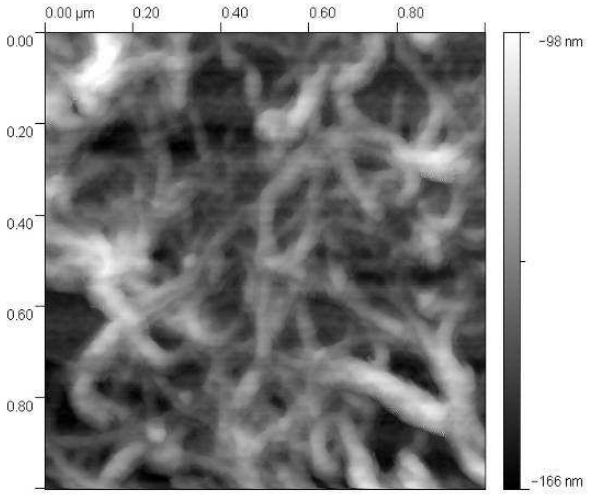


Diameter:
1-2 nm









FM Toma and D Scaini, 2011 unpublished

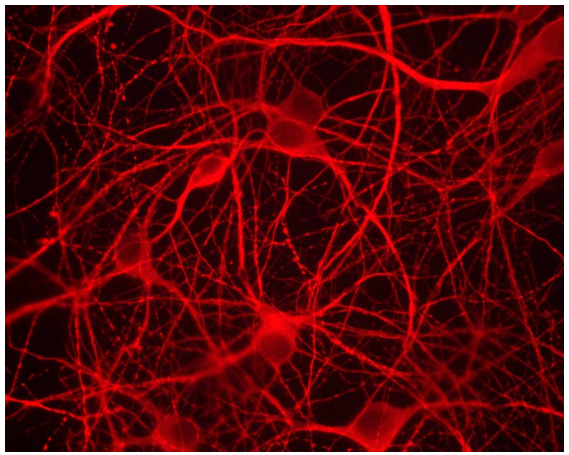
CNTs represent biocompatible substrates (Mattson et al., 2000; Hu et al., 2004; Lovat et al., 2005; Ni et al. 2005; Galvan-Garcia et al. 2007: Mazzatenta et al., 2007; Cellot et al. 2009; Cellot et al 2011)

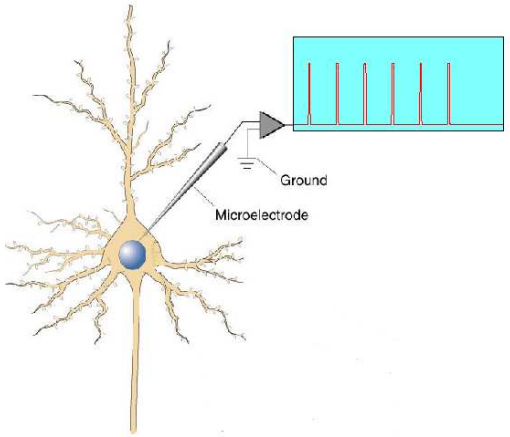
CNTs can be functionalized with polymers to modulate neuronal survival and growth (Bekyarova et al., 2005; Ni et al. 2005; Malarkey et al. 2009; Lu et al. 2010)

CNTs nanoscale dimensional environment allows direct molecular interactions with the nervous system (Silva, 2006)

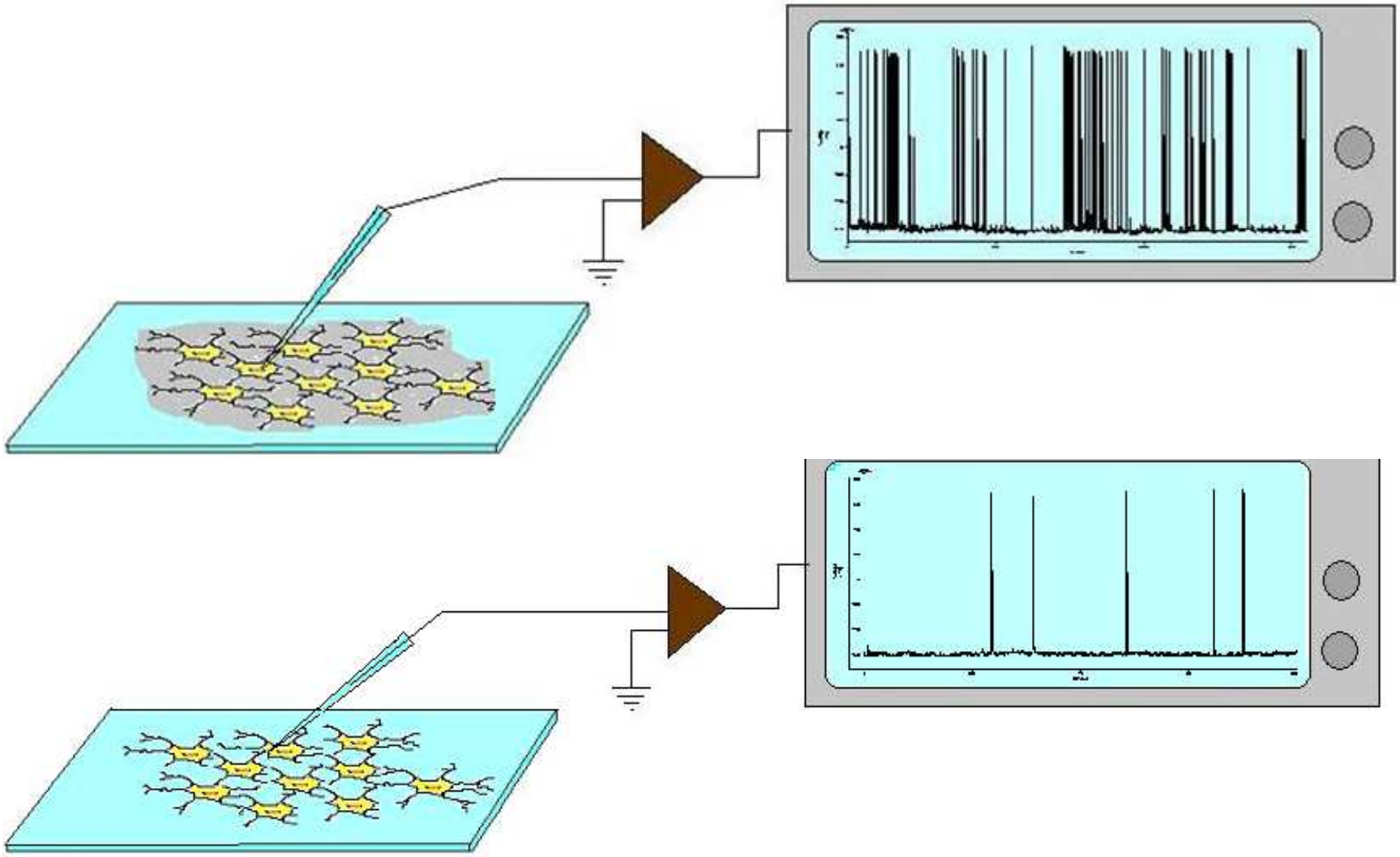
CNT are an ideal material for long-term neural implants, as they enhance electrical recording of neurons in culture and in living animals by reducing the impedence between devices and cell membranes (Keefer et al., 2008; Shoval et al., 2009)

Future developments of nanotube-neurons devices require investigating the potentiality of CNT in interfacing neurons and neuronal activity. How specific is this interaction? Do hybrid systems impact on neuronal activity, and can we exploit such an impact in designing interfaces or assistive devices?

