



Published in final edited form as:

J Magn Reson Imaging. 2025 April ; 61(4): 1597–1617. doi:10.1002/jmri.29575.

Neuromodulation in small animal fMRI

Li-Ming Hsu, PhD^{1,2,4}, Yen-Yu Ian Shih, PhD^{1,2,3}

¹Center for Animal Magnetic Resonance Imaging, The University of North Carolina at Chapel Hill

²Biomedical Research Imaging Center, The University of North Carolina at Chapel Hill

³Departments of Neurology, The University of North Carolina at Chapel Hill

⁴Departments of Radiology, The University of North Carolina at Chapel Hill

Abstract

The integration of functional magnetic resonance imaging (fMRI) with advanced neuroscience technologies in experimental small animal models offers a unique path to interrogate the causal relationships between regional brain activity and brain-wide network measures—a goal challenging to accomplish in human subjects. This review traces the historical development of the neuromodulation techniques commonly used in rodents, such as electrical deep brain stimulation, optogenetics, and chemogenetics, and focuses on their application with fMRI. We discuss their advantageous roles in uncovering the signaling architecture within the brain and the methodological considerations necessary when conducting these experiments. By presenting several rodent-based case studies, we aim to demonstrate the potential of the multimodal neuromodulation approach in shedding light on neurovascular coupling, the neural basis of brain network functions, and their connections to behaviors. Key findings highlight the cell-type and circuit-specific modulation of brain-wide activity patterns and their behavioral correlates. We also discuss several future directions and feature the use of mediation and moderation analytical models beyond the intuitive evoked response mapping, to better leverage the rich information available in fMRI data with neuromodulation. Using fMRI alongside neuromodulation techniques provide insights into the mesoscopic (relating to the intermediate scale between single neurons and large-scale brain networks) and macroscopic fMRI measures that correlate with specific neuronal events. This integration bridges the gap between different scales of neuroscience research, facilitating the exploration and testing of novel therapeutic strategies aimed at altering network-mediated behaviors. In conclusion, the combination of fMRI with neuromodulation techniques provides crucial insights into mesoscopic and macroscopic brain dynamics, advancing our understanding of brain function in health and disease.

Keywords

fMRI; optogenetics; chemogenetics; deep brain stimulation

INTRODUCTION

This review explores the integration of functional magnetic resonance imaging (fMRI) with advanced neuromodulation techniques in small animal models. We begin with an overview of fMRI techniques in rodent models, followed by detailed discussions of three primary neuromodulation methods: electrical deep brain stimulation (DBS), optogenetics, and chemogenetics. For each technique, we trace its historical development, examine key fMRI studies, highlight applications in rodent models, and discuss technical considerations. We then explore integrative analysis approaches, including mediation and moderation models, for leveraging the rich data obtained from these combined techniques. Throughout the review, we emphasize how these methods contribute to our understanding of brain function, neurovascular coupling, and the neural basis of behavior. We conclude by discussing future directions and the potential impact of these approaches on neuroscience research and therapeutic strategies.

Complementary Neuromodulation and fMRI Techniques

Understanding the complex mechanisms underlying brain function and dysfunction has been a central goal of neuroscience research. While fMRI offers unparalleled insights into the brain's spatial and temporal dynamics, its full potential is unlocked when combined with complementary neuroscience technologies. Performing fMRI in rodent models offers a platform to investigate the topological and functional basis of behavior and neurological diseases. This review focuses on integrating fMRI with cutting-edge neuromodulation methodologies, including electrical DBS, optogenetics, and chemogenetics, which offer time-locked and/or cell-type-specific controls of information flow within the brain.

DBS modulates neural circuits, with its versatility in parameter selection, offering a direct bridge between animal studies and human clinical applications (1). Optogenetics provides an unmatched level of specificity, manipulating neurons with light to parse out neural circuitry dynamics and their consequent behavioral influences (2). Chemogenetics allows for non-invasive and remote cellular modulation through receptor signaling, enabling researchers to study the sustained effects of modulation (3). Each of these techniques contributes uniquely to interrogating distinct aspects of brain signaling and has the potential to precisely control behaviors or provide interventions for neurological and psychiatric conditions.

Overview of fMRI Techniques in Rodent Models

fMRI has revolutionized the field of neuroscience, offering a non-invasive window into the complex dynamics of brain activity patterns in health and disease across species. Though fMRI experiments can be conducted using several specialized acquisition techniques that measure cerebral blood flow, cerebral blood volume, and cerebral metabolic rate of oxygen (CMRO₂), the technique primarily relies on the Blood Oxygen Level Dependent (BOLD) contrast (4). The BOLD contrast is a measure of magnetic field susceptibility effects based on the changes in the amounts of oxygenated and deoxygenated blood across the brain, serving as an indirect indicator of neuronal activity. This technique has evolved into various research strategies, including, but not limited to, stimulus-evoked (task-related or event-related) fMRI, which captures brain activity in response to task execution and

discrete events or stimuli with precise temporal resolution (5), its variant for observing brain responses to drug administration (6), and resting-state fMRI for examining intrinsic coupling of activity dynamics across brain regions (7). Amidst these advances, naturalistic fMRI emerges as a promising frontier, reflecting a shift towards studies that mimic real-life scenarios to understand the brain's functioning in environments that closely resemble those encountered in the natural world (8). This approach is increasingly recognized for its potential in human neuroimaging research. However, the application of naturalistic fMRI in animal studies remains relatively unexplored but holds significant promise for future research. Techniques such as zero echo time (ZTE) (9) and multi-band SWeep Imaging with Fourier Transformation (MB-SWIFT) (10) have shown promises in advancing this direction, offering reduced acoustic noise and susceptibility/motion artifacts, which are useful for accurately mapping brain activity in more naturalistic settings.

As an extended method, pharmacological fMRI (phfMRI) extends the utility of fMRI into the realm of pharmacology, allowing for assessment of drug effects on brain function. phfMRI can elucidate the brain-wide dynamics and spatial site of action for various compounds, offering valuable data on the efficacy and potential side effects of drugs. In rodent studies, this approach is crucial for preclinical drug development, providing a bridge between molecular pharmacology and behavioral neuroscience (6, 11-13). Unlike direct brain stimulation methods such as electrical, optogenetic, or direct pharmacological injections (14, 15), which induce comparatively rapid, localized changes in neuronal activity, phfMRI often involves the systemic administration of pharmacological agents. This difference means that phfMRI generally observes slower, more widespread responses that cannot be repeated as rapidly due to the systemic distribution and metabolic clearance of drugs. Analytically, phfMRI emphasizes changes in functional connectivity or drug-induced network alterations, contrasting with the assessment of immediate, task-evoked brain activity (16). This requires models that account for the extended temporal effects of drugs on brain activity.

Analyzing spontaneous fMRI signals offers a unique window into the brain's intrinsic coupling of activity (or often referred as functional connectivity), without the need for specific tasks or stimuli (7, 17). During the "rest state", the spontaneous fluctuations over time can classically be used to examine temporal correlation focusing on low frequency (0.01–0.1 Hz) BOLD fluctuations between spatially distinct brain regions (18, 19). Emerging studies indicate that signals at frequencies higher than 0.1 Hz also contain useful information (18-21). The term "resting-state fMRI" may sometimes be a misnomer, particularly in studies where the brain is not in a typical "resting" condition. Therefore, we believe that associating fMRI with the terms such as functional connectivity, intrinsic activity, or coupling could be more encompassing, reflecting the broad spectrum of studies that explore changes in correlated brain activity, whether during various behaviors or under experimental neuromodulation, as described in this article. In both humans and rodents, fMRI has revealed large-scale brain networks involved in critical functions such as auditory processing, motor function, visual processing, memory, and executive functioning. In rodents, the default mode network (DMN), anchoring at the retrosplenial cortex, was first demonstrated by Lu et al. (22) with its constituent (23) in rats, and then also identified by Liska et al. in mice (24) with detailed anatomical architecture reported more recently

(25). Its functional implications have since then been increasingly investigated (26-29). The salience network (SN), anchoring at the ventral anterior insular cortex, was also identified and investigated in rodent models (20, 28, 30, 31). Tsai et al. demonstrated that the rat SN reacts to conditioned cues and shows increased functional connectivity with the DMN during conditioned heroin withdrawal, emphasizing its crucial role in mediating attentional and emotional processes related to addiction (30).

With the advent of high spatial resolution fMRI (32, 33), researchers are now able to discern functional activity at the layer-specific level within the cortex, revealing patterns of neural engagement. This advancement, coupled with high-resolution studies at ultra-high magnetic fields (34), allows for detailed mapping of neural circuits and their contributions to overall brain function. On the temporal front, advancements in fast fMRI techniques (35) have significantly improved the ability to capture dynamic brain activities, offering a window into the rapid changes in neural communication. A pivotal shift in fMRI research methodologies is the transition from anesthetized to awake rodent studies (36-39). This transition addresses the confounding effects of anesthetics on brain activity measurements. Awake rodent fMRI studies present a more accurate reflection of natural brain states and behaviors, facilitating a closer approximation to human fMRI studies, which are predominantly conducted without anesthetics. As fMRI technology and analytical methods continue to evolve, especially in the realm of awake imaging, the future of rodent fMRI research is poised for significant breakthroughs. Emphasizing awake imaging as a key direction aligns with the imperative to mitigate anesthetic confounds, enabling a more authentic exploration of the brain's functional architecture and its relationship with behavior and cognitive processes. Below we review the technical evolution of DBS, optogenetics, and chemogenetics, highlight key progress made in the field of animal fMRI, and discuss technical considerations and future opportunities.

ELECTRICAL DEEP BRAIN STIMULATION (DBS)

Historical Development of DBS

DBS allows testing our understanding of neural circuits and exploring potential circuit-based treatment for neurological and neuropsychiatric disorders. Initially approved by the FDA for Parkinson's Disease in 1997 (40), DBS has expanded its reach to include dystonia (2003), obsessive-compulsive disorder (2009), and epilepsy (2018) (41). DBS offers easily adjustable stimulation parameters (e.g., frequency, pulse-width, and amplitude), enabling the study of brain function and facilitating more seamless translation of research finding between animals and humans (1). In fact, the concept of DBS was initially explored in rodent models, where its potential therapeutic effects on movement disorders were examined on the subthalamic nucleus (STN) based on knowledge of the basal ganglia circuitry in movement control (42, 43). These early successes laid the groundwork for its application in humans. This translation was significantly propelled by the work of Benabid and Pollak (44). Although their initial study did not focus directly on the STN, their seminal discoveries of the thalamic DBS effects have demonstrated the efficacy of high frequency DBS in alleviating movement disorders. Since the establishment of DBS as a clinical procedure in the 1990s (45), the integration of DBS with brain mapping technologies, such as fMRI, has

emerged as a key area of interest. This synergy is particularly crucial for researchers aiming to decipher the brain's information flow or to interrupt pathological signals at the frequency and fidelity that are difficult to achieve by other competing neuromodulation techniques.

DBS fMRI studies

The integration of DBS and fMRI technologies enables researchers to visualize and quantify the immediate effects of DBS on brain activity in action, enhancing our understanding of the underlying mechanisms of DBS therapy. The convergence of DBS with fMRI in small animals began in the early 2000s as researchers aimed to navigate the technical challenges inherent in integrating these methods (46-48). A notable step was made in 2004, when Shyu et al. reported the use of a bended glass-coated carbon fiber electrode underneath a surface coil at 4.7T to demonstrate reliable thalamic DBS-induced changes in BOLD activity and c-Fos expression within the downstream ipsilateral anterior cingulate cortex (46). Building on this, Dunn et al. introduced a protocol for coating carbon fiber electrodes with polymer solution for chronic applications at 9.4T (47), which was among the initial studies to illustrate the feasibility of using chronically implanted electrode to monitor evoked BOLD activities within the brain. Further research expanded the range of “MR-compatible” electrode materials, exploring alternatives such as platinum–iridium (Pt–Ir) alloy electrodes (48-53), which, despite their translational practicality and accessibility, introduce comparatively stronger susceptibility artifacts. Tungsten microwire electrodes, available commercially (54-58) or as custom fabrications (46), present smaller susceptibility artifacts. Recent advances have introduced designs using graphene fiber electrodes (Fig. 1) (59) and thin-film polyimide-based gold-copper electrodes (60-63) further minimizing susceptibility artifacts and enabling the acquisition of more comprehensive fMRI maps without signal loss. The continuous advances of thin-film electrodes, notable for their superior biocompatibility, flexible manufacturing for variable channel counts, inherent MR-compatibility, and broad usage in research beyond MRI, indicate a promising increase in their use alongside fMRI in the foreseeable future.

From the perspective of imaging sequences, the innovative applications of ZTE (9) or MB-SWIFT techniques, as initially demonstrated by Lehto et al. (10) and subsequently elaborated in multiple follow-up studies (64-70), have shown significant potential. Owing to the sequences' insensitivity to T2* effects, these methods provide fMRI maps nearly free of susceptibility artifacts, thereby enabling the use of a wider range of electrode materials in high magnetic field environment. Importantly, these techniques enable the visualization of activity changes near an implanted lead – a capability that proves challenging with conventional gradient-echo-based fMRI methods. They also facilitate studies of DBS effects in awake animals, free from confounding effects caused by anesthetics, thanks to the reduced acoustic noise.

Applications in Rodent Models

While continuing advances in MR-compatible electrodes and imaging sequences have notably improved the ability to perform DBS during fMRI, with only a few exceptions (55), rats are predominantly used over mice due to their larger brain size in affording electrode susceptibility artifacts and more stable physiology. DBS-fMRI studies of the hippocampus

and its related pathways (48, 49, 51, 67, 71-73) have shown significant modulation of brain networks involved in memory and cognition. This suggests potential applications in enhancing memory performance and offering therapeutic benefits for neurological conditions like Alzheimer's disease and temporal lobe epilepsy. A seminal study by Canals et al. (51) demonstrated the pioneering use of hippocampal DBS to map long-term potentiation (LTP), showing that LTP induces a global reorganization of activity patterns beyond hippocampus, enhances interhemispheric communication, and engages limbic and neocortical circuits due to changes in hippocampal synaptic strength (Fig. 2). DBS of the STN and internal globus pallidus has demonstrated frequency-dependent responses in the rat brain, particularly affecting the ipsilateral motor cortex (74). DBS in the ventroposterior thalamus has allowed for repetitive sampling of thalamocortical responses, revealing consistent and specific BOLD responses in the somatosensory cortex. This highlights the potential of using DBS-fMRI to study brain plasticity and the dynamics of specific neural circuits over time (56). Similarly, DBS of the substantia nigra pars reticulata (SNr) and external globus pallidus (GPe) revealed extensive functional connectivity within the basal ganglia, uncovering unexpected connectivity patterns and evoked responses. These findings suggest a more complex role for the SNr and GPe in modulating neural networks (75). DBS of the nucleus accumbens (NAc) has been demonstrated to modulate the prefrontal cortex and limbic areas, potentially affecting emotional, cognitive, and reward processing (57). DBS in the infralimbic cortex leads to specific alterations in amygdala activity, showcasing the utility of precision neuromodulation in mapping and modulating brain responses (64). DBS of the lateral habenula (LHb) in rat models of depression demonstrated an immediate alleviation of depressive-like symptoms, with fMRI revealing DBS-induced activation of multiple regions within the LHb's afferent and efferent circuitry (76). Furthermore, DBS-fMRI studies targeting the infralimbic cortex suggest sex-specific neural dynamics in anxiety, highlighting the need for gender-informed therapeutic strategies (77). DBS targeting the ventral tegmental area (VTA) has proven to be a consistent method for eliciting dopamine release. This neurostimulation approach has significantly contributed to the advancement and empirical validation of integrated techniques combining fast-scan cyclic voltammetry (FSCV) with fMRI. Such simultaneous FSCV-fMRI methodologies enable real-time monitoring and mapping of dopamine neurotransmission dynamics in response to VTA stimulation, offering valuable insights into dopaminergic signaling mechanisms (Fig. 3) (52). Central thalamic DBS (CT-DBS) has emerged as a promising intervention for enhancing cognitive functions and addressing social deficits in neurological and psychiatric disorders, including autism spectrum disorder (ASD), with fMRI findings illustrating CT-DBS's role in modulating the corticostriatal, corticolimbic, and thalamocortical networks (60, 78). DBS in the STN of Parkinsonian rats revealed robust BOLD responses across the basal ganglia-thalamocortical network, indicating that STN-DBS modulates both motor and non-motor pathways, potentially through both orthodromic and antidromic signal propagation (Fig. 1) (59). These examples collectively underscore the pivotal role of integrating DBS and fMRI in advancing our understanding of neurological diseases in animal models, facilitating a deeper comprehension of neural circuitry and pioneering innovative treatment approaches, thereby setting the stage for future clinical applications.

