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Optogenetics and its Applications in Psychology: Manipulating the Brain Using Light

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abstract

In a broad sense, optogenetics uses genetically addressable photosensitive tools to monitor and control activity of living cells and tissue. This paper focuses on causal manipulation of neural populations by delivering light to light-sensitive ion channels or other proteins called microbial opsins. This enables refined manipulation of specific types or compartments of neurons with millisecond precision, whereas traditional electrical brain stimulation affects all neurons in a given area. Additionally, intracellular pathways can be studied using opto-XRs which could aid psychopharmacological research. Recent studies have applied optogenetics to psychology, leading to new experiments and yielding interesting results. Thus, this paper attempts to make optogenetics accessible to psychologists to enrich existing psychological research methods.

Keywords: Electrical brain stimulation, microbial opsins, optogenetics.

For many years humans have tried to understand their psyche and behavior, which both seem to stem from our brains. In a quest to unravel how the brain works, scientists have designed diverse experiments and observed many phenomena. These have ranged from electrically stimulating the squid giant axon to understand the electrical properties of neurons (Hodgkin & Huxley, 1952), to studying the memory of a patient with amnesia due to hippocampal lesions (Corkin, 1984). However, the mechanisms by which the brain produces behavior and the psyche are still poorly understood, since the nervous system is one of the most complex pieces of machinery known. In 2005, Boyden, Zhang, Bamberg, Nagel nad Deisseroth successfully used a new method to study the mammalian brain. This new method, called optogenetics, uses genetically addressable photosensitive tools, i.e. tools that are light sensitive (Dugué, Akemann, & Knöpfel, 2012). These tools can be used to both monitor and manipulate the cellular and molecular activity of living cells and tissues. More specifically, Boyden et al. (2005) used light to manipulate the activity of neurons. Over the years optogenetics has proven to be an excellent tool for testing a range of hypotheses which previous methods could not address. This paper will explain the mechanisms underlying optogenetics and will attempt to demonstrate why optogenetics is useful for psychological research.

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Neurons

To understand how the brain works it is important to begin with its fundamental building blocks - neurons (Figure 1). Neurons are complex pieces of electrochemical machinery (Purves et al., 2012). Like most other cells, neurons have cell bodies which contain organelles and a cell nucleus. However, neurons also possess other structures such as dendrites, which look like branches of a tree and are generally used to receive signals from other neurons. Signals can be sent using the axon, a relatively long projection. When a signal (also known as an action potential) is propagated through the axon, it eventually reaches an axon terminal; an extremity of the neuron usually connecting to dendrites of other neurons. However, the axon terminals and dendrites are not physically connected. A small gap called the synapse exists between the two neurons. The neuron sending the signal into the synapse known as the presynaptic neuron, whereas the receiving neuron is called the postsynaptic neuron.

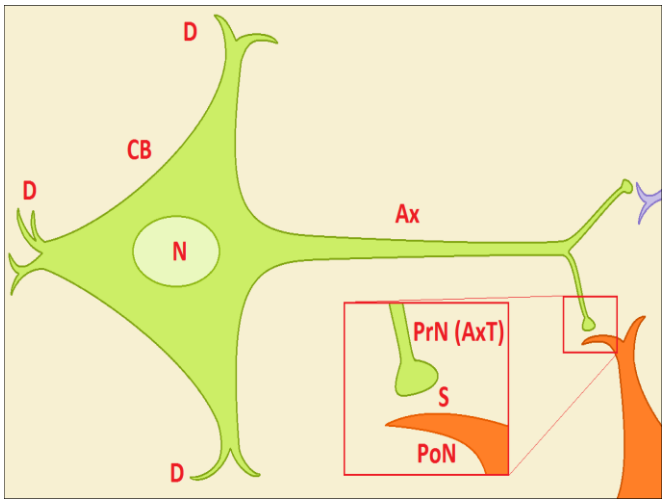

Figure 1. A schematic neuron. CB = cell body, D = dendrites, N = nucleus, Ax = axon, PrN = presynaptic neuron, AxT = axon terminal, S = synapse, PoN = postsynaptic neuron.

Electrochemical signaling between several billions of neurons determines how we think and behave (Purves et al., 2012). To do this, neurons communicate with each other by using their electrical and chemical properties. A neuron differs in electrical potential from its environment with a potential difference of approximately -70 mV. This difference is also referred to as the membrane potential. If the membrane potential near the beginning of the axon depolarizes (i.e. goes up) to a threshold of approximately -55 mV, sodium channels in the membrane of the beginning of the axon open allowing an influx of positively charged sodium ions. Within a millisecond the membrane potential increases to a positive value. The depolarizing current generated by the movement of the positive charges propagates through the axon, causing distant sodium channels to reach their threshold potential as well. This chain of depolarization known as an action potential, travels along the axon eventually reaching the axon terminal. In response, the terminal releases neurotransmitters into the synapse. The postsynaptic neuron has receptors on its membrane, which bind to receptors on the cell membrane of the postsynaptic neuron.

