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Optogenetics

Discovery of a neuronal switch

The mysteries of the brain have intrigued humanity for centuries. How does an organ composed of such bewildering neuronal diversity, wired together into vast circuits, seamlessly orchestrate everything from a fleeting memory to a deliberate step? Bridging the gap between this intricate circuitry and the causal control of behavior is a century-old challenge. In a technological and conceptual tour de force, this year's Laureates of the Nobel Prize in Physiology or Medicine, Karl Deisseroth, Peter Hegemann and Georg Nagel have transformed the field of neuroscience. What began as a fundamental curiosity about the phototactic behavior of algae, culminated in the birth of optogenetics. The discovery of channel-rhodopsins as light-gated ion channels marked a pivotal moment, finally providing the long-sought, millisecond-precise switch for neuronal activity. This breakthrough has empowered the field of neuroscience with unprecedented ability to move from passively recording brain activity to actively controlling it, finally illuminating the precise causal relationships that dictate how the brain encodes cognition, emotion and behavior.

The brain is the most complex organ in biology, with an intricate organization of its cellular and circuit architecture. Across the animal kingdom, behavioral, emotional and cognitive functions emerge through the action of neural circuits composed of intermingled excitatory, inhibitory, and modulatory cell types connected through intertwined axons and dendrites. The contributions of these diverse cell types depend on their molecular identity, connectivity, projection targets, and moment-to-

moment activity dynamics. Neural activity unfolds in milliseconds across circuits where neighboring neurons often drive opposing functions or are involved in distinct behaviors. Together, these features make neural circuits very difficult to disentangle experimentally.

Our ability to decode how neural circuits generate behavior has historically been constrained by available technology. For much of modern neuroscience, the central challenge was not to observe the brain, but to intervene in it with sufficient precision to establish causal relationships. Traditional methods like electrophysiology, lesion studies, pharmacology, and imaging were powerful to establish correlations between neural activity and perception, movement, emotion, and cognition. However, they lacked the precision to manipulate specific cell types within intact circuits on the millisecond timescale relevant to neural computation. Moving systems neuroscience from an observational discipline to one grounded in causal intervention required methods to control neuronal activity that were simultaneously spatially targeted, genetically selective, and temporally matched to natural neuronal firing.

Francis Crick explicitly anticipated this requirement in his early perspectives (Crick, 1979; Crick, 1999). He framed the brain as one of biology's central unsolved problems and argued that deciphering brain function demanded the ability to perturb defined neuronal populations with high spatial and temporal precision. Crick proposed that a

transformative method would be one where *“all neurons of just one type could be activated or inactivated, leaving the others more or less unaltered”*. He speculated that to *“turn the firing of one or more types of neurons on and off in the alert animal in a rapid manner ... the ideal signal would be light”* — an idea he acknowledged was *“rather far-fetched”* at the time. This vision effectively outlined the central premise of optogenetics, using genetically targeted optical control of neuronal activity to interrogate neural computation and behavior (Crick, 1999).

Dawn of optical control – early engineering strategies

Optogenetics ultimately fulfilled this vision, enabling light-driven control of genetically defined neurons with millisecond precision. However, its emergence relied on decades of conceptual and technical developments. Successive early attempts to make neurons light-sensitive gradually clarified the stringent design principles required for a practical optical actuator. Such an actuator needed genetic encodability, compactness, robust membrane expression, fast kinetics, reversibility, and the ability to use photons directly to induce membrane currents.

Before microbial channelrhodopsins were discovered and deployed in mammalian neurons, neuroscientists had already begun engineering systems to achieve cell-type specific remote control over neural activity. As early as 1971, Richard Fork exploited the weak endogenous light sensitivity of neurons in *Aplysia* to stimulate them with laser light (Fork, 1971). Ira Farber and Amiram Grinvald (1983) continued these efforts by staining the membranes of leech neurons with a fluorescent dye (Farber and Grinvald, 1983). Exciting this dye with a laser produced a reversible depolarization, which successfully generated action potentials in the neuron. However, these early approaches were ultimately abandoned

because the intense lasers and exogenous dyes caused phototoxic damage to the cells.

Decades later, solutions to this challenge arose from two principal engineering strategies. The first relied on introducing heterologous receptors, either by reconstituting native metazoan phototransduction cascades or pairing ion channels with photo-uncageable ligands, while the second used synthetic, light-sensitive chemical photoswitches tethered to engineered channels. These approaches demonstrated feasibility, but also revealed why a simpler, faster, single-component actuator would be transformative.