DBS fMRI Considerations

Considering the future of DBS in neuromodulation research, it is imperative to address both the ethical and practical challenges associated with its use. The capacity of DBS to drive both incoming and outgoing axons as well as neurochemical activity raises important questions about the specificity and precision of DBS in modulating neural circuits (1, 79-82). This effect complicates the delineation of DBS-induced changes, necessitating advanced analytical methods to unravel the complex neural interactions at play. In addition, the ethical considerations of subjecting animal models to invasive procedures for the sake of research demand rigorous justification. The pursuit of less invasive neuromodulation approaches remains a critical area of research, promising to expand our toolkit for studying and modulating brain function with fewer ethical concerns and practical limitations.

In response to these challenges, the field is witnessing the emergence of ultrasound, a non-invasive technique that is promising for modulating activities deep in the brain. This approach leverages the use of focused ultrasound waves from multiple transducers, offering a potential solution to the limitations posed by traditional DBS and promising to expand toolkit for studying and modulating brain function (83-85). We anticipate more relevant studies to emerge in the small animal fMRI space.

The synergy between DBS and fMRI holds potential to offer insights into neurological and neuropsychiatric disorders that do not present immediate behavioral feedback such as that in movement disorders. The potential for personalized or closed-loop therapy based on fMRI activity and connectivity metrics is significant (86), urging continued efforts to overcome technical hurdles and foster interdisciplinary collaboration. Specifically, the individual differences in DBS treatment responses are broadly documented in humans but not typically accounted for in small animal fMRI experiments. Future studies examining how baseline brain state and connectivity patterns predict neuromodulation outcomes may shed light on this issue.

OPTOGENETICS

Introduction to Optogenetics

Optogenetics has emerged as an essential tool for understanding the roles of cell types, circuits, and systems under both normal and pathological conditions (2). This revolutionary technique originated from the ingenious integration of light-sensitive proteins, known as opsins, into cells, allowing for the precise control of these cells upon light exposure. The conceptual foundation for optogenetics was laid in a 1979 *Scientific American* article by Nobel laureate Francis Crick, who articulated the paramount challenge in neuroscience: the need to control specific types of cells within the brain, while leaving others unaffected (87). In 1970s, Oesterhelt and Stoekenius made a breakthrough by discovering bacteriorhodopsin, a light-sensitive protein in microorganisms (88). Building on this foundation, Hegemann, Nagel, and colleagues identified and characterized channelrhodopsin in the early 2000s (89), a critical step towards the development of optogenetics. The field reached a seminal milestone with the work done in Karl Deisseroth's lab (90, 91) which enabled the introduction of channelrhodopsin-2 (ChR2) into mammalian neurons.

ChR2, when activated by light, opens channels in the membrane of neurons, leading to depolarization and the initiation of an action potential. The core technologies underpinning optogenetics include the genetic engineering methods used to express opsins in targeted cells, and the optical systems designed to deliver light of specific wavelengths to activate or inhibit cells. Notably, excitatory opsins like the ChR2 variants (90, 92) depolarize neurons to initiate firing, while inhibitory opsins such as archaerodopsin (Arch) (93) and halorhodopsin (NpHR) (94) hyperpolarize neurons, effectively silencing neuronal activity. These tools have been continually improved over the years and complemented by advances in fiber optics and light delivery systems (95-97), allowing precise spatial and temporal control of neuronal activity in vivo (98-101). Moreover, the development of a diverse array of opsin proteins, each with unique properties regarding light sensitivity, ion selectivity, and activation kinetics, enables more nuanced control over neuronal activity (95, 96).

Optogenetic fMRI Studies

The advent of optogenetics paired with fMRI technology has heralded a new era in the exploration of neural circuitry and functional connectivity in animal models. The pioneering work by Lee et al. initiated this journey, revealing how optogenetic stimulation of excitatory neurons targeted by CaMKII α promoter leads to modulation of BOLD signals (102). This effort allowed for the mapping of complex brain networks and laid the foundation for subsequent explorations into the intricacies of brain function. Kahn et al. built on this, examining the linearity of BOLD responses, further cementing fMRI's reliability as a gauge of neuronal activity (103). In followed up studies, Kahn et al. correlated optogenetic activation of neocortical neurons with fMRI signals (104), suggesting a link between neural firing and BOLD responses, whereas Yu et al. advanced the measurement of optogenetically driven fMRI responses from mesoscopic to microscopic, single vessel scale (Fig. 4) (33). Chen et al. innovatively combined optogenetic manipulation of neuronal Ca²⁺ signaling with single-vessel BOLD and CBV-fMRI, uncovering precise hippocampal hemodynamic responses related to specific neuronal activities (105). This study significantly enhances fMRI resolution, offering detailed insights into the hippocampus, a region often limited by conventional fMRI techniques. Collectively, the studies above have paved the way towards a better understanding of neural activation and its coupling with local and downstream vascular responses (106-108). Beyond demonstration of the roles of excitatory neuron stimulation (15, 102-104, 109-112), the precise selectivity of optogenetics has further elucidated the role of GABAergic, dopaminergic, serotonergic, cholinergic, and norepinephrine systems in shaping brain-wide activity patterns and behaviors. Studies examining GABAergic neurons, where optogenetic stimulation reveals a generally lower oxygen demand compared to excitatory activities, suggesting a nuanced metabolic landscape (113, 114). While excitatory neuron stimulation typically induces positive BOLD responses, inhibitory GABAergic neuron stimulation can lead to positive, negative, or biphasic responses, revealing the intricate dynamics between neuronal activity and cerebral hemodynamics (15, 115-123). For example, Cerri et al. delineate the contrasting fMRI responses with optogenetically induced elevated striatal neuronal activity: negative responses from stimulating GABAergic neurons in striatum and several of their relevant projections, versus positive responses from dopaminergic neuron stimulation (Fig. 5), highlighting the critical role of neurochemicals in modulating fMRI signal polarity

(15). Several studies focusing on optogenetic stimulation of dopaminergic neurons have collectively shed light on the precise topological landscapes of these neurons in striatal and prefrontal regions (124-126), linking their relevance to reward-seeking behaviors (124), as well as the impact of pairing dopamine stimulation on strengthening the representation of a discrete sensory stimulus (125). Recent studies employing optogenetics in conjunction with fMRI have significantly advanced our understanding of the serotonin, cholinergic, and norepinephrine systems. These studies have revealed the complex roles these neurotransmitter systems play in brain function and behavior. Research utilizing this combined approach has detailed the serotonergic system's brain-wide effects in response to acute stress and fluoxetine, a selective serotonin reuptake inhibitor (127). This exploration has unveiled the intricate, region-specific dynamics of the serotonergic system, illuminating its role in modulating brain function and stress responses through its extensive projections and receptor distributions. Significant advancements have been made in understanding the cholinergic system's role in modulating large-scale brain networks (39). Though cholinergic neurons comprise only a fraction of the basal forebrain's neuronal population, their activation via optogenetics significantly alters functional connectivity and BOLD signal amplitude within key neural networks such as the default-mode-like network. This alteration contrasts with effects observed from other neuron types in the basal forebrain, highlighting the cholinergic neurons' unique contribution to modulating attention, learning, memory, and emotional regulation. Their findings also reveal that cholinergic and non-cholinergic neurons collaboratively modulate global functional networks, driving behavior preferences towards either internally or externally oriented states. Further studies into the locus coeruleus-norepinephrine system have elucidated how its various firing patterns uniquely modulate brain activity, dynamics, and behavioral states (128). Tonic firing, with slower frequencies, affects prefrontal and limbic areas, influencing cognitive performance and arousal in a nonlinear manner. High-frequency bursts is linked to increased alertness and responsiveness to stimuli, enhancing network integration and facilitating brain state transitions. This study highlights the LC-NE system's critical role in brain flexibility and behavioral adaptation, demonstrating its complex influence on global brain dynamics through specific activity patterns. These findings highlight the regulatory role of the locus coeruleus in brain function, emphasizing its importance in facilitating transitions between brain states and optimizing cognitive performance.

The variety of viral expression strategies enables researchers to achieve multiplexed control of neural circuit activity with high specificity (96). For example, using retrograde vectors, recombinase-, or promoter-dependent strategies allows for the manipulation of selected cell types within a mixed population, whereas the point of light illumination at somata or axonal terminals in downstream brain regions offers different levels of pathway specificity. These approaches have been used to increase throughput in a single experimental session, where mixed populations can be manipulated or multiple regions can be independently targeted and controlled using distinct wavelengths of light. These multiplexing strategies can further incorporate naturalistic or pharmacological stimuli and enable simultaneous or sequential activation or inhibition of different cell populations, allowing for complex experimental designs. For example, Jung et al. exemplifies the controlled silencing of circuits downstream of somatosensory activity using fMRI. This study unveils a technique for suppressing

specific brain regions and reveals how interconnected regions contribute to information flow (129). A study by Cerri et al. demonstrates the concurrent use of optogenetics with intracranial pharmacology and fMRI, and showcases the utility of optogenetics to initiate antidromic spiking through terminal stimulation, while studying the effects of various pharmacological compounds on manipulating the evoked fMRI activity (15).

Applications in Rodent Models

Beyond research in neurovascular coupling and neurochemical influences of fMRI signals, several studies have leveraged optogenetic stimulation to map the causal relationship between selected nodes and functional network dynamics. Several studies have initially focused on the relation to sensory/motor functions and memory consolidation, highlighting the importance of large-scale spatiotemporal patterns in neural signal transmission, significantly advancing our understanding of systems-level brain network (101, 130-136). For example, frequency-dependent modulation of brain networks by distinct hippocampal stimulations has revealed how different stimulation frequencies can elicit varied network responses (137-140). Lee et al. provide a comprehensive analysis on how the anterior cingulate cortex contributes to the modulation of sensorimotor pathways in chronic pain, underscoring the role of this brain region in orchestrating movement avoidance behaviors in response to pain (141). Their findings elucidate the increased network strength of the anterior cingulate cortex in a chronic pain model, highlighting a critical aspect of how pain is not merely a sensory experience but deeply integrated with motor responses to form a cohesive defensive behavior. Adding to this, the work of Bernal-Casas et al. used dynamic causal modeling for optogenetic fMRI data to elucidate the cell-type-specific interactions within the basal ganglia-thalamocortical circuit, a key player in motor control. This circuit, comprising various brain regions and neuronal types, including D1- and D2-receptor-expressing medium spiny neurons, orchestrates complex motor behaviors through distinct pathways (142). A recent study by Menon et al. represents a reverse-translation case using rodent models to examine a hypothesis derived from human fMRI data modeling. Specifically, Menon et al. tackled the functional causal influence of anterior insular cortex on its related major network nodes, demonstrating feedforward optogenetic stimulation of insular neurons induces dynamic suppression and decoupling of the DMN (143). Interestingly, beyond the translational insights into anterior insular cortex's role in network switching, this study suggests that prelimbic and cingulate cortices, commonly identified as the rodent DMN nodes, may also involve in the SN. This implies distinct levels of network segregation between rodents and humans that warrants further investigations.

Optogenetic fMRI Considerations

As the field of optogenetics-fMRI advanced, the significance of addressing light-induced heating artifacts to avoid confounding fMRI signals has been underscored (144), alongside the necessity of recognizing and mitigating unspecific artifacts to preserve data integrity (145). Furthermore, the light-induced vasodilation, as indicated by Rungta et al. (146), introduces additional complexity, emphasizing the necessity to include essential controls. We expect the application of optogenetic fMRI to be extended to awake animals, as initially demonstrated by Liang et al. (110), to offer insights into the anesthetics-free brain dynamics. Along the same vein, Cover et al. (147) developed a method to image whole-brain

dynamics during optogenetic self-stimulation in mice, enabling concurrent observation of brain activity related to motivated behavior. Further, the innovative work by Kim et al. on whole-brain mapping of effective connectivity via fMRI with cortex-wide patterned optogenetics marks a significant step forward (Fig. 6) (101). It represents an exciting opportunity for modulating large-scale networks at high spatiotemporal precision beyond single-node interrogations as seen in conventional studies.

CHEMOGENETICS

Introduction to Chemogenetics

Chemogenetics, also known as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), represents an innovative technique that leverages the integration of receptor engineering with pharmacology to remotely control neuronal activity in experimental animals (3). This concept was initially used to describe how mutations affect enzyme activity and substrate specificities, exemplified by the chalcone isomerase in *Dianthus caryophyllus* flowers (148). Distinguished from optogenetics, which uses light and light-sensitive opsins for neuronal modulation, chemogenetics, as described by Bryan Roth et al. (3, 149), enable control over selected cellular functions with less invasive procedures, offering prolonged and sustained modulations. This proves especially advantageous for studying behaviorally relevant neural circuits similar to pharmacological approaches but with improved specificity. The development of DREADDs technology was pioneered by Armbruster et al. in the laboratory of Bryan Roth's (150), showing the engineered G-protein-coupled receptors (GPCRs) can be potently activated by an inert ligand. This innovation not only showcased the feasibility of neuromodulation using engineered receptors but also introduced clozapine-N-oxide (CNO) as a synthetic ligand that activates DREADDs. Subsequent enhancements in DREADD technology have broadened its application to various intracellular G-protein pathways (Gq, Gi, or Gs), enabling more sophisticated regulation of cellular responses beyond simple excitation or inhibition (151-153).

