# Pearson Edexcel International Advanced Level

# Biology

## Advanced

## Unit 5: Energy, Exercise and Coordination

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**Scientific Article for use with Question 7**

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**Scientific article for use with Question 7**

## Optogenetics

1. Despite the enormous efforts of clinicians and researchers, our limited insight into psychiatric disease (the worldwide-leading cause of years of life lost to death or disability) hinders the search for cures and contributes to stigmatization. Clearly, we need new answers in psychiatry. But as philosopher of science Karl Popper might have said, before we can find the answers, we need the power to ask new questions. In other words, we need new technology.

2. Developing appropriate techniques is difficult, however, because the mammalian brain is beyond compare in its complexity. It is an intricate system in which tens of billions of intertwined neurons – with multitudinous distinct characteristics and wiring patterns – compute with precisely timed, millisecond-scale electrical signals, as well as with a rich diversity of biochemical messengers. Because of that complexity, neuroscientists lack a deep grasp of what the brain is really doing – of how specific activity patterns within specific brain cells ultimately give rise to thoughts, feelings and memories. By extension, we also do not know how the brain's physical failures produce distinct psychiatric disorders such as depression or schizophrenia. The ruling paradigm of psychiatric disorders – casting them in terms of chemical imbalances and altered levels of neurotransmitters – does not do justice to the brain's high-speed electrical neural circuitry. And psychiatric treatments have historically been largely serendipitous: helpful for many but rarely illuminating, and suffering from the same challenges as basic neuroscience.

3. In a 1979 *Scientific American* article Nobel laureate Francis Crick suggested that the major challenge facing neuroscience was the need to control one type of cell in the brain while leaving others unaltered. Electrical stimuli cannot meet this challenge because electrodes are too crude a tool: they stimulate all the circuitry at their insertion site without distinguishing between different cell types, and their signals cannot turn off neurons with precision. Drugs are not specific enough either, and they are much slower than the natural operating speed of the brain. Crick later speculated in lectures that light might have the properties to serve as a control tool because it could be delivered in precisely timed pulses, but at the time no one had a strategy to make specific cells responsive to light.

4. Meanwhile, in a realm of biology as distant from the study of the mammalian brain as might seem possible, researchers were working on microorganisms that would only much later turn out to be relevant. At least 40 years ago biologists knew that some microorganisms produce proteins that directly regulate the flow of electric charge across cell membranes in response to visible light. These proteins, which are produced by a characteristic set of "opsin" genes, help to extract energy and information from the light in the microbes' environments. In 1971 Walther Stoeckenius and Dieter Oesterhelt, both then at the University of California, San Francisco, discovered that one of these proteins, bacteriorhodopsin, acts as a single-component ion pump that can be briefly activated by photons of green light – a remarkable all-in-one molecular machine. Later identification of other members of this family of proteins – the halorhodopsins in 1977 and the channelrhodopsins in 2002 – continued this original theme from 1971 of single-gene, all-in-one control.

5. In 20/20 hindsight, the solution to Crick's challenge – a potential strategy to dramatically advance brain research – was latent in the scientific literature even before he articulated the challenge. Yet it took more than 30 years, until the summer of 2005, for these fields to come together in a new technology (optogenetics) based on microbial opsin genes.

6. Optogenetics is the combination of genetics and optics to control well-defined events within specific cells of living tissue. It includes the discovery and insertion into cells of genes that confer light responsiveness; it also includes the associated technologies for delivering light deep into organisms as complex as freely moving mammals, for targeting light-sensitivity to cells of interest, and for assessing specific readouts, or effects, of this optical control.

7. What excites neuroscientists about optogenetics is control over defined events within defined cell types at defined times – a level of precision that is most likely crucial to biological understanding even beyond neuroscience. The significance of any event in a cell has full meaning only in the context of the other events occurring around it in the rest of the tissue, the whole organism or even the larger environment. Even a shift of a few milliseconds in the timing of a neuron's firing, for example, can sometimes completely reverse the effect of its signal on the rest of the nervous system. And millisecond-scale timing precision within behaving mammals has been essential for key insights into both normal brain function and into clinical problems such as parkinsonism.