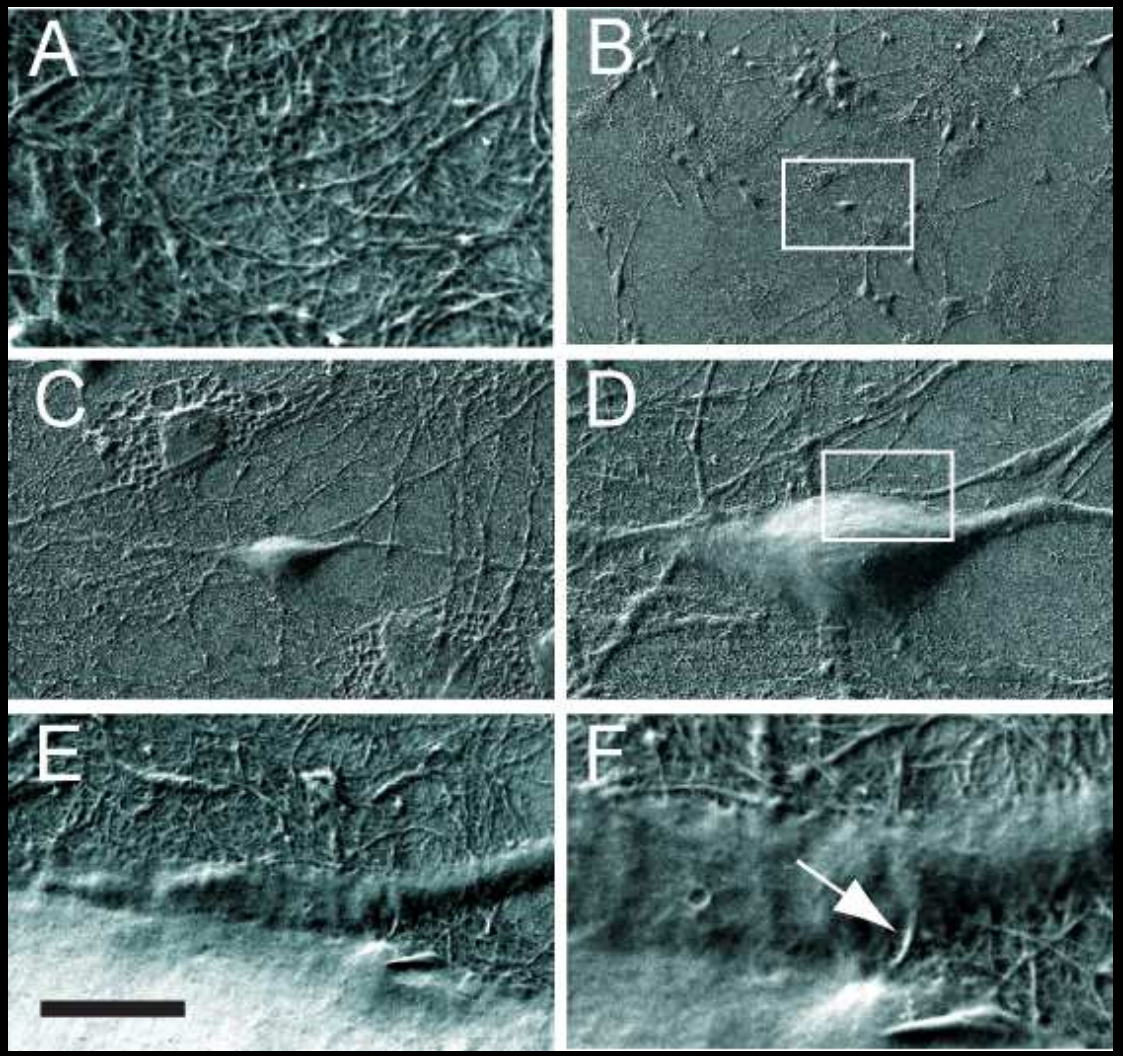




- We designed a simple experimental strategy to investigate the reciprocal influence of purified CNT and neurons
- cultured neurons and electrophysiology



Cellot et al J Neurosci 2011



Mazzatenta et al., J Neuroscience 2007

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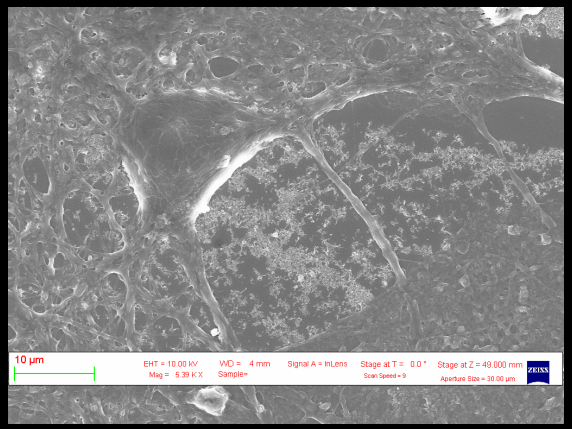
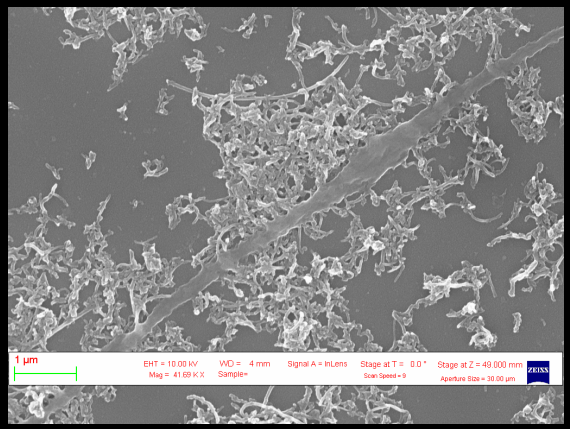
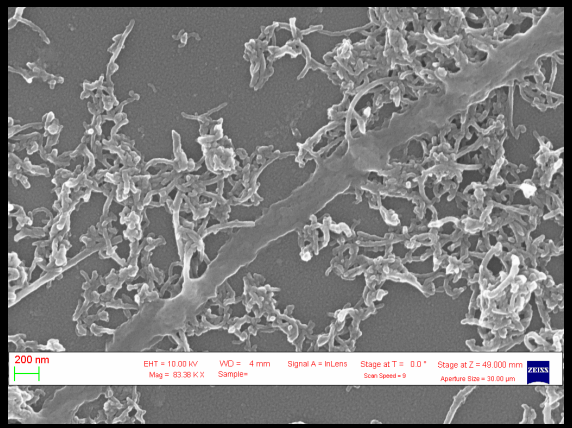
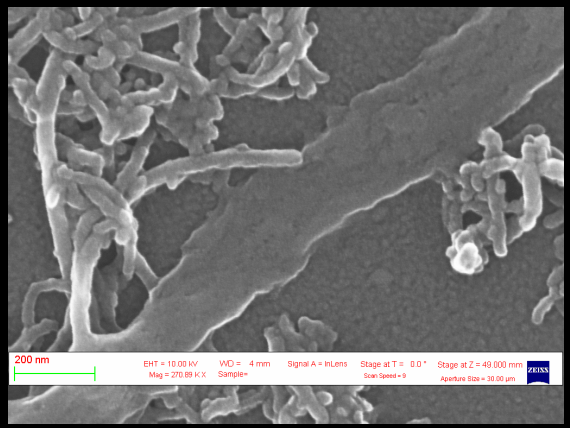
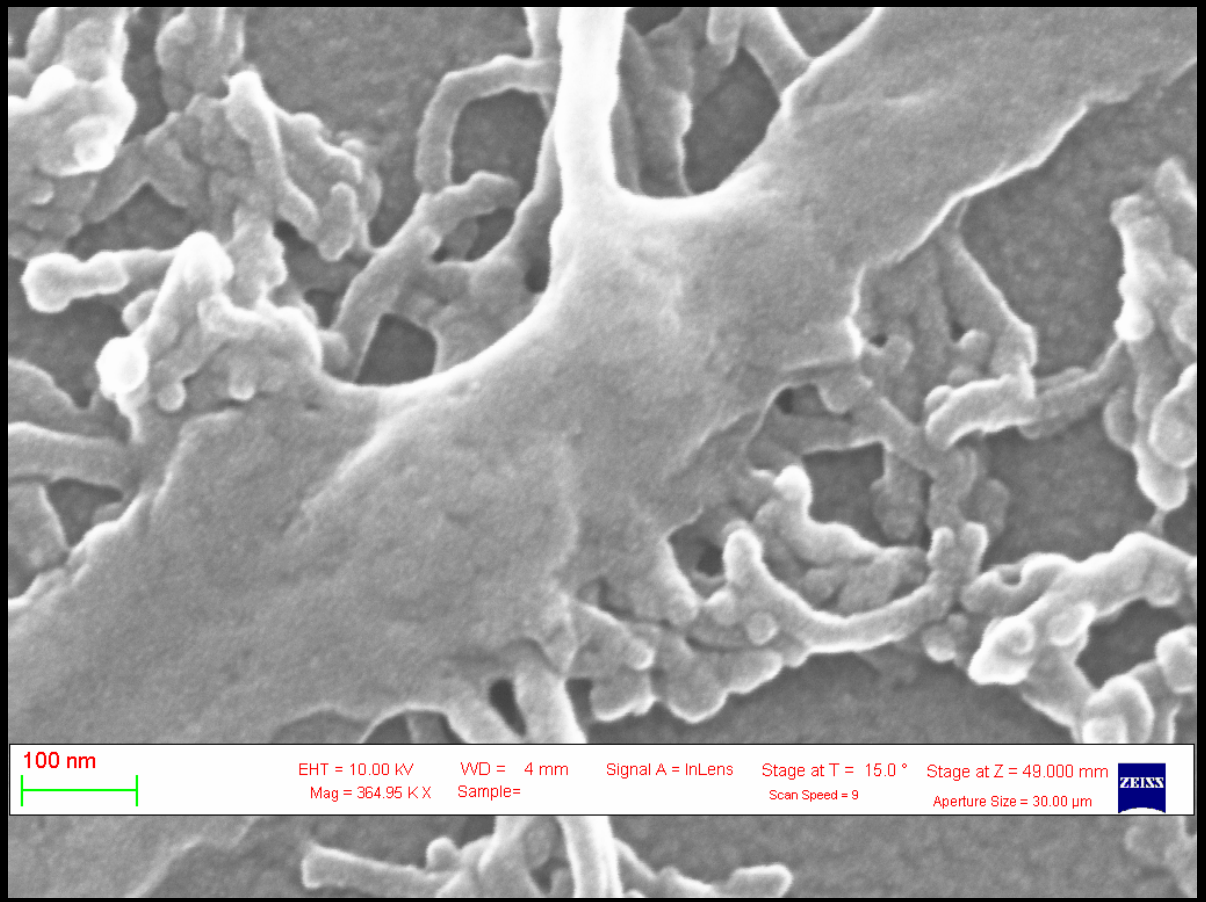