Gero Miesenböck and colleagues achieved the first genetic sensitization to light of non-photoreceptive neurons in 2002 (Zemelman et al., 2002). Their chARGe system utilized a multi-gene strategy to co-express three distinct proteins from the *Drosophila* visual cascade, including arrestin-2, rhodopsin, and the Gαq subunit of the G-protein. When introduced into cultured mammalian neurons, chARGe harnessed native intracellular G-protein-coupled receptor signaling cascades to open endogenous ion channels upon illumination, eliciting sustained action potential firing. In a subsequent study from the Miesenböck laboratory (Lima and Miesenböck, 2005), they expanded on this multi-component strategy by expressing the ionotropic purinergic receptor P2X2 in command neurons in *Drosophila*. By combining this with photoactivatable (“caged”) ATP, they were able to trigger escape responses through light stimulation and modulate locomotor movements by activating dopaminergic neurons. While both chARGe and the P2X2 system were key proof-of-concept demonstrations, neither system was fully genetically encoded, relying on co-injected factors (retinal or photoactivatable ATP). Furthermore, these approaches were too slow. Relaying the signal through several inter-mediate or relying on ligand diffusion loosened the coupling between stimulus and response, rendering the timing of

action potentials unreliable (reviewed in Miesenböck, 2004). Ultimately, these methods were unable to resolve the millisecond-scale dynamics of neural coding.

A parallel approach bypassed delays caused by diffusion and intermediate signaling by merging genetic targeting with synthetic chemistry. Developed by Ehud Isacoff, Richard Kramer, Dirk Trauner, and colleagues, this strategy relied on photoswitchable tethered ligands to create light-regulated ion channels. An endogenous ion channel, such as the Shaker K⁺ channel, was genetically engineered to create a chemical gate that confers light sensitivity to the channel (Banghart et al., 2004). This gate included a functional group for selective conjugation to the channel, a pore blocker and a photoisomerizable azobenzene. A synthetic chemical photoswitch was then exogenously applied, covalently bonding to the engineered channel. Illumination at specific wavelengths caused the azobenzene core of the switch to reversibly isomerize between *cis* and *trans* conformations, physically repositioning the ligand to modulate channel activity. These synthetic photoisomerizable azobenzene-regulated K⁺ channels allowed rapid, precise and reversible control over neuronal firing (Banghart et al., 2004). Later photoswitchable receptor strategies, such as light-activated glutamate receptors, extended this principle (Volgraf et al., 2006). Variants of this approach, such as light-activated potassium channels and related photoswitchable channel systems, successfully controlled cellular excitability in cultured cells and *in vivo* model organisms (Szobota et al., 2007).

Both approaches encountered bottlenecks that restricted their widespread adoption. These heterologous receptor systems required the co-delivery of chemicals, and often the coordinated expression of multiple large genes, complicating delivery in intact brains. Similarly, chemical photoswitches required the exogenous delivery of synthetic compounds

into living tissue, introducing challenges with spatial delivery gradients, variable labeling efficiencies, and risks of metabolic degradation. The ideal tool needed to bypass these multi-component hurdles. A single protein had to do the job, one that could be delivered genetically and couple photon absorption directly to ionic current with millisecond kinetics.

Microbial rhodopsins

Found across archaea, eubacteria, and eukaryotic microalgae, microbial rhodopsins harness light for cellular bioenergetics and sensory behaviors. They can directly convert photon energy into transmembrane ion flux (reviewed in Govorunova et al., 2017). The historical foundation of this field began with the discovery of bacteriorhodopsin in the halophilic archaeon *Halobacterium halobium* by Dieter Oesterhelt and Walther Stoeckenius (Oesterhelt et al., 1971). Subsequent studies demonstrated that this protein functions as a light-driven proton pump (Oesterhelt and Stoeckenius, 1973).

Further studies soon revealed that microbial ecosystems use solar energy to move a variety of ions across membranes. In 1977, Akemi Matsuno-Yagi and Yasuo Mukohata observed light-induced hyperpolarization in a bacteriorhodopsin-deficient mutant strain (Matsuno-Yagi and Mukohata, 1977), leading to the isolation of halorhodopsin (Mukohata and Kaji, 1981). A prominent variant of halorhodopsin from the haloalkaliphilic archaeon *Natronomonas pharaonis* was later identified as a light-driven chloride pump (Bivin and Stoeckenius, 1986; Lanyi et al., 1990; Lanyi, 1995). The discovery of these archaeal pumps proved that light could move specific ions across biological membranes via a single protein.