Two important types of postsynaptic receptors exist: ionotrophic receptors and metabotrophic receptors (Stahl, 2008). Ionotrophic receptors can depolarize (e.g. glutamate receptors) or hyperpolarize (e.g. GABA receptors) the postsynaptic neuron. Depolarization can induce action potentials activating subsequent neurons in a network, whereas hyperpolarization lowers the membrane potential making it harder to reach the threshold for action potentials. Metabotrophic receptors activate intracellular signal pathways, to transcription of genes, activation of enzymes, strengthening of the synapse, and many other important functions. They can also mediate depolarization and hyperpolarization by modulating channels through intracellular second messenger cascades.

Electrical Brain Stimulation and Pharmacology

Causal manipulation of the brain can be used to understand how the brain works. Two techniques have been used abundantly in neuroscience: electrical brain stimulation (EBS) and neuropharmacology.

Electrical Brain Stimulation

EBS exploits the electrical properties of neurons (Pinel, 2009). After opening the skull an electrode is placed against the neural tissue. By discharging an electrical current, the neural tissue changes its behavior and depending on the properties of the current, neurons will either depolarize or hyperpolarize. For example, stimulating the area of the motor cortex responsible for left hand motor control can cause jerk movements in the corresponding hand. Another reason for the success of EBS is the temporal precision of mere milliseconds, thus allowing stimulation of neurons for precise periods of time.

For more than a century, electrical stimulation has been used to study both fundamental and clinical aspects of the nervous system. Bartholow (1874) made the first report of placing an electrode against a brain *in vivo*. Stimulating the parietal cortex caused the kind of motor behavior as described above. Ever since, EBS has significantly aided our understanding of the brain. For example the motor cortex and its topographical layout (Penfield & Boldrey, 1937), the functioning and topographical structure of the visual cortex (Brindley & Lewin, 1968), conscious perception of sensations in the somatosensory cortex (Libet et al., 1964), and many other topics. In short, EBS has fundamentally changed our understanding of the brain.

Moreover, EBS is also applied as a treatment. Electroconvulsive therapy has been used for several decades and consists of placing electrodes against the scalp to induce seizures which in turn alleviate symptoms of clinical depression (Rudorfer, Henry & Sackeim, 2003). More recently, deep brain stimulation (DBS) has gained popularity as a treatment for the motor symptoms of Parkinson's disease, dystonia and clinical depression (Perlmutter & Mink, 2006). In DBS, an electrode is permanently implanted inside the brain. The electrode is connected to a neuropacemaker, similar to a pacemaker for the heart, allowing the patient to live a normal life while the electrode stimulates the brain. Although more research is needed, DBS is rapidly gaining interest from both clinicians and scientists.

However, EBS also has limitations. First, it is non-specific for the type of neuron. Since all neurons have electrical properties, the neurons within a given area will react to the electrode. This property hinders the targeted stimulation of specific types of neurons, for example dopaminergic but not serotonergic neurons. Consequently, brain areas with diffuse types of neurons are impractical to study with EBS. Second, EBS cannot differentiate between different neuron structures. Neurons have long axons that will pass through many regions. This means that any given area consists of neurons residing in that area, but also axons of distant neurons that do not necessarily share the same function as the neurons in that area. If a region is stimulated electrically, both the neurons residing in that region and the axons simply passing through that region will be activated. This makes it hard to ascribe the outcome of the stimulation solely to the stimulated area, as the outcome may have been influenced by distal neurons whose axons were stimulated.

Neuropharmacology

Neuropharmacology studies how drugs affect the nervous system. Since the nervous system in itself relies on chemicals such as neurotransmitters, pharmacology has significantly expanded the fields of neuroscience and psychology (Stahl, 2008). The main advantage of this approach is that it allows cell-type specific manipulation. Some drugs are specifically involved in GABA systems, whereas others affect only serotonin systems. In addition, the systems can be manipulated in several ways (Stahl, 2008). For example, agonists increase the activity of systems, while antagonists decrease their activity. L-Dopa, a medicine used for Parkinson's disease, increases the production of dopamine causing more dopamine to bind with postsynaptic dopamine receptors. In contrast, antipsychotics are dopamine antagonists and inhibit dopamine binding with postsynaptic receptors. Thus, pharmacology allows for targeted manipulation of specific chemical systems in the brain.

