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Fiber Photometry for Measuring Neuronal Activities in the Subcortical Regions of the Central Auditory Pathway

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Abstract: Fiber photometry has become a prominent tool for measuring population-level neuronal activity in the central auditory system (CANS). Yet, its application in subcortical regions, such as the inferior colliculus (IC) and medial geniculate body (MGB), remains underexplored. This review critically examines the limitations of in vivo fiber photometry in studying these regions, focusing on three key constraints: (1) low temporal resolution, which obscures millisecond-scale auditory responses; (2) lack of cell-type specificity, conflating excitatory and inhibitory signals; and (3) susceptibility to confounding variables (e.g., motion artifacts, GECI leakage). By synthesizing empirical studies from the IC and MGB, this review highlights how these limitations compromise data fidelity, particularly in the IC, where electrophysiology dominates due to its precision and accuracy. In the MGB, fiber photometry struggles with subregion-specific signal isolation and directional ambiguity in thalamocortical circuits. This review proposes that integrating fiber photometry with complementary techniques (e.g., optogenetics, electrophysiology) could mitigate these weaknesses. This review underscores the need for methodological innovations to enhance fiber photometry's utility in auditory neuroscience while clarifying its current constraints.

Keywords: Fiber Photometry; Inferior Colliculus; Medial Geniculate Body; Genetically Encoded Calcium Indicators (GECIs); Internal Validity.

1. Introduction

The auditory sense is a palpable sensory modality that species use to elicit sound information from external stimuli. Exploring the auditory sense allows researchers to gain a clearer understanding of how we process music; to identify biomarkers for hearing disorders; and to develop state-of-the-art technologies like artificial hearing systems and brain-machine interfaces (BMI). However, the intricacies behind anatomical details make the auditory sense challenging to study. Briefly, the central auditory nervous system (CANS) begins with the cochlea to the brain stem, to the middle brain, and ultimately reaches the auditory cortex (AC), where each brain region along the CANS has a different function and analyzes diverse aspects of a sound stimulus.

As mathematician and philosopher Rene Descartes said, "divide each difficulty into as many parts as is feasible and necessary to resolve it", one plausible strategy for the research of CANS is to measure the neuronal activities of each brain subregion in CANS when sound stimuli are given. This can be achieved by adapting " in vivo fiber photometry" into the research of CANS.

Fiber photometry is an optical technique where light triggers and measures fluctuations in fluorescence that arise from conformational change to an expressed biosensor [1]. In vivo fiber photometry means that this procedure is conducted on live animals (often rodents). Specifically, excitation light of a specific wavelength is delivered through an implanted optical fiber in a particular brain region, and fluorescence intensity from the region returns through the same fiber to reach a photodetector. Subsequently, the photodetector can convert the temporal fluorescence signals from the optical fiber into a spatial display through an analogue-to-digital converter (ADC). This allows the fluctuation in fluorescence to be presented on a time (x-axis) vs. fluorescence intensity (y-axis) graph, which is useful for statistical analysis.

Genetically encoded calcium indicators (GECIs) are where the fluorescence signals originate, and they can mark the activity of various neuronal elements, such as neurotransmitters, somas, and overall brain regions. Ca^{2+} concentration indicates the magnitude of activity in brain regions or neuronal elements. GECIs function in a way where calcium ion binding can cause conformational change so that when the GECI is activated by optics with a specific wavelength, it can generate fluorescence according to the concentration of calcium ions. The brain does not naturally express GECIs, though: researchers must artificially express the GECIs in the brain. Meanwhile, expressing too few GECIs leads to no signals, while expressing too much can lead to cell death. Thus, neuroscientists apply either viral expression or transgenic mouse models to achieve optimal GECI expression in animal models.

The ability of fiber photometry to capture real-time activity of specific brain regions in vivo paved the way for the research on CANS. Hitherto, fiber photometry has a variety of usages in the investigation of CANS, not limited to longitudinal research involving neuroplasticity in the CANS, such hearing loss; fear conditioning; how different frequencies in music affect activity of particular brain region within the CANS coding; rhythmic entrainment, which is the tendency of the neuronal firing rates in the CANS to align with the rhythm of sound stimuli; and the dynamic relationship between several CANS brain regions.

Nevertheless, fiber photometry carries limitations that can impair the depth and accuracy of research findings about the CANS. The review takes a closer look at why fiber photometry is vulnerable to these issues and how it affects experimental data. Admittedly, fiber photometry is a

convenient technique to collect neuronal activity in live animals. Nevertheless, recognizing major weaknesses of fiber photometry—and how they are expressed in studies— can steer researchers to develop more robust data collection methods to output research findings with high predictive power and applicability to real-life. Thereby, this review aims to reflect upon the limitations of fiber photometry to measure neuronal activities in the CANS.

2. Methodology

This review focuses on two subcortical regions of the CANS, the inferior colliculus (IC) and the medial geniculate body (MGB). Limitations of fiber photometry in each brain region are discussed theoretically and empirically. The theoretical side focuses on the shortcomings of fiber photometry locally, while the empirical side shows how the limitations of fiber photometry can influence experimental findings. Ultimately, this review will holistically evaluate the use of fiber photometry for the IC and MGB.

The scientific works used in this review were selected from science journal databases PubMed, ScienceDirect, eLife, Wiley, and Google Scholar.

3. Inferior Colliculus

3.1. Anatomy and Function of IC

The IC is a paired structure in the midbrain lying rostral to the trochlear nerve and caudal to the superior colliculus (SC) [2] It is composed of three regions, including the central nucleus, the dorsal cortex, and the external cortex.

In the auditory pathway, the central nucleus of the IC (CNIC) serves as an important relay point for auditory information as it travels from the inner ear to the primary auditory cortex [3]. Specifically, it receives signals from the brainstem auditory nuclei, such as the superior olivary cortex (SOC) and the cochlear nuclei (CN), as well as top-down signals from thalamic regions like the MGB [4]. Meanwhile, the CNIC shoots out efferent signals through the brachium to the MGB ipsilaterally [3], making it a major computational hub in the central auditory pathway.

