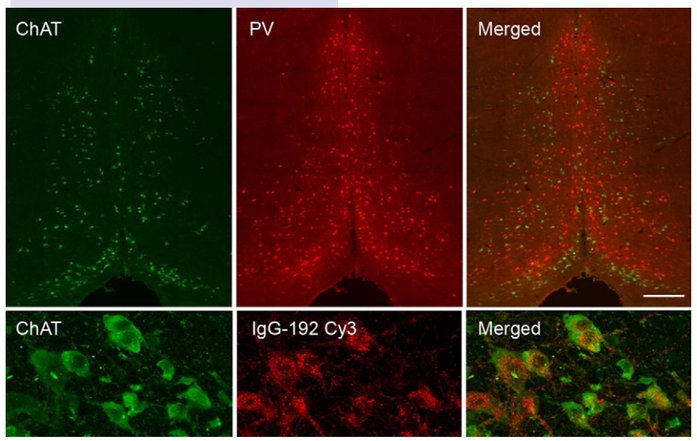
Delineating the mechanisms of synaptic transmission for the development of targeted neurotherapeutics

The successful development of innovative neurotherapies to restore synaptic functions and neural communication affected by disorders (e.g. chronic pain, neurodegenerative and neuropsychiatric conditions) requires an in-depth understanding of cellular and molecular mechanisms involved. Dr James Oliver Dolly, Science Foundation Ireland (SFI) Professor of Neurotherapeutics at the International Centre for Neurotherapeutics (ICNT), Dublin City University (DCU), and Dr Saak Victor Ovsepian, Professor of Neurobiology from the National Institute of Mental Health (NIMH), Prague, and Adjunct Professor at ICNT, have collaborated over a decade to develop and validate bio-therapeutics that are effective in the treatment of diseases of the nervous system.

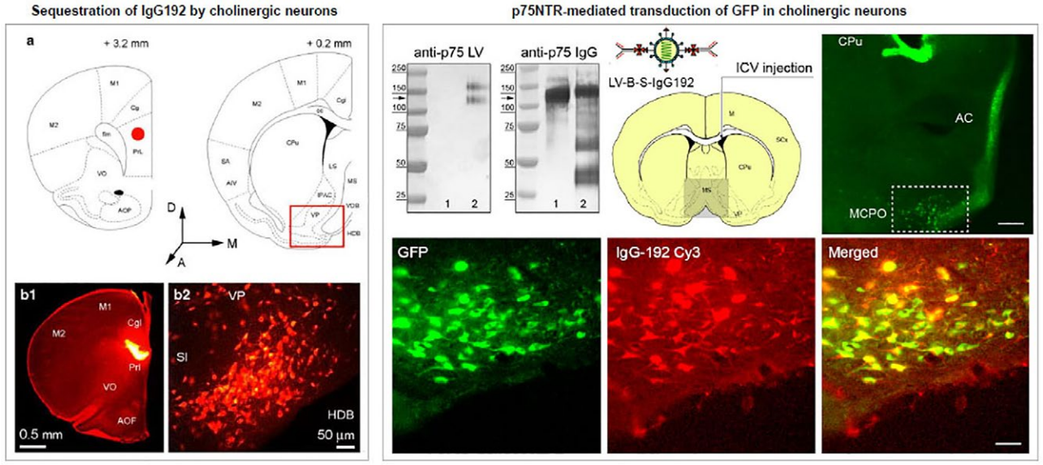
Dr James Oliver Dolly, Professor of Neurotherapeutics at the ICNT, DCU, Ireland and Dr Saak Victor Ovsepian, Professor of Neurobiology at the NIMH, Czech Republic, and adjunct Professor of Neuroscience at DCU, have conducted pioneering work and disseminated knowledge in many areas of Neurobiology and Neuropharmacology, individually and in collaboration. The focus of their collaborative research has been in synaptic transmission, a fundamental process where signals are exchanged between neurons or from a neuron to another effector, such as a muscle or gland. Deciphering the cellular and molecular mechanisms of neural communication and impairments is essential for developing effective therapeutics for the treatment of nervous system diseases.

