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Neuroscience Research: Before and After the Evolution of Optogenetics

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Abstract

In an article titled “Thinking about the Brain,” published in a special issue of Scientific American devoted to the Brain in September 1979, Francis H.C. Crick wrote: “Reflecting on itself, the human brain has uncovered some marvelous facts. What appears to be needed for understanding how it works is new techniques for examining it and new ways of thinking about it.” He made several important observations about the technological limitations of the time, noting that electrodes cannot readily distinguish different types of neurons. Light could be an ideal signal to turn firing on and off in an alert animal (Crick 1999; Crick 1979). This conceptual blueprint was eventually realized experimentally through the groundbreaking work of Hegemann, Nagel, Deisseroth, and their colleagues, who harnessed the microbial channelrhodopsins to achieve fast, genetically targeted, light-driven control of neuronal activity (Boyden et al. 2005; Nagel et al. 2002; Nagel et al. 2003). Over the past two decades, optogenetics has become a mainstay of circuit neuroscience and is now emerging as a potential therapeutic approach. In light of the 2026 Nobel Prize awarded to optogenetics, this perspective narrates the evolution of neuroscience research before and after the advent of optogenetics, and summarizes how this technology has transformed our approaches to studying the brain.

Main

In the years preceding the advent of optogenetics, neuroscientists relied heavily on physical, pharmacological, and genetic tools to discern the working principles of the brain. Early insights derived from clinical case studies by Broca and Wernicke, such as brain lesions, paved the way for the very primal, albeit coarse, functional map linking brain regions to behaviors (Broca 1861; Geschwind 1979; Wernicke 1874). The seminal work of Cajal and Golgi provided fundamental insights into the cellular organization of the brain, revealing diverse neuronal types and their potential connections (Cajal 1889; DeFelipe 2025; Golgi 1873). While Adrian’s work established key principles of action potential generation and neuronal signaling, Sherrington studied the spinal reflexes in detail and introduced the concept of synapse (Adrian 1914; Adrian and Zotterman 1926; Levine 2007; Sherrington 1906). The pioneering work of Dale and Loewi established the role of chemical neurotransmission in neuronal communication, demonstrating how nerve stimulation can trigger the release of chemical messengers that transmit signals to the other (Bechtel 2023; Dale 1914; Loewi 1921).

The modern history of ion channel physiology and neuronal signaling started with the transformative studies done by Hodgkin and Huxley. They elucidated the ionic mechanisms underlying action potential generation and propagation, establishing a quantitative framework for understanding neuronal excitability (Hodgkin and Huxley 1939, 1952; Schwiening 2012). This also laid the foundation for the invention of patch-clamp technique by Erwin Neher and Bert Sakmann, which enabled the recording of currents through single ion channels in neurons and earned them the 1991 Nobel Prize in Physiology or Medicine (Hamill et al. 1981; Neher and Sakmann 1976). Later, Roderick MacKinnon and his colleagues succeeded in demonstrating the structure of potassium ion channel to deepen our knowledge of single-channel physiology (Doyle et al. 1998). Eric Kandel's research group’s innovative work using Aplysia

demonstrated how activity-dependent modifications in synaptic strength and intracellular signaling facilitate learning and memory, thus connecting cellular physiology to behavior (Carew et al. 1981; Hawkins et al. 1983). Building on this foundational knowledge of brain physiology, researchers started with pharmacological perturbations of neuronal populations during behavior, such as the focal application of tetrodotoxin (Bermudez-Rattoni et al. 1991), lidocaine (Packard and McGaugh 1996), or muscimol (Majchrzak and Di Scala 2000), to investigate causal roles. Although these approaches provided better structure-function relationships, methods for rapidly and selectively manipulating neuronal circuits based on molecular identity of specific neuronal subtypes remained limited.