**Optogenetics, medicine and psychiatry**

8. Work from the World Health Organization has shown that psychiatric disease is the leading source of disability worldwide in terms of years of life lost to death or disability. Even a single psychiatric disease, major depression, is the leading cause of disability worldwide in women aged 15 to 44. But much stigma remains (which may relate to why hearing about this epidemiology is so surprising to many people). Why the stigma? A major reason is our collective lack of understanding. Just as a cancer diagnosis once carried more stigma than it does now (perhaps because of confusion over what cancer really "is," over concerns for contagion or even over blame for the cancer on personality features of the patient), so too does lack of insight into psychiatric disease contribute to stigmatization, further slowing progress in this enormous problem for global human health. This lack of insight, sadly, is universal: throughout the global community, from members of the general public to the most influential and advanced psychiatrists, we don't know what psychiatric disease "is" at a fundamental level.

9. As one example: What is depression? Unlike the case with heart failure, for example, we don't have good models for what organ dysfunction depression represents. The heart is a pump, and its dysfunction (to a first-order approximation) relates to its pumping, which can be readily understood, measured, modeled and tuned. But we lack deep understanding of what the brain is really doing, which of course means that we don't understand its failure modes.

10. I come face-to-face with this challenge continually. In addition to running a research laboratory in a bioengineering department, I am also a practicing psychiatrist, and I treat patients regularly using combinations of medication, therapy, and electrical or magnetic brain stimulation. After my undergraduate years at Harvard University, I had obtained my MD and PhD degrees at Stanford University, focusing on synaptic electrophysiology and optical studies of mammalian neural circuitry. I then completed my psychiatry residency and postdoctoral fellowships at Stanford, where I developed as a physician and developed skills in the study of animal behavior. Although as a physician I employ modern tools (such as transcranial magnetic stimulation), these tools are still not good enough and, most important, do not provide deep insight into the diseases, only highlighting (as do the patients) our limitations. I remember a brilliant young college student suffering from psychotic depression and terrified by the incomprehensible voices and uncontrollable bizarre ideas in his mind. I remember a retired woman so severely depressed that she was unable to smile, barely able to eat and unresponsive to her grandchildren. My inability to explain these changes in a scientific way and the unfortunately failed responses to treatments these patients experienced have never left my mind.

11. As a principal investigator and psychiatrist at Stanford in 2004 (and supported by a new grant from the National Institute of Mental Health), I was able to put together and launch a research team to address the technological challenge of precise neural control. And as so often happens in science, our collective need for new ideas has helped drive the development of new technology. Being asked to reflect on our optogenetics work here also provides an opportunity to consider broader implications of the scientific process.

**Casting light on life**

12. Biology has a tradition of using light to intervene in living systems. Researchers have long employed a light-based method called CALI to destroy, and thus inhibit, selected proteins; lasers have also been used to destroy specific cells, for example, in the worm *Caenorhabditis elegans*. Conversely, Richard L. Fork of Bell Laboratories (in the 1970s) and Rafael Yuste of Columbia University (in 2002) reported ways to stimulate neurons with lasers that partially disrupted cell membranes. More recently, the laboratories of Gero Miesenböck, then at Memorial Sloan-Kettering Cancer Center, and of Ehud Isacoff, Richard H. Kramer and Dirk Trauner, then all at the University of California, Berkeley, employed multicomponent systems for modulating targeted cells with light. They introduced, for example, both a protein that regulates neurons and a chemical that would spur the protein into action when triggered by ultraviolet light.

13. Yet destroying proteins or cells of interest obviously limits one's experimental options; and methods that depend on multiple components, although elegant and useful, entail practical challenges and have not experienced broad applicability or utility in mammals. A fundamental strategic shift to a single-component strategy was necessary. As it turned out, this single-component strategy was not able to build on any of the parts or methods from earlier approaches, but instead employed the remarkable all-in-one light-activated proteins from microbes: proteins now called bacteriorhodopsins, halorhodopsins and channelrhodopsins.