While archaeal pumps were being characterized, researchers were also investigating motile eukaryotic microalgae. Most flagellated green algae possess a visual

system that allows them to orient and swim dynamically relative to a light source, a behavior known as phototaxis (Foster and Smyth, 1980). Early studies in the biflagellate model organism *Chlamydomonas reinhardtii* confirmed that this light-guided navigation relies on a rhodopsin-like chromophore to generate rapid transmembrane photoreceptor currents (Foster et al., 1984; Uhl and Hegemann, 1990).

The discovery of channelrhodopsin

The quest to uncover the molecular mechanisms underlying this phototactic behavior took a pivotal turn when Peter Hegemann, having recently completed his PhD with Dieter Oesterhelt, joined the laboratory of Kenneth Foster. He sought to understand the biophysical basis of these rapid photoreceptor currents in *Chlamydomonas*. This work ultimately led to the discovery of channelrhodopsin – a breakthrough that unfolded sequentially, driven by the convergence of algal physiology, molecular genetics, and electrophysiology.

driving the phototactic behavior was extraordinarily fast, with a latency of only microseconds after flash stimulation (Figure 1; Harz and Hegemann, 1991). These kinetics were difficult to reconcile with classical plant or animal phototransduction cascades that inherently involve diffusible secondary messengers and multiple downstream biochemical steps. Hegemann's team therefore proposed that the rhodopsin photoreceptor and the ion channel must be intimately linked and perhaps exist as a single protein complex (Harz et al., 1992; Holland et al., 1996). This inference was conceptually important because it shifted the search from signaling pathways to molecular identity.

The definitive proof that algal rhodopsins functioned directly as ion channels required the identification of responsible genes, a breakthrough accelerated by the availability of the *Chlamydomonas* expressed sequence tag database (Asamizu et al., 2000). In the early 2000s, three research groups, led by Peter Hegemann, John Spudis, and Toshio



Figure 1 | Light sensitivity and rapid electrical response of the *Chlamydomonas reinhardtii* eyespot. (A) Illustration of light-induced migration (phototaxis) in *Chlamydomonas*. (B) Recording photocurrents in wall-deficient *Chlamydomonas* mutants using the suction pipette technique (Harz and Hegemann, 1991). Ey, eyespot; Fl, flagella. (C) Rapid photocurrents in *Chlamydomonas* (Holland et al. 1996).

Early functional evidence came from Peter Hegemann and colleagues using suction-electrode recordings, a technique previously developed by Felix Litvin, Oleg Sineshchekov, and colleagues in *Haematococcus pluvialis* (Litvin et al., 1978; Sineshchekov et al., 1990). They characterized these photoactivated currents in the green alga *Chlamydomonas* and discovered that the photosensitive current

Takahashi, independently identified cDNA sequences encoding proteins homologous to archaeal bacteriorhodopsin.

Hegemann's laboratory identified the first of these sequences based on its homology to the retinal-binding site and proton-conducting pathways of known opsins. They named this protein channelopsin-1 (Chop1, GenBank

Accession No. AF385748) and hypothesized that it might function as a light-driven ion channel (reviewed in Hegemann et al 2001). Hegemann and colleagues subsequently identified a second homologous sequence, naming it channelopsin-2 (Chop2, GenBank Accession No. AF461397).

of the photocurrents (Suzuki et al., 2003; Mittelmeier et al., 2013).

The decisive functional breakthrough emerged when Peter Hegemann contacted Georg Nagel whose strong electrophysiological expertise in studying ion channels and pumps in

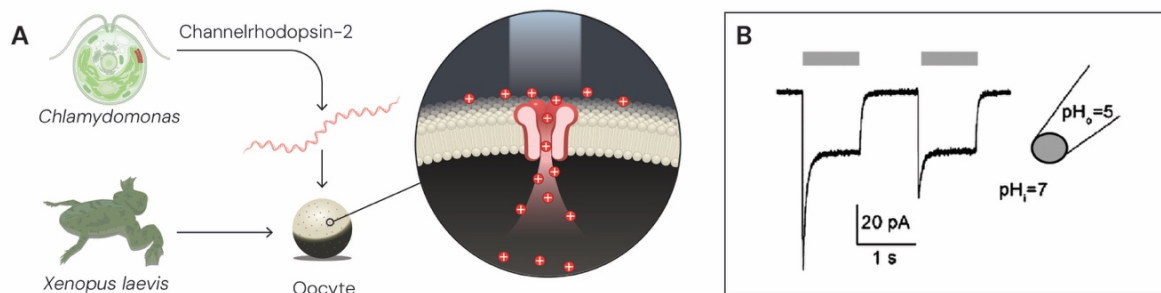


Figure 2 | Heterologous expression of channelrhodopsin-2 in *Xenopus laevis* oocytes. (A) Illustration of the experimental system. (B) Recording of photocurrents induced by light pulses (grey bars) in oocyte membrane patches in the presence of all-trans retinal (Nagel et al. 2003).