Similar to optogenetics, chemogenetics also allows cell body versus projection-specific targeting (154). For the later case, different levels of precision can be achieved by using viral vectors and Cre-recombinase strategies (155), such as injecting Cre-dependent DREADD vectors to the cell bodies of the targeted circuit and injecting retrograde Cre-expressing vectors in projection terminals, allowing DREADDs to be expressed in a specific projection. Alternatively, localized, intracranial injection of a DREADD actuator during experiments can also approach projection-specific effects. Similar to optogenetics, chemogenetics enables multiplexed modulation when distinct DREADDs are expressed in a cell-type-specific manner within different neuron populations. For example, (e.g., Gq-DREADDs (for activation) and KOR-DREADDs (for inhibition using a distinct actuator salvinorin B) can be expressed within the same subjects and be activated by different actuators (156). Importantly, at the time of data acquisition, the minimal invasive nature of chemogenetics allows its seamlessly integration with optogenetics or other neuromodulation methods. This integration facilitates the simultaneous or sequential activation or inhibition of multiple neural pathways with less space constraints, facilitating intricate experimental manipulations

of brain circuits during behavior or during fMRI. These advancements in chemogenetics, alongside the continuous development of DREADD actuators with improved affinity and specificity, pave the way for a simplified integration of selective neuromodulation technique with fMRI and other neuroscience tools in animal models.

Chemogenetic fMRI Studies

Integrating chemogenetics with fMRI offers the most simplified, and perhaps most efficient setting to examine cell type and circuit specific modulations on brain networks. This methodology marries the precise control over neuronal activity afforded by chemogenetics with the capability to visualize the spatiotemporal effects across the entire brain via fMRI. A foundational study in this field by Gozzi et al. showcased the potential of this integrative approach (157). Their work highlighted how the early variant of chemogenetic inhibition within the central nucleus of the amygdala impacts conditioned fear responses in mice, unveiling specific brain circuits involved in fear expression and modulation. This study established a direct connection between targeted neuronal inhibition and changes in brain-wide activity and behavior. Building on this foundational work, further research introduced chemogenetic fMRI to examine the brain-wide effects of serotonergic transmission (158). This design allowed for the selective activation of serotonin-producing neurons in mice, leading to the identification of specific regional brain activations. Such studies underscore the targeted action of endogenous neurotransmission systems and their unique functional signatures across the brain. Subsequently, Roelofs et al. showcased a chemogenetic and fMRI approach to map the mesocorticolimbic system in rats, revealing how activating VTA-to-NAc core or VTA-to-mPFC projections influences global brain dynamics and behavior (159). By using DREADDs combined with the concept pioneered in pHMRI, their study demonstrated significant BOLD responses not just in targeted areas but also across remote neural circuits, aligning these changes with behavioral outcomes like locomotor activity. This work highlights the effects of specific neural pathway activations on broad neural network activity. A landmark paper demonstrated that the chemogenetic activation of the locus coeruleus (LC) noradrenergic neurons could rapidly reconfigure the functional connectome (Fig. 7)(160). This study highlights how neurotransmitter systems, specifically norepinephrine, critically modulate brain function and network dynamics, emphasizing the locus coeruleus' role in enhancing certain connectivity patterns.

Applications in Rodent Models

Building upon these insights, a deeper understanding has emerged on how manipulations of specific neural pathways can profoundly influence brain dynamics and behavior. The regulation of BOLD dynamics and brain-wide connectivity, for example, has been significantly advanced through studies targeting specific neural circuits. Research into the striato-thalamo-cortical circuit has unveiled its crucial role in regulating these aspects, particularly through the manipulation of D1 medium spiny neurons (161). The unilateral activation of D1 medium spiny neurons can influence bilateral brain networks, highlighting the striato-thalamo-cortical circuit's significant role in brain dynamics (162). Similarly, the exploration of the hippocampal regions' differential roles has been associated with pain management, revealing novel strategies that could potentially transform therapeutic approaches to neuropathic pain (163). Kargl et al. explored affective learning mechanisms

through a cortico-limbic circuit involving the insular cortex and amygdala (164). Their research illuminates the integration of affective value, dissecting it into salience and valence within this circuitry, which plays a role in behavioral conditioning. This work addresses how specific neural pathways contribute to complex behaviors and also suggests avenues for therapeutic interventions. Li et al. demonstrated that chemogenetic suppression of specific brain hubs, such as the sensory cortex and subcortical areas, significantly impacts memory consolidation in mice, suggesting the role of distributed networks beyond the hippocampus in this process (165). The research into brain network dynamics and memory underscored the essential role of network integration in memory consolidation dynamics.

The role of the DMN and its nodes has been of particular interest in recent years, notably highlighted by Peeters et al. (2020). Their study on the anterior cingulate area of mice, using chemogenetic silencing, revealed the involvement of this area in modulating functional network activity and connectivity (166). Tu et al. discovered that inactivating the dorsal anterior cingulate cortex disrupts brain network organization, affecting resilience, segregation, and behavior without compromising overall network integration (167). This chemogenetics and resting-state fMRI study highlights the DMN's crucial role in complex behaviors and the interconnectedness of brain networks. A recent study revealed that inhibiting activity in specific brain regions like the medial prefrontal cortex can paradoxically enhance fMRI connectivity (Fig. 8) (168), suggesting a counterintuitive increase in network connectivity through altered oscillatory processes. The paradoxical phenomenon is further elucidated by in vivo electrophysiology findings, which show that such chemogenetic inhibition augments low-frequency (0.1–4 Hz) oscillatory power. These findings challenge conventional understanding and indicate that decreased regional brain activity can in fact enhance overall network connectivity by modifying the dynamics of oscillatory brain processes. Furthermore, the application of chemogenetic manipulations to study the default mode-like network and the locus coeruleus has provided critical insights into the interplay between neuromodulation, network connectivity, and behavior. Oyarzabal et al. (Fig. 9) (26) utilized a multifaceted approach, incorporating fMRI, PET, and spectral fiber photometry, to elucidate the locus coeruleus's role in modulating the DMN through chemogenetic modulation. Specifically, this study revealed that tonic activation of locus coeruleus noradrenergic neurons notably reduces cerebral blood volume and PET-derived glucose uptake while concurrently enhancing synchronous low-frequency fMRI activity as well as calcium-dependent neuronal activity within the DMN's frontal cortices. This observation of NE-induced vasoconstriction alongside increased neuronal firing and network connectivity highlights the importance to consider additional neurochemical-related vasomotor activity when interpreting fMRI data. Aside from the noradrenergic system, cholinergic modulation on DMN has also been examined by chemogenetic fMRI. Peeters et al. (169) provided insights into the interplay between cholinergic neuromodulation and the DMN-like network through unilateral stimulation of cholinergic neurons. More recently, Sanda et al. explored the neural mechanisms by which cholinergic modulation affects the resting-state activity in the brain, particularly focusing on the DMN (170). Utilizing computational models based on rodent and human connectomes, they demonstrated that alterations in excitability and synaptic connections—precipitated by cholinergic influences—can lead to significant changes in resting-state dynamics.

Chemogenetic fMRI Considerations

The chemogenetic technique has established itself as a broadly utilized approach for neuromodulation. Yet, understanding the procedural nuances of this technique is crucial to enhancing its application in fMRI. Rapidly toggling activity on and off with multiple repetitions, common in DBS and optogenetic fMRI studies, is not feasible with chemogenetics. Consequently, this prompted most studies to leverage the prolonged data sampling to examine functional connectivity changes. Drift in the fMRI signal can pose challenges in chemogenetic fMRI data analysis. This drift may be due to changes in animal physiology and/or MRI gradient heating and can be confounded with the slow pharmacodynamics of DREADD-induced activity changes. The sensitivity of fMRI to such drifts suggests the need for meticulous experimental design to discern changes from chemogenetic modulations, often necessitating a sufficiently long baseline period and appropriate controls. Control experiments are especially warranted due to the side effects of actuator drugs. For instance, CNO and its analogs may interact with endogenous receptors, representing a critical point of consideration. Off-target effects can obscure interpretations of results, as changes in neural activity or behavior may not solely indicate the activation of DREADDs but also the broader pharmacological impact of the actuators. A study by Gomez et al. investigated the use of CNO in chemogenetic studies, suggesting the potential confounding effect of its rapid in vivo conversion to clozapine, a metabolite with high affinity and potency for DREADD activation (171). Specifically, it was suggested that the assumed direct activation of DREADDs by CNO may be confounded by its rapid in vivo conversion to clozapine, a metabolite of CNO with high affinity and potency for DREADD activation. Over the past few years, the search for alternative actuators capable of more specific modulation of DREADDs has led to the identification and increasing use of ligands such as Compound 21 (C21) (172) and Deschloroclozapine (DCZ) (173), with the latter now being the most increasingly utilized. These advancements have laid the groundwork for enhancing the utility of this technique in animal fMRI, and it is conceivable that the ongoing development of DREADD actuators will further improve specificity and reduce the systemic effects associated with this technique.

It is important to note that the potential future applications of DREADDs are promising for studies extending beyond neurons, offering insights into other brain cell types essential for neurovascular coupling, such as astrocytes. Unlike neurons, astrocytes don't fire action potentials but signal through GPCRs. By utilizing DREADDs to selectively modulate astrocytic signaling, one can parse out their distinct contributions to fMRI signals and deepen our understanding of fMRI contrast mechanisms. Ultimately, the capacity for sustained neuromodulation afforded by chemogenetics epitomizes pharmacological mimicry, delivering a level of specificity superior to that of traditional drugs. This could lead to alterations in behaviorally relevant brain states, facilitating causal studies with a precision previously unattainable. We anticipate that the integration of chemogenetics and fMRI will be instrumental in elucidating the mechanisms underlying brain network transitions.

INTEGRATIVE ANALYSIS IN NEUROMODULATION FMRI STUDIES

In the pursuit of understanding complex neurobehavioral phenomena through neuromodulation studies in small animal fMRI, researchers may choose from a spectrum of sophisticated analytical models. Among these options, causal inference models, like mediation and moderation analyses, serve as suggestive tools rather than prescriptive methods, offering a detailed approach to dissecting the interplay between neuromodulatory inputs, brain network measures, and behavioral outcomes. Central to these models is the concept of causal inference (174), which involves statistical methods aimed at determining whether a cause-and-effect relationship exists between a manipulation and an outcome. Causal inference is the backbone of understanding how and why certain effects occur in the brain and behavior, going beyond mere associations to predict how changes in one variable will produce changes in another. Causal inference allows us to construct and test theoretical models that specify the cause-and-effect pathways among neuromodulatory techniques (such as electrical DBS, optogenetics, and chemogenetics), observed changes in brain networks via fMRI, and alterations in animal behavior.

Mediation and Moderation Models

Causal inference models typically include mediation analysis (Fig. 10) (26, 134, 175), where the model can be set to investigate whether the relationship between a neuromodulatory input (the independent variable) and behavioral outcomes (the dependent variable) passes through an intermediate brain network variable (the mediator). These models excel in distinguishing direct effects from those mediated by alterations in brain network activity. Their capacity to handle scenarios with multiple mediators (176) enhances the understanding by allowing for the simultaneous consideration of various brain network measures as mediators. This capability is crucial for delving into whether behavioral changes induced by interventions like optogenetic stimulation are direct or mediated through modifications in specific fMRI-derived brain network measures, such as functional connectivity within and between multiple networks. Furthermore, these causal inference models are adaptable, enabling researchers to extend their analyses to consider network measures not just as mediators but also as outcomes, with activities from other network nodes acting as mediators. The application of mediation analysis goes beyond mere analytical choices; they are integral to weaving a comprehensive narrative that links disparate scales of data. Positioning behaviors or network measures as independent variables in the model allows researchers to address fundamentally different questions. For instance, when behaviors are set as the dependent variables, the focus is on elucidating the pathways through which neuromodulation influences behaviors via hypothesized mediators. Conversely, when network measures are deemed the dependent variables, the analysis shifts towards understanding how the mediators might alter network strengths or connectivity patterns. This approach not only enriches our insight into the specific mechanisms of action behind neuromodulatory interventions but also underscores the importance of carefully considering the theoretical and methodological underpinnings of our analytical models in neuroimaging research.

On the other hand, moderation analysis (Fig. 10) allows us to understand the conditions under which certain neuromodulatory effects on fMRI signals are strengthened or weakened, providing insights into the contextual dependencies of neural circuit manipulations (177). For instance, the efficacy of electrical deep brain stimulation may vary depending on the baseline activity level of the targeted network, a relationship that moderation analysis can quantify. Furthermore, considering factors like sex and age is increasingly recognized as crucial in brain studies, as these variables can significantly influence both the baseline state of neural networks and their response to neuromodulatory inputs. As an example, a recent work (178) demonstrates that the intrinsic FC between the anterior insula and the retrosplenial cortex before cocaine exposure serves as a moderator for the impact of cocaine self-administration on FC changes between these regions during abstinence in rats. This finding highlights the critical role of pre-existing network configurations in influencing the neural and behavioral outcomes following neuromodulatory interventions. Another example that demonstrates the influence of pre-existing conditions on neuromodulatory outcomes comes from the work of Lee et al (31). Their research into acute alcohol exposure reveals how it differentially affects the functional connectivity within the lateral cortical network in rats, depending on their age. Specifically, they found that acute alcohol induces a greater dose-dependent increase in FC within this network in adult rats compared to adolescents. This age-dependent sensitivity to alcohol underscores the critical role of developmental stages in determining the brain's response to neuromodulatory substances.

Causal Inference in Neuromodulation Research

While both mediation and moderation analyses are useful toolkit for analyzing neuromodulatory effects, it is critical to recognize their unique contributions and requirements. Mediation analysis provides a framework for understanding causality, mapping the pathway from an independent variable through a mediator to a dependent variable. This approach assumes a specific sequence: that the independent variable can cause changes in the mediator, which in turn affects the dependent variable. Such a model requires a strong theoretical foundation to hypothesize and subsequently test the direct and indirect effects within the causal chain. This is particularly useful in neuromodulation studies for delineating the process by which neuromodulatory techniques influence behavior through changes in brain network activity, allowing researchers to pinpoint the role of specific neural circuits in mediating behavioral outcomes. On the other hand, moderation analysis focuses on the conditions or contexts that modify the effect of the independent variable on the dependent variable. A moderator variable is one that affects the strength or direction of the relationship between the independent and dependent variables, without being in the causal pathway. Moderation can reveal how different baseline characteristics, again, such as the sex and age of animals or the baseline activity level of targeted neural networks, can influence the outcomes of neuromodulatory interventions. This analysis does not imply causality between the moderator and the outcome; instead, it provides insights into the variability of effects across different conditions or subgroups. The distinct roles of mediation and moderation analyses in causal inference highlight the importance of carefully designing neuromodulation studies. Researchers must clearly define their hypotheses regarding causal pathways and moderating factors to apply the appropriate analytical models.

These analytical models underscore the importance of a holistic view of the data, one that recognizes the complexity of interactions between neural activity, brain networks, and behavior. It provides one of the worth noting structured methodology to dissect these complex interactions, paving the way for our use of fMRI technique towards a comprehensive understanding of the brain's modulatory landscapes and their behavioral correlates.