Genetic approaches introduced molecular specificity and illuminated the contributions of specific genes, RNAs, and proteins to learning, memory, and activity-dependent synaptic plasticity (Chen and Tonegawa 1997). However, the precise temporal control of activity in intricate neuronal networks remained elusive. In the late 1980s, Capecchi, Evans, and Smithies achieved gene targeting in mice through homologous recombination, a breakthrough that earned them the 2007 Nobel Prize in Physiology and Medicine (Doetschman et al. 1987; Evans and Kaufman 1981; Smithies et al. 1985; Thomas and Capecchi 1987). This enabled the scientific community to carry out molecularly specific studies across neuroscience and other disciplines. These advances progressively deepened our understanding of brain function, as demonstrated by discoveries of impaired long-term potentiation in hippocampal neurons and correlated loss of spatial memory in an α-CaMKII-knockout mouse model (Silva et al. 1992). Homologous recombination in embryonic stem cells enabled the generation of constitutive knockout mice, revealing critical roles for ion channels, receptors, and signaling molecules in synaptic transmission and plasticity (Gerlai 2016; Thomas and Capecchi 1987). However, the interpretation of these findings was often complicated by developmental compensatory mechanisms and, in some cases, reduced viability following whole-organism gene deletion.

Subsequently, the discovery of site-specific recombination machinery using bacteriophage P1 enabled conditional gene knockout studies in neuroscience research, overcoming the limitations associated with embryonic lethality following the constitutive deletion of essential genes (Gaveriaux-Ruff and Kieffer 2007; Sauer and Henderson 1988). Cre/loxP recombination allowed cell-type-specific gene excision/inversion, while the Tet-Off/Tet-On systems provided temporal control over transgene expression through doxycycline administration (Gossen and Bujard 1992; Tsien et al. 1996a). The conditional knockout of NR1 subunit of *NMDA* receptor specifically in the hippocampal CA1 region using the Cre/loxP system provided evidence for the essential role of the hippocampus in spatial memory (Tsien et al. 1996b). Viral vectors, including lentiviruses, adenoviruses, and herpes simplex viruses (HSVs), extended the spatial and temporal selectivity of genetic manipulation in adult animals, enabling region-specific overexpression, dominant-negative interference, or reporter expression without germline modification (Davidson and Breakefield 2003). Pharmacogenetic approaches, beginning with Receptors Activated Solely by Synthetic Ligands (RASSLs), and later advancing to Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), enabled the selective and reversible modulation of genetically defined neuronal populations through systemic administration of synthetic ligands, complementing

faster but more technically demanding optogenetic approaches (Armbruster et al. 2007; Coward et al. 1998; Roth 2016).

Although these technological advances propelled neuroscience toward breakthroughs, they also highlighted a critical gap: the lack of methods capable of manipulating defined neuronal subtypes with millisecond-scale temporal precision and circuit-level spatial specificity, as required to control fast network activity (Deisseroth 2015).

Understanding the functional principles underlying brain and behavior is essential for disease prognosis, therapeutic interventions, and palliative care for patients with neurological and psychiatric disorders. Brain dysfunctions are inherently complex because of the extensive interconnectivity of neuronal networks and multifactorial aetiologies, including genetic predispositions, environmental stressors, and other biological factors (Abraham et al. 2025; Abraham and Pardasani 2022; Bhowmik et al. 2023; Pandey et al. 2024). The human brain contains billions of neurons interconnected through trillions of synapses, with substantial molecular heterogeneity across neuronal subpopulations. Owing to this extensive connectivity, local perturbations rarely remain confined to their site of origin; instead, they can propagate along connected circuits and alter activity in both upstream and downstream regions. Thus, any lesion or molecular defect can generate a plethora of comorbidities. Many brain disorders can therefore be conceptualized as “network disorders,” in which disruptions in interconnected circuits contribute to diverse sensory, cognitive, autonomic and motor impairments (Fornito et al. 2015; Stam 2014).