Meanwhile, in John Spudich's laboratory, Kwang-Hwan Jung together with Oleg Sineshchekov used RNA interference to silence the expression of these genes and analyzed photoreceptor currents and phototactic responses in the resulting transformants. They demonstrated that the encoded proteins, which they named *Chlamydomonas* sensory rhodopsins A and B (CSRA and CSRB), were responsible for generating photoreceptor currents (Sineshchekov et al., 2002). While they noted that the kinetics of CSRB-generated current and its low light saturation suggested a transduction chain involving diffusional biochemical steps (Sineshchekov et al., 2002), the fast kinetics of the CSRA-mediated current led Spudich and colleagues to propose that CSRA might itself form an ion channel or be tightly coupled to one, echoing Hegemann's earlier hypothesis (Harz et al., 1992; Holland et al., 1996). Concurrently, a group led by Tetsuo Takahashi identified the same sequences. Using immunofluorescence, they showed that these proteins localized precisely to the algal eyespot, providing anatomical validation for the origin

heterologous expression systems was exactly what the project needed. This successful collaboration built upon a long-standing connection. Nagel obtained his PhD under Ernst Bamberg at the Max Planck Institute of Biophysics in Frankfurt, where he first met Hegemann. After postdoctoral training at Yale and Rockefeller University, he returned to Frankfurt as an independent investigator. Nagel had used *Xenopus laevis* oocytes as a heterologous experimental system to characterize bacteriorhodopsin as a light-driven proton pump (Nagel et al., 1995). Together with Peter Hegemann, who provided the cloned *Chop1* and *Chop2* cDNA sequences, and Ernst Bamberg, Nagel used this system to express these genes and study their function (Nagel et al., 2002; Nagel et al., 2003; reviewed in Hegemann and Nagel, 2013). In 2002, he expressed mRNA encoding *Chop1*. By introducing all-trans-retinal and using two-electrode voltage-clamp recordings, he showed that green-light illumination induced inward photocurrents carried primarily by protons (Nagel et al., 2002). Truncated variants encoding only the core seven-transmembrane

domain preserved this light-dependent conductance, demonstrating that the protein itself was sufficient for channel function. This biophysical fingerprint provided the first direct evidence of a light-gated ion channel, prompting its formal designation as channelrhodopsin-1 (ChR1).

The second, arguably more consequential breakthrough came the following year. The same collaborative team, led by Georg Nagel, expressed *Chop2* in both *Xenopus* oocytes and mammalian human embryonic kidney cells (Nagel et al., 2003). They demonstrated that this second protein, named channelrhodopsin-2 (ChR2), functioned distinctly from ChR1. ChR2 exhibited a blue-shifted activation spectrum and operated as a broad, non-selective cation channel (Figure 2). A truncated fragment of just the first 315 amino acids was sufficient to preserve light-gated conductance. ChR2 photocurrents displayed unique biophysical properties, characterized by a prominent transient initial peak current that decayed to a lower, stable steady-state plateau during prolonged illumination. By measuring shifts in the reversal potential under varying extracellular ionic balances, Nagel and colleagues confirmed that the channel mediated a passive, inward-directed cation flux down the electrochemical gradient (Nagel et al., 2003).

Optogenetics – translating light into action

Finding a microbial protein that could act as a single unit, simultaneously as a photoreceptor and an ion channel, provided the exact molecular logic needed for fast, genetically targetable optical activation of neurons. Unlike the previously discovered archaeal light-driven pumps, channelrhodopsins are *bona fide* ion channels. While pumps move ions slowly against electrochemical gradients, ion channels allow ion flow rapidly down their gradients,

perfectly matching the timescale of action potentials. The discovery that ChR1 and ChR2 were single-component, light-gated ion channels provided the precise tools that neuroscience had been looking for.

