Optogenetics: Breaching the Boundary of Biology's Black Box - "A Very Complicated Place"

John Snape

It contains an estimated 10^{11} individual cells, each being one of 10^4 different cell types and making 10^3 connections with other cells (1, 2). It weighs just 1.4 kg, and yet it is the site of every thought and feeling you've ever had (3); Professor Karl Deisseroth (Stanford University) says it is "wonderfully, and unfortunately, a very complicated place" (4). This structure is the human brain, and the cells are neurones. They maintain a 'resting potential' across the cell membrane, with the cell interior negatively charged compared to the exterior. Neurones transmit signals called 'action potentials' when protein channels in the cell membrane open and allow positively charged particles ('cations') to enter the cell, making the interior more positively charged and perturbing the resting potential. This occurs sequentially along the length of the cell, and is followed by the opening of another type of protein channel that allows a different cation out of the cell, making the exterior more positive to restore the resting potential (5).

It's not too outlandish to venture that the brain represents something of a "black box" in biology. Though we can observe its inputs and outputs, up to now studies of the internal workings of the living brain have been limited by an inability to directly generate action potentials in specific neurones. Though it is possible to surgically insert electrodes into the brain to electrically trigger action potentials, this procedure is invasive and stimulates every neurone around the electrode. Though it is also possible to chemically alter the brain using drugs, this is slow and even less precise than electrical stimulation. As early as 1979, the need for greater precision in neurological studies was highlighted by Francis Crick (Nobel laureate for the discovery of the DNA double helix) (6, 7).

Controlling Neurones with Light

The boundaries limiting investigation of the brain were pushed back significantly in 2005 (6) with the conception of 'optogenetics' – a wide variety of technologies that involve modifying a cell's genome to produce 'reporter proteins' for monitoring neural activity or 'actuator proteins' for influencing neural activity. Optogenetics is perhaps most associated with 'opsins' (8), channels in the membranes of cells that transport cations upon exposure to light (8). Opsins are used to mimic the action potential generation or inhibition as described above, by allowing cation flow into or out of neurones to modify

the resting potential.

How Optogenetics Works

Opsins are inserted into neurones via genes – DNA sequences that cells can use to create the opsin. The microbial gene is modified so that it has an additional DNA sequence at the start called a 'promoter', only useable by specific neurons in the brain. The modified gene is commonly incorporated into a virus – essentially a piece of DNA or RNA encased in protein – that infects and introduces the gene into the neurones. The virus most often used adeno-associated virus (AAV), which does not cause disease and enables fast integration of the gene into the DNA of the infected neurone (1, 9, 10). Each neurone exposed to the virus receives the modified gene, but only those neurones that recognise the promoter can use the gene to make opsins. Hence, a small number of neurones can be controlled – as few as two in 200,000 (11) – leaving all other neurones unaffected.

Upon receiving light, the light-absorbing component of the opsin transiently changes shape such that the channel opens and permits cation flow. A second type of opsin responding to a different colour of light can be inserted into the same cell, to inhibit action potential generation by permitting cation flow out of (or negatively charged 'anion' flow into) the cell (8).

In order to deliver light to neurones in a model organism, such as a mouse, a light-emitting optical fibre – as thin as a human hair and as light as 2 g – is inserted into the brain (4, 8).

Precision and Speed

Optogenetics allows the stimulation of a selected subset of neurones in milliseconds, providing extremely high precision without sacrificing speed of stimulation. Optogenetics has fewer side effects due to this precision, and the procedures for optogenetic modification are much less invasive than those for electrode insertion (1, 4). The great precision of optogenetics – its "exquisite cell specificity in the intact animal" – earned it the award of Method of the Year 2010 from *Nature Methods* (12).

However, the experimental use of opsins is associated with drawbacks. Being foreign proteins, opsins could induce non-physiologic states in cells by, for example, changing the properties of the cell membrane (13). Furthermore, the viruses used to insert the opsin genes into neurones can only hold a total of approximately 15 kb of added DNA (8). These issues are being addressed by developing non-viral methods of gene transfer; genes can be inserted into large

loops of bacterial DNA called 'plasmids' that move through a cell membrane made temporarily leaky with electrical pulses (14).

Potential Scientific Applications

The use of optogenetics in scientific investigation is already bearing fruit. Professor Gero Miesenböck (Oxford University) has used optogenetics to validate a model of neural feedback; in this model 'actor' neurones give rise to a behaviour that is evaluated by both environmental feedback and by 'critic' neurones that fire when a particular behaviour is unfavourable. By placing an optogenetically-modified fly into a small chamber containing two partitioned odours, and stimulating the critic neurones each time the fly enters one of those odours, the investigator is able to make the fly remain in the region containing the other odour. By adding promoters to the opsin genes that different cells recognise, Miesenböck was able to narrow down the identity of the critic neurones to just 12 cells (11).

These experiments hint at a tantalising model in which intelligent behaviour may arise from physical interactions between cells – a model that could explain more complex phenomena such as personality and memory. This is yet another aspect of the boundary-shattering potential of optogenetics – breaking down barriers not only to research, but also between traditionally separate scientific disciplines like biology and physics.

Potential Clinical Applications

The rise of optogenetics has consequences beyond basic science alone; the boundaries of clinical medicine stand to be redrawn by the manifold uses of this powerful technology.
It is estimated that some 26% of Americans aged over 18 experience a psychiatric disorder in a given year (15). An example is posttraumatic stress disorder (PTSD) – an anxiety disorder characterised by re-experiencing (or avoiding stimuli associated with) an intensely traumatic event, causing hyperalertness and insomnia (16). PTSD can be treated using drugs like sertraline hydrochloride that chemically interact with the brain, but these are often associated with side effects such as headaches and nausea (17). A different approach was taken by the laboratory of Professor Edward Boyden (MIT), where mice were conditioned to display a fear response to a sharp audible tone associated with a brief (painless) shock. After the tone is associated with fear, just 10 minutes of repeated optogenetic stimulation of specific neurones at the same time as the tone is heard appears to override the fear response – Boyden suggests that this could one day be used as a treatment for PTSD (1).