Drugs have contributed to our understanding of neuronal functioning. For example, action potentials have been studied by blocking the ion channels involved in depolarization and hyperpolarization. In one experiment, sodium channels were blocked using tetrodotoxin (Narahashi, Moore, & Scott, 1964), whereas in another experiment tetraethylammonium was used to block potassium channels (Armstrong & Binstock, 1965). These experiments showed how sodium and potassium channels determine the characteristics of the action potential. In addition, some drugs act specifically on ionotrophic or metabotrophic receptors (Stahl, 2008). For example, benzodiazepines act as agonists on the ionotrophic $GABA_A$ receptor allowing for negative chloride ions to flow into the neuron causing hyperpolarization of the postsynaptic neuron. Similarly, gamma-hydroxybutyric acid, also known as GHB, is an agonist for the metabotrophic $GABA_B$ receptor. Via intracellular pathways, $GABA_B$ activation causes the opening of potassium channels, allowing for positive potassium ions to flow out of the neuron and thus hyperpolarizing the membrane potential. In short, numerous topics have been studied using neuropharmacology.

Some drugs have accidentally given insight into the brain. Antipsychotics are drugs used to alleviate psychotic symptoms but their effect was discovered by accident and drove research to elucidate the underlying mechanism (Stahl, 2008). Antipsychotics tipically inhibit the binding of dopamine to postsynaptic receptors, thus illustrating a link between dopamine and psychosis. Similarly, antidepressants were used without knowledge of its mechanisms (Stahl, 2008). After successfully applying antidepressants in the clinic, researchers discovered their effects on monoamines neurotransmitters such as serotonin, norepinephrine, and dopamine.

Although pharmacology is able to discriminate between specific types of neurons, psychopharmacological drugs are not ideal for studying the brain (Stahl, 2008). First, drugs are spatially non-specific affecting the whole brain rather than specific regions. This is impractical for localizing functions. Second, control on a temporal scale is low as drugs generally take effect after minutes, hours or even weeks and with a similar time period for the drug to degrade. However, action potentials work on a millisecond scale, and many psychological processes occur in the order of seconds. Despite these and several other disadvantages, psychopharmacology remains useful in studying chemical properties of neurons.

Optogenetics

History of Optogenetics

In recent years, optogenetics has proven to be an advantageous technique for studying the brain. As stated in the introduction, it applies genetically addressable photosensitive tools to measure and control cellular activity (Dugué et al., 2012). More specifically, it focuses on the manipulation of cellular activity using light (Deisseroth, 2011). To understand the underlying mechanisms, it is useful to understand their history. Although EBS and neurpharmacology are used in humans, both techniques were initially exclusively applied to animals and cell cultures. Similarly, optogenetics was first developed in animals such as fruit flies and mice.

One of the first attempts to control neuronal activity using light was the application of caged compounds (Figure 2; Kaplan, Forbush & Hoffman, 1978; Nerbonne, 1996). In a synapse of interest, a compound of choice (e.g. a neurotransmitter) is bound to photosensitive caging molecules. When bound to such molecules the compound is rendered inactive. However, when the right wavelength of light is applied to the caging molecules they detach from the compound, allowing the compound to function normally again, binding to postsynaptic receptors. Consequently, this technique allows the control of neurotransmitter activity in a synapse, simply by using light. However, the technique is limited in its use because the delivery of the caging molecules to a specific synapse only works for easily accessible networks. Caged compounds can be used to study neurons *in vitro* (i.e. isolated from an organism) or of a small organism such as

a fly, but the technique will not work for larger organisms such as mice and humans.

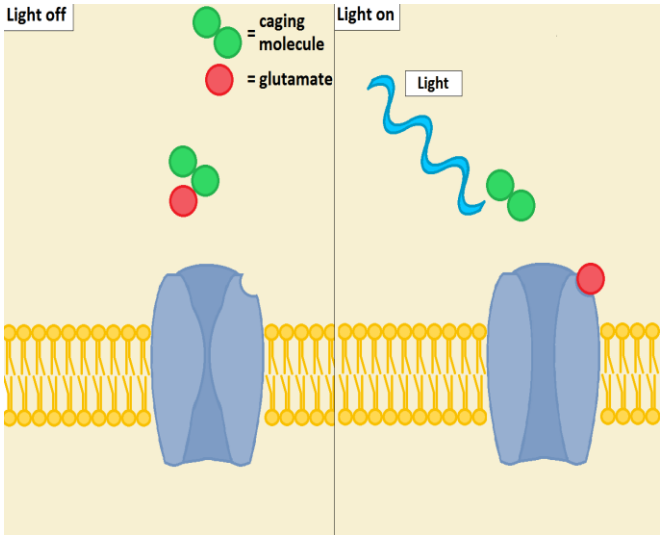

Figure 2. Schematic display of caging compounds. A caging molecule is attached to glutamate, making it unable to bind to the glutamate receptor (left image). When light is presented, the caging molecule separates from the glutamate, which in turn can bind to the glutamate receptor (right image).

