RESTORATION OF RESPIRATORY MOTOR FUNCTION THROUGH OPTOGENETIC NEURAL IMPLANTS

Ivo Lieberam, United Kingdom
Grant: € 80 000

Most 'higher' animals that live on land, including humans, breathe air through their lungs. The expansion and contraction of the lungs during breathing (respiration) is driven by a group of muscles that are attached to the rib cage. The breathing rhythm itself is generated in the brain and then conveyed to the muscles through a specialized type of nerve cells called 'respiratory motor neurons'. Patients suffering from Amyotrophic Lateral Sclerosis (ALS) lose the ability to breathe, because the disease destroys their respiratory motor neurons so that connection with the muscles involved in respiration is lost, leading to paralysis of these critical muscles. The resulting respiratory failure is the main cause of death from ALS.

Here, we propose to develop a novel kind of implant that consists of biological and electronic elements which will used to provide disconnected respiratory muscle with a new, artificial breathing rhythm.
This device will take advantage of two recent discoveries in biomedical science: i) Motor neurons can be generated directly from embryonic stem cells (ES cells), a type of immature cells that can be turned into any specific cell type in the body. Nerve cells derived from ES cells have been shown to survive and innervate muscles inside the body when implanted into a host nervous system. ii) Channelrhodopsins are molecular photosensors that algae and bacteria use to detect light. When a channelrhodopsin gene is integrated into human (or other) cells, then these cells gain the ability to be controlled by light. This photosensitization is crucial, because it will provide a communication interface between the biological component that connects to the muscle (grafted ES cell-derived motor neurons) and an electronic pacemaker that generates the breathing rhythm.

We recently have succeeded in producing ES cell-derived motor neurons that have a channelrhodopsin gene engineered into them. These neurons connect to muscle in a petri dish and can trigger muscle contractions when activated by light pulses. Our plan is to transfer the ES cell-derived motor neurons into degenerating respiratory nerves in mice that model ALS, let them reconnect to respiratory muscle and subsequently control breathing with light pulses. These regular light flashes will be produced by the pacemaker and transmitted to the motor neurons via optical fibre cables inside the body. In principle, our neural implant will be similar to traditional pacemakers for heart muscle, except that the connection to the muscle is provided by grafted artificial motor neurons, and the stimulus (light) is specific to these nerve cells and does not affect other tissue. If the tests in ALS mice are successful, we plan to adapt the technology for the use in humans. The therapy we propose here is designed to restore breathing by permanently replacing diseased nerve cells with healthy, artificially generated ones. Thus, the function of neural implant will not be affected by the progressive degeneration of the patients' own motor neurons.

Should we succeed, then the proposed approach will be used to alleviate the most life-threatening symptom of ALS (respiratory failure), irrespective of whether the disease is sporadic or familiar. In the long-run, a similar approach could be developed to restore other motor functions, such as walking.

Specific Aims

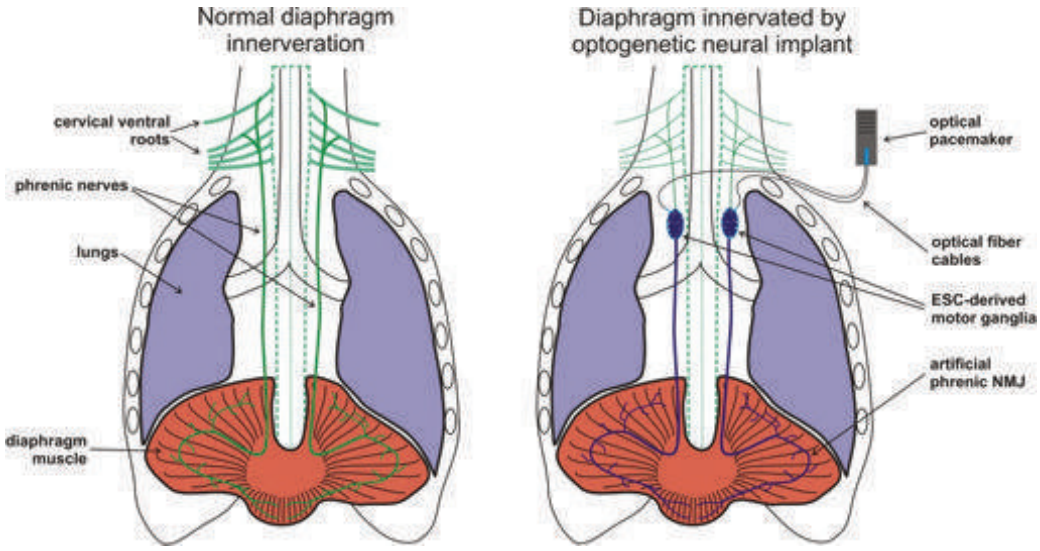
1) We plan to establish several stable mouse ESC lines that each carry a different candidate channelrhodopsin transgene, derive motor neurons from these cells and test the suitability of the channelrhodopsin variants by light stimulation *in vitro*.

2) We will develop methods for the differentiation and purification of astrocytes from ESCs, which we intend to use as disease-free support cells for ESC-derived motor neurons in *in vivo* grafts.