The first successful demonstration that ChR2 could be expressed in neurons came from the Karl Deisseroth laboratory (Figure 3). Deisseroth, a trained psychiatrist who was then beginning to establish his own laboratory at Stanford University, used a construct provided by Georg Nagel. This construct included a reporter fluorescent protein to monitor expression and localization (described by Boyden, 2011a, b). In preliminary experiments, they transfected cells differentiated from mammalian central nervous system progenitor cells. Using the phosphorylation of the transcription factor CREB as a proxy for neuronal activity, he observed that the expressed protein responded to blue light (described by Deisseroth, 2015). After subsequent optimization of lentiviral gene delivery protocols in hippocampal neurons in culture, a stable, membrane-localized ChR2 expression was achieved without apparent toxicity (described by Boyden, 2011b; Deisseroth, 2015).

However, the definitive functional proof required direct measurement of electrical activity in confirmed neurons. Electrophysiological recordings in cultured neurons demonstrated that blue-light activation of ChR2 could trigger precise, millisecond-scale action potentials (Boyden et al., 2005). This success relied on a fortuitous observation by Georg Nagel that exogenous retinal was unnecessary since endogenous trace levels were sufficient (Boyden et al., 2005). This first demonstration of the capability of ChR2 to faithfully drive action potentials in neurons represents a milestone for optogenetics (Figure 3).

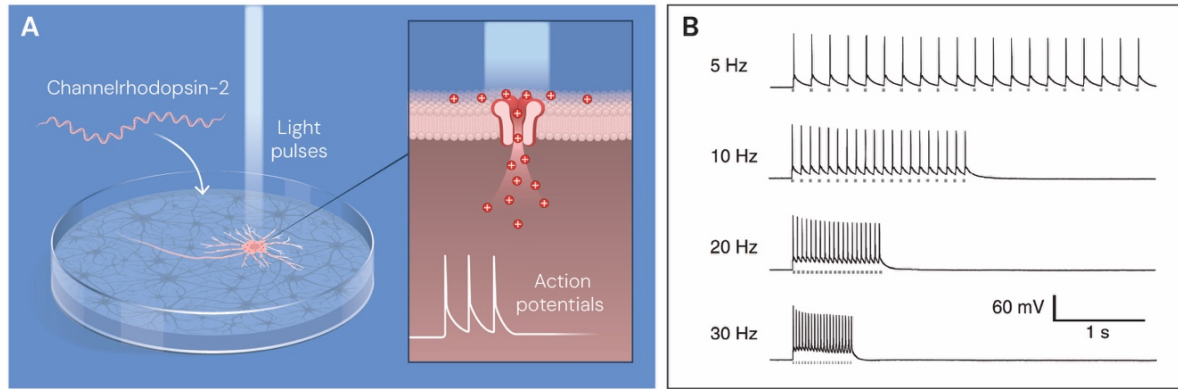


Figure 3 | Optogenetic control of neuronal action potentials using light. (A) Illustration of the experimental system. (B) Trains of action potentials induced by light pulses at increasing frequencies (Boyden et al. 2005).

Other independent studies published within a year confirmed the efficacy of ChR2 in controlling the activity of hippocampal neurons (Li et al., 2005), motor activity in the embryonic chick spinal cord (Li et al., 2005), behavior in *C. elegans* (Nagel et al., 2005), responses in the retina (Bi et al., 2006), and reinforcement learning in *Drosophila* during classical conditioning (Schroll et al., 2006). Building on these studies and aided by the fact that no exogenous retinal was required, this capability was subsequently extended to intact brain tissue *in vivo* using viral delivery (Bi et al., 2006; Ishizuka et al., 2006; Zhang et al., 2006).

By delivering depolarizing cation currents with millisecond temporal control, ChR2 transformed the speculative idea that light could control activity of defined neurons into an experimentally tractable reality. To encapsulate the unique synergy of integrating

optical actuators with targeted genetic expression, Karl Deisseroth, Guoping Feng, Ania Majewska, Gero Miesenböck, Alice Ting and Marc Schnitzer formally coined the term "optogenetics" in a 2006 review (Deisseroth et al., 2006).