To circumvent the need to deliver the photosensitive components to the synapse, genetic engineering was applied to rhodopsin (Khorana, Knox, Nasi, Swanson, & Thompson, 1988). Rhodopsin is a photosensitive protein found in the eye that allows us to perceive light. Additionally, rhodopsin functions as a metabotrophic receptor and when exposed to light, it activates intracellular pathways. Khorana et al. (1988) isolated the gene for rhodopsin from a cow. Using viral delivery (which is explained in the following section) they delivered the rhodopsin gene to frog oocytes. Eventually, the oocytes started expressing the cow rhodopsin, which in turn reacted to light. Zemelman, Lee, Ng, and Miesenböck (2002) used the same principle to develop a technique called ChARGe (Figure 3). They used a different rhodopsin called NinaE, but also incorporated genes for intracellular pathways which would be activated by NinaE. When the rhodopsin was exposed to light, the intracellular pathways eventually led to the activation of ion channels allowing positive ions to flow into the cell and cause depolarization. Due to advances in genetic engineering, which are explained later in this paper, ChARGe - also be restricted to specific types of neurons - only dopamine neurons become photosensitive while all other neurons remain insensitive to light. Thus, Zemelman et al. (2002) were capable of controlling the neuronal activity of specific neurons using genetically incorporated photosensitive chemicals.

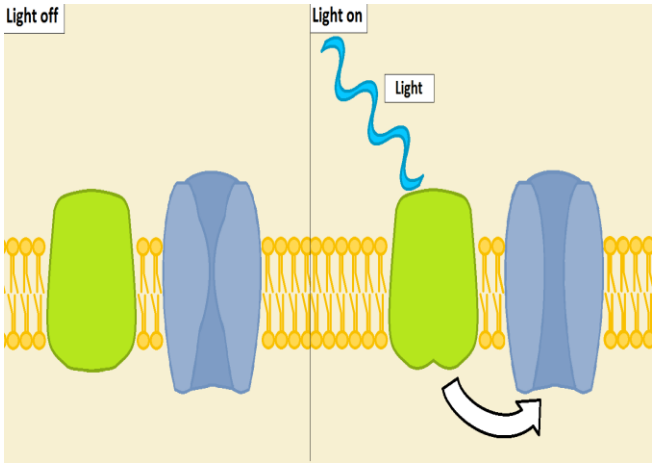

Figure 3. Schematic display of ChARGe. A closed channel (blue) is shown next to NinaE (green). When light is absent, the channel is closed (left image). When light is present, NinaE activates an intracellular pathway, ultimately opening the channel and allowing the flow of ions or other chemicals.

The intracellular pathways incorporated by Zemelman et al. (2002) take several seconds to activate the cation channels. However, neurons interact on a millisecond scale. This problem was solved by applying microbial opsins instead of rhodopsins (Boyden et al., 2005). Microbial opsins are photosensitive proteins found in microorganisms such as bacteria (Oesterhelt & Stoeckenius, 1973). More specifically, Nagel et al. (2003) discovered a new kind of opsin in the green alga Chlamydomonas reinhardtii. The opsin, called channelrhodopsin-2 (ChR2), functions as an ion channel rather than a metabotrophic receptor like rhodopsin. The channel opens when exposed to blue light immediately allowing an influx of positive ions (e.g. sodium ions) leading to depolarization of the neuron and a likely action potential (Figure 4). Thus, where ChARGe requires intracellular pathways to activate ion channels, ChR2 is itself an ion channel and immediately depolarizes the neuron when activated. In 2005, Boyden et al. successfully implemented these channels into mammalian neurons.

First, they cultured hippocampal tissue from mice. Second, they isolated the genetic code for ChR2 and infected the neural tissue with viruses to deliver the gene to neurons. After infection, ChR2 was expressed on the membranes of the neurons. By stimulating the tissue with blue light and simultaneously recording electrical activity, they found that the light caused action potentials in the neuron, milliseconds after stimulation. Thus, Boyden et al. (2005) were the first to succeed in controlling neuronal activity of mammalian brain tissue with millisecond precision by using light; a feat that has since been shown to be useful to study the brain.