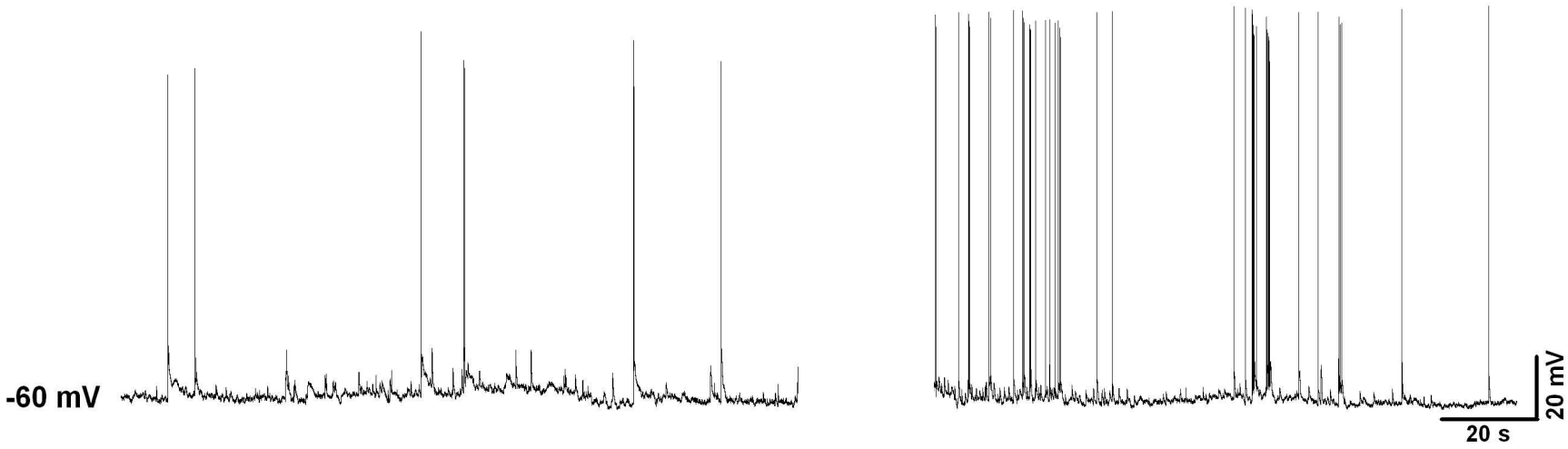
Fabbro et al, in Nanomedicine and the Nervous System, Science Publishers, 2012 in press

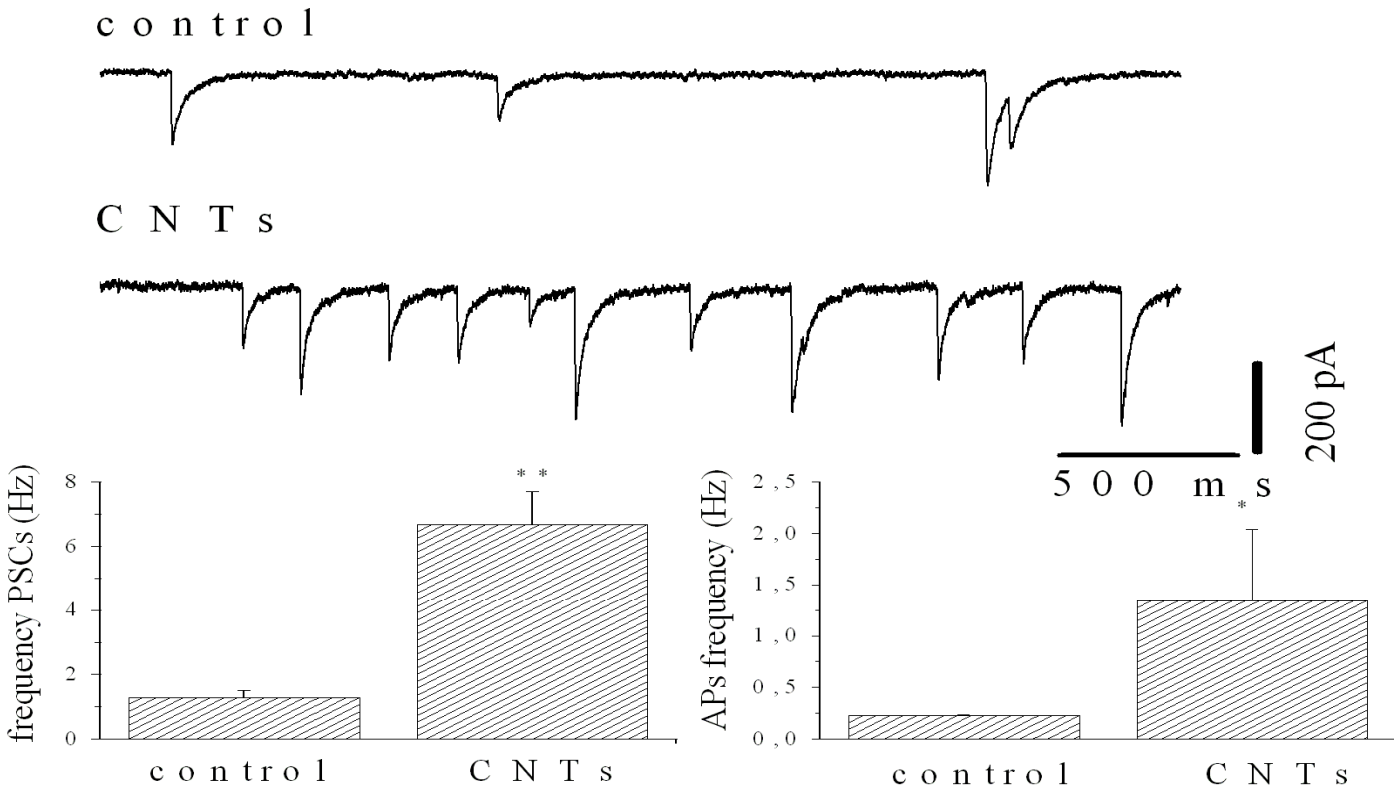


Fabbro et al, in Nanomedicine and the Nervous System, Science Publishers, 2012 in press