Confocal images of rat basal forebrain cholinergic (ChAT) and GABAergic neurons (PV) and merged image (top). Higher power images of ChAT neurons expressing p75NTR labeled with IgG-192 Cy3.



Dr Ovsepian is a prolific Neurobiologist, whose main interest has been cellular biology and synaptic physiology, neuromodulation and neurodegeneration. His early work made a pioneering contribution to understanding the evolution of synaptic transmission mechanisms in vertebrates, as well as in various forms of communication between neurons. This research has led to the hypothesis that chemical synapses might have evolved from primordial structures that existed before the rise of neurons and multicellular organisms. He also helped to elucidate mechanisms of cholinergic modulation of neurotransmission and synaptic plasticity, with the discovery of a new, homeostatic role of the central cholinergic system, in regulating the production and clearance of amyloid β peptide, a key cause of Alzheimer's disease (Ovsepian, O'Leary et al. 2016). Dr Ovsepian formulated the "brain's dark matter hypothesis", proposing a massive redundancy of neurons in the mammalian brain (Ovsepian 2019). According to this model, the great majority of nerve cells in the intact brain are rudimentary and maintained in a permanently silent state at a very high energetic cost. Their re-activation can contribute to the manifestation of 'savant skills' – a range of traits, sometimes related to autism, which are manifested as an extraordinary brilliance in a certain field – as well as the onset of various neurological and neuropsychiatric diseases.

Dr Dolly is a world-class authority in Molecular Neurobiology, having pioneered the purification and biochemical characterisation of acetylcholine receptors in skeletal muscle,



Schematic of rat brain (a, left) showing the site of tracer injection and imaging (red circle and box). Images of the same plane showing injection site and labeled neurons in basal forebrain (b1 and b2). Verification of a newly-made viral vector and testing in living rat (right). (Top) Production and ventricular injection of virus encoding GFP with its expression verified using confocal imaging.

and the Kv1 sub-family of voltage-sensitive K^+ channels from the brain (Rettig, Heinmann et al. 1994). Other major contributions include elucidation of the inhibition of acetylcholine release by botulinum toxins, entailing their selective binding to motor nerve terminals, acceptor-mediated endocytosis, and translocation to the cytosol where they proteolytically inactivate SNARE proteins essential for exocytosis (reviewed in Ovsepian, O'Leary et al. 2019). These discoveries aided the development of botulinum toxins for the successful clinical treatment of numerous conditions due to hyper-activity of motor and autonomic nerves, which include dystonias, spasticity, cerebral palsy, sialorrhoea, hyperhidrosis, as well as cosmetic applications. Investigations of the K^+ channels in the central nervous system unveiled an abnormal distribution of a subtype on demyelinated axons in a rodent model of multiple sclerosis. The resultant defective nerve conduction could be normalised by a specific inhibitor designed for the culprit K^+ channel species (Al-Sabi, Daly et al. 2017).

NEUROTOXINS: THEIR ROLE IN DRUG DISCOVERY AND PATIENT TREATMENT

Toxins and venoms contain a large variety of neuroactive molecules and peptides that offer a vast bank of raw resources for drug discovery. Hence, neurotoxins have attracted widespread investigations that revealed their considerable potential as probes for synaptic transmission mechanisms,as well as being of medical benefit. Drs Dolly and Ovsepian have designed and/or characterised a range of bacterial and animal toxins targeting the presynaptic secretory machinery (Ovsepian, O'Leary et al. 2019). In particular, botulinum toxins have highlighted the medical utility of bio-toxins.