The IC holds the major function of integrating sounds by analyzing sound signals based on amplitude, frequency, and time course [5] (Jen et al., 1998), which is largely influenced by the overall inhibitory projections in the IC. The neural interaction in the IC involves the inhibitory neurotransmitters gamma-aminobutyric acid (GABA) and glycine. All neurons in the IC are under the influence of GABAergic or glycinergic inhibitory input [6]. Inhibition can filter out noise factors from a set of sound stimuli, suppress weak or irrelevant signals, and enhance salient auditory cues [7]. Inhibition also shapes the selectivity of neurons in the IC to specific frequencies and types of sounds. This helps to accentuate dissonant, non-harmonic, or dangerous sounds, making IC play a vital role in auditory fear conditioning.

3.2. Fiber Photometry's Theoretical Utility in IC

Fiber photometry can theoretically aid the investigation of the IC in a variety of ways. Applying in vivo fiber photometry by implanting optical fibers in the IC can measure the activity of the IC over time while rodents (s) receive sound stimuli. It can track IC activity during natural behaviors such as monitoring how the IC encodes threatening sounds; tracing rodents' habituation and sensitization to sounds; and studying afferent and efferent pathways involving the IC, such as simulating the SOC while measuring the activity of the IC.

3.3. Temporal Resolution Limitations

While fiber photometry can theoretically achieve many research goals, it has low temporal resolution. Fiber photometry relies on Ca2+ imaging (100ms resolution) that can obscure the millisecond-scale responses to sound stimuli. Synaptic latency measurements in vivo have shown that descending excitation reaches IC neurons roughly 5 to 7 milliseconds after spike initiation in auditory cortex [8]. Action potentials in the IC fire in the duration of 0.5-1 millisecond [9] with a first spike latency to sound at 5-30 milliseconds [10]. Therefore, fiber photometry's reliance on Ca^{2+} kinetics at the timesteps around hundred milliseconds is too slow to capture the millisecond-scale spiking of IC neurons. Instead, fiber photometry blurs rapid dynamics into population averages. Hence, using fiber photometry to measure activity in the IC risks losing data precision.

3.4. Confounding Variables in Freely Moving Paradigms

Moreover, fiber photometry in the IC is especially vulnerable to confounding variables, as the rodent is allowed to move. The freedom of movement permits the rodent to exhibit more irrelevant behaviors concerning the tested variables. These behaviors can likely influence the activity of IC, as IC receives top-down control from various brain regions, such as the auditory cortex (AC) [8] [11], superior colliculus (SC) [12], and the locus coeruleus (LC) [13]. For example, the superior colliculus (SC), which is dorsally adjacent to the IC, regulates spatial navigation based on auditory and visual cues. Locomotion triggers SC activity [14], which, in turn, sends descending signals to the IC to alter sound responses. This alters the activity of IC. Speaking of the SC-IC pathway, one predominant factor that causes locomotion is fear: the more fearful rodents are, the more sensitive they are to auditory and visual cues for threat, and this can intensify activity in the SC-IC pathway even more, resulting in overestimated IC activity. This can potentially harm the internal validity of research involving the measurement of IC activity.

3.5. Cell-Type Specificity Challenges

Another weakness of fiber photometry that can potentially appear in the measurement of IC activity is the lack of cell type specificity. Commonly, fiber photometry uses intracellular Ca^{2+} concentration as an indicator of cellular activity. Knowing that calcium-driven conformational change—in general--triggers the release of neurotransmitters into the synaptic gap [15], and that the firing of action potentials in both excitatory and inhibitory neurons triggers the influx of Ca^{2+}, fiber photometry cannot represent excitatory/inhibitory activity in the nervous system. Meanwhile, the dynamical relationship between excitatory and inhibitory signals in the IC is crucial for sound localization and selectivity. Specifically, the inhibitory neurotransmitters GABA and glycine play vital roles in regulating binaural hearing by filtering out noise factors from sound stimuli [16]. Moreover, the dynamical relationship between GABA and the excitatory neurotransmitter glutamate (Glu) regulates tonal selectivity of neurons [17][7][18].

Table 1. where ABS = Auditory Brainstem, AC = Auditory Cortex, BS = Brainstem, Co = Cochlea, IC = Inferior Colliculus, MB = Midbrain, MGB = Medial Geniculate Body, PVN = Paraventricular Nucleus, PVT = Paraventricular Thalamus, SPFTN = Subparafascicular Thalamus Nucleus, TH = Thalamus