Given this extensive connectivity, circuit-level mapping of molecularly defined neuronal populations is the way forward in neuroscience research. This becomes particularly important when patients share a molecular risk factor but differ in the neural circuits and functions affected. Therefore, without circuit-level resolution, therapeutic interventions may be misdirected, especially in disorders characterized by mixed symptomatology. Moreover, the cortical-subcortical networks rely on a finely regulated balance between excitatory and inhibitory signaling (Gschwend et al. 2015; Pandey et al. 2025; Pardasani et al. 2023). This balance shapes circuit dynamics, enhances the signal-to-noise ratio, and facilitates the propagation of precisely tuned signals to interconnected brain regions, thereby orchestrating sensory perception and behavior (Abraham et al. 2010; Mahajan et al. 2025). Given these complexities, precise targeting and functional perturbation of specific neural circuits without affecting the neighbouring cells, passing axon bundles, or unrelated circuits has long been a central challenge in neuroscience research. This created a pressing need for tools capable of addressing these challenges in causal circuit dissection by combining cell-type-specific targeting with rapid and reversible control at the level of individual neuronal activity. Francis Crick highlighted the methodological limitations of the tools available at the time for investigating neural circuit functions and proposed optical control of defined neuronal populations. He envisioned a future in which specific cell types could be rendered genetically light-sensitive, enabling their rapid and selective modulation with light while leaving other cells unperturbed (Crick 1999). At the time, he could scarcely have anticipated the extent to which this idea would transform both basic and clinical neuroscience.

Optogenetics, recognized by the 2026 Nobel Prize in Physiology or Medicine, builds on a long history of microbial rhodopsin research. This trajectory began with the isolation of bacteriorhodopsin from *Halobacterium* membranes in 1970, establishing the existence of light-driven ion pumps in microbes (Oesterhelt and Stoeckenius 1971). The subsequent identification of halorhodopsin, a light-activated chloride pump, expanded the known functional repertoire of microbial opsins beyond proton transport (Lanyi and MacDonald 1977; Schobert and Lanyi 1982). In the revolutionary studies published by Georg Nagel, Peter Hegemann, and their colleagues identified the microbial channel-forming rhodopsins ChR1 and ChR2, present in the eyespot membrane of green alga *Chlamydomonas reinhardtii.* These were identified in a follow-up of the phototaxis behavior shown by Chlamydomonas (Harz et al. 1992; Nagel et al. 2002). Further, they expressed these microbial opsins in frog oocytes and demonstrated that these proteins function as light-gated cation channels capable of depolarizing cells in a heterologous system (Nagel et al. 2003).

A key concern was whether these light-sensitive microbial opsins, which require all-trans retinal as a light-absorbing cofactor, would function effectively in mammalian neurons. Crucially, mammalian neurons possess the necessary chromophore, all-trans retinal, a vitamin A derivative produced through endogenous retinoid pathways. In a landmark study published in 2005, Deisseroth and colleagues successfully expressed ChR2 in cultured hippocampal neurons and demonstrated that light illumination could alter neuronal excitability, establishing the feasibility of integrating microbial opsins into neuronal systems (Boyden et al. 2005). Owing to its relatively large photocurrents, ChR2 was readily adapted for reliable, optogenetic modulation of neuronal activity and rapidly became the most widely used actuator for light-induced neuronal excitation. In subsequent years, ChR2/NpHR/Arch systems were successfully implemented in mammalian models to demonstrate bidirectional control of neuronal activity using different wavelengths of light (Chow et al. 2010; Zhang et al. 2007). These advances established optogenetics as a powerful method for investigating neural circuits underlying behavior. In 2010, Nature Methods named optogenetics as “Method of the Year,” recognizing its transformative impact on circuit interrogation with millisecond-scale temporal precision, which is evidenced by the annual citation landscape of optogenetics (2011) (Fig. 1).

The ensuing years witnessed an explosion in the development of engineered and naturally occurring opsin variants designed to improve light sensitivity, increase photocurrents, enhance tissue penetration, and facilitate multiplexed experiments combining optogenetic manipulation with techniques such as calcium imaging (Fenno et al. 2011; Kim et al. 2017; Klapoetke et al. 2014; Lin et al. 2013a). These advances included red-shifted channelrhodopsins, such as Crismson and ReaChR, inhibitory ion pumps, and anion-conducting channels (Chow et al. 2012; Chow et al. 2010).