14. Well after bacteriorhodopsin and halorhodopsin had become known to science, in 2000 the Kazusa DNA Research Institute in Japan posted online thousands of new gene sequences from the green algae *Chlamydomonas reinhardtii*. While reviewing them, Peter Hegemann, then at the University of Regensburg in Germany, who had predicted that *Chlamydomonas* would have a light-activated ion channel, noticed two long sequences similar to those for bacteriorhodopsin. He obtained copies of them from Kazusa and asked Georg Nagel (then a principal investigator in Frankfurt) to test if they indeed coded for ion channels. In 2002 Hegemann and Nagel described their finding that one of these sequences encoded a single-protein membrane channel responsive to blue light: when hit by blue photons, it regulated the flow of positively charged ions. The protein was consequently dubbed channelrhodopsin-1, or ChR1. The following year Nagel and Hegemann (along with their colleagues, including Ernst Bamberg in Frankfurt) explored the other sequence and named the encoded protein "channelrhodopsin-2," or ChR2. Almost simultaneously, John L. Spudich in Houston provided evidence that those genes were important to the light-dependent responses of *Chlamydomonas*. But these channelrhodopsins – a third type of single-component light-activated ion-conductance protein – did not immediately translate into an advance in neuroscience any more than the discoveries of bacteriorhodopsins and halorhodopsins in previous decades had. Several years passed uneventfully after 2002, as they had since 1971.

15. A number of scientists have confided to me that they had considered inserting bacterial or algal opsin genes into neurons and trying to control the altered cells with light but had abandoned the idea. Indeed, anything is possible in biology, but what can actually be made to work is another story indeed. With challenges in funding, the need for low-risk projects to support trainee careers, and other issues there is a very high threshold for risk-taking in modern academic science. Animal cells were unlikely to manufacture these microbial membrane proteins efficiently or safely, and the proteins were virtually certain to be too slow and weak to be effective. Furthermore, to function, the proteins would require an additional cofactor – a vitamin A-related compound called all-*trans* retinal to absorb the photons. The risk of wasting time and money was far too great.

16. Nevertheless, for the bioengineering research team I had assembled at Stanford University, the motivation to improve our understanding of the brain in psychiatric disease states was more than enough to justify the extremely high risk of failure. As a principal investigator at Stanford beginning in 2004, I formed a team that included the extraordinarily talented graduate students Edward Boyden and Feng Zhang (both now assistant professors at the Massachusetts Institute of Technology) to address this challenge. I introduced channelrhodopsin-2 into mammalian neurons in culture by the well-established techniques of transfection – that is, by splicing the gene for ChR2 and a specific kind of on switch, or promoter, into a vector (like a benign virus) that ferried the added genetic material into the cells. Promoters can ensure that only selected kinds of neurons (such as those able to secrete the neurotransmitter glutamate) will express, or make, the encoded proteins.

17. Against all odds, the experiments worked shockingly well. Using nothing more than safe pulses of visible light, we attained reliable, millisecond-precision control over the cells' patterns of firing of action potentials – the voltage blips, or impulses, that enable one neuron to convey information to another. In August 2005 my team published the first report that by introducing a microbial opsin gene, we could make neurons precisely responsive to light. Channelrhodopsins (and, eventually as we found, the bacteriorhodopsin from 1971 and the halorhodopsins, too) all proved able to turn neurons on or off, efficiently and safely in response to light. They worked in part because mammalian tissues contain naturally robust quantities of all-trans retinal – the one chemical cofactor essential for photons to activate microbial opsins – so nothing beyond an opsin gene needs to be added to targeted neurons. Microbial opsin genes provided the long-sought single-component strategy.