CONCLUSION:

This review addresses how the integration of fMRI with neuromodulation technologies in rodents has led to advances in neuroimaging. Given the vast number of outstanding studies in this field, it is impractical to summarize all relevant papers comprehensively. Through our review of representative studies investigating neurovascular coupling and functional brain networks, we discuss the findings and challenges faced when integrating DBS, optogenetic, and chemogenetic technologies with fMRI and share our perspectives for future directions and analytical considerations.

The combination of these neuromodulation techniques with fMRI has offered unique insights into brain circuit function and dynamics that are difficult to achieve through other methods. These studies have revealed the complex interplay between different neurotransmitter systems and their effects on brain-wide activity patterns, significantly advancing our understanding of the neural basis of various behaviors and neurological disorders in rodent models. Each neuromodulation technique presents its own set of advantages and challenges when used in conjunction with fMRI, emphasizing the importance of careful experimental design and interpretation.

Looking towards the future, the field shows promise in several directions. The development of more specific and efficient neuromodulation tools, such as new opsins or DREADD actuators, may further enhance the precision and reliability of these studies. Advances in fMRI technology, including higher resolution imaging and faster acquisition techniques, could provide even more detailed insights into brain function when combined with neuromodulation. The increasing use of awake animal fMRI studies may bridge the gap between animal models and human research, offering more translatable insights.

Furthermore, the application of advanced analytical methods, such as machine learning algorithms for pattern recognition and dynamic network analysis, to neuromodulation fMRI data may uncover new patterns and relationships in brain function. The combination of multiple neuromodulation techniques in a single study, such as simultaneous optogenetic stimulation and chemogenetic inhibition, could provide a more comprehensive understanding of brain circuit dynamics and causality.

By providing an overview of progress in the field, we advocate for the importance of a multidisciplinary approach in advancing animal fMRI applications. As these technologies continue to evolve, they promise to yield even deeper insights into brain function, potentially leading to new therapeutic strategies for neurological and psychiatric disorders.

Grant Support:

National Institute of Biomedical Imaging and Bioengineering (R01EB033790), National Institute of Mental Health (R01MH126518, RF1MH117053, S10MH124745), National Institute of Neurological Disorders and Stroke (R01NS128278, R21NS133913), National Institute on Alcohol Abuse and Alcoholism (P60AA011605, U01AA020023), National Institute on Drug Abuse (R21DA057503), and NIH Office of the Director (S10OD026796)

References:

1. Kringsbach ML, Jenkinson N, Owen SLF, Aziz TZ: Translational principles of deep brain stimulation. *Nat Rev Neurosci* 2007; 8:623–635. [PubMed: 17637800]
2. Deisseroth K: Optogenetics: 10 years of microbial opsins in neuroscience. *Nat Neurosci* 2015; 18:1213–1225. [PubMed: 26308982]
3. Roth BL: DREADDs for neuroscientists. *Neuron* 2016; 89:683–694. [PubMed: 26889809]
4. Kim S-G, Ogawa S: Biophysical and physiological origins of blood oxygenation level-dependent fMRI signals. *J Cereb Blood Flow Metab* 2012; 32:1188–1206. [PubMed: 22395207]
5. Rosen BR, Buckner RL, Dale AM: Event-related functional MRI: past, present, and future. *Proc Natl Acad Sci USA* 1998; 95:773–780. [PubMed: 9448240]
6. Jenkins BG: Pharmacologic magnetic resonance imaging (phMRI): imaging drug action in the brain. *Neuroimage* 2012; 62:1072–1085. [PubMed: 22495143]
7. Canario E, Chen D, Biswal B: A review of resting-state fMRI and its use to examine psychiatric disorders. *Psychoradiology* 2021; 1:42–53. [PubMed: 38665309]
8. Lahnakoski JM, Glerean E, Salmi J, et al. : Naturalistic FMRI mapping reveals superior temporal sulcus as the hub for the distributed brain network for social perception. *Front Hum Neurosci* 2012; 6:233. [PubMed: 22905026]
9. Shih YY, MacKinnon MJ, Ma Y: Methods and systems for functional magnetic resonance imaging with a zero echo time pulse-sequence. US Patent App 17 ... 2023.
10. Lehto LJ, Idiyatullin D, Zhang J, et al. : MB-SWIFT functional MRI during deep brain stimulation in rats. *Neuroimage* 2017; 159:443–448. [PubMed: 28797739]
11. Wellman PJ, Clifford PS, Rodriguez JA, et al. : Brain reinforcement system function is ghrelin dependent: studies in the rat using pharmacological fMRI and intracranial self-stimulation. *Addict Biol* 2012; 17:908–919. [PubMed: 22017465]
12. Klomp A, Tremoleda JL, Schrantee A, Gsell W, Reneman L: The use of pharmacological-challenge fMRI in pre-clinical research: application to the 5-HT system. *J Vis Exp* 2012.
13. Shih Y-YI, Li G, Muir ER, De La Garza BH, Kiel JW, Duong TQ: Pharmacological MRI of the choroid and retina: blood flow and BOLD responses during nitroprusside infusion. *Magn Reson Med* 2012; 68:1273–1278. [PubMed: 22183830]
14. Zaldivar D, Rauch A, Logothetis NK, Goense J: Two distinct profiles of fMRI and neurophysiological activity elicited by acetylcholine in visual cortex. *Proc Natl Acad Sci USA* 2018; 115:E12073–E12082. [PubMed: 30510000]
15. Cerri DH, Albaugh DL, Walton LR, et al. : Distinct neurochemical influences on fMRI response polarity in the striatum. *Nat Commun* 2024; 15:1916. [PubMed: 38429266]
16. Leslie RA, James MF: Pharmacological magnetic resonance imaging: a new application for functional MRI. *Trends Pharmacol Sci* 2000; 21:314–318. [PubMed: 10918638]
17. Biswal B, Yetkin FZ, Haughton VM, Hyde JS: Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med* 1995; 34:537–541. [PubMed: 8524021]
18. Thompson GJ, Pan W-J, Keilholz SD: Different dynamic resting state fMRI patterns are linked to different frequencies of neural activity. *J Neurophysiol* 2015; 114:114–124. [PubMed: 26041826]
19. Pais-Roldán P, Biswal B, Scheffler K, Yu X: Identifying Respiration-Related Aliasing Artifacts in the Rodent Resting-State fMRI. *Front Neurosci* 2018; 12:788. [PubMed: 30455623]
20. Lee S-H, Broadwater MA, Ban W, et al. : An isotropic EPI database and analytical pipelines for rat brain resting-state fMRI. *Neuroimage* 2021; 243:118541. [PubMed: 34478824]

21. Pan W-J, Billings JCW, Grooms JK, Shakil S, Keilholz SD: Considerations for resting state functional MRI and functional connectivity studies in rodents. *Front Neurosci* 2015; 9:269. [PubMed: 26300718]
22. Lu H, Zou Q, Gu H, Raichle ME, Stein EA, Yang Y: Rat brains also have a default mode network. *Proc Natl Acad Sci USA* 2012; 109:3979–3984. [PubMed: 22355129]
23. Hsu L-M, Liang X, Gu H, et al. : Constituents and functional implications of the rat default mode network. *Proc Natl Acad Sci USA* 2016; 113:E4541–7. [PubMed: 27439860]
24. Liska A, Galbusera A, Schwarz AJ, Gozzi A: Functional connectivity hubs of the mouse brain. *Neuroimage* 2015; 115:281–291. [PubMed: 25913701]
25. Whitesell JD, Liska A, Coletta L, et al. : Regional, Layer, and Cell-Type-Specific Connectivity of the Mouse Default Mode Network. *Neuron* 2021; 109:545–559.e8. [PubMed: 33290731]
26. Oyarzabal EA, Hsu L-M, Das M, et al. : Chemogenetic stimulation of tonic locus coeruleus activity strengthens the default mode network. *Sci Adv* 2022; 8:eabm9898. [PubMed: 35486721]
27. Chao T-HH, Lee B, Hsu L-M, et al. : Neuronal dynamics of the default mode network and anterior insular cortex: Intrinsic properties and modulation by salient stimuli. *Sci Adv* 2023; 9:eade5732. [PubMed: 36791185]
28. Mandino F, Vrooman RM, Foo HE, et al. : A triple-network organization for the mouse brain. *Mol Psychiatry* 2022; 27:865–872. [PubMed: 34650202]
29. Gozzi A, Zerbi V: Modeling brain dysconnectivity in rodents. *Biol Psychiatry* 2023; 93:419–429. [PubMed: 36517282]
30. Tsai P-J, Keeley RJ, Carmack SA, et al. : Converging structural and functional evidence for a rat salience network. *Biol Psychiatry* 2020; 88:867–878. [PubMed: 32981657]
31. Lee S-H, Shnitko TA, Hsu L-M, et al. : Acute alcohol induces greater dose-dependent increase in the lateral cortical network functional connectivity in adult than adolescent rats. *Addiction Neuroscience* 2023; 7.
32. Yu X, Qian C, Chen D, Dodd SJ, Koretsky AP: Deciphering laminar-specific neural inputs with line-scanning fMRI. *Nat Methods* 2014; 11:55–58. [PubMed: 24240320]
33. Yu X, He Y, Wang M, et al. : Sensory and optogenetically driven single-vessel fMRI. *Nat Methods* 2016; 13:337–340. [PubMed: 26855362]
34. Shim H-J, Im GH, Jung WB, et al. : Protocol for mouse optogenetic fMRI at ultrahigh magnetic fields. *STAR Protocols* 2022; 3:101846. [PubMed: 36595930]
35. Cabral J, Fernandes FF, Shemesh N: Intrinsic macroscale oscillatory modes driving long range functional connectivity in female rat brains detected by ultrafast fMRI. *Nat Commun* 2023; 14:375. [PubMed: 36746938]
36. Gutierrez-Barragan D, Singh NA, Alvino FG, et al. : Unique spatiotemporal fMRI dynamics in the awake mouse brain. *Curr Biol* 2022; 32:631–644.e6. [PubMed: 34998465]
37. Mandino F, Vujic S, Grandjean J, Lake EMR: Where do we stand on fMRI in awake mice? *Cereb Cortex* 2024; 34.
38. Gao Y-R, Ma Y, Zhang Q, et al. : Time to wake up: Studying neurovascular coupling and brain-wide circuit function in the un-anesthetized animal. *Neuroimage* 2017; 153:382–398. [PubMed: 27908788]
39. Zou Y, Tong C, Peng W, et al. : Cell-type-specific optogenetic fMRI on basal forebrain reveals functional network basis of behavioral preference. *Neuron* 2024; 112:1342–1357.e6. [PubMed: 38359827]
40. Medtronic Activa Tremor Control System [<https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P960009>]
41. Lozano AM, Lipsman N, Bergman H, et al. : Deep brain stimulation: current challenges and future directions. *Nat Rev Neurol* 2019; 15:148–160. [PubMed: 30683913]
42. Milner KL, Mogenson GJ: Electrical and chemical activation of the mesencephalic and subthalamic locomotor regions in freely moving rats. *Brain Res* 1988; 452:273–285. [PubMed: 3401734]

43. Hammond C, Rouzaire-Dubois B, Féger J, Jackson A, Crossman AR: Anatomical and electrophysiological studies on the reciprocal projections between the subthalamic nucleus and nucleus tegmenti pedunculo-pontinus in the rat. *Neuroscience* 1983; 9:41–52. [PubMed: 6308507]
44. Benabid AL, Chabardes S, Mitrofanis J, Pollak P: Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *Lancet Neurol* 2009; 8:67–81. [PubMed: 19081516]
45. Gardner J: A history of deep brain stimulation: Technological innovation and the role of clinical assessment tools. *Soc Stud Sci* 2013; 43:707–728.
46. Shyu B-C, Lin C-Y, Sun J-J, Chen S-L, Chang C: BOLD response to direct thalamic stimulation reveals a functional connection between the medial thalamus and the anterior cingulate cortex in the rat. *Magn Reson Med* 2004; 52:47–55. [PubMed: 15236366]
47. Dunn JF, Tuor UI, Kmech J, et al. : Functional brain mapping at 9.4T using a new MRI-compatible electrode chronically implanted in rats. *Magn Reson Med* 2009; 61:222–228. [PubMed: 19097225]
48. Canals S, Beyerlein M, Murayama Y, Logothetis NK: Electric stimulation fMRI of the perforant pathway to the rat hippocampus. *Magn Reson Imaging* 2008; 26:978–986. [PubMed: 18479870]
49. Van Den Berge N, Vanhove C, Descamps B, et al. : Functional MRI during Hippocampal Deep Brain Stimulation in the Healthy Rat Brain. *PLoS ONE* 2015; 10:e0133245. [PubMed: 26193653]
50. Alvarez-Salvado E, Pallarés V, Moreno A, Canals S: Functional MRI of long-term potentiation: imaging network plasticity. *Philos Trans R Soc Lond B Biol Sci* 2014; 369:20130152. [PubMed: 24298154]
51. Canals S, Beyerlein M, Merkle H, Logothetis NK: Functional MRI evidence for LTP-induced neural network reorganization. *Curr Biol* 2009; 19:398–403. [PubMed: 19230667]
52. Walton LR, Verber M, Lee S-H, Chao T-HH, Wightman RM, Shih Y-YI: Simultaneous fMRI and fast-scan cyclic voltammetry bridges evoked oxygen and neurotransmitter dynamics across spatiotemporal scales. *Neuroimage* 2021; 244:118634. [PubMed: 34624504]
53. Shih Y-YI, Yash TV, Rogers B, Duong TQ: FMRI of deep brain stimulation at the rat ventral posteromedial thalamus. *Brain Stimulat* 2014; 7:190–193.
54. Krautwald K, Mahnke L, Angenstein F: Electrical Stimulation of the Lateral Entorhinal Cortex Causes a Frequency-Specific BOLD Response Pattern in the Rat Brain. *Front Neurosci* 2019; 13:539. [PubMed: 31191231]
55. Lai H-Y, Albaugh DL, Kao Y-CJ, Younce JR, Shih Y-YI: Robust deep brain stimulation functional MRI procedures in rats and mice using an MR-compatible tungsten microwire electrode. *Magn Reson Med* 2015; 73:1246–1251. [PubMed: 24798216]
56. Chao T-HH, Chen J-H, Yen C-T: Repeated BOLD-fMRI imaging of deep brain stimulation responses in rats. *PLoS ONE* 2014; 9:e97305. [PubMed: 24825464]
57. Albaugh DL, Salzwedel A, Van Den Berge N, Gao W, Stuber GD, Shih Y-YI: Functional magnetic resonance imaging of electrical and optogenetic deep brain stimulation at the rat nucleus accumbens. *Sci Rep* 2016; 6:31613. [PubMed: 27601003]
58. Helbing C, Angenstein F: Frequency-dependent electrical stimulation of fimbria-fornix preferentially affects the mesolimbic dopamine system or prefrontal cortex. *Brain Stimulat* 2020; 13:753–764.
59. Zhao S, Li G, Tong C, et al. : Full activation pattern mapping by simultaneous deep brain stimulation and fMRI with graphene fiber electrodes. *Nat Commun* 2020; 11:1788. [PubMed: 32286290]
60. Lin T-C, Lo Y-C, Lin H-C, et al. : MR imaging central thalamic deep brain stimulation restored autistic-like social deficits in the rat. *Brain Stimulat* 2019; 12:1410–1420.
61. Lee S, Albaugh DL, Chen Y-Y, Shih Y-YI: On- and off-target circuit effects of high frequency electrical deep brain stimulation at the subthalamic nucleus. *ISMRM*; 2016.
62. Yang P-F, Chen Y-Y, Chen D-Y, Hu JW, Chen J-H, Yen C-T: Comparison of fMRI BOLD response patterns by electrical stimulation of the ventroposterior complex and medial thalamus of the rat. *PLoS ONE* 2013; 8:e66821. [PubMed: 23826146]