These developments expanded the original family of microbial rhodopsins into a diverse, continually growing repertoire of tools for the precise spatiotemporal control of neural activity. Opto-GPCRs represent a further advancement in optogenetic technology. This enables precise, cell-type–specific optical control of G-protein-coupled receptor signaling involved in neuromodulation, synaptic plasticity, and state-dependent circuit dynamics-processes that cannot be fully investigated using ion-channel–

based optogenetics alone. Thus, Opto-GPCRs address a critical gap by allowing researchers to examine neuromodulatory signaling with the spatial and temporal precision afforded by channelrhodopsins (Mahn et al. 2021; Wietek et al. 2024)

A major advantage of optogenetics is that it can be combined with Cre-lox recombination system and synapse-crossing viral vectors to target connected neuronal ensembles within a circuit (Nectow and Nestler 2020). By combining anterograde or retrograde viral vector strategies with cell-type-specific genetic tools, researchers can restrict opsin expression to neurons defined by their projection targets or connectivity (Packer et al. 2013; Sjulson et al. 2016). This enables the selective manipulation of inputs to, and outputs from, a brain region rather than indiscriminately targeting neurons at the manipulating site. Such approaches facilitate causal studies on how specific synaptic connections contribute to network dynamics and behavior, establishing optogenetics as a powerful tool for the functional dissection and manipulation of defined neural circuit architectures.

Following the successful application of optogenetics to excite hippocampal neurons, Karl Deisseroth's laboratory conducted numerous in vivo studies to investigate neural circuit mechanisms underlying brain functions in health and disease. As examples, they dissected out the hypothalamic circuit involved in arousal and demonstrated that optogenetic stimulation of afferent axons in the basal ganglia could alleviate Parkinsonian symptoms in an animal model of the disease (Adamantidis et al. 2007; Gradinaru et al. 2009). Furthermore, a 2011 study published in Nature dissected the specific amygdala circuit involved in regulating anxiety levels (Tye et al. 2011). During 2012 to 2016, Susumu Tonegawa's lab used optogenetics to tag and manipulate memory engram cells, demonstrating that their optogenetic reactivation could result in creating a false memory in mice (Ramirez et al. 2013). Around the same time, structural studies published in Science elucidated the molecular architecture of channelrhodopsins, providing insights that facilitated the engineering of red-shifted opsins with improved properties, including faster kinetics and deeper tissue penetration (Volkov et al. 2017) (Fig. 2).

From 2017 to 2026, optogenetics expanded into the studies of systemic physiology. Notably, a 2023 study published in Nature reported the use of optogenetics to modulate cardiac activity, providing insights into the relationship between cardiac acceleration and anxiety (Hsueh et al. 2023). As many fundamental research findings have been made using this technology, it also paved the way for translational applications, leading to clinical trials using optogenetics to vision restoration in patients with retinitis pigmentosa (RP), a genetic disorder characterised by progressive vision loss. A landmark study, published in Nature Medicine in 2021, demonstrated partial recovery of visual function in a blind patient using the red-shifted opsin ChrimsonR in combination with light-stimulating goggles (Sahel et al. 2021; Suleman 2025). In recognition of its transformative contributions to neuroscience, optogenetics won the 2026 Nobel Prize in Physiology or Medicine, and continues to attract considerable scientific attention.