Researchers	Year of Publication	Title	DOI	Brain Regions Studied	Technique for Brain Recording
Zhou et al.	2025	A midbrain circuit mechanism for noise-induced negative valence coding	10.1038/s41467-025-59956-z	IC	Fiber photometry + Optogenetics
Lee et al.	2025	Emergence of a brainstem somatosensory tonotopic map for substrate vibration	10.1038/s41593-024-01821-1	BS	In vivo electrophysiology + optogenetics imaging
Sitek et al.	2022	Structural Connectivity of Human Inferior Colliculus Subdivisions Using in vivo and post mortem Diffusion MRI Tractography	10.3389/fnins.2022.751595	IC	In vivo and postmortem diffusion MRI tractography
Yu et al.	2020	Fiber Photometry of Neural Activity in Specific Neural Circuit	10.1007/978-1-0716-1146-3_16	PVT, PVN	Fiber photometry
Yanagawa et al.	2016	Commissural functional topography of the inferior colliculus assessed in vitro	10.1016/j.heares.2015.08.011	IC	In vitro electrophysiology
Devore et al.	2013	Dual sensitivity of inferior colliculus neurons to ITD in the envelopes of high-frequency sounds: experimental and modeling study	10.1152/jn.00450.2013	IC	Extracellular electrophysiological recording + computational modeling
Oberle et al.	2021	Synaptic mechanisms of top-down control in the non-lemniscal inferior colliculus	10.7554/eLife.72730	IC	In vivo electrophysiology + optogenetics
Blackwell et al.	2020	Auditory cortex shapes sound responses in the inferior colliculus	10.7554/eLife.51890	AC, IC	In vivo electrophysiology + optogenetics
Xie et al.	2021	Transcriptomic encoding of sensorimotor transformation in the midbrain	10.7554/eLife.69825	MB	RNA sequencing and in situ hybridization
Gilad et al.	2020	Learning-related population dynamics in the auditory thalamus	10.7554/eLife.56307	MGB	In vivo electrophysiology + optogenetics
Mellot et al.	2014	Excitatory and inhibitory projections in parallel pathways from the inferior colliculus to the auditory thalamus	10.3389/fnana.2014.00124	IC, MGB	Tracing + Electrophysiology
Burghard et al.	2016	The contribution of inferior colliculus activity to the auditory brainstem response (ABR) in mice	10.1016/j.heares.2016.08.008	IC, ABS	In vivo electrophysiology
Richter et al.	2011	Spread of cochlear excitation during stimulation with pulsed infrared radiation: inferior colliculus measurements	10.1088/1741-2560/8/5/056006	IC	In vivo electrophysiology
Tan et al.	2015	Temporal properties of inferior colliculus neurons to photonic stimulation in the cochlea	10.14814/phy2.12491	IC, Co	In vivo electrophysiology
Baumhoff et al.	2018	Intracochlear near infrared stimulation: Feasibility of optoacoustic stimulation in vivo	10.1016/j.heares.2018.11.003	Co	In vivo optoacoustic stimulation + electrophysiology
Hu and Fei	2022	An inferior-superior colliculus circuit controls auditory cue-directed visual spatial attention	10.1016/j.neuron.2021.10.004	IC, SC	In vivo electrophysiology + optogenetics
Yasui et al.	1990	The subparafascicular thalamic nucleus of the rat receives projection fibers from the inferior colliculus and auditory cortex	10.1016/0006-8993(90)90378-o	SPFTN	Tracing + electrophysiology
Ledoux et al.	1987	Topographic organization of convergent projections to the thalamus from the inferior colliculus and spinal cord in the rat	10.1002/cne.902640110	TH, IC	Tracing + electrophysiology
Cheng and Liang	2013	Neural interactions in unilateral colliculus and between bilateral colliculi modulate auditory signal processing	10.3389/fncir.2013.00068	IC	In vivo electrophysiology + optogenetics
Malmerica and Manuel	2021	Corticothalamic Pathways in Auditory Processing: Recent Advances and Insights From Other Sensory Systems	10.3389/fncir.2021.721186	TH, AC	Tracing + electrophysiology
Sun et al.	1998	Corticofugal regulation of auditory sensitivity in the bat inferior colliculus	10.1007/s003590050291	AC, IC	In vivo electrophysiology
Naumov et al.	2019	Analysis of excitatory and inhibitory neuron types in the inferior colliculus based on Ih properties	10.1152/jn.00594.2018	IC	In vivo electrophysiology + immunohistochemistry
Anderson and Malmerica	2012	The effect of auditory cortex deactivation on stimulus-specific adaptation in the inferior colliculus of the rat	10.1111/ejn.12018	AC, IC	In vivo electrophysiology + optogenetics
Ayala et al.	2015	Differences in the strength of cortical and brainstem inputs to SSA and non-SSA neurons in the inferior colliculus	10.1038/srep10383	IC	In vivo electrophysiology + optogenetics
Krishna and Semple	2000	Auditory Temporal Processing: Responses to Sinusoidally Amplitude-Modulated Tones in the Inferior Colliculus	10.1152/jn.2000.84.1.255	IC	In vivo electrophysiology

Knowing that fiber photometry cannot distinguish between excitatory and inhibitory signals and only measures populational signals, the encoding of both sound localization and frequencies (tonal selectivity) might be displayed as an increase in Ca^{2+} level, displayed as spikes that look similar. Such ambiguity makes it hard for researchers to identify the

function that the IC is performing and fail to accurately reflect the true functional mechanisms of neuronal activity in the IC for sound analysis. Hence, without cell-type specificity, applying sole fiber photometry to measure activities in the IC may risk conflating data.

To resolve this, Zhou et al., a recent study, employed Cre-expressing mice (Vglut2-Cre and Vgat-Cre) to restrict GCaMP6m expression to either glutamatergic or GABAergic IC neurons [19]. This approach disentangled their contributions: In Vglut2-Cre mice, photometry selectively captured CNIC Glu activity, revealing intensity-dependent responses to aversive sounds. In Vgat-Cre mice, it isolated GABAergic signals, which were suppressed during noise exposure. By pairing Cre-dependent targeting with pathway-specific manipulations, the study circumvented fiber photometry's inherent ambiguity, linking noise-induced aversion to glutamatergic (not GABAergic) transmission. This strategy exemplifies how cell-type-specific tools can recover functional precision lost in population-level recordings, particularly in circuits like the IC where excitation and inhibition encode divergent auditory features. Hence, if an experiment is investigating a specific function related to the IC, it is recommended to identify the neuromodulation or neurotransmission mechanism behind it and use Cre-expressing mice to target particular neurotransmitters.

3.6. Overview of Studies

Table 1. shows 26 relevant studies yielded after searching for the terms "Inferior colliculus fiber photometry", "GCaMP Inferior colliculus", "Optical fiber implantation inferior colliculus", "Fiber photometry IC", "Midbrain Central Auditory Fiber Photometry" with no constraints for publication year.

Databases used include PubMed, Google Scholar, and ResearchGate.

Out of these 26 relevant studies, 1 study (Zhou et al., 2025) involved implanting optical fibers in the IC for fiber photometry. A few studies—like Hu and Fei, Sitek et al.—focused on cortical pathways including IC (such as the SC-IC, AC-IC pathway and fiber photometry was applied; however, they did not implant optical fiber in the IC for fiber photometry.

3.6.1. Zhou et al.

Zhou et al. examined the neural pathways that encode unpleasant sounds and engender negative emotional responses to the sounds. By using fiber photometry, the researchers identified a specific circuit involving glutamatergic neurons in the CNIC, which relay aversive information to GABAergic neurons in the ventral tegmental area (VTA) via the cuneiform nucleus (CnF). Specific to the CANS, the researchers found that CNIC glutamatergic neurons (CNICglu) respond not merely to loud noise but also to aversive stimuli—electric shock, for example, suggesting a role in encoding negative sensory information.