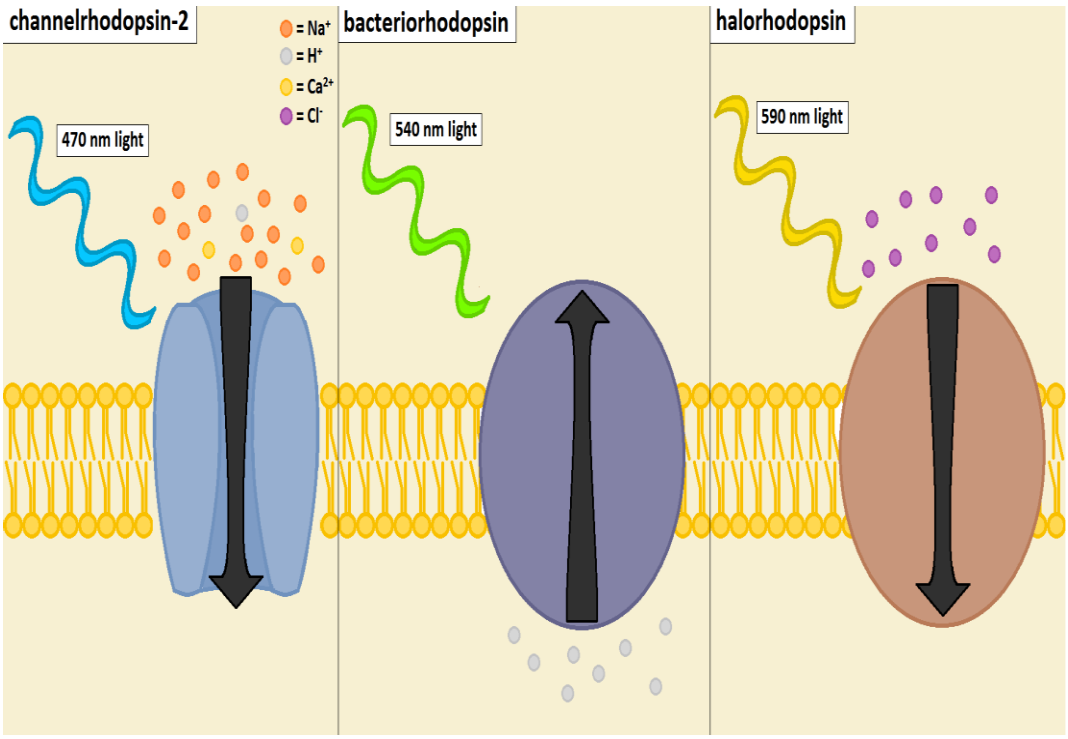

Figure 4. Schematic display of microbial opsins.

Opsins

Since the initial finding of ChR2 (Nagel et al., 2003), several other types of opsins have been discovered and/or engineered and are collectively referred to as microbial opsins (Yizhar, Fenno, Davidson, Mogri & Deisseroth, 2011a). Alongside opsins that excite cells, such as ChR2, several classes of opsins inhibit cells. One example is the ion pump bacteriorhodopsin (BR), which can actively pump protons (H^+) out of the cell (figure 4; Racker & Stoeckenius, 1974). Protons have a positive charge meaning their outflow causes the cell to hyperpolarize. Similarly, an archaebacteria named *Natronomonas pharaonis* expresses the ion pump halorhodopsin (NpHR), which hyperpolarizes cells by pumping chloride ions (Cl^-) into the cells decreasing the membrane potential (Figure 4; Essen, 2002). Note that these mechanisms of excitation and inhibition are distinctly different from EBS, which applies current, yet they can both achieve similar effects. More recently, special types of proteins have been engineered (Kim et al., 2005). These are called opto-XRs, which are created by fusing of metabotrophic receptors with vertebrate rhodopsin and can be used to control any intracellular pathway (Airan, Thompson, Fenno, Bernstein, & Deisseroth, 2009). The XR in opto-XR denotes the specific receptor it is composed of, for example opto-β_2AR is made of and functions as the β_2-adrenergic

receptor. Due to its novelty, opto-XRs have not been studied extensively. Thus this paper will focus on the other classes of opsins.

When opsins are implemented in the mammalian brain they offer interesting mechanisms to manipulate neural networks. First, electrical properties of neurons can be manipulated by applying different types of opsins as described above (Yizhar et al., 2011a). Second, different opsins are activated by different wavelengths of light (Fenno, Yizhar & Deisseroth, 2011). For example, ChR2 shows optimal activation at 470 nm (blue light), while NpHR activity peaks at 590 nm (green light). Multiple opsins can be expressed in a single neuron thus enabling both the activation and inhibition of that neuron by using multiple light sources. Third, optogenetics differentiates between different types of neurons since opsins can selectively be expressed in specific types of neurons (For example: dopaminergic neurons Rein & Deussing, 2011). If the opsins are delivered during a certain stage of embryonic development, it is also possible to target specific layers of the cortex allowing differentiation between different projections of a region (Fenno et al., 2011). Fourth, optogenetics enables the separate stimulation of cell bodies or projecting axons within a given brain area (Yizhar et al., 2011a). Transported by a virus, a gene for an opsin can be delivered to a specific brain region. Some viruses will only be taken up by cell bodies but not by axons. This way, only the neurons with their cell body in that region will recieve the opsin gene. Conversely, some viruses are only taken up by axon terminals and not by cell bodies. This way, only the neurons with their axon terminals in that region will recieve the opsin gene. In addition, axons simply passing through the region (i.e. without an axon terminal in that region) will not be affected by the virus. In conclusion, optogenetics offers specific neural manipulation with both high temporal and spatial resolution.