Looking ahead, optogenetics is expected to enable increasingly precise neural circuit manipulations, extending to the subcellular level through the integration of millisecond-scale temporal control and

closed-loop stimulation strategies. The development of subcellular optogenetic tools has enabled researchers to dissect the molecular and cellular mechanisms underlying brain functions with enhanced spatiotemporal precision (Karunarathne et al. 2015; Rost et al. 2017). Control of synaptic release has become possible through the development of optogenetic tools such as pHoenix and chromophore-assisted light inactivation (CALI) (Lin et al. 2013b; Rost et al. 2015). Furthermore, large-scale electrophysiological and optical recordings can be integrated with closed-loop optogenetic stimulation systems to modulate neuronal activity dynamically, thereby improving the precision in controlling neural circuit dynamics (Shang et al. 2024; Zaaimi et al. 2023; Zhang et al. 2018). Recent optogenetic studies targeting inhibitory networks have also revealed mechanisms underlying the integration of multimodal sensory information within a single sensory system (Mahajan et al. 2025). Such approaches will facilitate the investigation of pathological circuit dynamics and support the development of targeted interventions for neurological and psychiatric disorders (Lüscher et al. 2025). Ultimately, the integration of molecular specificity, temporal precision, and activity-dependent closed-loop control could establish optogenetics as an increasingly powerful platform for investigating the neural circuit dynamics underlying diverse behaviors and identifying novel therapeutic strategies. However, clinical implementation will require continued advances in optical delivery systems, safety, scalability, and long-term efficacy.

Declarations

Declaration of Generative AI and AI-assisted technologies in the writing process: During the preparation of this work the authors used ChatGPT in order to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication. Citation contexts and mentioning/contrasting classifications were evaluated using Smart Citations via scite.ai for making Figure 1.

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Author Contributions

N.M.A. and S.P. wrote and edited the manuscript.

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Figures

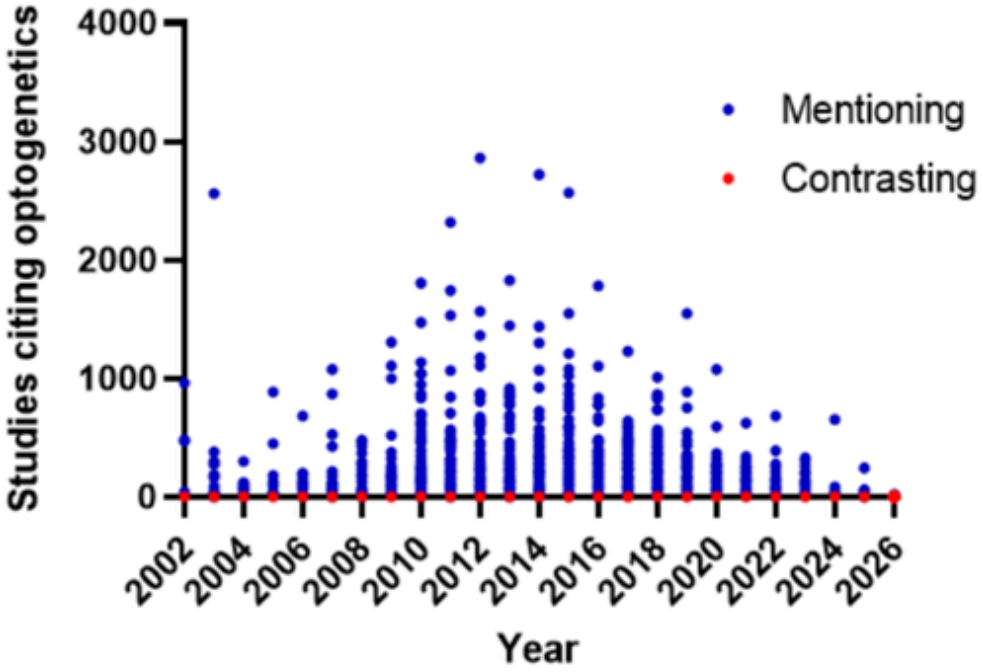


Figure 1

Annual citation landscape for optogenetics (2005–2026). The plot shows the year-wise number of publications that cite work on optogenetics, as retrieved from Scite AI using the query “optogenetics.” Data points represent total citations mentioning optogenetics (Blue) and citations that contrast optogenetic approaches (Red). This pattern underscores the growing importance and influence of optogenetics in neuroscience research.