3.6.2. Fiber Photometry in Zhou et al.

Fiber photometry was used extensively in the study to monitor the dependency between CNIC and CnF glutamatergic neurons and VTA GABA. To measure the activity of the CNICglu in particular, Zhou et al. used Vglut2-Cre mice, restricting genetic manipulation to CNICglu. Meanwhile, Cre-dependent AAVs (e.g., AAV-Flex-GCaMP6m) were injected into the CNIC so that the viruses only express GCaMP6m in VGlut2+ neurons.

By analyzing how fiber photometry data CNICglu change in response to sensory and nociceptor stimuli, researchers found that CNICglu responded to various intensities of white noise in a dose-dependent manner. Meanwhile, the same population of CNICglu also responded to other aversive—especially nociceptive--sensory stimuli. Collectively, these data suggest that the CNIC functions as a node integrating various sensorimotor modalities in a highly interconnected manner, complementing its well-established role in auditory perception.

The use of Cre-expressing mice for fiber photometry is an obvious strength of this study because this allows researchers to record fiber photometry data from neurons releasing particular neurotransmitters (Glu and GABA) that they are interested in. This reduces the chance of conflating calcium intensities from neurons releasing other neurotransmitters like serotonin and dopamine. By eliminating more extraneous variables, Zhou et al. establish a robust causal relationship between nociceptive stimuli and the activity of CNICglu. That being said, confounding variables such as the effect of mice movement might still interfere with the calcium level recording from CNICglu, especially when the mice are exposed to nociceptive stimuli that can trigger escape responses such as rapid body movements. On a different lens, the use of viral tools reflects methodological reductionism, where complex sensory processes are dissected into isolated components. Cre-lox systems (e.g., VGlut2-cre) constrain sensors to predefined neuronal populations (e.g., CNICglu), which ignores the interactions between the dynamical relationship between neurotransmitters. Nevertheless, as transmitter interactions can generate complex additive, subtractive, non-linear, or emergent effects [20], it is difficult to study without advanced mathematical modeling tools which are vulnerable to assumptions and inaccuracy. Overall, despite inevitable movements of the mice, fiber photometry added with Cre-expressing mice has provided robust empirical evidence to support the aim of Zhou et al.

3.7. Holistic View

Based on the theoretical knowledge and empirical evidence about the use of fiber photometry in measuring IC activity, it is clear that fiber photometry has inherent limitations in cellular and temporal specificity; however, as a convenient recording technique, the specificity it can achieve is already legitimate. Moreover, recent research and development paved the way for fiber photometry to target specific neurotransmitters by using Cre-expressing mice as experimental subjects. Hence, for the most part, fiber photometry is indeed useful for the research on the IC. That said, as shown in Table 1, very limited studies have used fiber photometry to measure IC activity. In other words, the effect of theoretical concerns of fiber photometry on experimental data about IC activity is weakly substantiated.

4. Medial Geniculate Body

4.1. Anatomy and Function of MGB

After an auditory signal passes through the IC, it reaches the MGB. The MGB is located in the thalamus. It receives signals from both up and downstream auditory regions, including the auditory brainstem, inferior colliculus, and top-down control from the auditory cortex and thalamic reticular nucleus (TRN) [21]. Meanwhile, the projections include regions of the auditory cortex and TRN. The MGB comprises several subnuclei, such as the ventral, dorsal, and medial

nuclei.

Each subdivision of the MGB contributes to different aspects of sound decomposition. The ventral division of the MGB (MGV) regulates the rapid transmission of auditory information sharply tuned for frequency and responds to fast temporal modulations of sounds [22]. MGV receives IC input mainly from the CNIC. Moreover, MGV processes sound with short tone latency (10ms) and has a narrow receptive field. MGV has tonotopic organization but sends signals to the A1for more precise tonotopic mapping. On the other hand, the dorsal division of MGB (MGD) often synchronizes with broad, multi-peaked [23], and complex sound stimuli with longer tone latency (20-50 ms). MGD receives IC inputs from the dIC. That being said, MGD does not contain tonotopic mapping [22]. Finally, the medial nucleus of the MGB (MGM), in comparison with the previous nuclei has more disparate functions: some neurons synchronize with rapid modulation frequencies whereas others synchronize with slower modulations. It processes both high and low sound frequencies. Though not as precise as MGV, the MGM also contains tonotopic mapping. In terms of innervations with the IC, the MGM receives IC input from the ECIC.

The neuronal signaling in the MGB is more complicated on the neuronal level, as different subregions of the MGB contain different neuronal types. Specifically, tufted neurons (typically have a single primary dendrite that branches in a tufted pattern) are often found in the MGV [24] and are mostly glutaminergic (excitatory). Its basic structure can reflect its ability to process simple sound features like frequency and intensity; bipolar neurons (two primary dendrites emerging from opposite poles of the soma) are found in both MGD and MGM divisions. The fact that it receives information contralaterally allows it to integrate multisensory modalities such as auditory-somatosensory inputs in the MGM. Then, stellate cells (dendrites radiating in various directions) are predominantly located in the MGD [25]. By synthesizing multiple stimuli as well as balancing excitatory and inhibitory (E/I) ratios, stellar cells play a critical role in cross-frequency integration, complex sound processing (e.g. music, speech), and top-down modulation.

4.2. Theoretical Utility of Fiber Photometry on MGB

Given how protean and intricate the MGB is, it is worthwhile to apply fiber photometry to study how different subsections of the MGB respond to varying external stimuli. Possible applications include learning about fear conditioning; comparing MGB responses to simple vs. complex sounds, natural and artificial sounds, or consonant vs. dissonant sounds; and recording MGB responses (MGM in specific) during cross-modal tasks.

4.3. GECI Leakage Between MGB Subregions

As seen before, the MGB consists of adjacent subregions MGV, MGM, and MGD that each contain different types of neurons and respond differently—maybe even oppositely—to audio stimuli. Therefore, the calcium signal collected from different subsections of the brain may vary abundantly. Meanwhile, virally injected GECIs inside the brain can diffuse within a radius of 0.5 to 1.5 millimeters depending on the virus used to carry the GECIs and how much GECIs are injected. As evidence, one study found GCaMP6 expression carried by adeno-associated viral vector 1 (AAV1) in mouse cortex spreads 0.8 to 1.2 millimeters from the site it is injected [26]. In the MGB, GCaMP leakage between tonotopically organized MGV and non-tonotopic MGD could obscure subnucleus-specific sound processing [22]. Additionally, a 1 mm spread of calcium indicators could capture and average out signals from several MGB subregions. In this case, if a researcher targets at measuring the activity of a specific subregion of the MGB to sound stimulus, then the spread of GECIs will introduce extraneous variable and random noise to the data collection, reducing the causal relationship between the independent variable the researcher is testing and the activity of the targeted MGB subregion. As measurements of populational activity might conflate heterogeneous activities from several subregions, using fiber photometry to record MGB activity theoretically hinders the internal validity of research on the MGB.