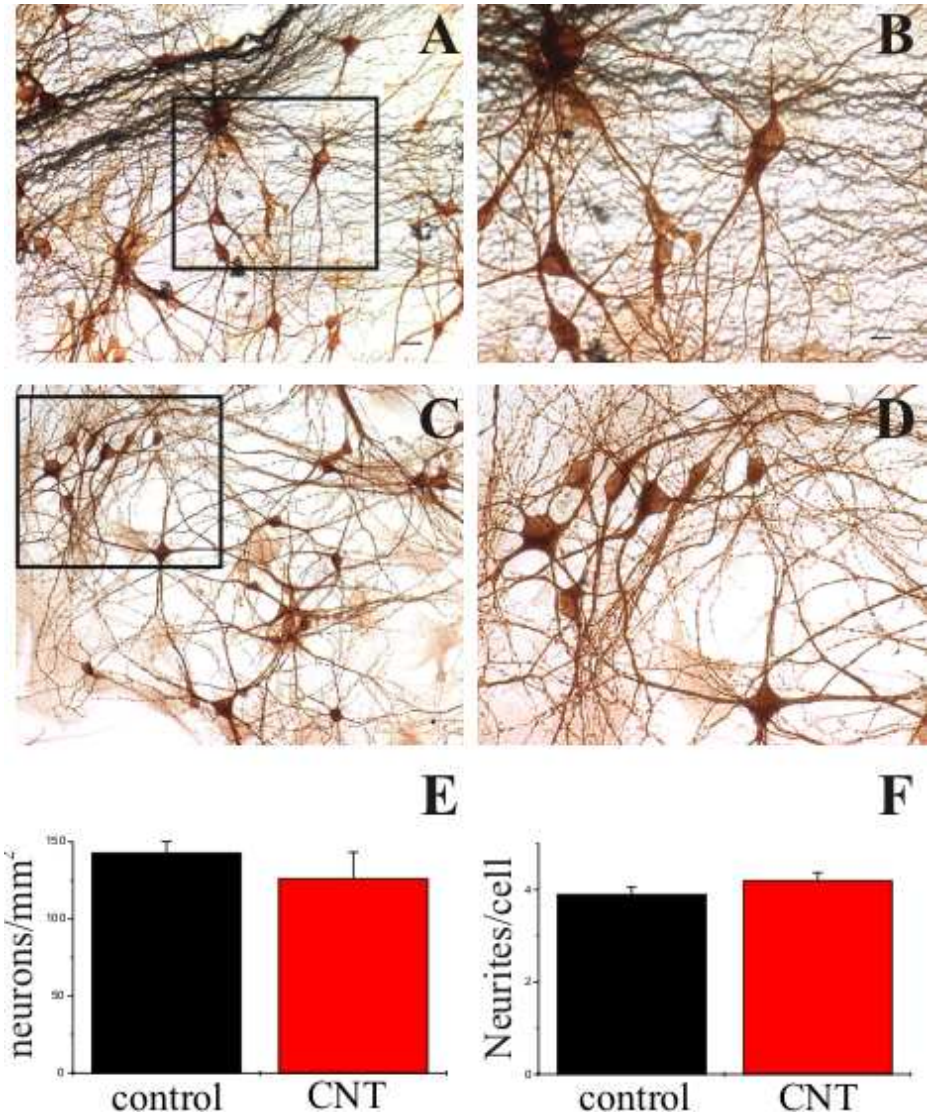
GLASS

CNT

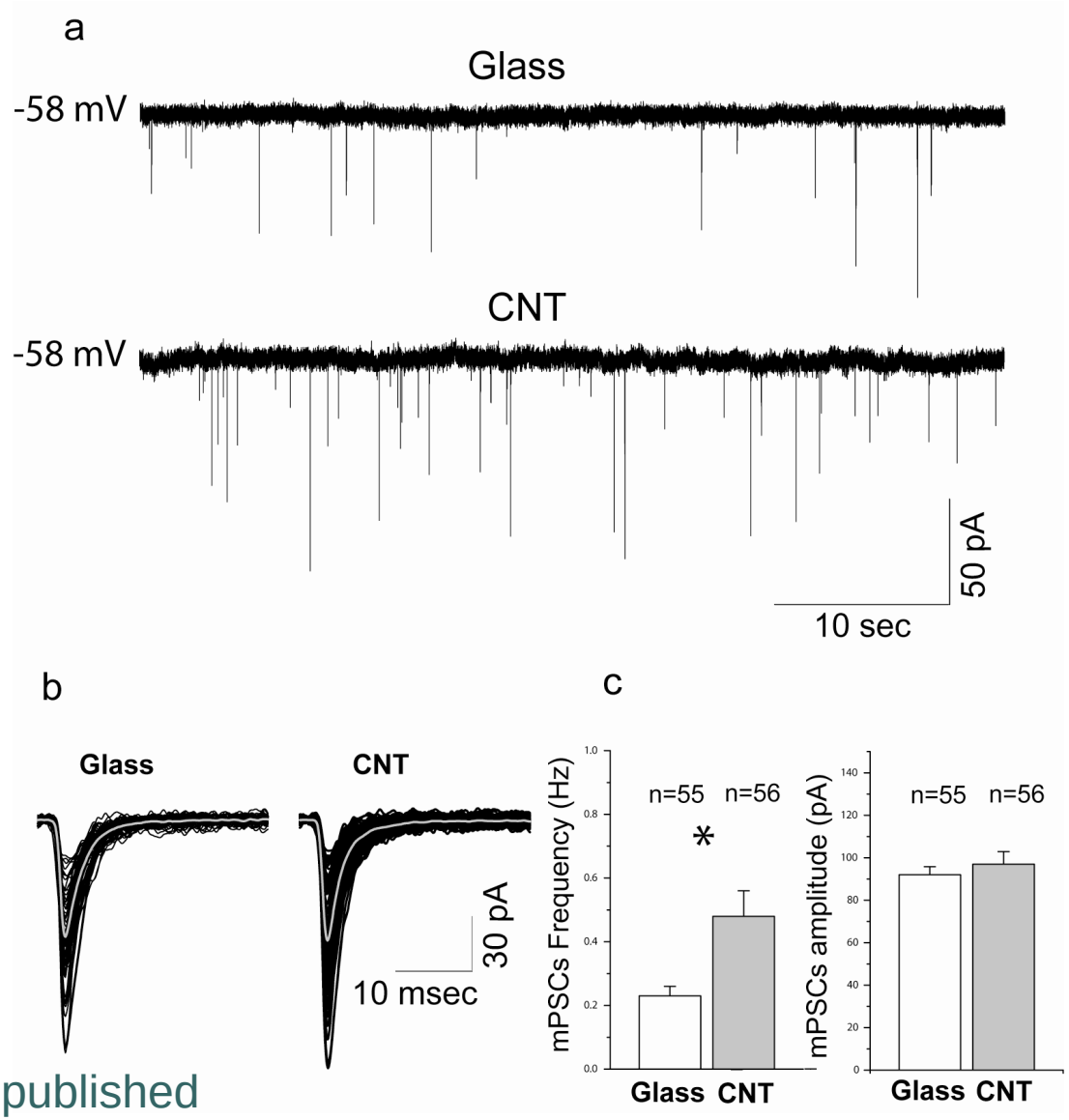




Lovat et al., NanoLetters 2005

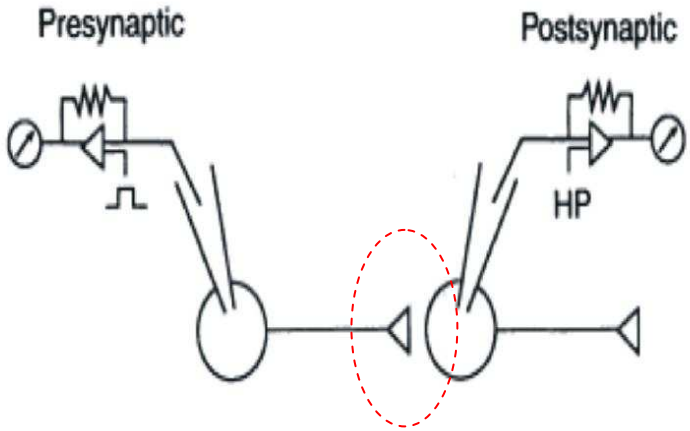


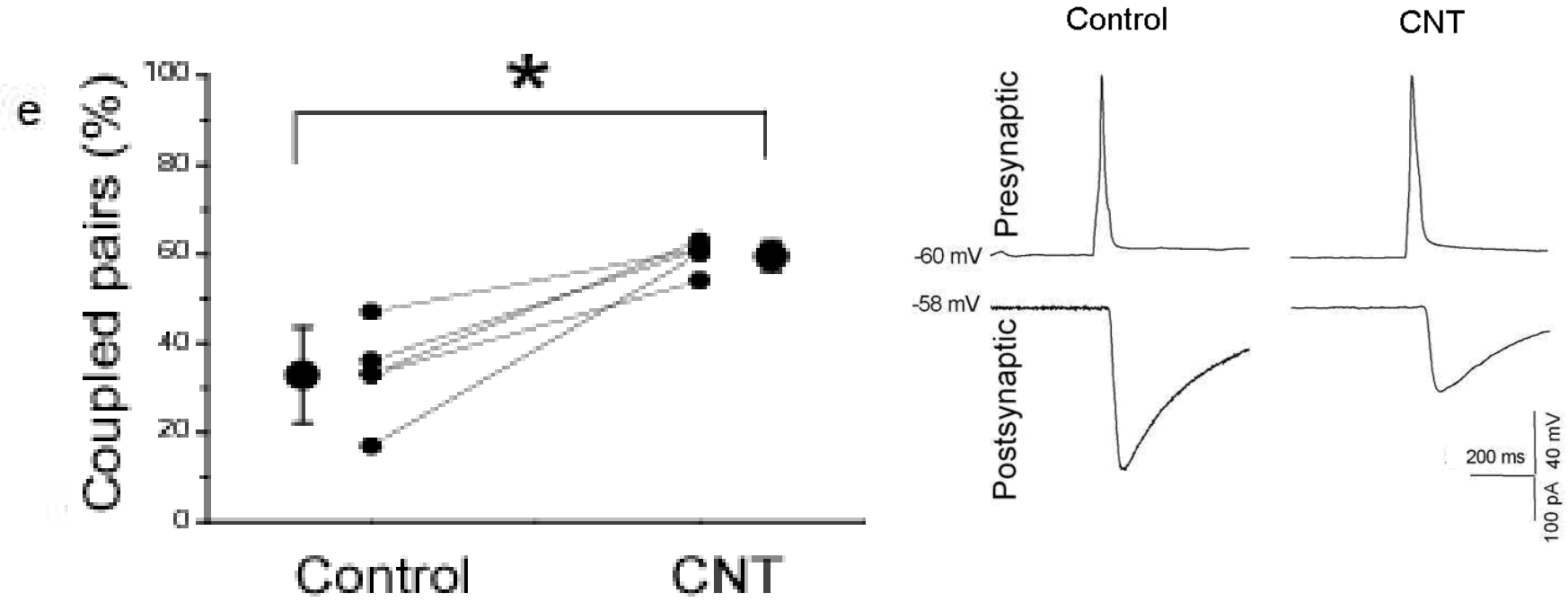
Lovat et al., NanoLetters 2005



G. Cellot, 2011 unpublished

- CNT are biocompatible substrates for neuronal growth
- CNT boosted neuronal signaling
- Enhanced network activity was apparently related to an increase in synaptic function and an increase in the number of synapses

