NEUROPHAGE

Phage-based targeted neural stimulation in neurodegenerative disease

Poster number: 15
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Aims, research questions, and working hypothesis
With more than 10 million people affected worldwide, Parkinson's Disease (PD) is one of the most common neurodegenerative diseases with major psychological, social, and economic impacts. PD is characterized by the progressive degeneration of the nigrostriatal dopaminergic pathway that physiologically facilitates the initiation of voluntary movements. This results in the imbalance of striatal outputs and impairment of the striatal D1- dopaminoceptive "direct pathway", leading to akinesia. The current pharmacological approaches control the symptoms in the early phase but still suffer from long-term complications and drug-resistance issues. Among alternative methods, current implant technologies using microelectrodes for delivering stimuli (DBS) meet several problems that limit the performance and safety of the implants, while optogenetics, a breakthrough in optical stimulation, requires gene therapy and implanted fiber optics. We have recently shown that photovoltaic polymer nanoparticles (NPs) are able to efficiently stimulate denervated neurons on demand. As an alternative to invasive DBS or optogenetics, NEUROPHAGE aims at delivering these polymeric NPs using engineered brain-permeable M13 phages as nanocarriers for specific targeting and activation of D1-dopaminoceptive neurons in the striatum. To this aim, M13 phages will bear a docking system to recognize specific neuronal epitopes and an orthogonal cargo of polymeric NPs eliciting neuronal activation in response to light stimulation. The NEUROPHAGE innovation, distinct from existing NP-based technologies, consists of using biological biocompatible and harmless vectors to bring active organic NPs in close proximity to selected target neurons. With the proven capability of the phage system to efficiently cross the blood-brain barrier (BBB) and the possibility to tune the NP properties to respond to NIR light stimulation, our project holds great promise for an effective, cell-specific, minimally invasive strategy to drive specific basal ganglia activation and rescue PD symptoms.

Means/methods implemented by the consortium
This interdisciplinary project brings together expertise from seven research institutes across six European countries to tackle the development of such an innovative therapeutic strategy for PD and other neurodegenerative diseases. The Consortium includes the Center of Synaptic Neuroscience and Technology led by Fabio Benfenati at the clinical research hospital IRCCS Ospedale Policlinico San Martino in Genova, specialized in smart interfaces for neuronal activation, and two world-renown laboratories for research on basal ganglia physiopathology and therapy for Parkinson's disease, namely the INSERM Unit led by Jean-Antoine Girault in Paris and the Karolinska Institutet Unit led by Gilberto Fisone in Stockholm. The Consortium is enriched by the presence of excellent centers in the field of bionano interactions as the Center for BioNano Interactions (NUI-UCD) led by Kenneth A. Dawson, the Nanoscalic system group (HZDR) represented by Kristof Zarschler, the NanoBio Interface Lab of Matteo Calvaresi and the advanced preclinical imaging center (CAPI) directed by Luděk Šefc. The methods implemented by the consortium involve phage production, purification, and retargeting, a palette of characterization techniques specifically adapted for the phage hybrid systems, bioconjugation strategies, transfection in vitro and in vivo, blood-brain barrier models of increasing complexity, and in vivo electrophysiological and behavioral analyses.

What are the outcomes of the project?
With this project, we have demonstrated that M13 bacteriophages are able to cross the BBB, keeping their structural integrity and functionality, including retargeting ability, by exploiting non-conventional trafficking pathways specific to the interaction with barrier cell type. The M13 phage has been both genetically and chemically modified in an orthogonal fashion with diverse functional nanomaterials of choice. Interestingly, the M13 phage does not undergo hard coronation by the plasma proteins, and it is able to provide efficient targeting even in a crowded "in vivo-like" biological context. We also developed a robust methodology to follow M13 within the body after intravenous injection without affecting its colloidal stability, providing a very long circulation time. Our building blocks are now being translated in an in vivo model of transgenic mice for specific targeting of D1-dopaminoceptive neurons in the striatum.

Significance and impact of the work on the field
We propose an alternative, non-invasive, nanotechnology-based therapeutic approach for PD by targeted stimulation of D1R-expressing neurons of the direct pathway in a widely recognized murine experimental model of PD. In contrast to DBS, the excitation will focus on the pathway that is directly inactivated by neurodegeneration. The use of conjugated polymers that are able to respond to light makes this methodology minimally invasive, as the stimulation source can be applied at the skull surface. Given the existing knowledge and the persistent therapeutic need, PD provides an ideal condition for evaluating the efficacy of the NEUROPHAGE approach. In addition, the modulation of neural activity via wireless stimulation with the specific targeting capability and high temporal/spatial resolution would represent an unprecedented technological advancement applicable to a broad range of neurodegenerative diseases.

Next steps and future challenges
Our next steps are focused on understanding what features of the M13 phages hybrid construct will promote a more efficient translocation to the brain and what is the best configuration for the delivery of active nanoparticles in close contact with the neuronal membrane. These studies will pave the way for a very flexible platform based on novel hybrid bio-nano interfaces applicable in vivo to rescue neural functions that are lost in neurodegeneration.