63. Lai H-Y, Liao L-D, Lin C-T, et al. : Design, simulation and experimental validation of a novel flexible neural probe for deep brain stimulation and multichannel recording. *J Neural Eng* 2012; 9:036001. [PubMed: 22488106]
64. Lehto LJ, Filip P, Laakso H, et al. : Tuning neuromodulation effects by orientation selective deep brain stimulation in the rat medial frontal cortex. *Front Neurosci* 2018; 12:899. [PubMed: 30618544]
65. Lehto LJ, Canna A, Wu L, et al. : Orientation selective deep brain stimulation of the subthalamic nucleus in rats. *Neuroimage* 2020; 213:116750. [PubMed: 32198048]
66. Canna A, Lehto LJ, Wu L, et al. : Brain fMRI during orientation selective epidural spinal cord stimulation. *Sci Rep* 2021; 11:5504. [PubMed: 33750822]
67. Wu L, Canna A, Narvaez O, et al. : Orientation selective DBS of entorhinal cortex and medial septal nucleus modulates activity of rat brain areas involved in memory and cognition. *Sci Rep* 2022; 12:8565. [PubMed: 35595790]
68. Paasonen E, Paasonen J, Lehto LJ, et al. : Event-recurring multiband SWIFT functional MRI with 200-ms temporal resolution during deep brain stimulation and isoflurane-induced burst suppression in rat. *Magn Reson Med* 2022; 87:2872–2884. [PubMed: 34985145]
69. Gureviciene I, Laakso H, Narvaez O, et al. : Orientation selective stimulation with tetrahedral electrodes of the rat infralimbic cortex to indirectly target the amygdala. *Front Neurosci* 2023; 17:1147547. [PubMed: 37214391]
70. Paasonen J, Laakso H, Pirttimäki T, et al. : Multi-band SWIFT enables quiet and artefact-free EEG-fMRI and awake fMRI studies in rat. *Neuroimage* 2020; 206:116338. [PubMed: 31730923]
71. Schwaderlapp N, Paschen E, LeVan P, von Elverfeldt D, Haas C: Probing hippocampal stimulation in experimental temporal lobe epilepsy with functional MRI. *Frontiers in Neuroimaging* 2023.
72. Angenstein F: A Combined fMRI and Electrophysiological Approach to Study Signal Processing and Signal Propagation in the Rodent Hippocampus. In *Handbook of Behavioral Neuroscience*. Volume 28. Elsevier; 2019:425–439. [Handbook of Behavioral Neuroscience]
73. Jiang Y, Liu D-F, Zhang X, Liu H-G, Zhang C, Zhang J-G: Modulation of the rat hippocampal-cortex network and episodic-like memory performance following entorhinal cortex stimulation. *CNS Neurosci Ther* 2022; 28:448–457. [PubMed: 34964261]
74. Lai H-Y, Younce JR, Albaugh DL, Kao Y-CJ, Shih Y-YI: Functional MRI reveals frequency-dependent responses during deep brain stimulation at the subthalamic nucleus or internal globus pallidus. *Neuroimage* 2014; 84:11–18. [PubMed: 23988274]
75. Van Den Berge N, Albaugh DL, Salzwedel A, et al. : Functional circuit mapping of striatal output nuclei using simultaneous deep brain stimulation and fMRI. *Neuroimage* 2017; 146:1050–1061. [PubMed: 27825979]
76. Li G, Bo B, Wang P, et al. : Instantaneous antidepressant effect of lateral habenula deep brain stimulation in rats studied with functional MRI. *eLife* 2023; 12.
77. Vila-Merkle H, González-Martínez A, Campos-Jiménez R, et al. : Sex differences in amygdalohippocampal oscillations and neuronal activation in a rodent anxiety model and in response to infralimbic deep brain stimulation. *Front Behav Neurosci* 2023; 17:1122163. [PubMed: 36910127]
78. Li S-J, Lo Y-C, Lai H-Y, et al. : Uncovering the Modulatory Interactions of Brain Networks in Cognition with Central Thalamic Deep Brain Stimulation Using Functional Magnetic Resonance Imaging. *Neuroscience* 2020; 440:65–84. [PubMed: 32446855]
79. Herrington TM, Cheng JJ, Eskandar EN: Mechanisms of deep brain stimulation. *J Neurophysiol* 2016; 115:19–38. [PubMed: 26510756]
80. McIntyre CC, Savasta M, Kerkerian-Le Goff L, Vitek JL: Uncovering the mechanism(s) of action of deep brain stimulation: activation, inhibition, or both. *Clin Neurophysiol* 2004; 115:1239–1248. [PubMed: 15134690]
81. McIntyre CC, Anderson RW: Deep brain stimulation mechanisms: the control of network activity via neurochemistry modulation. *J Neurochem* 2016; 139 Suppl 1(Suppl 1):338–345. [PubMed: 27273305]

82. Price JB, Rusheen AE, Barath AS, et al. : Clinical applications of neurochemical and electrophysiological measurements for closed-loop neurostimulation. *Neurosurg Focus* 2020; 49:E6.
83. Elias WJ, Lipsman N, Ondo WG, et al. : A randomized trial of focused ultrasound thalamotomy for essential tremor. *N Engl J Med* 2016; 375:730–739. [PubMed: 27557301]
84. Ye PP, Brown JR, Pauly KB: Frequency dependence of ultrasound neurostimulation in the mouse brain. *Ultrasound Med Biol* 2016; 42:1512–1530. [PubMed: 27090861]
85. Yang P-F, Phipps MA, Jonathan S, et al. : Bidirectional and state-dependent modulation of brain activity by transcranial focused ultrasound in non-human primates. *Brain Stimulat* 2021; 14:261–272.
86. Kang W, Ju C, Joo J, Lee J, Shon Y-M, Park S-M: Closed-loop direct control of seizure focus in a rodent model of temporal lobe epilepsy via localized electric fields applied sequentially. *Nat Commun* 2022; 13:7805. [PubMed: 36528681]
87. Crick FH: Thinking about the brain. *Sci Am* 1979; 241:219–232. [PubMed: 115087]
88. Oesterhelt D, Stoeckenius W: Functions of a new photoreceptor membrane. *Proc Natl Acad Sci USA* 1973; 70:2853–2857. [PubMed: 4517939]
89. Nagel G, Ollig D, Fuhrmann M, et al. : Channelrhodopsin-1: a light-gated proton channel in green algae. *Science* 2002; 296:2395–2398. [PubMed: 12089443]
90. Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K: Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci* 2005; 8:1263–1268. [PubMed: 16116447]
91. Zhang F, Wang L-P, Brauner M, et al. : Multimodal fast optical interrogation of neural circuitry. *Nature* 2007; 446:633–639. [PubMed: 17410168]
92. Nagel G, Brauner M, Liewald JF, Adeishvili N, Bamberg E, Gottschalk A: Light activation of channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses. *Curr Biol* 2005; 15:2279–2284. [PubMed: 16360690]
93. Chow BY, Han X, Dobry AS, et al. : High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature* 2010; 463:98–102. [PubMed: 20054397]
94. Gradinaru V, Thompson KR, Deisseroth K: eNpHR: a *Natronomonas halorhodopsin* enhanced for optogenetic applications. *Brain Cell Biol* 2008; 36:129–139. [PubMed: 18677566]
95. Emiliani V, Entcheva E, Hedrich R, et al. : Optogenetics for light control of biological systems. *Nat Rev Methods Primers* 2022; 2.
96. Yizhar O, Fenno LE, Davidson TJ, Mogri M, Deisseroth K: Optogenetics in neural systems. *Neuron* 2011; 71:9–34. [PubMed: 21745635]
97. Beravat R, Wong GKL, Frosz MH, Xi XM, Russell PSJ: Twist-induced guidance in coreless photonic crystal fiber: A helical channel for light. *Sci Adv* 2016; 2:e1601421. [PubMed: 28138531]
98. Pisanello F, Mandelbaum G, Pisanello M, et al. : Dynamic illumination of spatially restricted or large brain volumes via a single tapered optical fiber. *Nat Neurosci* 2017; 20:1180–1188. [PubMed: 28628101]
99. Stujenske JM, Spellman T, Gordon JA: Modeling the spatiotemporal dynamics of light and heat propagation for in vivo optogenetics. *Cell Rep* 2015; 12:525–534. [PubMed: 26166563]
100. Pégard NC, Mardinly AR, Oldenburg IA, Sridharan S, Waller L, Adesnik H: Three-dimensional scanless holographic optogenetics with temporal focusing (3D-SHOT). *Nat Commun* 2017; 8:1228. [PubMed: 29089483]
101. Kim S, Moon HS, Vo TT, et al. : Whole-brain mapping of effective connectivity by fMRI with cortex-wide patterned optogenetics. *Neuron* 2023; 111:1732–1747.e6. [PubMed: 37001524]
102. Lee JH, Durand R, Gradinaru V, et al. : Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. *Nature* 2010; 465:788–792. [PubMed: 20473285]
103. Kahn I, Desai M, Knoblich U, et al. : Characterization of the functional MRI response temporal linearity via optical control of neocortical pyramidal neurons. *J Neurosci* 2011; 31:15086–15091. [PubMed: 22016542]

104. Kahn I, Knoblich U, Desai M, et al. : Optogenetic drive of neocortical pyramidal neurons generates fMRI signals that are correlated with spiking activity. *Brain Res* 2013; 1511:33–45. [PubMed: 23523914]
105. Chen X, Sobczak F, Chen Y, et al. : Mapping optogenetically-driven single-vessel fMRI with concurrent neuronal calcium recordings in the rat hippocampus. *Nat Commun* 2019; 10:5239. [PubMed: 31748553]
106. Katz BM, Walton LR, Houston KM, Cerri DH, Shih Y-YI: Putative neurochemical and cell type contributions to hemodynamic activity in the rodent caudate putamen. *J Cereb Blood Flow Metab* 2023; 43:481–498. [PubMed: 36448509]
107. Lee J-Y, You T, Woo C-W, Kim S-G: Optogenetic fMRI for Brain-Wide Circuit Analysis of Sensory Processing. *Int J Mol Sci* 2022; 23.
108. Lee JH, Liu Q, Dadgar-Kiani E: Solving brain circuit function and dysfunction with computational modeling and optogenetic fMRI. *Science* 2022; 378:493–499. [PubMed: 36327349]
109. Desai M, Kahn I, Knoblich U, et al. : Mapping brain networks in awake mice using combined optical neural control and fMRI. *J Neurophysiol* 2011; 105:1393–1405. [PubMed: 21160013]
110. Liang Z, Watson GDR, Alloway KD, Lee G, Neuberger T, Zhang N: Mapping the functional network of medial prefrontal cortex by combining optogenetics and fMRI in awake rats. *Neuroimage* 2015; 117:114–123. [PubMed: 26002727]
111. Wang M, He Y, Sejnowski TJ, Yu X: Brain-state dependent astrocytic Ca²⁺ signals are coupled to both positive and negative BOLD-fMRI signals. *Proc Natl Acad Sci USA* 2018; 115:E1647–E1656. [PubMed: 29382752]
112. Iordanova B, Vazquez AL, Poplawsky AJ, Fukuda M, Kim S-G: Neural and hemodynamic responses to optogenetic and sensory stimulation in the rat somatosensory cortex. *J Cereb Blood Flow Metab* 2015; 35:922–932. [PubMed: 25669905]
113. Dahlqvist MK, Thomsen KJ, Postnov DD, Lauritzen MJ: Modification of oxygen consumption and blood flow in mouse somatosensory cortex by cell-type-specific neuronal activity. *J Cereb Blood Flow Metab* 2020; 40:2010–2025. [PubMed: 31645177]
114. Lee HJ, Weitz AJ, Bernal-Casas D, et al. : Activation of Direct and Indirect Pathway Medium Spiny Neurons Drives Distinct Brain-wide Responses. *Neuron* 2016; 91:412–424. [PubMed: 27373834]
115. Krawchuk MB, Ruff CF, Yang X, Ross SE, Vazquez AL: Optogenetic assessment of VIP, PV, SOM and NOS inhibitory neuron activity and cerebral blood flow regulation in mouse somatosensory cortex. *J Cereb Blood Flow Metab* 2020; 40:1427–1440. [PubMed: 31418628]
116. Anenberg E, Chan AW, Xie Y, LeDue JM, Murphy TH: Optogenetic stimulation of GABA neurons can decrease local neuronal activity while increasing cortical blood flow. *J Cereb Blood Flow Metab* 2015; 35:1579–1586. [PubMed: 26082013]
117. Vazquez AL, Fukuda M, Kim S-G: Inhibitory neuron activity contributions to hemodynamic responses and metabolic load examined using an inhibitory optogenetic mouse model. *Cereb Cortex* 2018; 28:4105–4119. [PubMed: 30215693]
118. Moon HS, Jiang H, Vo TT, Jung WB, Vazquez AL, Kim S-G: Contribution of Excitatory and Inhibitory Neuronal Activity to BOLD fMRI. *Cereb Cortex* 2021; 31:4053–4067. [PubMed: 33895810]
119. Vo TT, Im GH, Han K, Suh M, Drew PJ, Kim S-G: Parvalbumin interneuron activity drives fast inhibition-induced vasoconstriction followed by slow substance P-mediated vasodilation. *Proc Natl Acad Sci USA* 2023; 120:e2220777120. [PubMed: 37098063]
120. Lee J, Stile CL, Bice AR, et al. : Opposed hemodynamic responses following increased excitation and parvalbumin-based inhibition. *J Cereb Blood Flow Metab* 2021; 41:841–856. [PubMed: 33736512]
121. Howarth C, Mishra A, Hall CN: More than just summed neuronal activity: how multiple cell types shape the BOLD response. *Philos Trans R Soc Lond B Biol Sci* 2021; 376:20190630. [PubMed: 33190598]