**Improving on nature**

18. The number of optogenetic tools, along with the diversity of their capabilities, has since expanded rapidly because of a remarkable convergence of ecology and engineering. Investigators are adding new opsins to their tool kits by scouring the natural world for novel ones; they are also applying molecular engineering to tweak the known opsins to make them even more useful for diverse experiments in a wider range of organisms.

19. In 2008, for instance, our genome searches led by Feng Zhang on a different algal species, *Volvox carteri*, revealed a third channelrhodopsin (VChR1), which responds to yellow light instead of blue as we showed together with Peter Hegemann. Using VChR1 and the other channelrhodopsins together, we can simultaneously control mixed populations of cells, with yellow light exerting one type of control over some of them and blue light sending a different command to others. And we now have found that the most potent channelrhodopsin of all is actually a hybrid of VChR1 and ChR1 (with no contribution from ChR2 at all). Our other modified opsins (created with Ofer Yizhar, Lief Fenno, Lisa Gunaydin and Hegemann and his students) now include "fast" and "slow" channelrhodopsin mutants that offer exquisite control over the timing and duration of action potentials: the former can drive action potentials more than 200 times per second, whereas the latter can push cells into or out of stable excitable states with single pulses of light. Our newest opsins can also now respond to deep red light that borders on the infrared, which stays more sharply focused, penetrates tissues more easily and is very well tolerated by subjects. Many groups are now also pushing opsin engineering forward, including those of Hiromu Yawo in Japan, Ernst Bamberg in Frankfurt and Roger Tsien in San Diego.

20. Many of the natural opsin genes now being discovered in various non-animal genomes encode proteins that mammalian cells do not make well. But Viviana Gradinaru in my group has developed a number of general-purpose strategies for improving their delivery and expression. For example, pieces of "trafficking" DNA can be bundled with the opsin genes to act as "zip codes" to ensure the genes are transported to the correct compartments within mammalian cells and translated properly into functional proteins. This generalizable approach has served to unlock the broad ecological repertoire of microbial opsin genes.

21. Molecular engineering has also extended optogenetic control beyond cells' electrical behaviors, to well-defined biochemical events. A large fraction of all approved medical drugs act on a family of membrane proteins called G-protein coupled receptors. These proteins sense extracellular signaling chemicals, such as epinephrine, and respond by changing the levels of intracellular biochemical signals, such as calcium ions, and thus the activity of the cells. By adding the light-sensing domain from a rhodopsin molecule to G-protein coupled receptors, early in 2009 Raag Airan and others in my laboratory published a set of receptors called optoXRs that respond rapidly to green light. When viruses are used to insert the single-component optoXR genes into the brains of lab rodents, the first cell type – specific fast optical control over defined biochemical pathways was enabled, working even in freely moving mammals. Optical control over defined biochemical events is now also being explored in many laboratories, and opens the door to optogenetics in essentially every cell and tissue in biology.

22. With fiber-optic tools we developed and published in 2006 and 2007, investigators can now deliver light for optogenetic control to any area of the brain – whether surface or deep – in freely moving mammals. And to enable simultaneous readouts of the dynamic electrical signals elicited by optogenetic control, we also have published millisecond-scale instruments that are integrated hybrids of fiber optics and electrodes ("optrodes"). A long-sought synergy can emerge between optical stimulation and electrical recording because the two can be set up to not interfere with each other. We can now, for instance, directly observe the changing electrical activity in the neural circuits involved in motor control at the same time that we are optically controlling those circuits with microbial opsins. The more rich and complex that both our optogenetic inputs and the electrical-output measures of neural circuits become, the more powerfully we can infer the computational and informational roles of neural circuits from how they transform our signals.

**The round-trip back to psychiatry**

23. The importance of optogenetics as a research tool, particularly in conjunction with other technologies, continues to grow rapidly. In addition to distributing these diverse engineered opsin genes to more than 700 laboratories worldwide (http://www.optogenetics.org), my students have worked hard over the past few years to develop and deliver optogenetics instruction. We have found that despite the unusual combination of technologies required for optogenetics, the fundamentals can be taught in focused hands-on courses in the laboratory, which accelerate the benefits of the technology. Scientists from all over the world come to practice optogenetics and return to their home institutions, where they serve as local sources of knowledge and wisdom, resulting in application to diverse settings and challenges across the globe.