Regulatory and Philosophical Issues

Often the removal of boundaries to scientific progress is concomitant with the identification of safety and ethical boundaries that must be considered. A European Medicine Agency reflection paper on AAV gene therapy (which could encompass the proposed optogenetic PTSD treatment above) lists a variety of concerns including the potential for unwanted transmission of the genes to future generations via inheritance and to other organisms via saliva and urine (18).

Moreover, the light inputs used to control neurones in optogenetics can be represented in binary code (e.g. '1' for on or '0' for off), which might one day be downloaded from or uploaded to the brain. Complex and personal emotions could be represented by a string of numbers, challenging the way concepts like love and cognition are defined and placing great demands on regulatory policy to specify how such data are protected and used.

Future Directions

Neural processes are not the only pathways that can be controlled using optogenetics. In 2009 Deisseroth replaced components of an opsin with those of a cellular signalling molecule, so that the light-sensitivity of the opsin could be used to trigger the biochemical pathway associated with the signalling molecule (10). A process already modified in this way is the movement of proteins within cells (12).

Opsins are now being developed that respond to infrared light or ultrasound frequencies, which are able to penetrate tissues deeper than visible light and could eliminate the need for implantation of optical fibres altogether (8, 10).

The optical fibre is also undergoing modification. Boyden has proposed the development of 3D arrays of optical fibres, each entering the brain to a different depth, to allow a form of 'high-throughput screening' of behaviours induced by stimulating different combinations of spatially separated neurons (1).

With the unveiling of the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) initiative in the US, putting $100 million towards uncovering new ways of treating, preventing and curing brain diseases, there are definite goals to be achieved by emerging technologies like optogenetics (19).

The Rise of Optogenetics

Optogenetics serves as a compelling example of new technological advancement removing longstanding boundaries to scientific progress. Its swift adoption by the scientific community is testament to the fulfilment of a previously unmet need. Optogenetics could radically change the way the brain is investigated and its disorders are treated, but it also presents profound scientific and ethical issues yet to be resolved. Clearly the intricacy of the boundaries surrounding studies of the brain will be reflected in the technology needed to cross them.

References

Boyden, E. S. (2011) A light switch for neurons (TEDTalks). http://www.youtube.com/watch?v=hupHAPF1fHY.

Marois, R., and Ivanoff, J. (2005) Capacity limits of information processing in the brain, *Trends Cogn. Sci. (Regul. Ed.) 9*, 296–305.

National Geographic, "Brain". http://science.nationalgeographic.co.uk/science/health-and-human-body/human-body/brain-article/.

Deisseroth, K. (2009) Controlling the Brain with Light (Stanford University). http://www.youtube.com/watch?v=C8bPbHuOZXg.

(2004) Action Potential, *The Concise Corsini Encyclopedia of Psychology and Behavioral Science*, Wiley, Hoboken, NJ, USA. Retrieved from http://www.credoreference.com/entry/wileypsych/action_potential.

Deisseroth, K. (2011) Optogenetics, *Nat Meth 8*, 26–29.

The Nobel Prize in Physiology or Medicine 1962, *Nobelprize.org*. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1962/.

Dugué, G. P., Akemann, W., and Knöpfel, T. (2012) A comprehensive concept of optogenetics, *Prog. Brain Res. 196*, 1–28.

Büning, H., Perabo, L., Coutelle, O., Quadt-Humme, S., and Hallek, M. (2008) Recent developments in adeno-associated virus vector technology, *J. Gene Med. 10*, 717–733.

Mei, Y., and Zhang, F. (2012) Molecular tools and approaches for optogenetics, *Biol. Psychiatry 71*, 1033–1038.

Miesenböck, G. (2010) Gero Miesenboeck reengineers a brain (TEDTalks). http://www.youtube.com/watch?v=EZ_f3Fc0ZRA.

(2011) Method of the Year 2010, *Nat. Meth. 8*, 1–1.

Knöpfel, T., Lin, M. Z., Levskaya, A., Tian, L., Lin, J. Y., and Boyden, E. S. (2010) Toward the second generation of optogenetic tools, *J. Neurosci. 30*, 14998–15004.

Tsong, T. Y. (1991) Electroporation of cell membranes, *Biophys. J. 60*, 297–306.

National Institute of Mental Health (NIMH) (2005) The Numbers Count: Mental Disorders in America. http://www.nimh.nih.gov/health/publications/the-numbers-count-mental-disorders-in-america/index.shtml.

(2011) Posttraumatic stress disorder, *Dorland's Illustrated Medical Dictionary*, Elsevier Health Sciences, Philadelphia, PA, USA. Retrieved from http://www.credoreference.com/entry/ehsdorland/posttraumatic_stress_disorder.

Electronic Medicines Compendium (eMC) (2012, October 19) SPC: Lustral 50mg film coated tablets (Pfizer Limited). http://www.medicines.org.uk/EMC/medicine/1467/SPC/Lustral+50mg+film+coated+tablets/.

Committee for Medicinal Products for Human Use (CHMP), European Medicines Agency (EMA) (2010, June 24) Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/07/WC500094345.pdf.

Grogan, K. (2013, April 3) Obama launches $100 million project to map brain, *PharmaTimes Online*. http://www.pharmatimes.com/Article/13-04-03/Obama_launches_100_million_project_to_map_brain.aspx.