122. Lee L, Boorman L, Glendenning E, et al. : Key aspects of neurovascular control mediated by specific populations of inhibitory cortical interneurons. *Cereb Cortex* 2020; 30:2452–2464. [PubMed: 31746324]
123. Iordanova B, Vazquez A, Kozai TD, Fukuda M, Kim S-G: Optogenetic investigation of the variable neurovascular coupling along the interhemispheric circuits. *J Cereb Blood Flow Metab* 2018; 38:627–640. [PubMed: 29372655]
124. Ferenczi EA, Zalocusky KA, Liston C, et al. : Prefrontal cortical regulation of brainwide circuit dynamics and reward-related behavior. *Science* 2016; 351:aac9698. [PubMed: 26722001]
125. Decot HK, Namboodiri VMK, Gao W, et al. : Coordination of Brain-Wide Activity Dynamics by Dopaminergic Neurons. *Neuropsychopharmacology* 2017; 42:615–627. [PubMed: 27515791]
126. Ioanas H-I, Saab BJ, Rudin M: Whole-brain opto-fMRI map of mouse VTA dopaminergic activation reflects structural projections with small but significant deviations. *Transl Psychiatry* 2022; 12:60. [PubMed: 35165257]
127. Grandjean J, Corcobá A, Kahn MC, et al. : A brain-wide functional map of the serotonergic responses to acute stress and fluoxetine. *Nat Commun* 2019; 10:350. [PubMed: 30664643]
128. Grimm C, Duss SN, Privitera M, et al. : Locus Coeruleus firing patterns selectively modulate brain activity and dynamics. *BioRxiv* 2022.
129. Jung WB, Jiang H, Lee S, Kim S-G: Dissection of brain-wide resting-state and functional somatosensory circuits by fMRI with optogenetic silencing. *Proc Natl Acad Sci USA* 2022; 119.
130. Leong ATL, Wang X, Wong EC, Dong CM, Wu EX: Neural activity temporal pattern dictates long-range propagation targets. *Neuroimage* 2021; 235:118032. [PubMed: 33836268]
131. Wang X, Leong ATL, Tan SZK, et al. : Functional MRI reveals brain-wide actions of thalamically-initiated oscillatory activities on associative memory consolidation. *Nat Commun* 2023; 14:2195. [PubMed: 37069169]
132. Chan RW, Leong ATL, Ho LC, et al. : Low-frequency hippocampal-cortical activity drives brain-wide resting-state functional MRI connectivity. *Proc Natl Acad Sci USA* 2017; 114:E6972–E6981. [PubMed: 28760982]
133. Chen Y, Sobczak F, Pais-Roldán P, Schwarz C, Koretsky AP, Yu X: Mapping the Brain-Wide Network Effects by Optogenetic Activation of the Corpus Callosum. *Cereb Cortex* 2020; 30:5885–5898. [PubMed: 32556241]
134. Li Y, Lee S-H, Yu C, et al. : Optogenetic fMRI reveals therapeutic circuits of subthalamic nucleus deep brain stimulation. *Brain Stimulation* 2024.
135. Jung WB, Im GH, Jiang H, Kim S-G: Early fMRI responses to somatosensory and optogenetic stimulation reflect neural information flow. *Proc Natl Acad Sci USA* 2021; 118.
136. Choe KY, Sanchez CF, Harris NG, Otis TS, Mathews PJ: Optogenetic fMRI and electrophysiological identification of region-specific connectivity between the cerebellar cortex and forebrain. *Neuroimage* 2018; 173:370–383. [PubMed: 29496611]
137. Han X, Cramer SR, Zhang N: Deriving causal relationships in resting-state functional connectivity using SSFO-based optogenetic fMRI. *J Neural Eng* 2022; 19.
138. Weitz AJ, Fang Z, Lee HJ, et al. : Optogenetic fMRI reveals distinct, frequency-dependent networks recruited by dorsal and intermediate hippocampus stimulations. *Neuroimage* 2015; 107:229–241. [PubMed: 25462689]
139. Salvan P, Lazari A, Vidaurre D, Mandino F, Johansen-Berg H, Grandjean J: Frequency modulation of entorhinal cortex neuronal activity drives distinct frequency-dependent states of brain-wide dynamics. *Cell Rep* 2021; 37:109954. [PubMed: 34731612]
140. Leong ATL, Chan RW, Gao PP, et al. : Long-range projections coordinate distributed brain-wide neural activity with a specific spatiotemporal profile. *Proc Natl Acad Sci USA* 2016; 113:E8306–E8315. [PubMed: 27930323]
141. Lee J-Y, You T, Lee C-H, et al. : Role of anterior cingulate cortex inputs to periaqueductal gray for pain avoidance. *Curr Biol* 2022; 32:2834–2847.e5. [PubMed: 35609604]
142. Bernal-Casas D, Lee HJ, Weitz AJ, Lee JH: Studying Brain Circuit Function with Dynamic Causal Modeling for Optogenetic fMRI. *Neuron* 2017; 93:522–532.e5. [PubMed: 28132829]

143. Menon V, Cerri D, Lee B, Yuan R, Lee S-H, Shih Y-YI: Optogenetic stimulation of anterior insular cortex neurons in male rats reveals causal mechanisms underlying suppression of the default mode network by the salience network. *Nat Commun* 2023; 14:866. [PubMed: 36797303]
144. Christie IN, Wells JA, Southern P, et al. : fMRI response to blue light delivery in the naïve brain: implications for combined optogenetic fMRI studies. *Neuroimage* 2013; 66:634–641. [PubMed: 23128081]
145. Schmid F, Wachsmuth L, Albers F, Schwalm M, Stroh A, Faber C: True and apparent optogenetic BOLD fMRI signals. *Magn Reson Med* 2017; 77:126–136. [PubMed: 26778283]
146. Rungta RL, Osmanski B-F, Boido D, Tanter M, Charpak S: Light controls cerebral blood flow in naive animals. *Nat Commun* 2017; 8:14191. [PubMed: 28139643]
147. Cover CG, Kesner AJ, Ukani S, et al. : Whole brain dynamics during optogenetic self-stimulation of the medial prefrontal cortex in mice. *Commun Biol* 2021; 4:66. [PubMed: 33446857]
148. Forkmann G, Dangelmayr B: Genetic control of chalcone isomerase activity in flowers of *Dianthus caryophyllus*. *Biochem Genet* 1980; 18:519–527. [PubMed: 7437010]
149. Lee H-M, Giguere PM, Roth BL: DREADDs: novel tools for drug discovery and development. *Drug Discov Today* 2014; 19:469–473. [PubMed: 24184433]
150. Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL: Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proc Natl Acad Sci USA* 2007; 104:5163–5168. [PubMed: 17360345]
151. Smith KS, Bucci DJ, Luikart BW, Mahler SV: DREADDs: Use and application in behavioral neuroscience. *Behav Neurosci* 2021; 135:89–107. [PubMed: 34060867]
152. Urban DJ, Roth BL: DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. *Annu Rev Pharmacol Toxicol* 2015; 55:399–417. [PubMed: 25292433]
153. Zhu H, Roth BL: DREADD: a chemogenetic GPCR signaling platform. *Int J Neuropsychopharmacol* 2014; 18.
154. Atasoy D, Sternson SM: Chemogenetic tools for causal cellular and neuronal biology. *Physiol Rev* 2018; 98:391–418. [PubMed: 29351511]
155. Campbell EJ, Marchant NJ: The use of chemogenetics in behavioural neuroscience: receptor variants, targeting approaches and caveats. *Br J Pharmacol* 2018; 175:994–1003. [PubMed: 29338070]
156. Vardy E, Robinson JE, Li C, et al. : A new DREADD facilitates the multiplexed chemogenetic interrogation of behavior. *Neuron* 2015; 86:936–946. [PubMed: 25937170]
157. Gozzi A, Jain A, Giovannelli A, et al. : A neural switch for active and passive fear. *Neuron* 2010; 67:656–666. [PubMed: 20797541]
158. Giorgi A, Migliarini S, Galbusera A, et al. : Brain-wide Mapping of Endogenous Serotonergic Transmission via Chemogenetic fMRI. *Cell Rep* 2017; 21:910–918. [PubMed: 29069598]
159. Roelofs TJM, Verharen JPH, van Tilborg GAF, et al. : A novel approach to map induced activation of neuronal networks using chemogenetics and functional neuroimaging in rats: A proof-of-concept study on the mesocorticolimbic system. *Neuroimage* 2017; 156:109–118. [PubMed: 28502844]
160. Zerbi V, Floriou-Servou A, Markicevic M, et al. : Rapid Reconfiguration of the Functional Connectome after Chemogenetic Locus Coeruleus Activation. *Neuron* 2019; 103:702–718.e5. [PubMed: 31227310]
161. Markicevic M, Sturman O, Bohacek J, et al. : Neuromodulation of striatal D1 cells shapes BOLD fluctuations in anatomically connected thalamic and cortical regions. *eLife* 2023; 12.
162. Nakamura Y, Nakamura Y, Pelosi A, et al. : fMRI detects bilateral brain network activation following unilateral chemogenetic activation of direct striatal projection neurons. *Neuroimage* 2020; 220:117079. [PubMed: 32585345]
163. Wei X, Centeno MV, Ren W, et al. : Activation of the dorsal, but not the ventral, hippocampus relieves neuropathic pain in rodents. *Pain* 2021; 162:2865–2880. [PubMed: 34160168]
164. Kargl D, Kaczanowska J, Ulonska S, et al. : The amygdala instructs insular feedback for affective learning. *eLife* 2020; 9.

165. Li Z, Athwal D, Lee H-L, Sah P, Opazo P, Chuang K-H: Locating causal hubs of memory consolidation in spontaneous brain network in male mice. *Nat Commun* 2023; 14:5399. [PubMed: 37669938]
166. Peeters LM, Hinz R, Detrez JR, et al. : Chemogenetic silencing of neurons in the mouse anterior cingulate area modulates neuronal activity and functional connectivity. *Neuroimage* 2020; 220:117088. [PubMed: 32592851]
167. Tu W, Ma Z, Ma Y, Dopfel D, Zhang N: Suppressing anterior cingulate cortex modulates default mode network and behavior in awake rats. *Cereb Cortex* 2021; 31:312–323. [PubMed: 32820327]
168. Rocchi F, Canella C, Noei S, et al. : Increased fMRI connectivity upon chemogenetic inhibition of the mouse prefrontal cortex. *Nat Commun* 2022; 13:1056. [PubMed: 35217677]
169. Peeters LM, van den Berg M, Hinz R, Majumdar G, Pintelon I, Keliris GA: Cholinergic modulation of the default mode like network in rats. *iScience* 2020; 23:101455. [PubMed: 32846343]
170. Sanda P, Hlinka J, van den Berg M, Bazhenov M, Keliris GA, Krishnan G: Cholinergic modulation supports dynamic switching of resting state networks through selective DMN suppression. *PLOS Computational Biology* 2024.
171. Gomez JL, Bonaventura J, Lesniak W, et al. : Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science* 2017; 357:503–507. [PubMed: 28774929]
172. Goutaudier R, Coizet V, Carcenac C, Carnicella S: Compound 21, a two-edged sword with both DREADD-selective and off-target outcomes in rats. *PLoS ONE* 2020; 15:e0238156. [PubMed: 32946510]
173. Nagai Y, Miyakawa N, Takuwa H, et al. : Deschloroclozapine, a potent and selective chemogenetic actuator enables rapid neuronal and behavioral modulations in mice and monkeys. *Nat Neurosci* 2020; 23:1157–1167. [PubMed: 32632286]
174. Pearl J: An introduction to causal inference. *Int J Biostat* 2010; 6:Article 7.
175. Taylor AB, MacKinnon DP, Tein JY: Tests of the three-path mediated effect. *Organizational Research Methods* 2008; 11:241–269.
176. Hsu L-M, Yang J-T, Wen X, et al. : Human thirst behavior requires transformation of sensory inputs by intrinsic brain networks. *BMC Biol* 2022; 20:255. [PubMed: 36357909]
177. Baron RM, Kenny DA: The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986; 51:1173–1182. [PubMed: 3806354]
178. Hsu L-M, Cerri DH, Lee S-H, Shnitko TA, Carelli RM, Shih Y-YI: Intrinsic Functional Connectivity between the Anterior Insular and Retrosplenial Cortex as a Moderator and Consequence of Cocaine Self-Administration in Rats. *J Neurosci* 2024; 44.

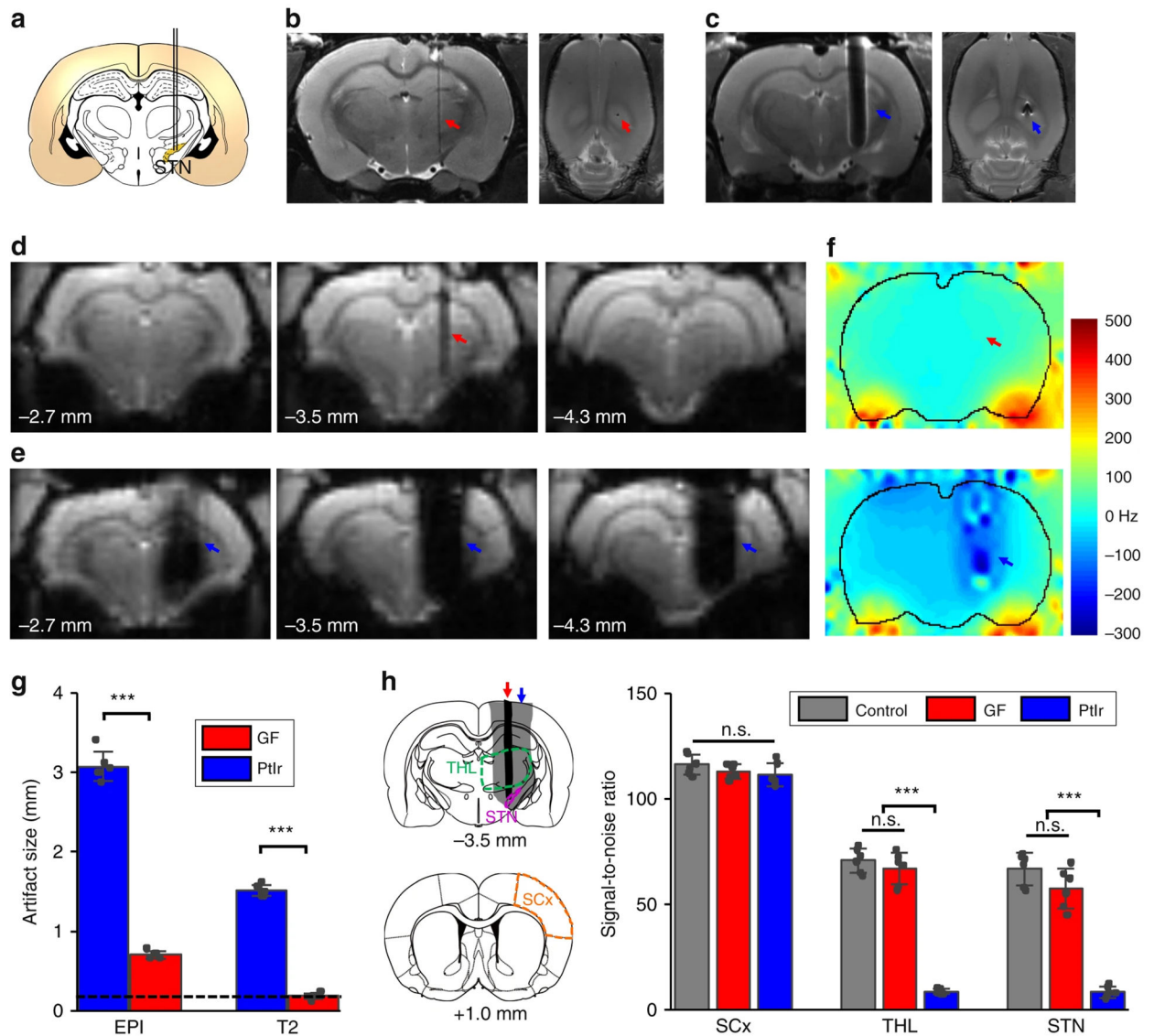


Figure 1. Comparative analysis of MRI artifacts induced by graphene fiber and PtIr electrodes in rat brain.