24. One example of an unexpected class of application involves brain imaging. In recent years, neuroscience has made many advances based on the brain-scanning technique called functional magnetic resonance imaging (fMRI). These scans are usually billed as providing detailed maps of neural activity in response to various stimuli. Yet strictly speaking, fMRI shows only changes in blood-oxygen levels in different areas of the brain; those changes are just a proxy for actual neural activity. Some nagging uncertainty has therefore always surrounded the question of whether these complex fMRI signals can be triggered by increases in local excitatory neural activity. This past May, however, my laboratory used a combination of optogenetics and fMRI (dubbed ofMRI) to verify that the firing of local excitatory neurons is fully sufficient to trigger the complex signals detected by fMRI scanners. In addition, ofMRI can map working neural circuits with an exactness and completeness not previously possible with electrodes or drugs. Optogenetics is thereby helping to validate and advance a wealth of scientific literature in neuroscience and psychiatry.

25. Optogenetics has also been employed to control a kind of neuron (the hypocretin cells) thought to be involved in the sleep disorder narcolepsy, in the first application of optogenetics to a freely moving mammal. Specific types of electrical activity in those neurons, we have found, set off the complex transition of awakening. Optogenetics has also been employed to help determine how dopamine-making neurons may give rise to feelings of reward and pleasure. In this work with Hsing-chen Tsai, Feng Zhang, Antonello Bonci, Garrett Stuber and Luis de Lecea, we optogenetically drove well-defined dopamine neurons in the mouse in different temporal patterns during free behavior, and found parameters that were sufficient to drive reinforced behavior (for example, in the absence of any other cue or reward, healthy animals simply chose to spend more time in places where they had received particular kinds of optogenetic bursting activity in dopamine neurons). This work is relevant to hedonic (pleasure-related) pathologies involved in depression (as in my depressed patient who could no longer even enjoy seeing her grandchildren, otherwise one of the most rewarding experiences known to humankind) and in substance abuse, as well as in healthy reward processes.

26. The optogenetic approach has also improved our understanding of Parkinson's disease, which involves a disturbance of information processing in certain motor-control circuits of the brain. Since the 1990s some Parkinson's patients have received relief via a therapy called deep-brain stimulation, in which an implanted device similar to a pacemaker applies carefully timed, oscillating electric stimuli to certain areas far inside the brain, such as the subthalamic nucleus. Yet the promise of this technique for Parkinson's (and indeed for a variety of other conditions) is partially limited because electrodes stimulate nearby brain cells unselectively and medical understanding of what stimuli to apply is woefully incomplete. Recently, however, we have used optogenetics to study animal models of Parkinson's and gained fundamental insight into the nature of the diseased circuitry and the mechanisms of action of therapeutic interventions. For example, we have found that deep-brain stimulation may be most effective when it targets not cells but rather the connections between cells – affecting the flow of activity between brain regions. And we have worked with Anatol Kreitzer of U.C.S.F. who has functionally mapped two pathways in brain movement circuitry: one that slows movements and one that speeds them up and can counteract the parkinsonian state.

**Recent developments**

27. Stimulating the brain using flashes of light might pave the way for a novel treatment for depression.

28. Herbert Covington at Duke University in Durham, North Carolina, and colleagues have reversed the effects of stress-induced depression in mice using optogenetics – a technique in which light is used to stimulate in the brains of genetically modified mice.

29. The team used *herpes simplex* virus to ferry the light-sensitive protein channel rhodopsin 2 (ChR2) into neurons in the medial prefrontal cortices of depressed mice: the mPFC is thought to orchestrate decision-making and social behaviour.