Optogenetics interrogation of brain circuits using optical fibers

The next critical step was to deploy optogenetics in deep brain structures in awake and behaving mammals. This required pairing genetically targeted ChR2 expression with new methods for delivering light to defined brain regions. Deisseroth and colleagues developed an optical neural interface using laser diodes coupled to optical fibers, which enabled precise illumination and control of defined deep brain structures in freely behaving animals (Aravanis et al., 2007). Importantly, they confirmed that, even in living animals, no exogenous retinal

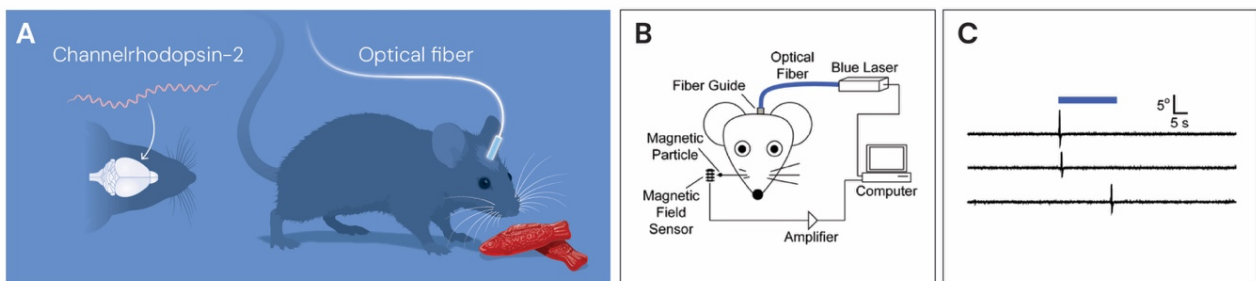


Figure 4 | In vivo optogenetics for control of animal behavior using light. (A) Virally delivered channelrhodopsin-2 is activated by light delivered through an optical fiber. (B) The whisker deflection assay for detecting barrel cortex activation in the rodent vibrissa system. (C) Whisker deflections induced by light stimulation (blue bar) in in vivo in mice (Aravanis et al. 2007).

was required at any time. They targeted the rodent vibrissal motor system and measured the resulting deflection of the whiskers (Figure 4). By expressing ChR2 in excitatory neurons of layer 5 of the vibrissal motor cortex, they successfully demonstrated optogenetic control over whisker deflections (Aravanis et al., 2007).

What began as an effort to understand the phototactic navigation of green algae had culminated in a powerful new technology, launching a rapidly expanding field that would soon reshape the landscape of modern neuroscience. At last, Francis Crick's theoretical vision had been realized, scientists could now control the firing of specific, genetically defined neurons in an alert animal using only light.

Expanding the optogenetic toolbox

Although channelrhodopsin provided a powerful mechanism to drive neuronal firing, fully interrogating neural circuits also required the ability to suppress activity selectively. In 2007, two independent studies, one by Feng Zhang and colleagues in Karl Deisseroth's laboratory (Zhang et al., 2007) and another led by Xue Han and Edward Boyden (Han and Boyden, 2007), achieved this goal by adapting halorhodopsin (NpHR) from the archaeon *Natronomonas pharaonis*. Expressed in mammalian neurons, NpHR actively pumped chloride ions into the cell upon illumination with yellow light, hyperpolarizing the membrane and silencing action potentials. Because NpHR operates at a red-shifted wavelength compared to blue-light-activated ChR2, this spectral separation enabled the first dual-color optogenetic experiments, allowing bidirectional control of neuronal activity within the same circuit.

However, light-driven chloride pumps like NpHR and its later engineered variants (such as eNpHR3.0; Gradinaru et al., 2010), together with outward proton pumps (Chow et al., 2010) relied on active transport, moving only a single ion per absorbed photon. Although ChRs were

successfully engineered through mutagenesis to conduct chloride (Berndt et al., 2014; Wietek et al., 2014), a true light-gated inhibitory channel remained a highly sought-after goal. In 2015, John Spudich's laboratory discovered a naturally occurring light-gated anion channels from the cryptophyte alga *Guillardia theta* (Govorunova et al., 2015). These *Guillardia theta* anion channelrhodopsins (GtACR1 and GtACR2) provided substantially greater light sensitivity and chloride conductance compared to pumps. By allowing chloride ions to flow rapidly and passively down their electrochemical gradients, GtACRs fundamentally upgraded the efficiency of optogenetic silencing.

Optogenetic decoding of behavioral causality in the brain

An early *in vivo* application of optogenetics probed the causal relationship between the activation of orexin/hypocretin neurons in the lateral hypothalamus and the transitions from sleep to wakefulness (Adamantidis et al., 2007). While these neurons were known to be essential for maintaining arousal stability (Adamantidis et al., 2007), whether their electrical activity was sufficient to drive awakening or merely correlated with the arousal state was unclear. In a collaboration between the laboratories of Karl Deisseroth and Luis de Lecea, ChR2 was targeted to orexin/hypocretin neurons using a viral vector. Fiber-optic illumination of the lateral hypothalamus *in vivo* increased the probability of transitions to wakefulness from both slow-wave and rapid-eye-movement sleep. The latency of these transitions depended strictly on the stimulation frequency. This work provided one of the first demonstrations that optogenetics could establish a direct causal link between the firing dynamics of a defined neuronal population and a specific mammalian behavior.