(A) Schematic of electrode placement in the rat STN. (B-C) T2 MRI with graphene fiber (GF) and PtIr electrodes. (D-E) EPI images showing anterior to posterior placement of GF and PtIr. (F) B0 maps indicating GF and PtIr artifact areas. (G) Artifact size comparison between GF and PtIr electrodes. (H) SNR comparison across brain regions in control, GF, and PtIr implanted rats. Reproduced from (59) with permission.

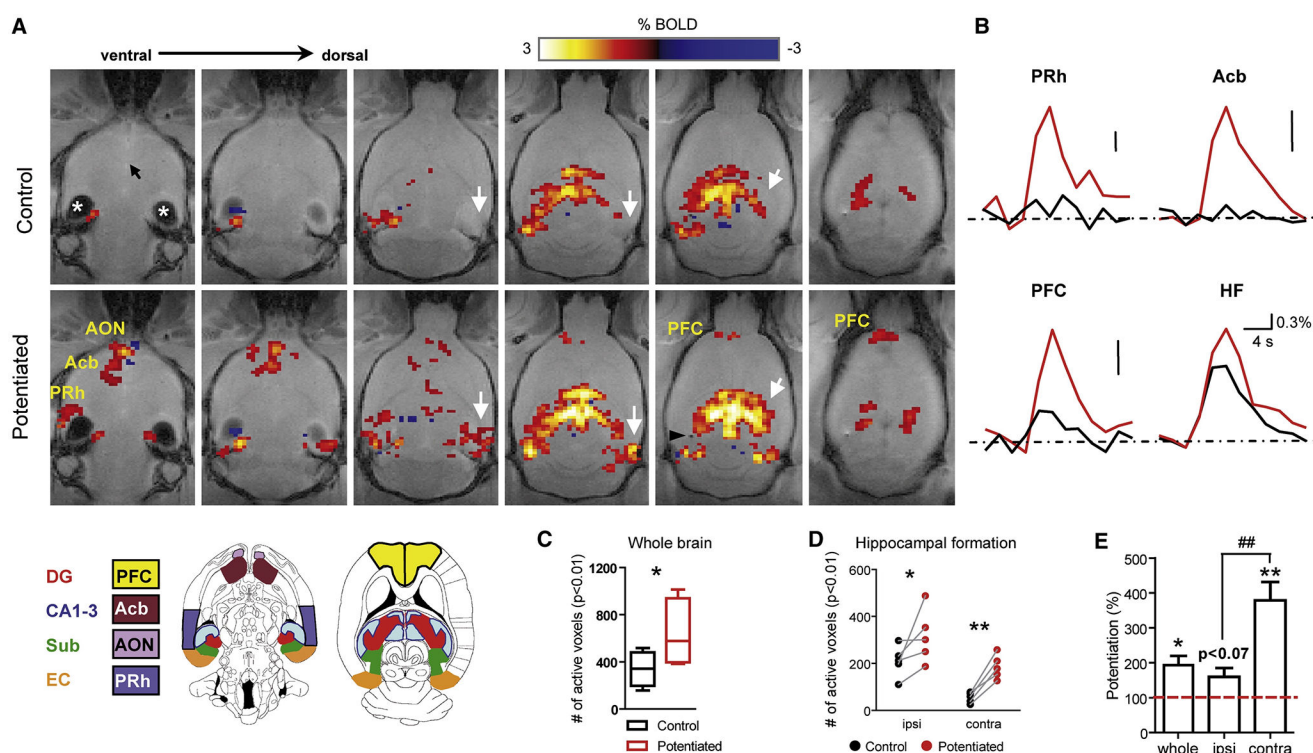


Figure 2. Neural circuit reorganization following hippocampal long-term potentiation (LTP) induction.

(A) MRI-derived brain activity during and after hippocampal stimulation, highlighting LTP effects. (B) BOLD signal shifts in targeted regions. (C-E) Increase in active voxels and altered activation patterns in hippocampal and associated brain regions post-LTP. Reproduced from (51) with permission.

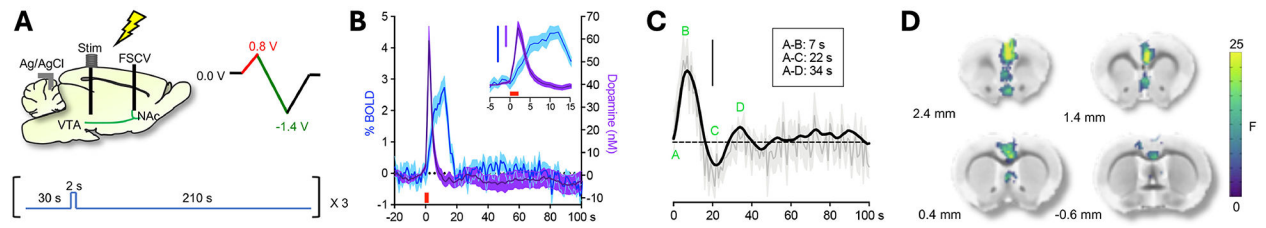


Figure 3. Multi-modal Approach for Mapping Dopamine Dynamics via DBS and FSCV-fMRI. (A) Setup showing VTA electrical stimulation and FSCV at the NAc. (B) Corresponding BOLD and dopamine signals. (C) Group hemodynamic response from dopamine activity. (D) BOLD fMRI data-based statistical maps, showing brain regions that preferentially encode dopamine amplitude differences rather than solely reflecting stimulus-evoked dopamine release. Reproduced from (52) with permission.

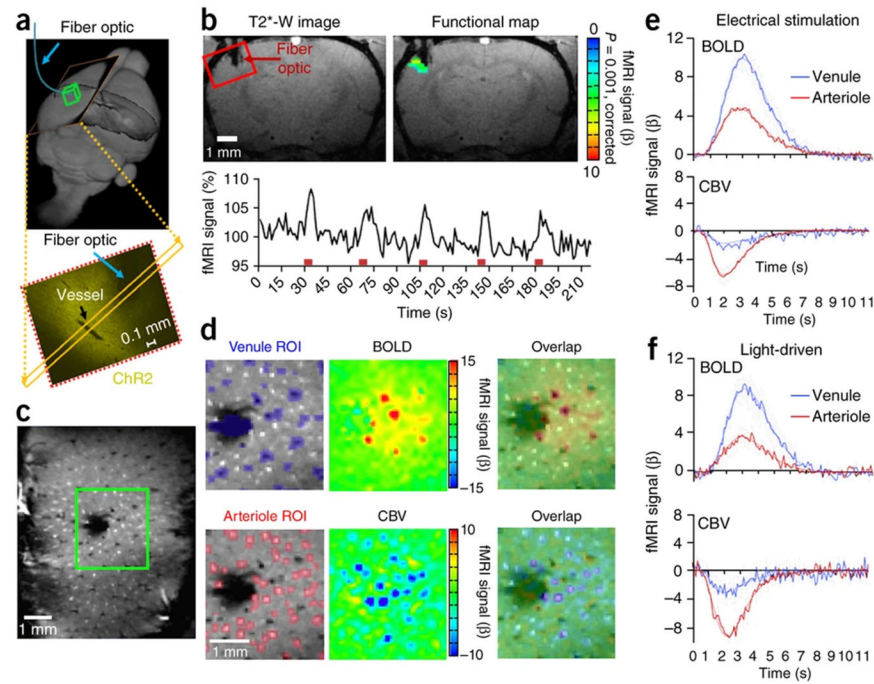


Figure 4. Optogenetically Driven Single-Vessel fMRI in the Rodent Brain.

(A) Experimental setup indicating ChR2 expression and fiber optic positioning. (B) fMRI signal change in the targeted cortical area. (C) Vascular mapping differentiating arterioles and venules. (D) BOLD and CBV changes in specific vascular regions. (E) Averaged BOLD and CBV responses to sensory stimulation of the forepaw. (F) Time courses of BOLD and CBV from targeted vessels during optogenetic stimulation. Reproduced from (33) with permission.

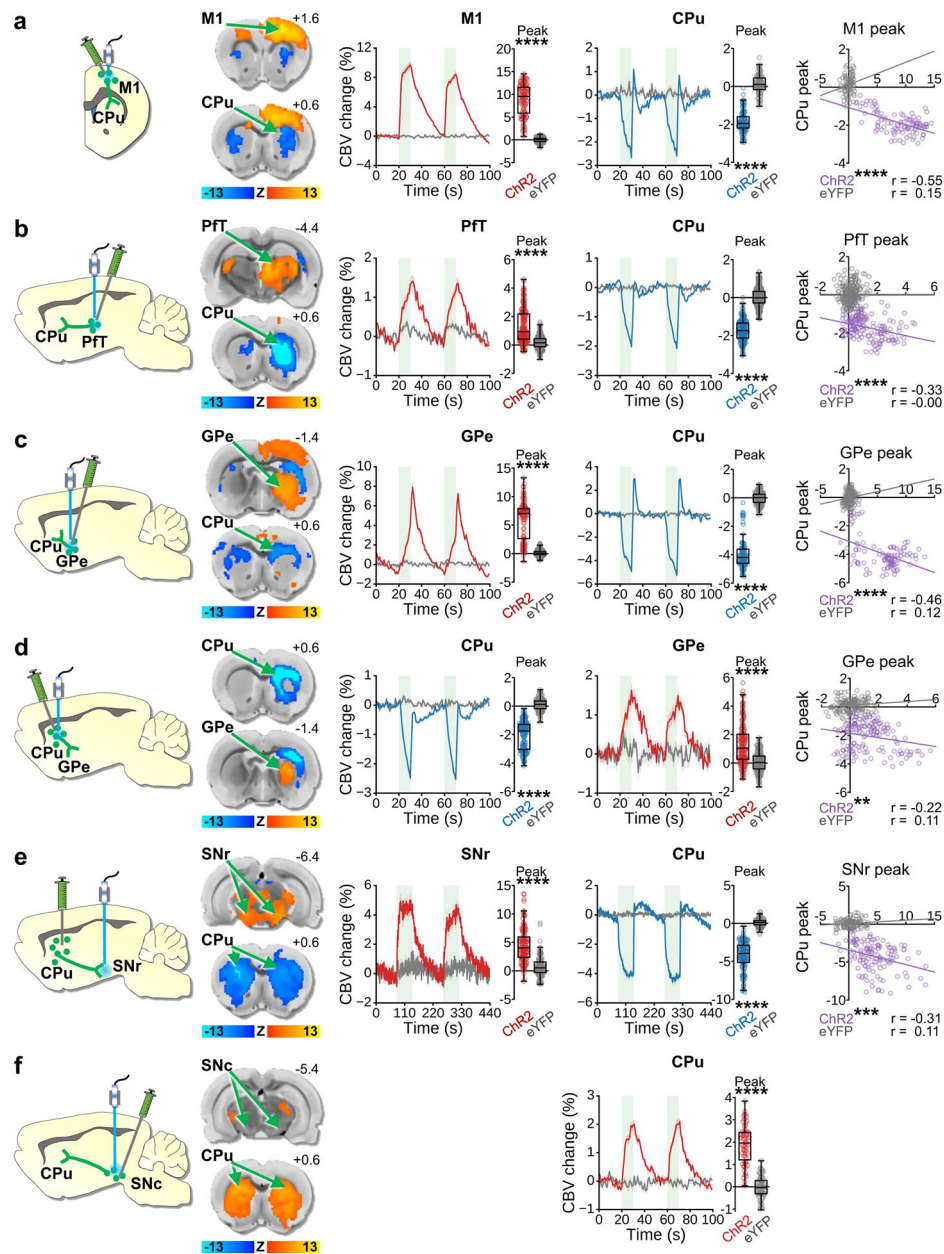


Figure 5. Differential fMRI Responses to Optogenetic Stimulation of Circuits Related to Striatum.

(a) to (f) depict the fMRI responses to optogenetic stimulation in various brain regions. The time-courses reveal peak changes during stimulation and correlation plots illustrate the relationships between stimulation targets and projections. Results indicate varied stimulus-evoked responses across circuits, reflecting the complexity of circuit-level contributions to fMRI signals. Reproduced from (15) with permission.

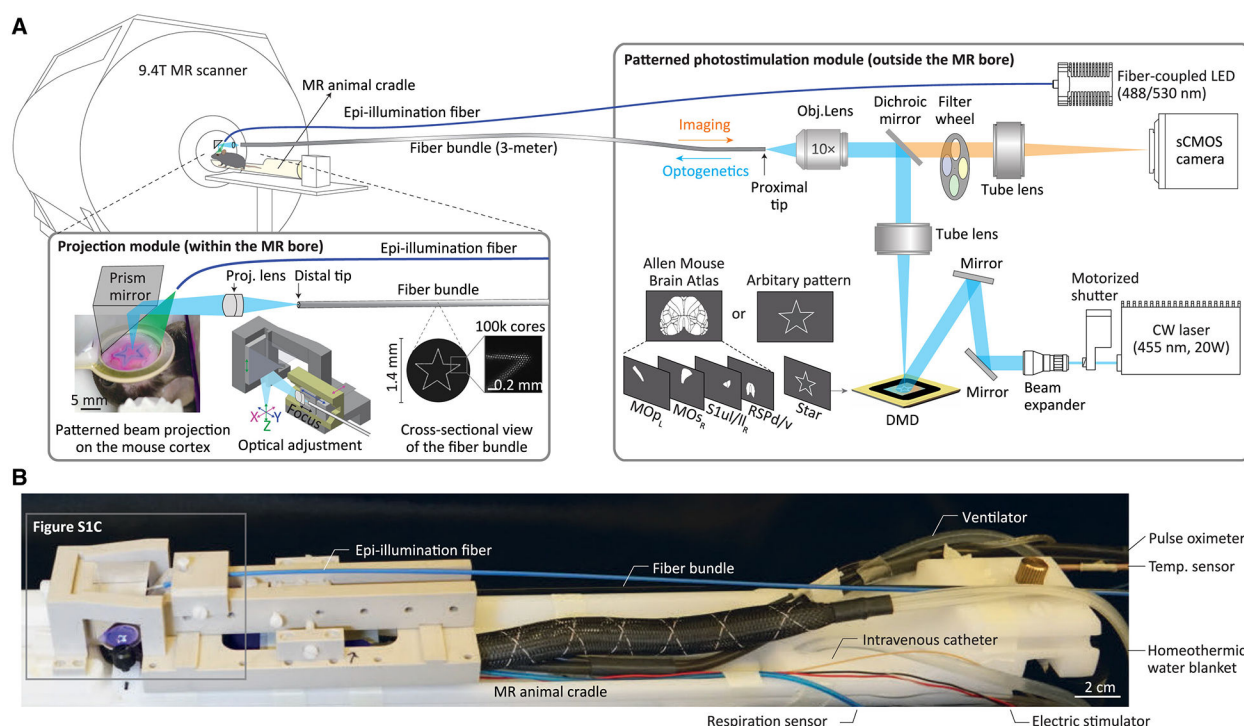


Figure 6. Cortex-wide Optogenetic fMRI Mapping System.