Applying Optogenetics in Mammals

Expression of microbial opsins in mammals can be achieved by using either viruses such as adeno-associated viruses, lentiviruses, or herpes simplex viruses. or germline transgenesis (Rein & Deussing, 2011). As Boyden et al. (2005) did in their experiment, the genetic code for microbial opsins can be built into viruses. More specifically, plasmids can be constructed, which are relatively short pieces of genetic code. These plasmids are introduced to a virus, which in turn is introduced to the organism of interest. The virus does not affect the whole brain, but only regions where it was injected. By infecting the organism with the virus, the plasmid enters the neurons of interest leading to the expression of opsins. Another method is germline transgenesis. The genetic code is build into stem cells, which are then injected into a pre-fertilized blastocyst (Manis, 2007). This leads to the development of an embryo, with the new genetic code in all its cells. Thus, all neurons in the brain will contain the gene for the opsin.

Depending on the construction of the vector and the type of organism used, the factor can be specifically expressed in certain kinds of neurons, for example only dopaminergic neurons or inhibitory neurons (Rein & Deussing, 2011). To start the cascade from gene transcription to opsin production, the gene first has to be transcribed. To initiate this process, proteins called transcription factors are required. There are many transcription factors, some of which are unique to certain types of neurons. By engineering a gene that requires a transcription factor only present in dopaminergic neurons, the gene will only be expressed in dopaminergic neurons as no other neuron has the required transcription factor to transcribe the gene. This allows expression of opsins in specific types of neurons.

Once the genetic code is delivered to the cells of interest and the opsins are expressed, light of the right wavelength has to reach the opsins (Bernstein & Boyden, 2011). In addition to extracted neural tissue as studied by Boyden et al. (2005), optogenetics can be applied *in vivo*. To deliver light to the brain, the skull is bypassed by making a hole into which optical fibers are inserted. These fibers are designed to transduce light from one end of the

fiber to the other. To keep the fibers in position, they are docked into a plate that is fixed onto the animal's head. These steps allow stimulation of photosensitive neurons by sending a laser through the optical fibers.

The field of optogenetics is rapidly developing and the limits of applying optogenetics are currently unknown. Yet there is one major obstacle for the field of psychology. The technique has been tested in many species, such as zebra fish, mice, rats, and more recently non-human primates (Fenno et al., 2011). However, psychology focuses on human behavior and cognition. Thus, it is unclear how optogenetics could be applied to humans. Genetic engineering in humans is very controversial and gene delivery using viruses may permanently change the structure of the brain. Even so, optogenetics has the potential to significantly increase our understanding of the brain, similar to the contributions of EBS and pharmacology over the last decades.

Due to the complex and technical nature of optogenetics, a substantial amount of information in this paper has been simplified. Interested readers are referred to Dugué et al. (2012) for an extensive review of optogenetics, which includes the history of optic and genetic techniques, properties of optogenetic tools, descriptions of technical aspects such as light delivery, and several future challenges.

Applications of Optogenetics

Some branches of psychology focus on the complex interplay of neural networks suggesting optogenetics could be an interesting technique to improve the understanding of the human brain. Over the last few years, optogenetics has been applied in many areas of neuroscience relevant to psychology, from basal functions such as breathing (Alilain et al., 2008), to the mechanism of antidepressants in the medial prefrontal cortex (Covington III et al., 2010). This following section has two objectives. First, it will give an overview of the extent to wich research has utilized optogenetics. Second, it aims to show how studies have applied optogenetics in their experimental designs.

Cognition and Psychological Functioning

Aversion learning. Schroll et al. (2006) demonstrated how drosophila (fruit fly) larvae learn aversive and appetitive associations. A previous study showed that larvae lacking dopamine expression are unable to learn by aversion, whereas the absence of a neurotransmitter called octopamine inhibited appetitive learning (Schwaerzel et al., 2003). However, inhibition of these neurotransmitters does not exclude the possibility that dopamine plays some role in appetitive learning, nor that octopamine influences aversive learning. To clarify this, Schroll et al. (2006) developed flies that were genetically engineered to express ChR2. First, the dopaminergic network was addressed. Dopaminergic neurons express a gene called TH while other neurons do not, so the expression of ChR2 was coupled to expression of TH. Stimulating dopaminergic neurons via ChR2 led the larvae to develop aversion for a specific scent, while never leading to attraction to a appetitive scent. Conversely, ChR2 in octopaminergic neurons was coupled to a gene called TDC2. Activation of these neurons could link appetitive learning to a scent, but never led to aversive learning of that scent. Thus, they demonstrated that the dopamine and octopamine networks function independently. Moreover, this study showed how psychologists could study the interaction of multiple neural networks.