The combination of engineered opsins, targeted genetic expression, and *in vivo* optical

interfaces allowed neuroscientists to perform experiments to rapidly control the activity of neurons. By modulating defined circuits with precise control over timing and pattern, testing the sufficiency and necessity of specific circuit elements is finally possible, thereby establishing definitive causal links between neural activity and behavior.

One profound application of this approach was the search for the memory engram, the physical cellular substrate encoding a specific memory. Historically, the engram had eluded causal demonstration due to the inability to selectively access the exact cells altered during learning (Josselyn and Tonegawa, 2020). Optogenetics bridged this gap through activity-dependent genetic tagging. In a study from the late Susumu Tonegawa's laboratory (Nobel Prize in Physiology or Medicine, 1987, for his discovery of the genetic principle underlying antibody diversity), researchers expressed ChR2 exclusively in hippocampal dentate gyrus neurons that were active during contextual fear conditioning. Subsequent optical stimulation of these labeled cells in a neutral context subsequently triggered a fear response, proving that activating a defined neuronal ensemble is sufficient to evoke memory recall (Liu et al., 2012). Researchers also applied the same logic to create artificial memories. Neurons naturally activated during the exploration of a safe context were optogenetically reactivated while mice underwent fear conditioning in a different environment. When mice were returned to the original, safe context, they froze despite never having been fear-conditioned there (Ramirez et al., 2013). This work demonstrated that memory content can be causally manipulated within neuronal ensembles that form engrams.

The ability to isolate specific functional ensembles also refined the understanding of neuromodulatory centers, moving away from the historical view of these regions as uniform broadcast systems. Midbrain dopamine

neurons had previously been implicated in generalized concepts of reward, aversion, and salience (Schultz, 2007; Bromberg-Martin et al., 2010; Ungless et al., 2010). Optogenetics resolved this ambiguity by demonstrating that functionally distinct dopaminergic populations are anatomically intermingled yet strictly segregated by their circuit connectivity. For example, specific dopamine neurons in the ventral tegmental area were shown to drive opposing states depending entirely on their routing. Afferents from the laterodorsal tegmentum drive reward, while inputs from the lateral habenula drive aversion (Lammel et al., 2012). Applying this projection-specific interrogation to the amygdala allowed researchers to disentangle overlapping emotional states such as fear and anxiety (Ciocchi et al., 2010; Haubensak et al., 2010; Johansen et al., 2010; Stuber et al., 2011; Tye et al., 2011; Adhikari et al., 2015). Notably, activating projections from the basolateral amygdala to the central amygdala produces a robust anxiolytic effect, reducing anxiety-related behavior (Tye et al., 2011). Activation of basolateral amygdala projections to the ventral hippocampus acutely and robustly increased anxiety-like behaviors (Felix-Ortiz et al., 2013). This projection-defined modularity of anxiety was further extended through work from Karl Deisseroth's laboratory. The anterodorsal bed nucleus of the stria terminalis parses into distinct pathways. Projections to the lateral hypothalamus mediate behavioral risk-avoidance, while those to the ventral tegmental area were associated with positive valence (Kim et al., 2013). These findings show that complex emotional states are not governed by brain regions as monolithic units. Instead, they are assembled from discrete, projection-defined circuits.

This principle of target-defined modularity extends across the brain, allowing seemingly unified innate behaviors to be parsed into discrete processing streams. In the ventrolateral subdivision of the ventromedial

hypothalamus, work from David Anderson's laboratory revealed that optogenetic stimulation of molecularly defined *Esr1*⁺ neurons can drive an intensity-dependent behavioral continuum (Lin et al., 2011; Lee et al., 2014). This behavior scales linearly from social interaction to mating and ultimately culminates in aggressive attacks. Similarly, Catherine Dulac and colleagues dissected the intricate choreography of maternal care into independent subroutines (Kohl et al., 2018). Within the medial preoptic area, discrete projection-defined subpopulations of galanin-expressing neurons independently govern specific components of parenting. One projection pool drives motor interactions with pups. Another controls the motivation to seek them out and a third regulates social interaction with other adults. Innate behaviors once treated as indivisible drives are built from separable, molecularly and anatomically defined modules.