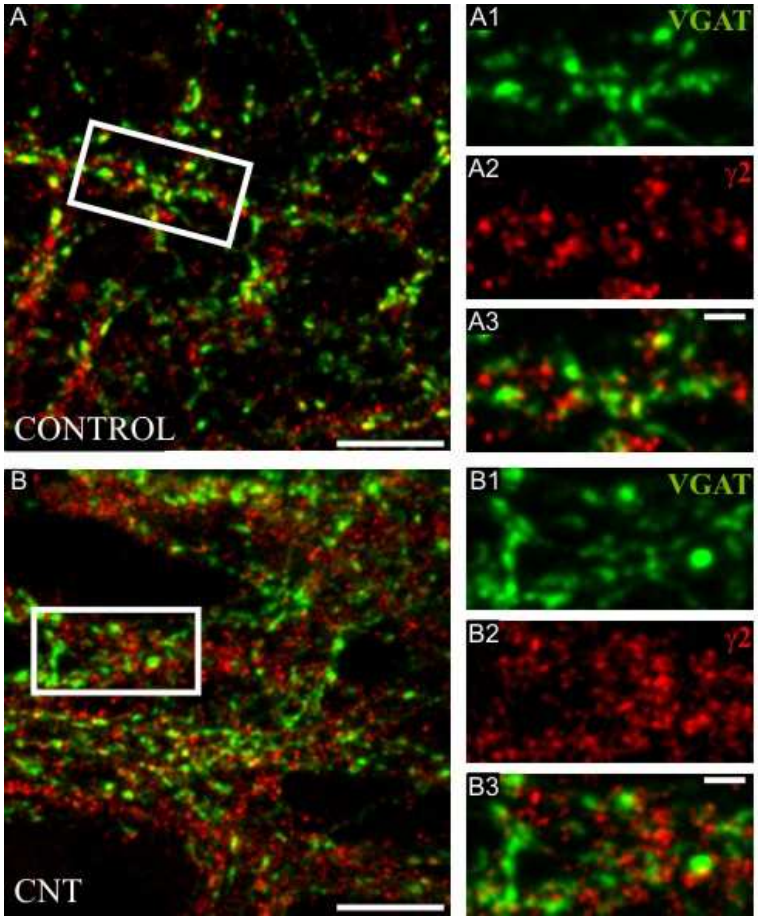

Presynaptic
Postsynaptic
HP

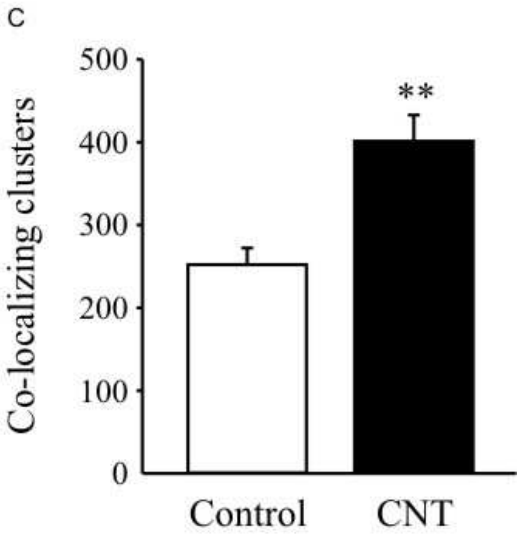


Cellot et al., J Neurosci 2011

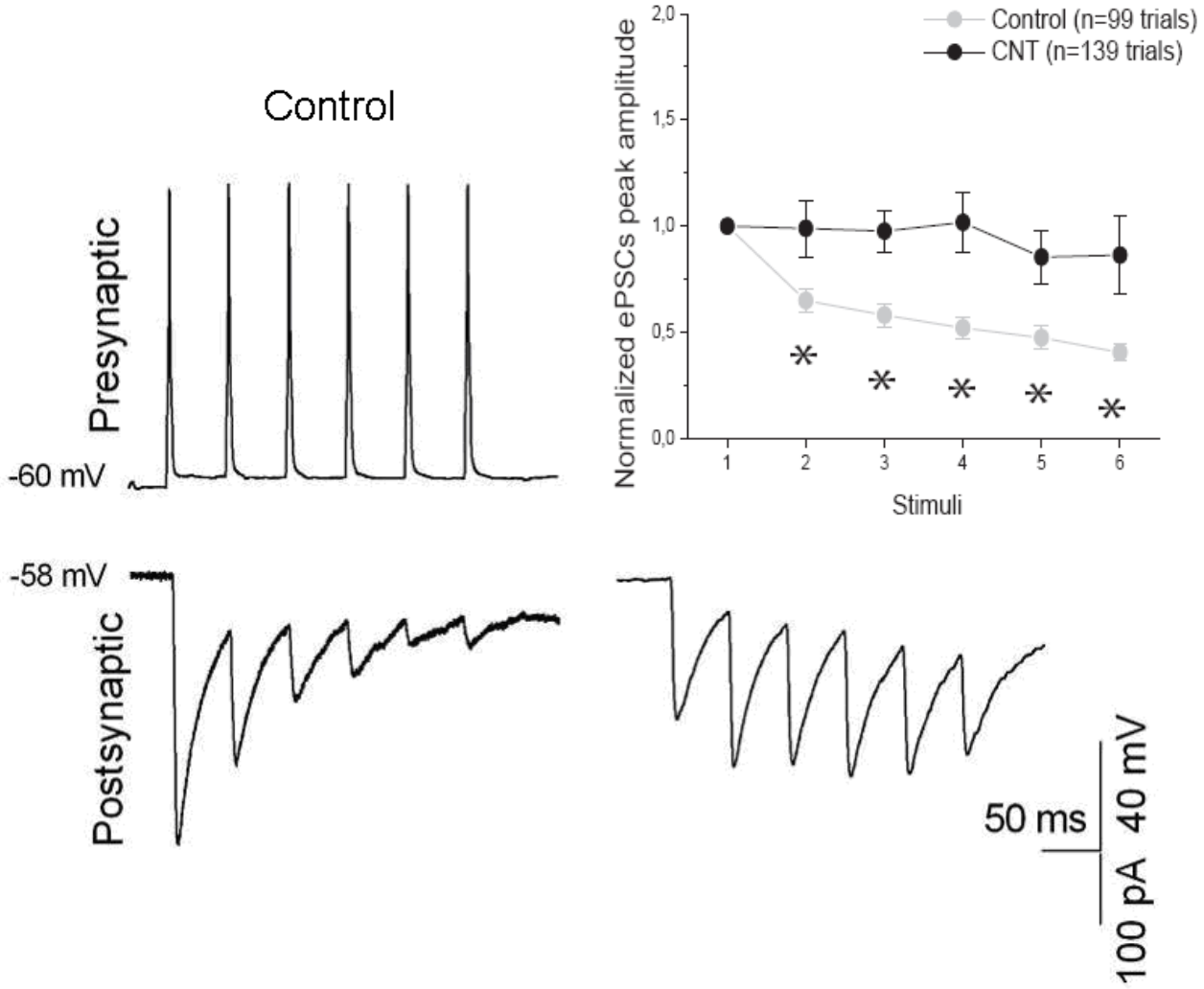
- To estimate whether changes in synaptic density may account for the enhanced coupling probability we co-immunostained for the γ2 subunits containing $GABA_A$ receptors and for VGAT, the vesicular GABA transporter considered a GABAergic pre-synaptic marker