    Next they shone blue lasers on the mice mPFC in 40-millisecond bursts every three seconds for five minutes, stimulating the neurons in this area. After treatment the mice no longer showed signs of depression, with restored levels of social interaction and ability to experience pleasure, as measured by a 15 per cent increase in their preference for drinking sugar water over plain water.

30. The team also discovered similar signatures of depression between the mice and humans. Examining the post-mortem brains of 20 people, Covington and colleagues found less messenger RNA for two genes present in the mPFCs of depressed people, indicating neurons fired less often there when they were alive. They found a similar result in depressed mice, suggesting that depression leaves similar footprints in mice and humans.

31. The results are the first demonstration that optogenetics can "drive an antidepressant effect", Covington says, but just as important is the improved understanding of the neurobiology of depression. While scientists knew that mPFC was important in depression, the causality was unclear. Now, there are signs that a change in the prefrontal cortex may influence a person's mental state, says Covington. The next step will be to understand how mPFC interacts with other brain regions to create or respond to depression, he adds.

32. That researchers are using an optogenetic method to study depression "is fantastic progress", says Helen Mayberg, a neuroscientist and clinical psychiatrist at Emory University in Atlanta, Georgia. "Nobody was looking at this region (in animal studies) despite constant begging for ten years," she says.

33. Pulses of light might one day restore normal muscle activity in people with cerebral palsy or paralysed limbs. That's the hope of researchers now using the technique to control the leg muscles of mice.

34. The work is part of a growing field called optogenetics, and used light-activated proteins from photosynthetic algae to switch nerve cells on and off. The latest study is the first to apply the technique to the peripheral nervous system, which controls voluntary movements.

35. Karl Deisseroth of Stanford University, and colleagues, inserted into mice the gene which codes for the algal protein ChR2, which caused the protein to attach itself to the surface of nerve cells. After anaesthetising each mouse, they optically stimulated its sciatic nerve – which runs from the lower back to the lower limb – using a cuff lined with light-emitting diodes. They measured the resulting contractions in the Achilles tendon.

36. Stimulating muscles with electrical impulses has allowed paralysed people to walk. But electrical signals activate large, fast-twitch nerve fibres before small slow-twitch ones – the opposite of what happens naturally. This makes people walk with jerky, robotic movements which quickly become exhausting.

37. In contrast, the light pulses reproduced the "natural firing order" of nerves in the mice, says team-member Scott Delp, also at Stanford. The team is hopeful that the technique could work in humans to restore movement to paralysed limbs, or counter the muscle spasticity characteristic of cerebral palsy.

38. Last year saw a new twist on optogenetics: magnetogenetics, or using a magnetic field to trigger the firing of genetically engineered neurons.

    Arnd Pralle's group at the State University of New York at Buffalo engineered neurons from the nematode, worm, *Caenorhabditis elegans*, to manufacture an ion channel that triggers electrical firing following a small rise in temperature. The neurons are heated with iron nanoparticles, which gently warm up in a magnetic field.

39. To test their idea, the team used the worm's instinctive reaction to back away from a heat source. They made the worm's sensory neurons in the tip of its "head" manufacture the ion channel, and chemically altered the nanoparticles so they became concentrated in the mucus protecting the worm's head.

40. When a magnetic field was turned on, the nanoparticles slowly warmed up, thus triggering electrical impulses in the neurons. "Once we switch on the field, most of the worms reverse course," says Pralle. "We could make them go back and forth." The technique could be used on larger animals by injecting the nanoparticles into the blood, says Pralle, as they are small enough to diffuse into the brain. The team is now testing the technique in genetically modified mice.

41. The advantage of magnetogenetics over optogenetics is that unlike visible light, a magnetic field can pass through the skull, removing the need to implant optical fibres in the brain. "This will allow us to penetrate much deeper into tissue," says Pralle.

42. On the other hand, optogenetics gives finer control over neuronal firing. The light source can be turned on and off almost instantaneously, while nanoparticles take several seconds to warm up. Pralle, however, predicts the process will get faster as the technique is honed.

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