

## P13 – Optical Tweezers as Means to Stimulate Hippocampal Neurons With BDNF Coated Beads

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Brain derived neurotrophic factor (BDNF) is a small secreted protein that acts as neurotrophin and plays important roles in the development of nervous system in vertebrates. Secretion of BDNF occurs from both dendrites and axons and is regulated in an activity-dependent manner. Its effects, mediated mainly by TrkB receptor, are partially contrasting since BDNF can induce both survival, growth, differentiation and cell death [1]. This dichotomy can be ascribed to the differences between neuron types, to the activation of different pathways, or to the fine regulation of its local availability (stimulation of soma or dendrites can produce opposite effects)[2]. To better understand this process and its molecular mechanisms, we used 1.5  $\mu\text{m}$  diameter silica beads functionalized with BDNF to stimulate precise domains of hippocampal rat neurons and transported them to the site of stimulation by means of optical tweezers [3]. We demonstrate that BDNF, even if it is covalently bound to the beads, preserves its biological activity and is able to activate TrkB receptor. BDNF-coated beads, in fact, induce the translocation into the nucleus of c-Fos (a transcription factor) and lead to increase in calcium levels both in the soma and in the dendrites [4]. In this work we show a novel application of optical tweezers for localized delivery and stimulation of neurons.

### Bibliography

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P13



## Optical Tweezers as means to stimulate hippocampal neurons with BDNF coated beads

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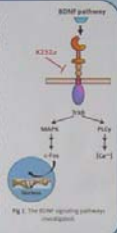


### ABSTRACT

Brain-derived neurotrophic factor is a secreted protein that has high morphoregulatory functions [1]. Binding of BDNF to its receptors (TrkB or p75NTR) can lead to very different outcomes, depending also on the site of stimulations [2]. In this work we demonstrate the possibility to reach a high spatial resolution delivery of BDNF using protein-coated beads manipulated by means of optical tweezers [3]. This allows the stimulation of the same cell with both control-bead and BDNF-coated bead, thus avoiding the inter-cellular differences, a big problem with that neuroscientists face.

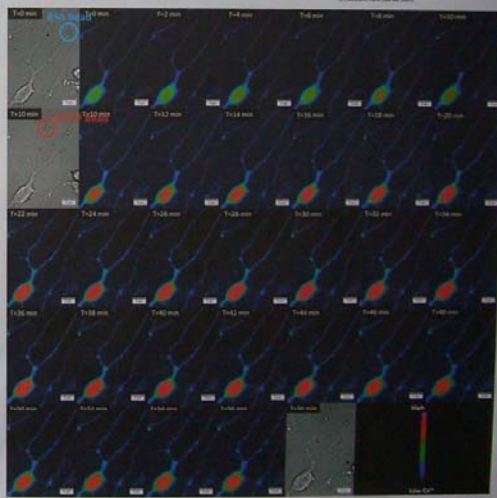
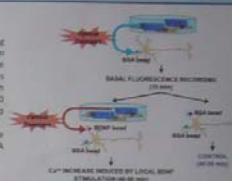
### INTRODUCTION

Neurons are excitable cells in the nervous system that process and transmit information by electrochemical signaling. Their survival, development and functions are regulated by a family of signaling proteins called neurotrophins. It has been demonstrated that different part of the same neuron respond in different way to the same neurotrophin [4]. Brain-derived neurotrophic factor (BDNF) in particular, can lead to opposite cellular responses according to the site of stimulation. These responses range from cell survival to cell death, from long term potentiation to long term depression. It is thus a matter of great interest local stimulation of neurons with BDNF in order to analyze the different neuronal outcomes. In this work we demonstrate: (i) the possibility to reach a high spatial resolution delivery of BDNF, (ii) the preservation of biological function of BDNF covalently bound to silica beads and manipulated with Optical Tweezers. BDNF-beads, in fact, activate TrkB receptors and in particular MAPK and PLC- $\gamma$  pathways.



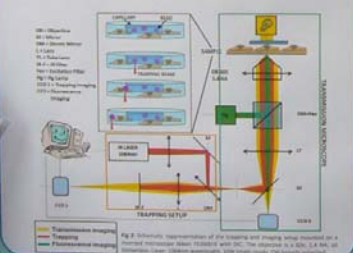
### RESULTS - Calcium levels

The PLC- $\gamma$  pathway was also investigated, with Calcium imaging technique. Calcium was visualized using Fluo-3 dye. Two capillaries filled with control- or BDNF-beads were used. First the BSA-bead was placed on a dendrite and basal fluorescence was recorded for 10 min, then the BDNF-bead was positioned on another dendrite and fluorescence was measured for about 40 min, taking images every 2min. In the control experiments two control beads were placed. BDNF-beads induced an increase in calcium levels both in the soma and in the dendrites of the stimulated cells, while BSA beads did not.



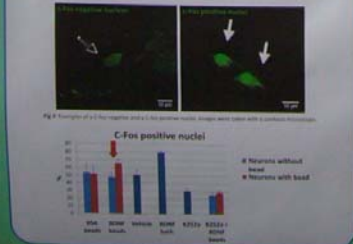
### METHODS

We used 1.5  $\mu$ m silica beads functionalized with BDNF. A capillary was filled with beads, that were trapped with Optical Tweezers ( $\lambda=1064$ nm) and, by moving the stage of the microscope, positioned on primary rat hippocampal neurons.



### RESULTS - C-Fos translocation

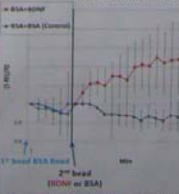
We analyzed the MAPK pathway, that is activated by TrkB, using an immunofluorescence assay. At the end of this pathway the transcription factor C-Fos translocates from the cytoplasm into the nucleus of the cell. We demonstrated that control beads coated with BSA (Bovine Serum Albumin) did not affect the percentage of C-Fos positive nuclei, while BDNF-coated beads induced the translocation of the transcription factor into the nucleus. This effect is specifically mediated by TrkB receptors because the use of K252a (an inhibitor of TrkB receptor's family) completely abolished C-Fos translocation.



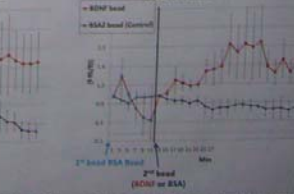
### CONCLUSIONS

We demonstrated that BDNF covalently bound to silica beads preserves its biological activity, indeed it is able to induce C-Fos translocation and an increase in calcium levels. Moreover we showed that we are able to reach a high spatial resolution (2.5  $\mu$ m) delivery of the neurotrophin to neuronal cells. We will now use this system to better analyze different cellular responses induced by Neurotrophins.

### Soma's Fluorescence



### Fluorescence second dendrite



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