- The putative co-localisation of pre-synaptic VGAT and post-synaptic γ2 (quantified by confocal analysis) reliably indicates a GABAergic connection





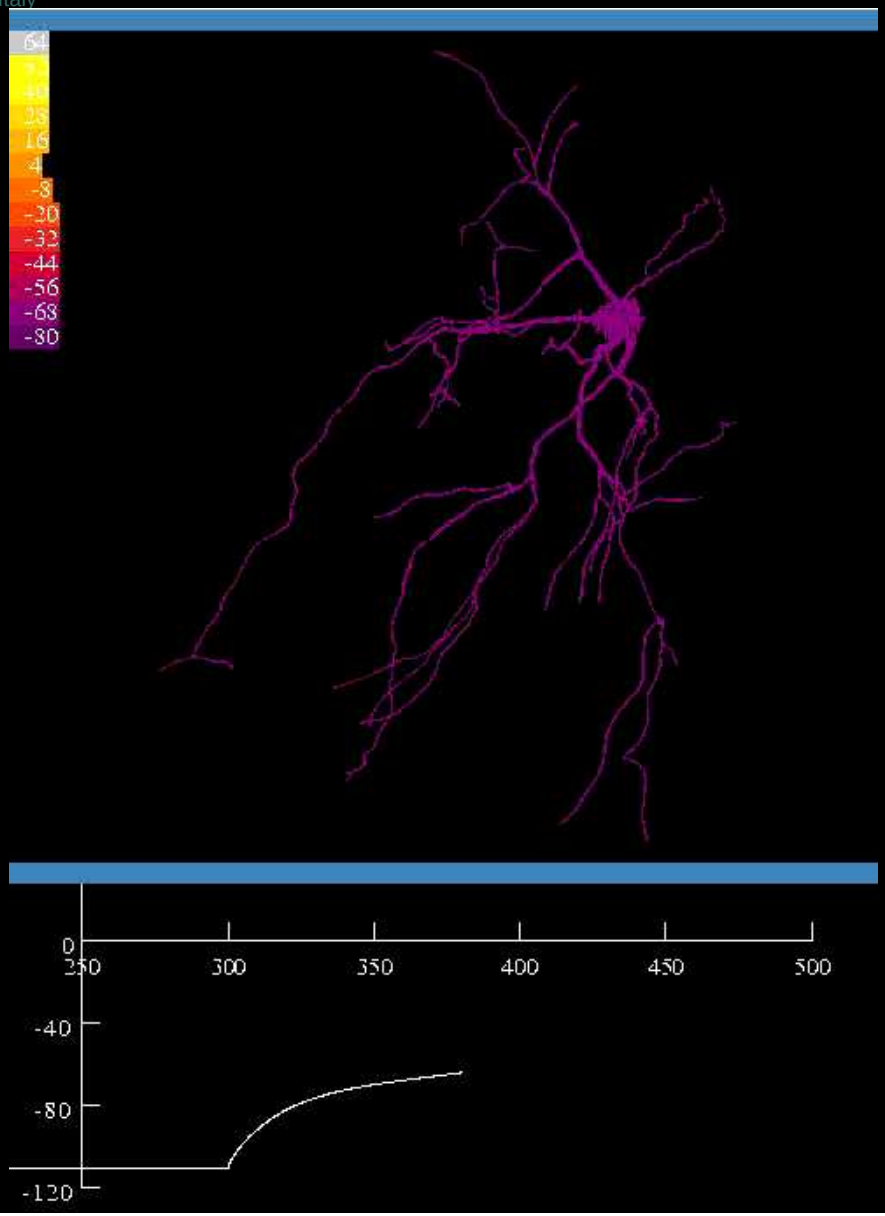
Cellot et al., J Neurosci 2011



Cellot et al., J Neurosci 2011

- Is this network enhancement ultimately linked to the nanoscale physical interactions between CNT and neurons?

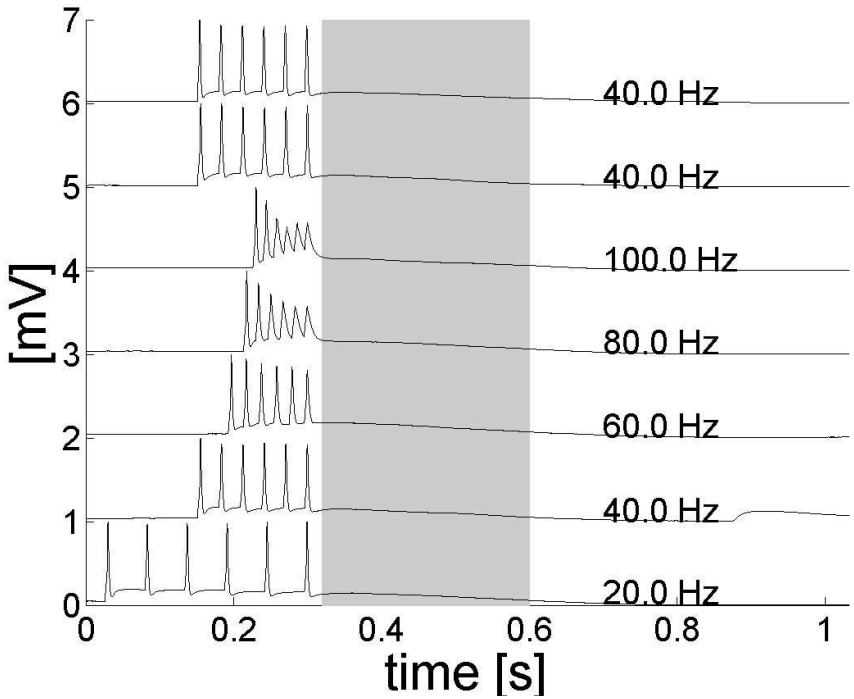
- We investigated whether CNT-substrate direct interactions with neuronal membranes might affect single dendrite activity

