

## NGF-INDUCED NEURITE OUTGROWTH INHIBITION AND PAIN CONTROL

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During development neurons extend numerous processes that differentiate into dendrites and axons. These processes, also termed neurites, are critical for communication between neurons. It has recently become apparent that the inability of damaged nerve fibers to regenerate is an active process under the control of molecules able to repulse and inhibit growing neurites and not a passive process as once was believed. Therefore much more effort has been done in CNS drug discovery trying to identify compounds affecting neurite outgrowth with therapeutic potential for neurodegeneration/ regeneration.

Peripheral nerve injury often leads to neuropathic pain, which might involve sympathetic postganglionic nerve fiber sprouting in the dorsal root ganglion (DRG). This sprouting is mediated by some factors whose availability is altered in DRG after nerve injury. A likely candidate is the nerve growth factor (NGF).

The over-stimulation of NGF receptors leads to a remodelling of pain pathways with an increase in the number of nociceptive fibres and pain receptors, such as ion channels.

It has been reported that local anesthetics (lidocaine, ropivacaine), by suppressing the sympathetic sprouting of DRGs, are effective in a rat neuropathic pain model. Moreover, they also suppress the NGF-mediated neurite outgrowth in PC12 cells, which is considered a cellular model of sympathetic sprouting. The inhibition of NGF-stimulated tyrosine kinase activity of TrkA (NGF signalling blockade) might be involved in the mechanism of suppression of neurite outgrowth induced by local anesthetics.

The aim of the present study was to develop an innovative, quantitative, plate-format, cell-based assay to measure the NGF-induced neurite outgrowth in PC12 cells. The method, using a fluorescent dye and transwell tissue culture chambers with an optical opaque membrane through which the neurons extend neurites, allows the physical separation of cell bodies from neurites making possible the automated fluorescent measurement only of neurite elongations without the interference of cell bodies. The new methodological approach was fully validated by testing several different reference compounds, such as lidocaine, ropivacaine, K252a and MDL-12330. The assay, in screening format, is suitable and will be useful to identify novel chemical entities able to inhibit the NGF-induced neurite outgrowth and therefore with therapeutic potential for pain treatment.