To address the confounding effects of GECI diffusion across MGB subregions (e.g., tonotopic MGV vs. non-tonotopic MGD), researchers can adopt two potential strategies. First, researchers should apply low-volume nano-injection to restrict expression to target subregions, which can minimize the spread of the GECI. However, this is expensive. Moreover, low-volume nano-injection means that the rodent must be tested quickly after the injection because GECIs like GCaMP may decay over time due to oxidation. One study, for instance, presented that GCaMP3 signals in the motor cortex declined approximately 30% over 3 weeks due to oxidative damage to the fluorophore, independent of photobleaching [27]. With less volume of GECI injected, the time taken for the GECI to decay is even shorter. If the experimenter waits for a long period until they begin using the rodent with a small volume of GECI, then the signals collected will be substantially weaker than the reality. That is, the amplitudes of calcium level will be scaled down, resulting in the underestimation of fluorescence intensity, and instigating systematic errors. Hence, if researchers decide to reduce the quantity of GECI used, it is recommended to experiment as soon as possible. If the experiment requires longitudinal observation, then it is not suggested to reduce of volume of injection. Another method that can mitigate the leakage of GECI in MGB subregions is, again, targeting brain regions based on precise chemical messengers by employing Cre-expressing rodents to limit GECI expression from being defined on irrelevant cell populations. In the context of cats and primates, MGV has an abundance of small GABAergic interneurons distributed amongst the lamina-forming principal cells. Therefore, it seems logical to directly target GABAergic neurons for the recording of MGV activities. However, in salient contrast, GABAergic neurons are absent in rats, illustrating the inconsistency of function across species [28].

4.4. Omission of Directionality of Neuronal Signal

As shown by the bipolar and multipolar neuronal cell types, the MGB contains neuronal signaling from different directions with different sets of purposes. For example, stellate cells in the MGD receive and synthesize various signals from the MGV neurons that are tonotopically mapped to analyze that complex sound as a whole. In this case, the signal travels from ventral to dorsal. Meanwhile, the MGB receives top-down control from cortical regions of the CANS such as the auditory cortex. Top-down descending corticothalamic projections to the MGB are more extensive than ascending reciprocal thalamocortical projections [29].

Top-down control to the MGB is relevant for speech recognition [30][31], sound discrimination [32], and processing complex tones [33].

However, fiber photometry, which relies on fluorescence intensity to measure overall neuronal activity, does not account for the directionality of signals. This means that using fiber photometry to measure activity in the MGB will hardly yield useful data to support the functions of the MGB relating to the direction of signals, showing how the use of fiber photometry can potentially limit the scope and depth of research on the functions of the MGB.

Nevertheless, in the MGB, neurons of the same type are in the same subregion, such that most unipolar neurons are positioned in the MGV while most multipolar neurons are in the MGM and MGD. Therefore, by applying fiber photometry to subregions of the MGB can theoretically operationalize the different functions of different neuronal cell types, as shown later in Li et al.; however, given the tendency of GECI leakage and the complex of ascending and descending signals in the MGB, the measurement remains coarse.

4.5. Overview of Studies

2 studies were found to use fiber photometry to measure MGB activity, including Gilad et al. and Li et al.

4.5.1. Gilad et al.

Gilad aimed at studying learning-related modulations, especially the discrimination of sound stimuli, in the MGB, targeting mainly the dorsal and medial regions of the MGB. To study this, Gilad et al chronically imaged MGB population responses to sounds as the mice learned a go/no-go auditory discrimination task, specifically, the mice were trained to lick in response to a "go" tone and withhold licking to a "no-go" tone. To operationalize the process of learning, researchers collected the responses from the MGB across different stages, such as the novice stage (when mice are learning and making choices) and expert stage (when the mice are gaining a reflex towards the go no-go sound discrimination task). After analyzing fiber photometry data using the area under the curve (AUC) method, researchers found that stimulus information is present in the MGB relatively early, whereas choice information develops relatively late: several hundred milliseconds after stimulus onset. That is to say, the MGB encodes sound stimulus early and choices late.

4.5.2. Fiber Photometry in Gilad et al.

To measure the populational calcium dynamics in the MGB as the mice learn this task, Gilad et al. applied fiber photometry: they injected AAV virus carrying the calcium indicator GCaMP6f into the dorsal or medial part of the MGB. From there, a 400 micrometer optical fiber was planted, enabling chronic imaging of population calcium responses from the MGB [34].

The use of fiber photometry aided Gilad et al. to visualize the difference in response latency between sound and choice-encoding. However, fiber photometry also introduced ambiguity to the results. Since the researchers were unable to decompose the fiber photometry signal into single-cell components, they can only speculate about the nature of cells encoding the choice. It is uncertain whether the difference in latency between sound and choice-encoding is due to the contribution of different neurons or the level of contribution of different neurons. Also, although most of the signal recordings were from the MGD and MGM, the feedback connection of MGB and higher cortex or signal leaking from the MGV are not excludable. Hence, with the use of fiber photometry, the findings from Gilad et al. can be challenged from two perspectives: first, neuronal signals are conflated, do not present cellular-level specifications; second, a considerable number of extraneous variables make the findings less robust.

4.5.3. Li et al.

Li et al., on the other hand, aimed to explore the role of the A1 in generating anticipatory motor responses to rhythmic sound stimuli in the process of rhythmic entrainment. Using a combination of techniques, including two-photon calcium imaging, fiber photometry, optogenetics, and behavioral assays, the authors demonstrated that neuronal ensembles in layers 2/3, 4, and 5 of A1 exhibit "echo responses" (activity at expected time intervals after rhythmic sound cessation). These responses correlated with anticipatory licking behavior in mice trained in an auditory task. The study concluded that A1 is essential for encoding predictive sensory information and transforming it into anticipatory motor actions.