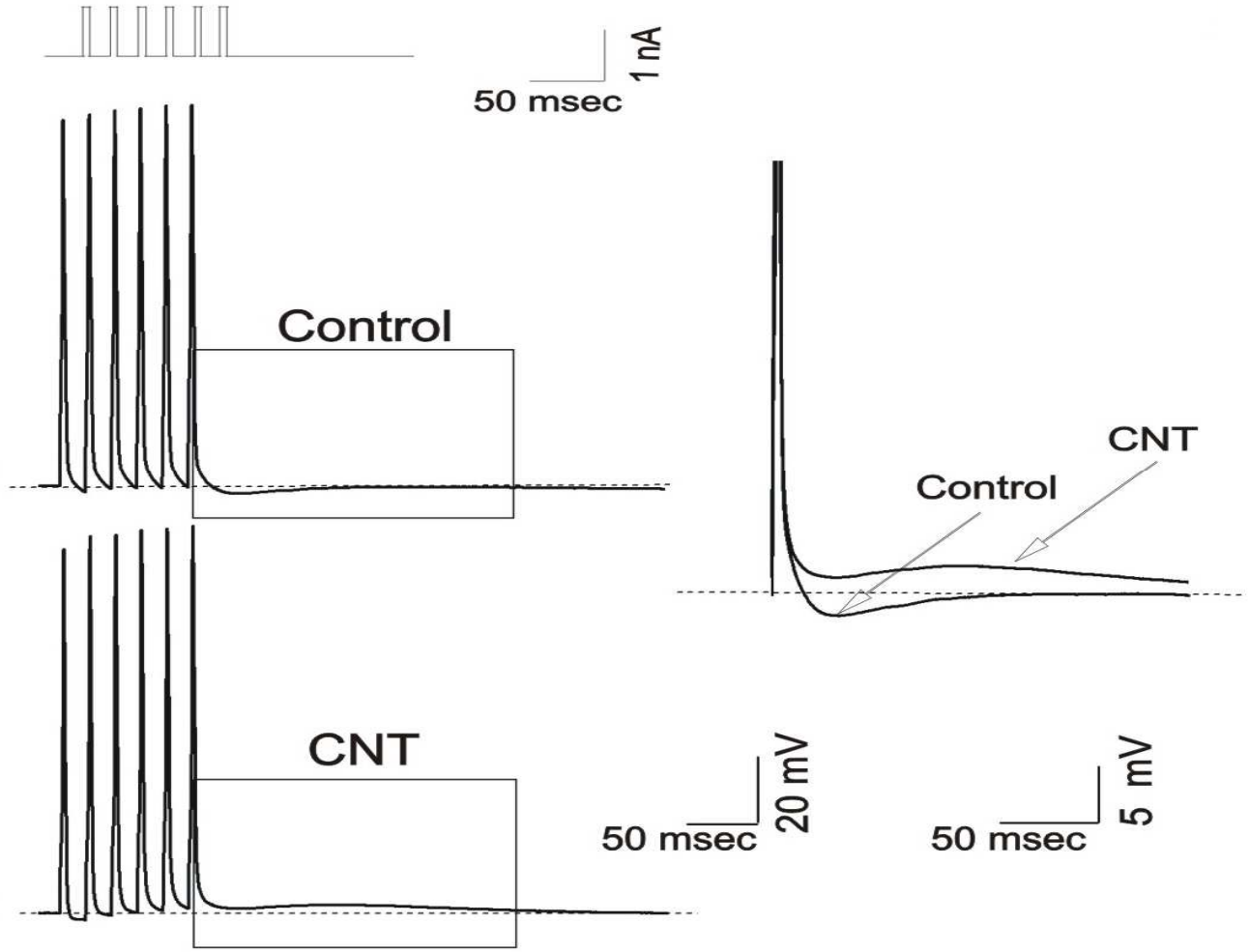


Destexhe et al. (1996) J Neurosci

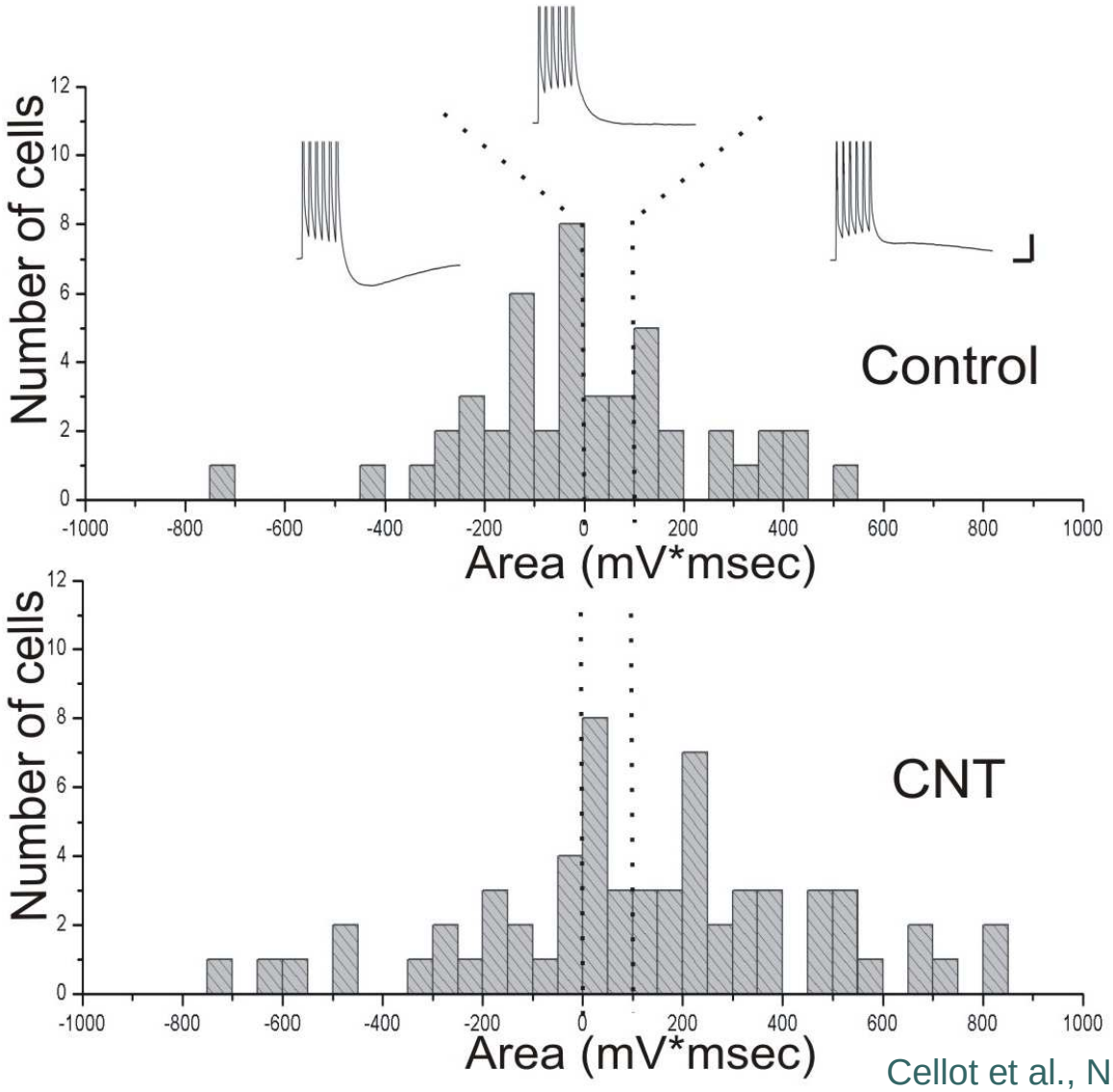
- To maximize the interactions between regenerative properties in proximal and distal areas of a single neuron, we adopted the critical frequency (CF) stimulating protocol (Larkum et al., 1999).

- Somatic current injection by brief pulses (4 ms) in trains of 6 spikes in frequency ranging from 20 to 200 Hz. We measured the appearance of additional somatic depolarization 20 ms after the last AP in the train