Researchers at ICNT engineered novel chimeras of two serotypes of botulinum neurotoxin that acquired an ability to reduce the release from sensory neurons of a pain mediator, if evoked by a noxious stimulus (Wang, Meng et al. 2011). This discovery prompted optimisation of an effective non-addictive analgesic (Wang, Casals et al. 2017), a much-needed goal due to the prevalence of chronic pain. An additional exploitation arose from the early creation and patenting of a protease-inactive mutant of botulinum neurotoxin type B (BoTIM/B), which allowed Dr Dolly to exemplify how such variants achieve targeted delivery of drugs into mouse motor nerves (Dolly, Aoki et al. 1995; Edupuganti, Ovsepian et al. 2012). Just this year, the specificity and capability of such targeted neuronal carriers were elegantly documented for counteracting botulism symptoms in animals, as highlighted by K. Servick in Jan. 2021 issue of *Science Magazine*. Evidence was accrued for an even more sophisticated application of BoTIM/B for endowing neurotropism on lentiviral vectors, for the delivery of a reporter and therapeutic genes (O'Leary, Ovsepian et al. 2011).

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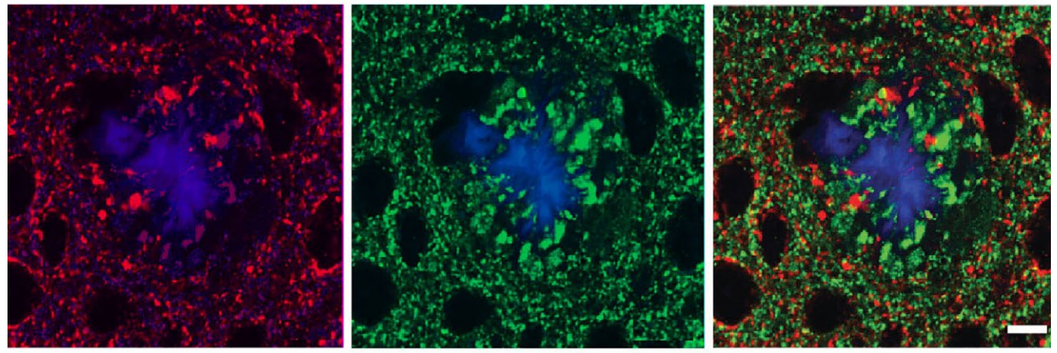
Another example of how a deadly poison can be turned into a remedy is given by detoxified tetanus toxin. The toxin crosses synapses and the active form blocks the release of neurotransmitters such as GABA and glycine, causing disinhibition of large motor neurons that leads to spastic paralysis. If untreated, tetanus can cause death, due to violent convulsions and breathing failure. The unique capability of tetanus toxin to target and enter motor nerve terminals and travel from there to the central nervous system has generated considerable interest for its potential in clinical applications. The toxin is the only known substance capable of overcoming flaccid muscles and muscle weakness caused by brain and spinal cord injuries (Fishman 2009). This unique property of the tetanus



Brain images of APP-PS1 Alzheimer's disease mouse model showing an amyloid plaque (blue) with related changes of surrounding inhibitory (red) and excitatory (green) axons.

toxin can be exploited for the potential treatment of several conditions related to acute and chronic muscle weakness.