4.5.4. Fiber Photometry in Li et al.

Fiber photometry in the MGB was used to show that the MGB does not contribute to auditory predictive coding. Specifically, the calcium indicator CTB-green was injected into MGV and MGD separately, accounting for the different functions they have: MGV is tonotopically mapped and processes rapid sound stimuli, while MGD is not tonotopically mapped and processes more complex sound stimuli as well as multisensory information. Across both regions of the MGB, the researchers found no echo responses (Fig. 1), supporting the conclusion that rhythmic entrainment happens in the cortex (A1), not the thalamus.

One important strength of using fiber photometry presented through Li et al. is that it allows researchers to visualize rhythmic entrainment, where spikes represent encoding of sound stimuli, which makes whether or not. Moreover, data triangulation was used to reduce the typical limitations of fiber photometry. Fiber photometry was not the sole neuroimaging technique used to reveal the absence of rhythmic entrainment in the MGB. Optogenetics was also applied, where the researchers turned the MGB of the rodents on and off and found insignificant levels of entrainment in both scenarios, illustrating the redundancy of MGB for predictive coding. Here, the use of optogenetics complemented the vulnerability of fiber photometry to extraneous variables. In other words, while fiber photometry correlates activity with behavior, optogenetics tests causality. By silencing MGB, the researchers can confirm MGB's dispensability for rhythmic entrainment; in other words, data triangulation has raised the internal validity of the experimental results from Li et al.

Moreover, by applying fiber photometry to measure the activities of both MGV and MGD, the researchers accounted for the functional heterogeneity in the MGB. Hence, the researchers can yield comprehensive data about the response of MGB to rhythmic sound stimuli. This compensates for the common weakness of fiber photometry to only measure neuronal activities in bulk. Overall, both studies have presented that fiber photometry is not ideal for demonstrating causality, as it is vulnerable to extraneous variables. In the case of Gilad et al., for example, the researchers admitted that signal leaking in the MGV and top-down signals from the high cortex to the MGB are inevitably present in the fiber photometry data they collected.

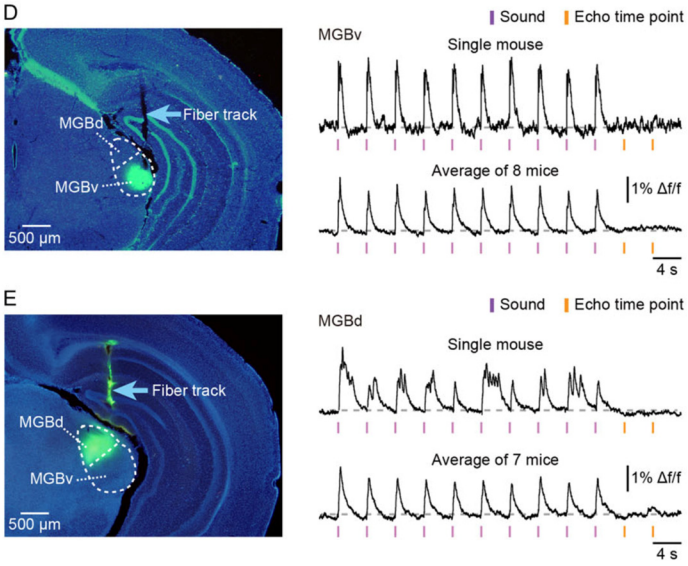

Figure 1. (D) Left: post hoc histology showing a fiber recording site in MGV. Right: single trial from this experiment (upper) and average of responses from 8 naïve mice (lower). (E) Left: a fiber recording site in MGD. Right: single trial from this experiment (upper) and average of responses from 7 naïve mice (lower) [38]

4.6. Holistic View

Given the vulnerability of fiber photometry to extraneous variables, fiber photometry theoretically only manifests correlations rather than causations, which introduces bidirectional ambiguity. For instance, MGB receives ascending input from the IC and descending input from the A1. In the case of Gilad et al., during auditory discrimination tasks, corticothalamic feedback and colliculo-thalamic feedforward both shape thalamic responses. Meanwhile, fiber photometry cannot distinguish whether it is MGB activity driving discrimination or merely reflecting cortical feedback about the discriminated sound stimuli. A similar issue applies to Li et al., where fiber photometry cannot discern whether it is MGB computing the prediction error or is it relaying feedback from the A1.

Nevertheless, as a convenient measuring tool, fiber photometry is still useful for presenting a rough trend for researchers studying sound encoding in the MGB. For more precise measurements, though, the triangulation of neuronal activity recording techniques such as fiber photometry, electrophysiology, and optogenetics--like the case of Li et al.—is more ideal than sole fiber photometry. Surprisingly, no studies have yet employed fiber photometry to investigate the MGM, a nucleus uniquely positioned to integrate multisensory inputs (auditory, somatosensory, vestibular) and project directly to non-auditory cortex. The pathway between MGM and S1 [35], for example, encapsulates a subcortical auditory hub bypassing A1 to influence somatosensation—making it a high-value target for fiber photometry. For instance, fiber photometry can display how MGM activity dynamically shifts during auditory-tactile threat conditioning or cross-modal plasticity. Accordingly, the use of fiber photometry for the research on the MGB is still restrained. Being a state-of-the-arts technique that is still undergoing development, fiber photometry is not merely a useful tool in measuring brain activities but can also engender new ideas in research. In conclusion, while fiber photometry can introduce shortcomings in the mapping of MGB trends, its true potential is unlocked through convergent methodologies.

5. Discussion

This review has critically examined the limitations of in vivo fiber photometry for measuring neuronal activity in key subcortical regions of the central auditory pathway (CANS), specifically the inferior colliculus (IC) and medial geniculate body (MGB). While fiber photometry offers significant advantages for longitudinal, population-level activity monitoring in behaving animals, its application in these auditory nuclei faces inherent constraints that can compromise data fidelity and interpretation.