Inducing memories. In 2009, Han et al. successfully erased specific fear memories. After applying fear conditioning to mice, they assessed neural expression of cAMP response element-binding (CREB), a transcription factor which is expressed during learning. Neurons with a high level of CREB were destroyed using a toxin, which lead to diminished memory of the fear conditioning, but not other memories. However, this did not prove that activation of these neurons leads to the fear memory. However, Liu et al. (2012) proved this concept by applying

optogenetics. During fear conditioning a protein called c-Fos is expressed in the hippocampus, thus ChR2 was coupled to c-Fos expression. Theoretically, the neurons involved in learning the fear memory would express ChR2 thus later stimulation with light would induce the fear memory. Indeed, stimulation led to more fear reaction. Moreover, light-stimulation only led to more fear reaction when the mice were placed in the same context but not other contexts. Further studies could lead to a more fundamental understanding of the relationship between memories and specific neurons.

Mating behavior & aggression. Although many functions such as aggressive and mating behavior are ascribed to the hypothalamus, it remains unknown which neurons in the hypothalamus are responsible for these functions and how these neurons interact. By using optogenetics, Lin et al. (2011) identified a group of neurons that control aggressive behavior. After delivering ChR2 virally to mice, they showed that light-driven stimulation of the ventromedial hypothalamus (ventrolateral subdivision, VMHvl) induces aggressive behavior, even towards inanimate objects However, activating the same region with EBS did not lead to aggressive behavior. This is because optogenetics only activates neurons with their cell bodies in the VMHvl, while EBS also affects axons of distant neurons that pass through the VMHvl. This shows that previous studies using EBS may have yielded false negative or false positive results due to this limitation.

Psychopathology and Psychiatric Treatments

Unconditioned anxiety. Tye et al. (2011) hypothesized a network in the amygdala that could explain unconditioned anxiety, that is generalized anxiety. The basolateral amygdala (BLA) excites the central lateral amygdala (CeL) via glutamate in turn inhibiting the central medial amygdala (CeM), which is responsible for autonomic and behavioral anxiety responses. In addition, the BLA projects to other brain regions that could be responsible for anxiety, such as the bed nucleus which is activated during threatening situations. Tye et al. (2011), wanted to see whether the BLA-CeL projections, but not projections of the BLA to other brain regions, could induce anxiety responses. They accomplished this by expressing ChR2 in the BLA of mice. In some mice, the light was directed at the BLA activating all BLA projections, whereas another group received stimulation of the CeL. By stimulating the CeL, only the axons of the BLA neurons in the CeL were activated, targeting the BLA-CeL projections exclusively. Indeed, stimulation of this pathway, but not all BLA neurons, caused anxiety responses in behavioral experiments. In addition, expression of NpHR to inhibit the BLA-CeL projections decreased anxiety-related behavior, whereas inhibition of all BLA neurons did not. In this study, optogenetics was able to identify the function of a specific neural projection, whereas EBS could not have achieved the same outcome.

Social dysfunction. Yizhar et al. (2011b) addressed social dysfunction, a core symptom in conditions such as autism. One explanatory hyphothesis is the excitation/inhibition (E/I) balance hypothesis, which states that the balance between excitatory and inhibitory activity in the prefrontal cortex is too high and caused by either too much excitatory, or too little inhibitory neural activity. Yizhar et al. (2011b) attempted to reveal the mechanism by stimulating excitatory neurons or silencing inhibitory neurons. Opsins were used in the medial prefrontal cortex. Excessive excitatory activity induced social dysfunction while reducing inhibitive activity did not thus lending support to the E/I balance hypothesis.

Deep brain stimulation. Although widely accepted as an effective treatment for Parkinson's disease, the mechanisms underlying DBS are poorly understood (Liu, Postupna, Falkenberg & Anderson, 2008). Gradinaru, Mogri, Thompson, Henderson & Deisseroth (2009) addressed this problem by optically deconstructing the relevant neural networks. DBS is an invasive, long-lasting treatment in which the patient's brain is stimulated by a pacemaker device. Stimulating the subthalamic nucleus

(STN) with DBS reduces the symptoms of Parkinson's disease significantly. Gradinaru et al. (2009) utilized mouse models with Parkinson's disease symptoms and modulated the neurons in their STN, but did not observe any relief of symptoms. Since DBS also elicits activity in afferent axons (axons that innervate the STN), therapeutic relief may rely on the afferent neurons rather than the neurons in the STN. Via sophisticated genetic engineering, the afferent neurons were tagged with ChR2, while the STN remained free of ChR2. Light stimulation subsequently ameliorated symptoms, thus giving insight into the mechanisms of DBS. Similar strategies could also be applied to explaining the mechanisms of transcranial magnetic stimulation and other therapeutic tools.