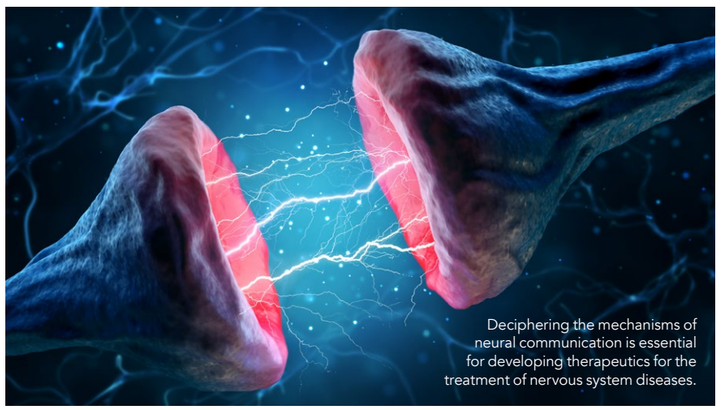
AMYLOID PLAQUES AND THE RELEASE OF GLUTAMATE IN ALZHEIMER'S DISEASE

Dr Ovsepian contributed to elucidating the synaptic pathology in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's. With renowned experts Drs Haass, Willem, and Herms from the Centre for Neurodegenerative Disease Research in Munich, the work lead to the discovery of a new mechanism of action for amyloid precursor protein, relevant to the pathobiology of AD. Presynaptic pathology caused by intracellular Aβ has been addressed (Ovsepian, O'Leary et al. 2018). It emphasised reports of numerous cases of severe dementia with low Aβ load or, vice versa, mild cognitive impairment with high Aβ levels in the brain. Nevertheless, there is a large body of evidence for focal damage caused by amyloid plaques through the localised rise of extracellular glutamate, and oxidative stress leading to mitochondrial dysfunctions. Moreover, intra-vital two-photon imaging showed that the presence of plaques correlates with impairments of dendritic spines, changes in synaptic and neuronal activity. Axons and presynaptic terminals are particularly sensitive to the presence of plaques, which cause areas of swelling known as dystrophies. With Dr Herms, Dr Ovsepian showed that axonal dystrophies associated with amyloid plaques might present hotspots for the non-synaptic release of glutamate. The uncontrolled build-up of glutamate would contribute to increased seizures in AD patients, and aggravate the pathophysiology and functional changes in their brain.

FUTURE DIRECTIONS

The delivery of therapeutics to central neurons across the blood-brain barrier (BBB) is certainly a desirable objective for the potential treatment of neuropathologies, but also a challenging one. The BBB, a system of selectively permeable vascular barriers, allows the entry of nutrients and metabolites while denying access to pathogens and toxins. The neuromuscular junction presents a unique gateway for delivery of therapeutics to the CNS when coupled to protease-inactive tetanus toxin via retrograde transport, thereby acting as a nano-carrier (Dolly, Aoki et al. 1995, Ovsepian, O'Leary et al. 2019). It interacts with certain lipids on the surface membranes with low affinity, followed by avid binding to synaptic vesicle proteins that affords internalisation into central neurons. It would be very attractive to target curative agents to motor and sensory neurons, for possibly alleviating muscular dysfunctions and chronic drug-resistant pain, provided that further studies confirm that such agents are capable to circumvent the action of the immune system against them. With the advancement of new approaches and instrumentation for non-invasive stimulation and brain imaging under development, supported by a recent European Union JPND programme, Dr Ovsepian anticipates notable progress in the future.

The delivery of therapeutics to central neurons across the blood-brain barrier is a desirable but challenging objective for the potential treatment of neuropathologies.



Deciphering the mechanisms of neural communication is essential for developing therapeutics for the treatment of nervous system diseases.

Behind the Research

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Research Objectives

During their research careers, Profs Dolly and Ovsepian have led pioneering work in Synaptic Neurobiology.

Detail

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Bio

James Oliver Dolly is the SFI Professor of Neurotherapeutics, Director of ICNT at DCU. He received his PhD in Biochemistry, followed by a Faculty appointment at University of Maryland, and Imperial College London, as a Prof of Molecular Neurobiology. Later he joined DCU as an SFI Professor and established ICNT. In 2002, he received a DSc from University of London.

Saak Victor Ovsepian is a Neurobiology Professor, Director of the Experimental Neurobiology Program and Head of the Department of Experimental Neurosciences at the NIMH. He is also an adjunct Professor at the ICNT, DCU, Ireland. He worked as Head of Neuroimaging and Neurophysiology at ICNT, Dublin and DZNE, Munich, and as the lead neuroimaging specialist at IBMI, Munich.

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- Prof Vasilis Ntziachristos, Institute of Biological and Medical Imaging (IBMI) and Centre for Biomedical Imaging, Technical University of Munich, Germany
- Prof Jochen Herms, German Centre for Neurodegenerative Disease, Centre for Neuropathology and Prion Disease, Munich, Germany

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Personal Response

What excites you most about your research?

"1) Creating novel, effective but non-addictive neurotherapeutics for chronic pain. 2) Discovery of the neurobiology and molecular mechanisms of neuronal signaling and brain functions under normal and diseased conditions. 3) Developing innovative tools and methods to advance brain research, diagnostics, and imaging, to address the outstanding questions of fundamental neurobiology and translational neurosciences."

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