Three primary limitations were highlighted. Firstly, the low temporal resolution inherent in calcium imaging (approx.100 ms) obscures the millisecond-scale dynamics crucial for auditory processing, particularly in the IC where neuronal spiking and synaptic events occur rapidly. This renders fiber photometry inadequate for capturing the precise timing of sound encoding. Secondly, the lack of cell-type specificity conflates excitatory and inhibitory signals. This is especially problematic in circuits like the IC, where the dynamic balance between glutamate, GABA, and glycine is fundamental for functions such as sound localization, frequency selectivity, and noise filtering. Bulk calcium signals fail to disentangle these opposing contributions. Thirdly, fiber photometry is susceptible to confounding variables. Motion artifacts in freely moving paradigms, coupled with the IC's sensitivity to top-down modulation from regions like the superior colliculus (e.g., during fear-induced locomotion), can introduce significant noise. In the MGB, GECI leakage between functionally distinct subnuclei (MGV, MGD, MGM) averages heterogeneous responses, obscuring subregion-specific processing. Furthermore, fiber photometry inherently omits signal directionality, a critical flaw in the MGB where ascending (colliculo-thalamic) and descending (corticothalamic) pathways have divergent functions in sound analysis and predictive coding.

Empirical evidence underscores these concerns. Studies in the IC (e.g., Zhou et al., 2025) demonstrate that while Cre-dependent targeting can recover some specificity, temporal blurring and movement artifacts remain challenges. Research

in the MGB (e.g., Gilad et al., 2020; Li et al., 2017) reveals difficulties in isolating subnuclei signals and establishing causality due to bidirectional ambiguity. Crucially, the scarcity of fiber photometry studies specifically targeting these subcortical nuclei (only a handful identified) highlights the methodological hurdles and reliance on electrophysiology for precision.

Despite these limitations, fiber photometry remains a valuable tool for observing population trends in vivo. Its true potential in auditory subcortical research, however, is unlocked not in isolation, but through strategic integration with complementary techniques. Combining fiber photometry with optogenetics (for causal manipulation and pathway dissection) and electrophysiology (for high temporal resolution and single-unit specificity) offers a powerful triangulation approach, as exemplified by Li et al. Future advancements should focus on refining GECI targeting (e.g., improved viral vectors, novel promoters), developing methods to mitigate motion artifacts and GECI spread, and exploring the understudied MGM with convergent methodologies. Acknowledging fiber photometry's current constraints is essential for designing robust experiments and accurately interpreting data, paving the way for more nuanced investigations into the complex neural computations of the auditory subcortex.

References

[1] Simpson, E. H., Akam, T., Patriarchi, T., Blanco-Pozo, M., Burgeno, L. M., Mohebi, A., Cragg, S. J., & Walton, M. E. (2024). Lights, fiber, action! A primer on in vivo fiber photometry. Neuron, 112(5), 718–739. https://doi.org/10. 1016/j.neuron.2023.11.016.

[2] Driscoll, M. E., & Tadi, P. (2023). Neuroanatomy, Inferior Colliculus. In StatPearls [Internet]. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/sites/books/NBK554468/.

[3] Mei, H.-X., Cheng, L., & Chen, Q.-C. (2013). Neural interactions in unilateral colliculus and between bilateral colliculi modulate auditory signal processing. Frontiers in Neural Circuits, 7, 68. https://doi.org/10. 3389/ fncir. 2013. 00068.

[4] Sitek, K. R., Calabrese, E., Johnson, G. A., Ghosh, S. S., & Chandrasekaran, B. (2022). Structural Connectivity of Human Inferior Colliculus Subdivisions Using in vivo and post-mortem Diffusion MRI Tractography. Frontiers in Neuroscience, 16, 751595. https://doi.org/ 10.3389/ fnins. 2022. 751595.

[5] Jen, P. H., Chen, Q. C., & Sun, X. D. (1998). Corticofugal regulation of auditory sensitivity in the bat inferior colliculus. Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology, 183(6), 683–697. https://doi.org/10. 1007/s003590050291.

[6] Merchán, M., Aguilar, L. A., Lopez-Poveda, E. A., & Malmierca, M. S. (2005). The inferior colliculus of the rat: Quantitative immunocytochemical study of GABA and glycine. Neuroscience, 136(3), 907–925. https://doi.org/10. 1016/j. neuroscience. 2004.12.030.

[7] Pollak, G. D. (2013). The Dominant Role of Inhibition in Creating Response Selectivities for Communication Calls in the Brainstem Auditory System. Hearing Research, 305. https://doi.org/10.1016/j.heares.2013.03.001.

[8] Oberle, H. M., Ford, A. N., Dileepkumar, D., Czarny, J., & Apostolides, P. F. (2022). Synaptic mechanisms of top-down control in the non-lemniscal inferior colliculus. eLife, 10, e72730. https://doi.org/10.7554/eLife.72730.

[9] Lumani, A., & Zhang, H. (2010). Responses of neurons in the rat's dorsal cortex of the inferior colliculus to monaural tone bursts. Brain Research, 1351, 115–129. https://doi.org/10. 1016/j. brainres.2010.06.066.

[10] Valdés-Baizabal, C., Casado-Román, L., Bartlett, E. L., & Malmierca, M. S. (2021). In vivo whole-cell recordings of stimulus-specific adaptation in the inferior colliculus. Hearing Research, 399, 107978. https://doi.org/10.1016/j. heares. 2020. 107978.

[11] Suma Chinta, & Pluta, S. R. (2025). Whisking and locomotion are jointly represented in superior colliculus neurons. PLoS Biology, 23(4), e3003087–e3003087. https://doi.org/10.1371/ journal. pbio.3003087.

[12] Hu, F., & Dan, Y. (2022). An inferior-superior colliculus circuit controls auditory cue-directed visual spatial attention. Neuron, 110(1), 109-119.e3. https://doi.org/10.1016/j. neuron. 2021.10.004.

[13] Hormigo, S., e Horta Junior, J. de A. de C., Gómez-Nieto, R., & López García, D. E. (2012). The selective neurotoxin DSP-4 impairs the noradrenergic projections from the locus coeruleus to the inferior colliculus in rats. Frontiers in Neural Circuits, 6. https://doi.org/10.3389/fncir.2012.00041.

[14] Blackwell, J. M., Lesicko, A. M., Rao, W., De Biasi, M., & Geffen, M. N. (n.d.). Auditory cortex shapes sound responses in the inferior colliculus. eLife, 9, e51890. https://doi.org/10. 7554/ eLife.51890.