This parallel processing framework also maps onto brainstem motor control. By isolating the mesencephalic locomotor region based on projection targets, spatially overlapping neuronal populations have been resolved into distinct functional streams that independently control locomotor speed, body extension, and fine forelimb manipulation (Caggiano et al., 2018; Ferreira-Pinto et al., 2021; Arber and Costa, 2022; Leiras et al., 2022). These examples establish that parallel, projection-defined streams encode different motor actions.

Clinical frontiers – restoration of vision

Retinitis pigmentosa is a hereditary neurodegenerative disease caused by genetic mutations that lead to the progressive loss of photoreceptors (cones and rods), ultimately resulting in blindness. An early application of optogenetics was to express ChR2 in the retinal circuitry. Specifically, Zhuo-Hua Pan and colleagues showed that an intraocular injection of an adeno-associated viral vector encoding

ChR2 led to expression in retinal ganglion cells in rodents (Bi et al., 2006). These cells sit at the end of the retinal processing pathway and connect the retina to the brain via the optic nerve. Light depolarized the ChR2 expressing retinal ganglion cells, restoring retinal photosensitivity and allowing light signals to reach the visual cortex in a mouse model of retinitis pigmentosa (Bi et al., 2006). Expression of ChR2 in surviving retinal neurons could therefore serve as a potential strategy for restoring vision after rod and cone degeneration. Zhuo-Hua Pan and colleagues subsequently showed that functional ChR2 could also be expressed in surviving retinal cells of marmoset monkeys (Ivanova et al., 2010). Likewise, viral delivery of Chrimson, a red-shifted channelrhodopsin, produced high expression and light responsivity in retinal ganglion cells of Macaque monkeys (Gauvain et al., 2021). Botond Roska and colleagues adapted this approach and virally delivered Chrimson to retinal ganglion cells in a blind patient with retinitis pigmentosa (Sahel et al., 2021). Light pulses delivered through engineered goggles activated the transduced cells and produced a prolonged, partial restoration of vision. This patient regained the ability to distinguish high-contrast objects on a table, a change likely to be of meaningful benefit in daily life. Moreover, multichannel electroencephalographic recordings revealed object-related activity in the visual cortex during visual perception. Several clinical trials using optogenetic approaches to improve vision in patients with retinitis pigmentosa are presently underway.

A lineage of technological transformation

Across cognition, emotion, and movement, these discoveries and applications illustrate a profound shift. Behaviors previously understood as unified phenomena can now be experimentally deconstructed into discrete computational streams. By enabling temporally precise, pathway-specific interventions in intact brains, optogenetics has transformed

neuroscience by revealing the causal logic of brain function.

While optogenetics uniquely solved the problem of interrogating fast neural dynamics, chemogenetics soon thereafter matured as a complementary strategy. Pioneered largely by the Bryan Roth's laboratory with the development of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in 2007 (Armbruster et al., 2007), this approach relies on engineered G-protein-coupled receptors. DREADDs are insensitive to endogenous neurotransmitters but highly responsive to otherwise inert synthetic ligands like clozapine-*N*-oxide (Armbruster et al., 2007; Roth, 2016) or deschloroclozapine (Nagai et al., 2020). The slower time course and the ability to actuate receptors systemically via peripheral ligand injection make the tool exceptionally useful for experiments requiring prolonged neuromodulation (Sternson and Roth, 2014). Chemogenetics is also suitable for modulation of large and widespread brain circuits and may eventually be used in the human brain to counteract various diseases.

Across the history of neuroscience, progress in understanding brain function has been

inextricably linked to technological innovation. Advances in methodology have not only expanded experimental access to the brain but also reshaped the scientific questions that can be asked. Modern neuroscience itself began with a technological breakthrough – the 'black reaction' staining method developed by Camillo Golgi, which enabled Santiago Ramón y Cajal to formulate the neuron doctrine (Nobel Prize in Physiology or Medicine, 1906). Decades later, Erwin Neher and Bert Sakmann's patch-clamp technique transformed the study of neuronal and synaptic physiology by allowing unprecedented access to single ion channels (Nobel Prize in Physiology or Medicine, 1991). Optogenetics belongs firmly in this lineage of transformative methods. By using light to control defined neuronal types in behaving animals, the technique fulfilled an early theoretical proposal. Optogenetics is therefore much more than a technical breakthrough. The method has transformed neuroscience from observing and "reading" brain activity to actively "writing" into it, establishing a causal link between distributed, genetically specified circuit motifs and behavior, emotion and cognition.

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