Medical Applications

Activating muscles. Human muscles consist of motor units, each unit being a group of fibers innervated by a single neuron. During movement, smaller units are used more than larger units, as the latter are prone to muscle fatigue (Thrasher, Graham & Popovic, 2005). However, current electrical stimulation techniques to activate paralyzed muscles either recruit units randomly or recruit larger units more often than smaller units, leading to muscle fatigue after short periods of stimulation. The larger units are activated more easily because the axons innervating them are larger and thus more available to external electrical stimulation. To activate smaller units before larger units, Llewellyn, Thompson, Deisseroth & Delp (2010) used ChR2 in mice. They reasoned that ChR2 should be expressed on all axons of innervating neurons equally, regardless of size, thus avoiding unnatural stimulation of larger units. Indeed, optogenetic stimulation caused significantly less muscle fatigue enabling longer stimulation and activation of the paralyzed muscle when compared with conventional electrical stimulation. Thus, due to its increased accuracy of neuron stimulation, optogenetics circumvents obstacles that EBS cannot.

Restoring vision. The retina of the eye has a photosensitive rhodopsin layer visual information to be perceived (Baylor, 1996). However, in some disorders such as retinitis pigmentosa, the photosensitive layer deteriorates, potentially leading to full blindness (Shintani, Shechtman & Gurwood, 2009). Optogenetics could aid in restoring the photosensitive layer since they both utilize proteins reacting to light. Cones, photosensitive cells responsible for color and daytime vision, tend to remain present after becoming insensitive for light. Thus, reactivating these cones with optogenetics could reverse some symptoms of retinitis pigmentosa (Busskamp et al., 2010). As light intensity increases, cones hyperpolarize more. Since NpHR also causes hyperpolarization when exposed to light, it was selectively expressed in mice cones. Busskamp et al. (2010) implemented NpHR in the retinas of blind mice, who subsequently showed significant responses to different visual shapes and performed better than untreated mice during a task that requires light perception. More importantly, Busskamp and colleagues also tested *ex vivo* human retinas. Untreated retinas did not show any electrical response to light, whereas the ones expressing NpHR did. These experiments show that retinal degeneration could benefit from optogenetics, especially since expression in human retinas has been successful.

Inhibiting epilepsy. Temporal lobe epilepsy is characterized by seizures theorized to occur due to loss of inhibitory neurons. This loss is thought to lead to excessive excitatory activity in the hippocampus (De Lanerolle, Kim, Robbins & Spencer, 1989). In particular, the pyramidal neurons of the hippocampus seem to be involved. Moreover, approximately one in eight patients does not respond to available drug treatment (Picot, Baldy-Moulinier, Daurès, Dujols & Crespel, 2008). To test whether optogenetics could help these patients, Tønnesen et al. (2009) applied NpHR to hippocampal organotypic slice cultures, which were cell cultures of hippocampal mouse tissue that showed epileptic properties and did not respond to epilepsy medication. When electrically stimulated, the slice cultures showed bursts of action

potentials typical of epilepsy. By selectively expressing NpHR in the pyramidal neurons and stimulating the tissue with light, the bursts could be prevented. Moreover, NpHR activation did not cause any side effects in the tissue, despite excessive inflow of chloride ions into the neurons. This experiment demonstrates the potential of optogenetics in treating temporal lobe epilepsy. As of now, the finding has to be replicated in animal models *in vivo*. Future research could also focus on stimulating inhibitory neurons, which overall could have the same effect as inhibiting pyramidal neurons (Kokaia, Andersson & Ledri, 2012).

Concluding Remarks

Optogenetics promises new ways to study the nervous system. As illustrated, the technique allows for very precise manipulations that were impossible with other techniques such as EBS. The development of opsins could tackle many problems in psychology, such as revealing the mechanisms underlying the effects of antidepressant drugs (e.g. Stahl, 2008), validating mirror neurons and their role in empathy (e.g. Wicker et al., 2003), or gaining deeper insight in the neural correlates of higher cognition. Although optogenetics is grounded in complex organic biology and chemistry, psychologists should attempt to explore and pursue these advances in science. Optogenetics will prove to have limits similar to any technique, but causally studying the brain in this way allow scientists to conduct experiments that were previously considered impossible.

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