[15] Neher, E., & Sakaba, T. (2008). Multiple roles of calcium ions in the regulation of neurotransmitter release. Neuron, 59(6), 861-872. https://doi.org/10.1016/j.neuron.2008.09.004.

[16] Klug, A., Park, T. J., & Pollak, G. D. (1995). Glycine and GABA influence binaural processing in the inferior colliculus of the mustache bat. Journal of Neurophysiology, 74(4), 1701–1713. https://doi.org/10.1152/jn.1995.74.4.1701.

[17] Gittelman, J. X., Wang, L., Colburn, S. S., & Pollak, G. D. (2012). Inhibition shapes response selectivity in the inferior colliculus by gain modulation. Frontiers in Neural Circuits, 6. https://doi.org/10.3389/fncir.2012.00067.

[18] Pollak, G. D., Xie, R., Gittelman, J. X., Andoni, S., & Li, N. (2011). The dominance of inhibition in the inferior colliculus. Hearing Research, 274(1), 27–39. https://doi.org/ 10.1016/j. heares. 2010.05.010.

[19] Zhou, S., Zhu, Y., Du, A., Niu, S., Du, Y., Yang, Y., Chen, W., Du, S., Sun, L., Liu, Y., Wu, H., Lou, H., Li, X.-M., Duan, S., & Yang, H. (2025). A midbrain circuit mechanism for noise-induced negative valence coding. Nature Communications, 16(1), 4610. https://doi.org/10.1038/s41467-025-59956-z.

[20] Parker, D. (2022). Neurobiological reduction: From cellular explanations of behavior to interventions. Frontiers in Psychology, 13. https://doi.org/10.3389/fpsyg.2022.987101.

[21] Rees, A. (2009). Medial Geniculate Body. In M. D. Binder, N. Hirokawa, & U. Windhorst (Eds.), Encyclopedia of Neuroscience (pp. 2275–2279). Springer. https://doi.org/10. 1007/978-3-540-29678-2_3364.

[22] Bartlett, E. L. (2013). The organization and physiology of the auditory thalamus and its role in processing acoustic features important for speech perception. Brain and Language, 126(1), 29–48. https://doi.org/10.1016/j.bandl.2013.03.003.

[23] Anderson, L. A., & Linden, J. F. (2011). Physiological differences between histologically defined subdivisions in the mouse auditory thalamus. Hearing Research, 274(1), 48–60. https://doi.org/10.1016/j.heares.2010.12.016.

[24] Bartlett, E. L., & Smith, P. H. (1999). Anatomic, Intrinsic, and Synaptic Properties of Dorsal and Ventral Division Neurons in Rat Medial Geniculate Body. Journal of Neurophysiology, 81(5), 1999–2016. https://doi.org/10.1152/jn.1999.81.5.1999.

[25] Chen, T.-W., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., Schreiter, E. R., Kerr, R. A., Orger, M. B., Jayaraman, V., Looger, L. L., Svoboda, K., & Kim, D. S. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature, 499(7458), 295–300. https://doi.org/10.1038/nature12354.

[26] Reiff, D. F., Ihring, A., Guerrero, G., Isacoff, E. Y., Joesch, M., Nakai, J., & Borst, A. (2005). In Vivo Performance of Genetically Encoded Indicators of Neural Activity in Flies. The Journal of Neuroscience, 25(19), 4766–4778. https://doi.org/10. 1523/JNEUROSCI.4900-04.2005.

[27] Winer, J. A. (1992). The Functional Architecture of the Medial Geniculate Body and the Primary Auditory Cortex. In D. B. Webster, A. N. Popper, & R. R. Fay (Eds.), The Mammalian Auditory Pathway: Neuroanatomy (pp. 222–409). Springer. https://doi.org/10.1007/978-1-4612-4416-5_6.

[28] Morest, D. K. (1964). The neuronal architecture of the medial geniculate body of the cat. Journal of Anatomy, 98(Pt 4), 611-630.1.

[29] Mihai, P. G., Moerel, M., Martino, F. de, Trampel, R., Kiebel, S., & Kriegstein, K. von. (2019). Modulation of tonotopic ventral medial geniculate body is behaviorally relevant for speech recognition. ELife, 8. https://doi.org/10. 7554/ elife. 44837.

[30] Kriegstein, K. von, Patterson, R. D., & Griffiths, T. D. (2008). Task-Dependent Modulation of Medial Geniculate Body Is Behaviorally Relevant for Speech Recognition. Current Biology, 18(23), 1855–1859. https://doi.org/10.1016/j.cub. 2008.10.052.

[31] Guo, W., Clause, A. R., Barth-Maron, A., & Polley, D. B. (2017). A corticothalamic circuit for dynamic switching between feature detection and discrimination. Neuron, 95(1), 180-194.e5. https://doi.org/10.1016/j.neuron.2017.05.019.

[32] Homma, N. Y., Happel, M. F. K., Nodal, F. R., Ohl, F. W., King, A. J., & Bajo, V. M. (2017). A Role for Auditory Corticothalamic Feedback in the Perception of Complex Sounds. The Journal of Neuroscience, 37(25), 6149–6161. https://doi.org/10.1523/JNEUROSCI.0397-17.2017.

[33] Gilad, A., Maor, I., & Mizrahi, A. (2020). Learning-related population dynamics in the auditory thalamus. eLife, 9, e56307. https://doi.org/10.7554/eLife.56307.

[34] Li, J., Liao, X., Zhang, J., Wang, M., Yang, N., Zhang, J., Lv, G., Li, H., Lu, J., Ding, R., Li, X., Guang, Y., Yang, Z., Qin, H., Jin, W., Zhang, K., He, C., Jia, H., Zeng, S., … Chen, X. (2017). Primary Auditory Cortex is Required for Anticipatory Motor Response. Cerebral Cortex, 27(6), 3254–3271. https://doi. org/10.1093/cercor/bhx079.

[35] Antunes, F. M., & Malmierca, M. S. (2021). Corticothalamic Pathways in Auditory Processing: Recent Advances and Insights From Other Sensory Systems. Frontiers in Neural Circuits, 15. https://doi.org/10.3389/fncir.2021.721186.