(A) The schematic outlines the innovative system for cortex-wide optogenetic fMRI, showcasing a continuous wave laser that projects through a digital micromirror device to shape the light beam. (B) The setup within the MRI environment includes the animal life-support and monitoring apparatus. Reproduced from (101) with permission.

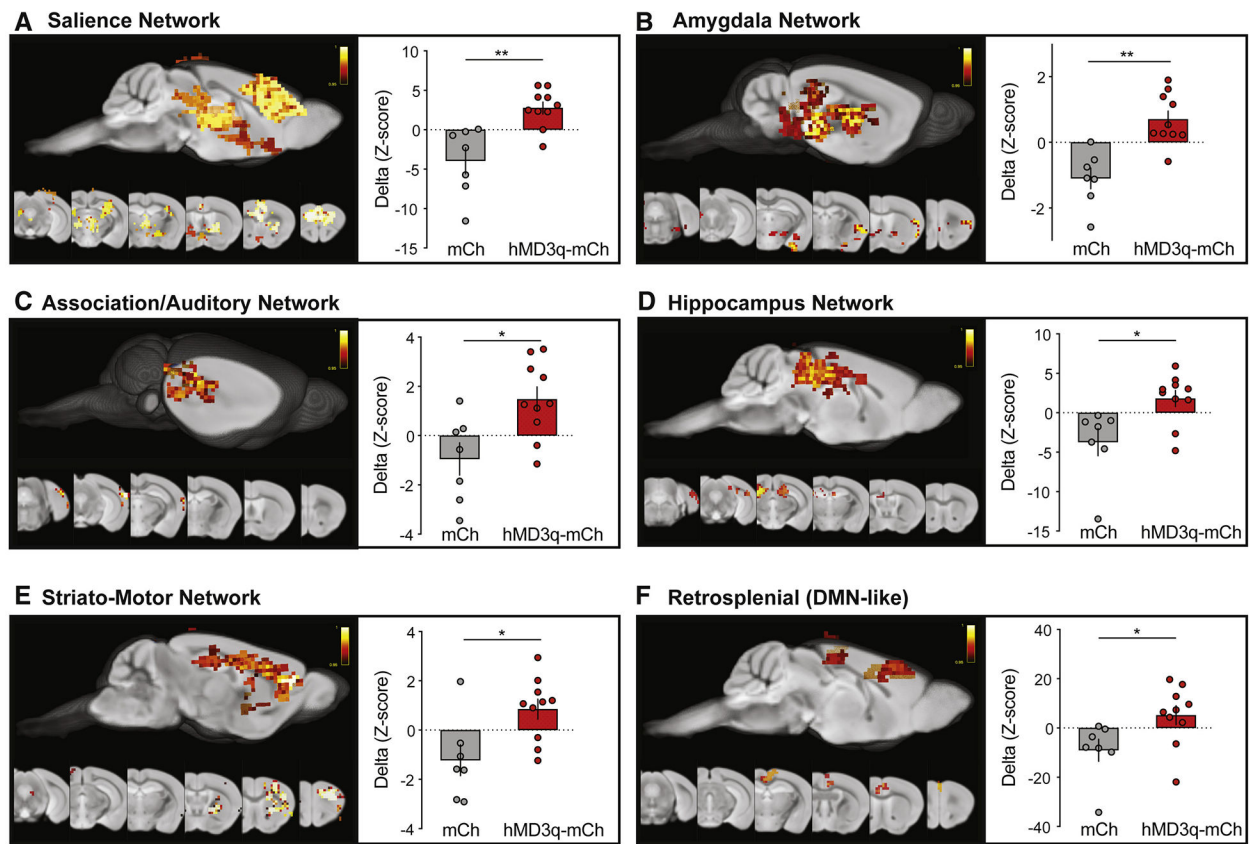


Figure 7. Impact of Locus Coeruleus Activation on Resting-State Network Connectivity.

Voxel-wise dual regression analysis identified changes within six key neural networks: (A) Salience, (B) Amygdala, (C) Association/Auditory, (D) Hippocampus, (E) Striato-Motor, and (F) Retrosplenial (DMN-like) networks. Increases in network strength post-clozapine administration were significant in mice expressing hMD3q-mCherry compared to control mice. Reproduced from (160) with permission.

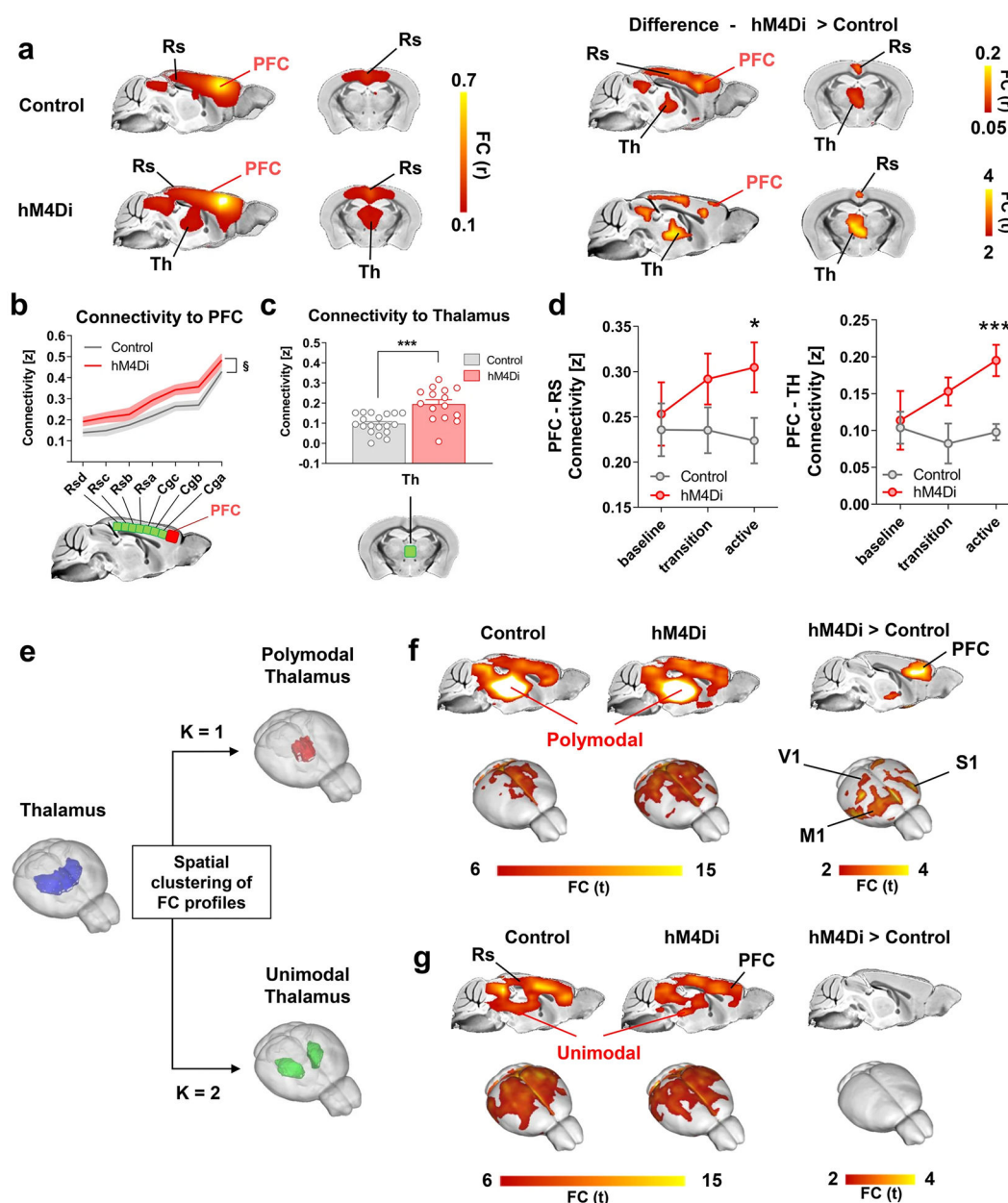


Figure 8. Effects of Medial Prefrontal Cortex Inhibition on Resting-State fMRI Connectivity
 (a) Changes in DMN connectivity with hM4Di expression. (b) Connectivity profiles along the DMN axis. (c) Thalamo-cortical synchronization increase in hM4Di mice. (d) Temporal connectivity changes in hM4Di mice. (e) Classification of thalamic regions by connectivity patterns. (f-g) Connectivity in polymodal and unimodal thalamic regions, with altered network dynamics evident in hM4Di mice. Reproduced from (168) with permission.

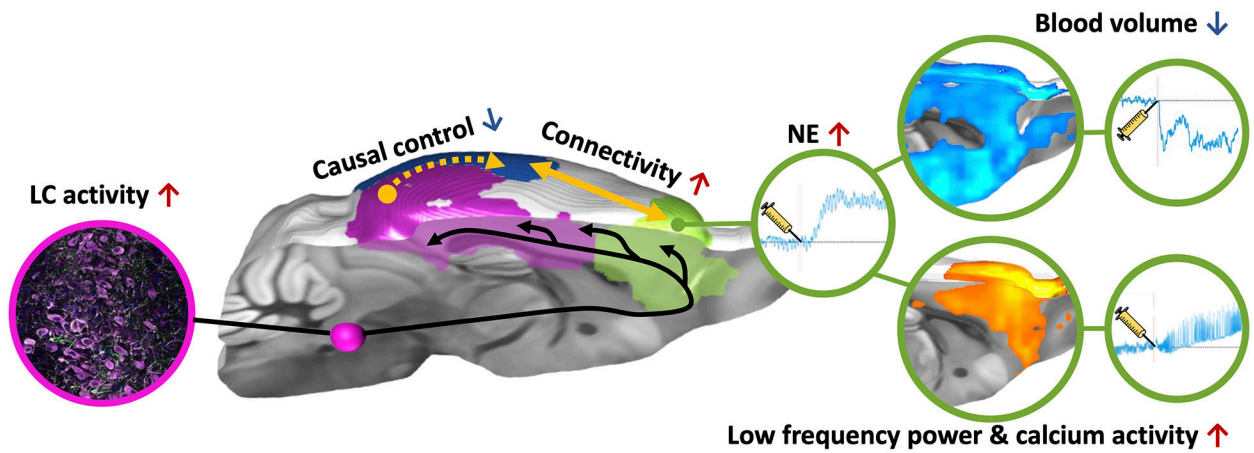


Figure 9. Chemogenetic Stimulation of Tonic Locus Coeruleus Activity Alters Frontal Cortical Activity and Connectivity.

Chemogenetic-induced tonic locus coeruleus activity decreases cerebral blood volume, increases synchronous low-frequency fMRI activity and calcium-weighted neuronal spiking. This stimulation also strengthens functional connectivity within the frontal DMN by reducing the modulatory inputs from the retrosplenial and hippocampal regions to the association cortices of the DMN. Summarized from (26) with permission.

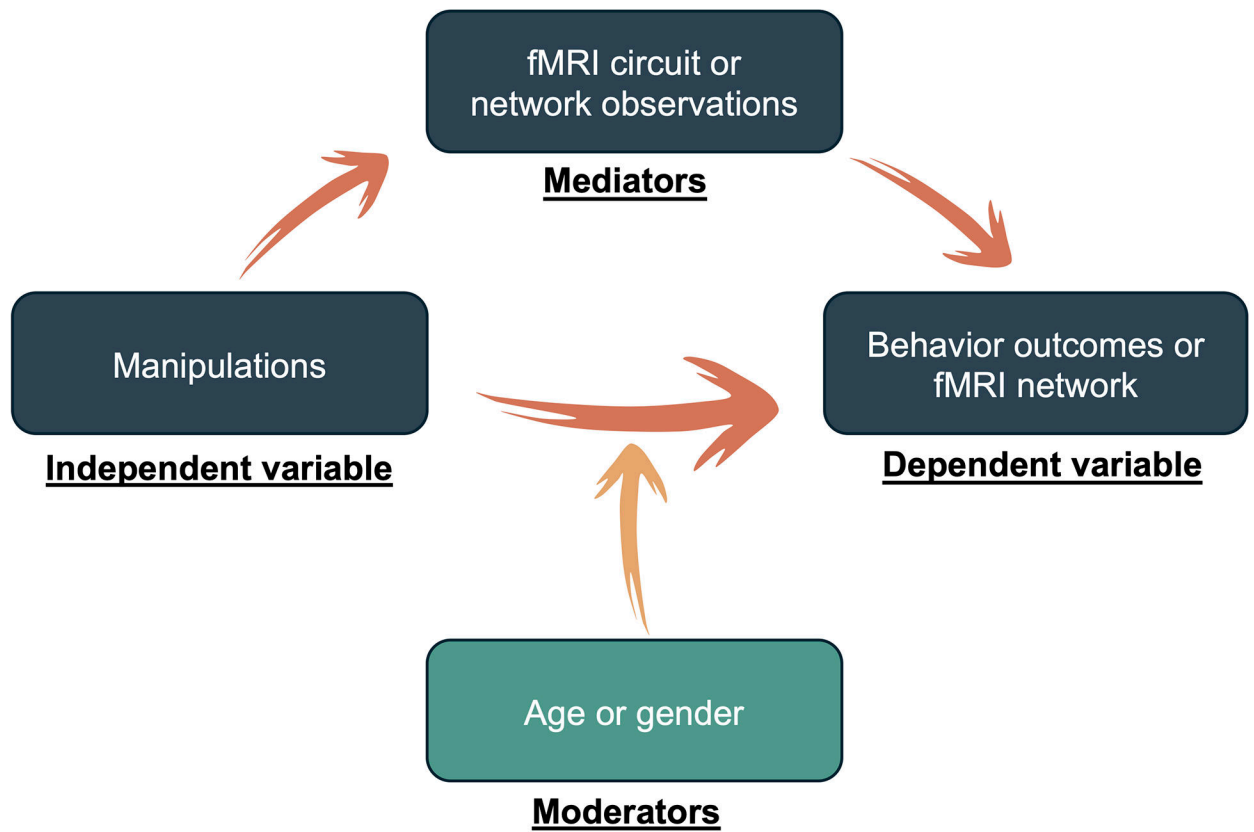


Figure 10. Mediation and Moderation Frameworks in Neuromodulation Studies.

The upper route illustrates the mediation model, positing that the impact of neuromodulatory interventions, such as DBS, optogenetics, and chemogenetics, on behavioral outcomes is intermediated by circuit or network changes, detectable via fMRI. The bottom route presents the moderation model, suggesting that individual characteristics such as age or gender can modulate the influence of neuromodulatory techniques on behavioral results. Together, these models emphasize the intricate relationships among experimental manipulations, neural circuitry, individual differences, and behavior in neuromodulation research.