Cellot et al., Nature Nanotechnology 2009



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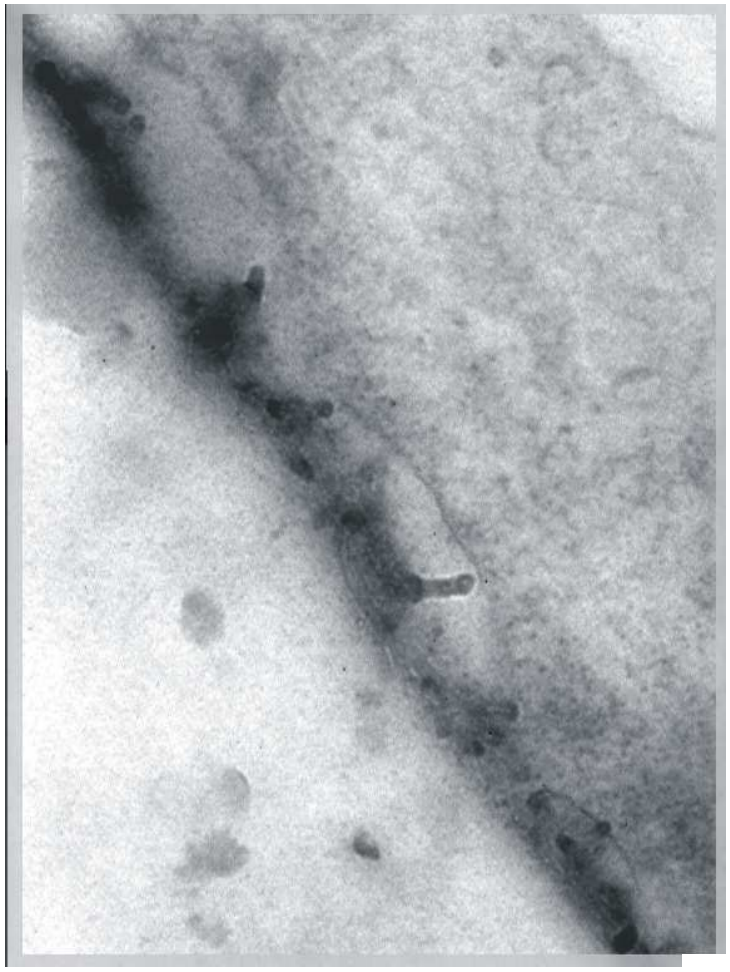
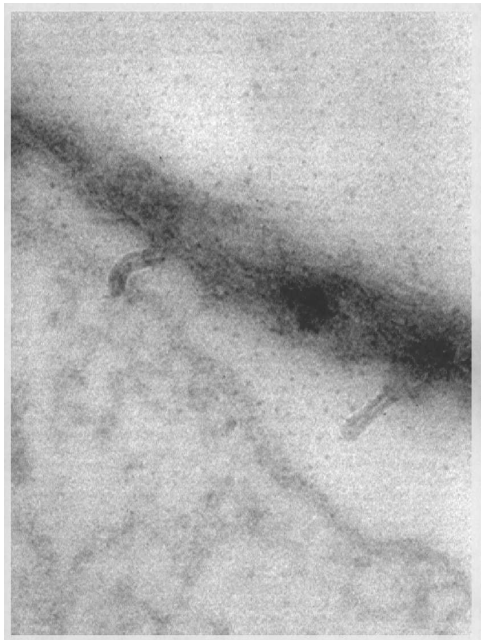
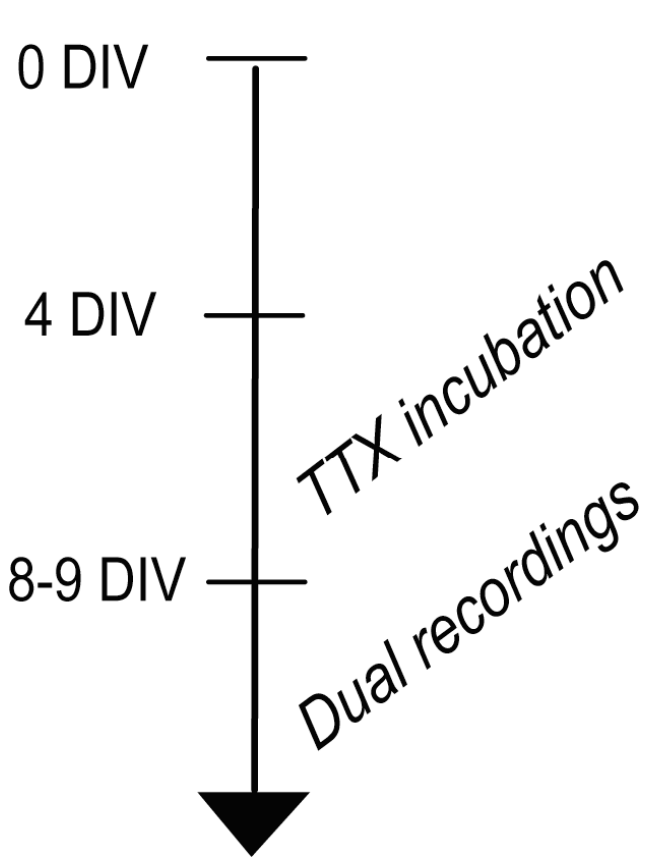
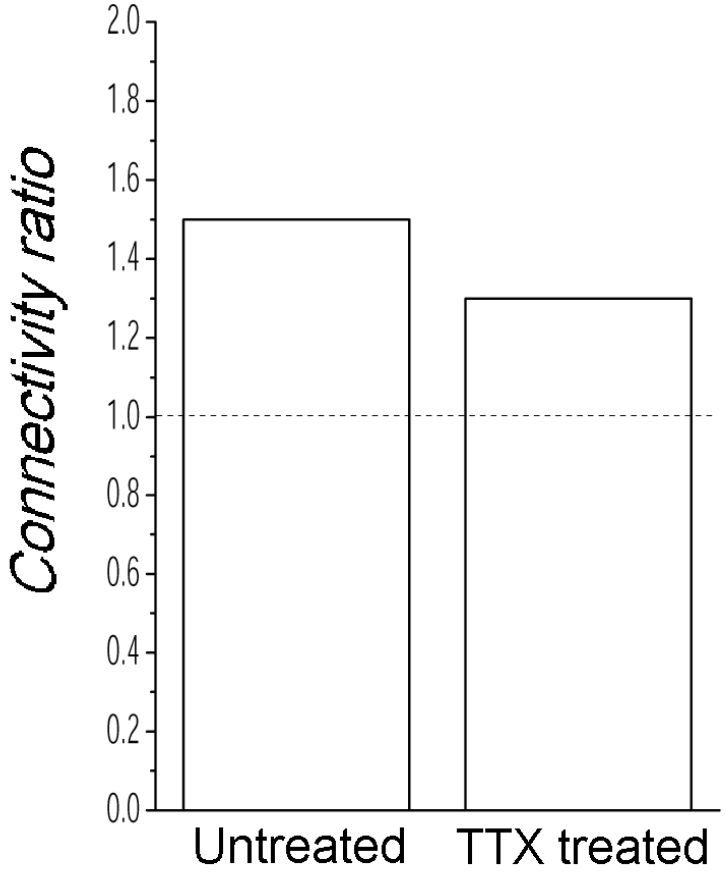


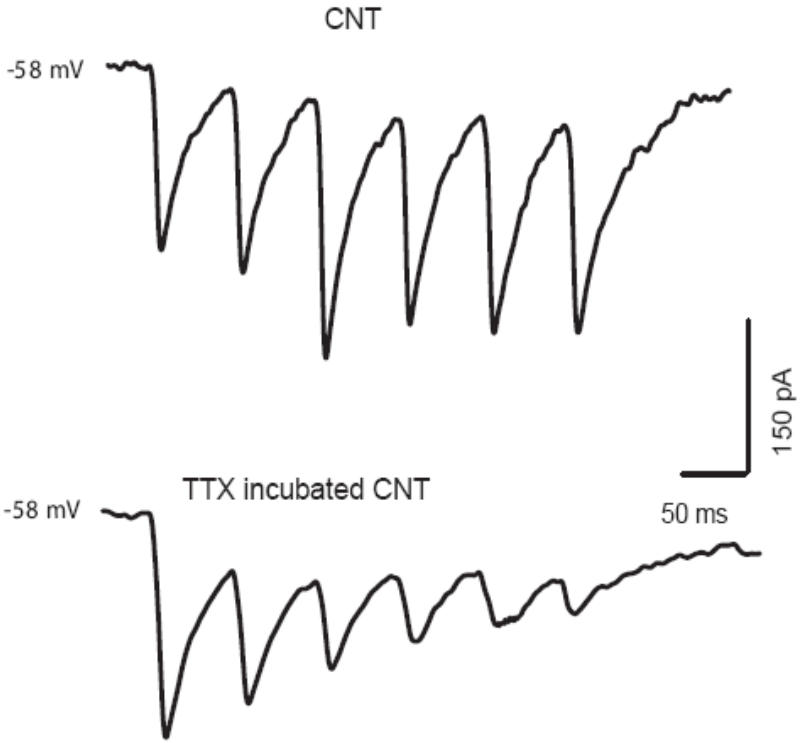


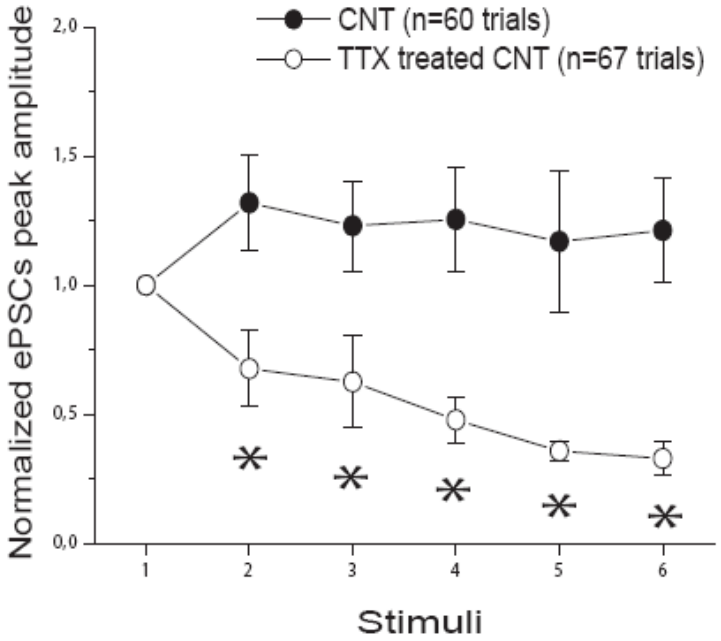
Cellot et al., Nature Nanotechnology 2009





Cellot et al., J Neurosci 2011





Cellot et al., J Neurosci 2011

- Our results show that hippocampal neurons on a carbon nanotube scaffold increase their global synaptic connectivity which has a clear anatomical counterpart in the increase in co-localization of clusters of pre and post synaptic proteins revealed by our confocal microscopy data

- The combination of these findings therefore indicates that true formation of synaptic contacts is promoted by carbon nanotubes, acting as unnatural biomimetic cues

- The synapses that are newly-formed in the presence of carbon nanotube are different in their short-term plasticity

- Synthetic materials at the nanoscale instruct cell-specific behaviors in the hippocampal cultured networks

- This may exploit the design of artificial submicroscopic man-designed devices that co-operate to neuronal network activity, generating hybrid structures able to cross the barriers between artificial devices and neurons

- Carbon nanotubes might affect neuronal information processing and improve neuronal performance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