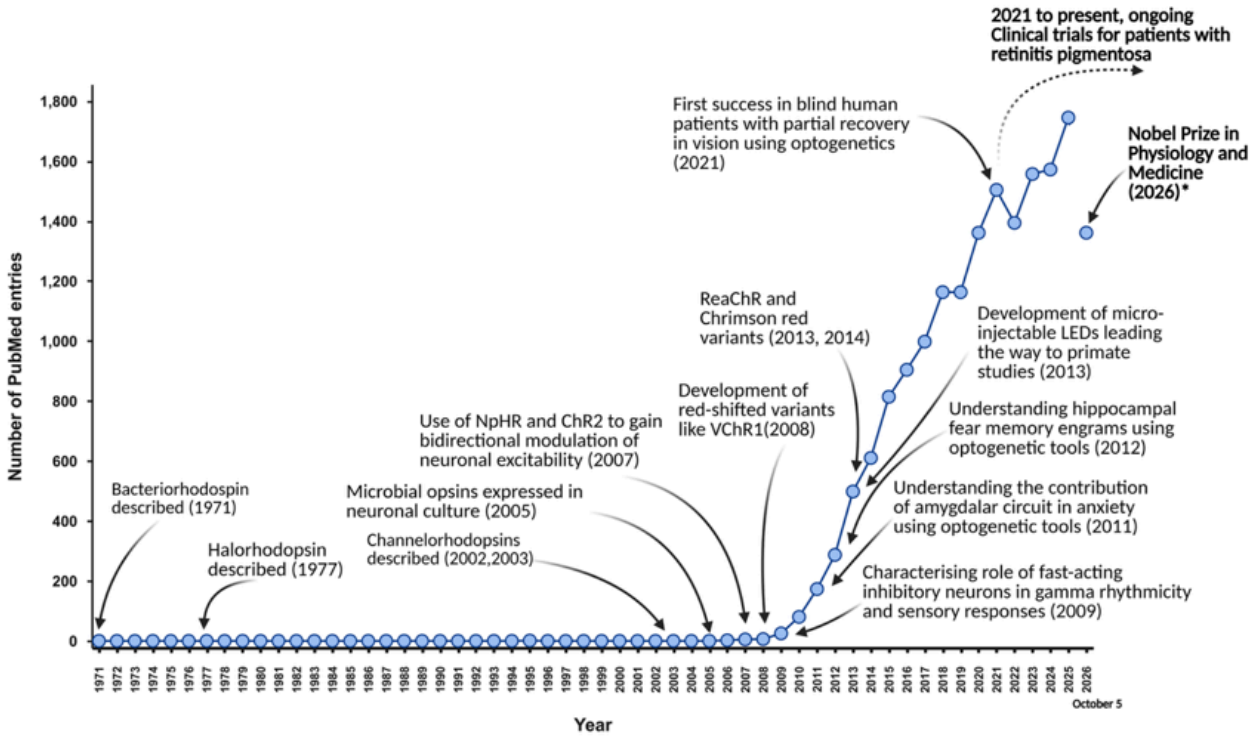


Figure 2

Timeline of key discoveries and breakthroughs in optogenetics (1971–2026). Landmark events include the identification of microbial rhodopsins (bacteriorhodopsin 1971 (Oesterhelt and Stoeckenius 1971), halorhodopsin 1976 (Matsuno-Yagi and Mukohata 1977), channelrhodopsin (2002 and 2003) (Nagel et al. 2002; Nagel et al. 2003), the first optogenetic control of neuronal spiking (2005) (Boyden et al. 2005), extension to freely moving animals (Zhang et al. 2007), engineering of red-shifted opsins (Zhang et al. 2008), interrogation of circuits underlying arousal (Adamantidis et al. 2007), memory engrams and anxiety using optogenetics (Ramirez et al. 2013; Tye et al. 2011), development of wireless and injectable micro-LED interfaces (Kim et al. 2013), and the translation to human vision-restoration trials (Sahel et al. 2021) culminating in the 2026 Nobel Prize in Physiology or Medicine (Adapted and modified from(Deisseroth 2011)).