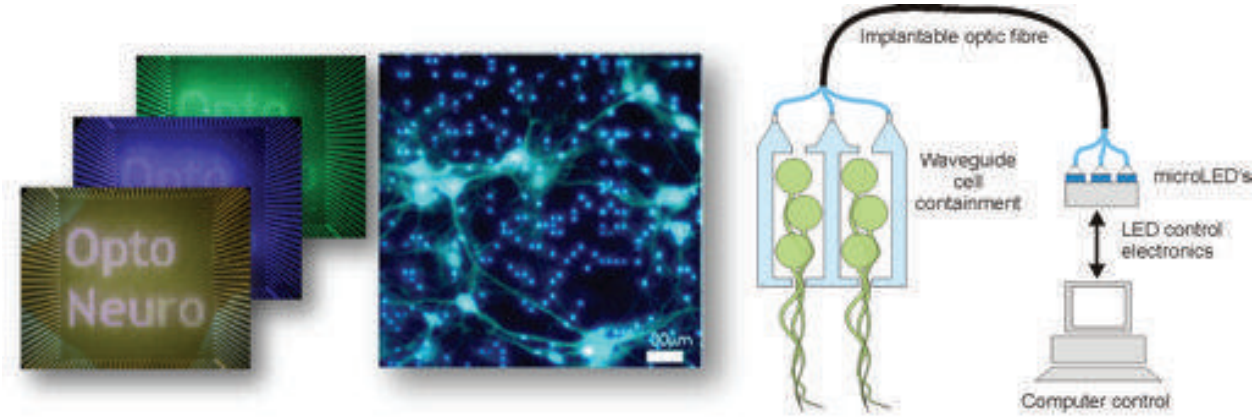
3) To establish and optimise the optogenetic implant technology, we will use a sciatic nerve lesion model to insert ESC-derived motor neurons into a degenerating nerve and determine whether the grafted neurons extend axons and establish synaptic contacts with the hindlimb muscles (eg gastrocnemius).

4) We will connect the sciatic nerve ESC-motor neuron graft to an optical pacemaker via fibreoptic cables and stimulate muscle contractions of the hindlimb muscle with light pulses to establish functional neuromuscular interaction between the grafted ESC-derived motor neurons and denervated host muscle.

5) Following optimisation of the optogenetic implant technology in the sciatic nerve model, we then plan to graft ESC-derived motor neurons into the phrenic nerve in adult rats in which the diaphragm has been denervated on one side and test the ability of the implanted cells to functionally innervate the denervated hemi-diaphragm. This model will be further developed to link the neural graft to an optical pacemaker *ex vivo*, and impose an optogenetic inspiratory rhythm on the diaphragm muscle.



Design of the optogenetic phrenic nerve neural implant. The Scheme on the left shows the normal anatomy of the thoracic cavity and the trajectory of the phrenic nerve as it projects to the diaphragm muscle. The scheme on the right depicts the neural implant that we plan to use in order to restore diaphragm function in ALS, after degeneration of the phrenic nucleus. NMJ: neuromuscular junction



The optoelectronic interface (Left, Centre) Dr. Degenaar's team has been developing optoelectronic stimulator arrays using microLED technology. These have been successfully used to stimulate neurons in both in-vitro culture and ex-vivo tissue (Right) We will develop a waveguide containment structure to prevent migration of the ESC-derived motor neurons and allow of even distribution of stimulation light.

TEAM

Ivo Lieberam (PhD in Genetics (Immunology) is since 2009 Lecturer, MRC Center for Developmental Neurobiology, King's College London, UK

Linda Greensmith (PhD in Neuroscience) is since 2009 Professor of Neuroscience, Institute of Neurology, UCL, UK

Patrick Degenaar (PhD Bioelectronics) is a senior lecturer in neuroprosthesis, School of Electrical, Electronic and Computer Engineering, Newcastle University, UK

BORG - Restoration of respiratory motor function through optogenetics neural implants

Call for projects 2010
Grant: 80 000 €
Project Duration: 18 months

Investigators: Ivo Lieberam, King's College London, United Kingdom

Updated results December 2012

Patients suffering from Amyotrophic Lateral Sclerosis (ALS) frequently show respiratory distress due to the degeneration of motor neurons that control breathing, and loss of respiratory function is the most common cause of death in ALS. The most crucial population of respiratory motor neurons in humans is the phrenic nucleus, which innervates the diaphragm, a key respiratory muscle. In our application for an 18-months pilot study supported by the Thierry Latran Foundation, we proposed to generate in vitro and in vivo proof-of-principle data for a new type of embryonic stem (ES) cell-based diaphragm pacemaker device. While restoration of normal breathing in ALS patients by artificially stimulating diaphragm contraction would not by itself represent a cure for the disease, such an intervention would dramatically increase the quality of life. In addition, once this technology is established, similar devices could be developed to restore other types of motor function, such as swallowing. The neural implants we propose will consist both of neural tissue directly derived from ES cells, namely motor neurons and glia, and of an electronic pacemaker device. The electronic component will generate a breathing rhythm and emit it as a sequence of light flashes, and the artificial neural components will receive the light signals, translate them into electrical impulses and relay the rhythm to the diaphragm muscle.

During the course of our pilot study, we: 1) Established a mouse ES cell line that carries several genetic modifications which allow us to i) magnetically enrich motor neurons from mixed in vitro cultures, ii) stimulate them with light though a molecular photosensor and iii) keep them alive in vitro and in vivo by expressing a neurotrophic factor. 2) Established a second mouse ES cell line which permits us to magnetically enrich astrocytes, the most abundant type of glia which supports motor neurons survival and maturation. 3) Implanted ES cell-derived motor neurons into a hindlimb nerve in mice to show that these cells survive in vivo for weeks after grafting and form connections to recipient muscle. The next two steps in this project will be to i) activate muscle with

light in vivo via the photosensitive ES cell-motor neuron graft and ii) introduce the same set of genetic tools into human stem cells to that we can produce artificial human neural tissue, which will be required for the adaptation of the technology to human patients.