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GRIN lens applications for studying neurobiology of substance use disorder

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Abstract

Substance use disorder (SUD) is associated with severe health and social consequences. Continued drug use results in alterations of circuits within the mesolimbic dopamine system. It is critical to observe longitudinal impacts of SUD on neural activity *in vivo* to identify SUD predispositions, develop pharmaceuticals to prevent overdose, and help people suffering from SUD. However, implicated SUD associated areas are buried in deep brain which makes *in vivo* observation of neural activity challenging. The gradient index (GRIN) lens can probe these regions in mice and rats. In this short communications review, we will discuss how the GRIN lens can be coupled with other technologies such as miniaturized microscopes, fiberscopes, fMRI, and optogenetics to fully explore *in vivo* SUD research. Particularly, GRIN lens allows *in vivo* observation of deep brain regions implicated in SUD, differentiation of genetically distinct neurons, examination of individual cells longitudinally, correlation of neuronal patters with SUD behavior, and manipulation of neural circuits.

Keywords

GRIN lens; Addiction; Substance Use Disorder; miniscope

1.1 Introduction

Substance use disorder (SUD) involves chronic drug use [1] and can result in inabilities to meet responsibilities, brain damage, and overdose [2]. Researchers have used animal models to explore neural correlates of SUD and identify hallmarks such as “titrating” internal drug

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level [3], escalation [4], withdrawal [5], and drug-seeking [6]. Chronic SUD alters circuits within the mesolimbic system [7] such as nucleus accumbens (NAc) [8], amygdala [9], prefrontal cortex (PFC) [10], and hypothalamus [11]. Ideal technology for *in vivo* recording necessitates observation of deep brain regions, differentiation of genetically distinct cell types, longitudinal single-neuron tracking, and correlation of activity with behavior. In this short communication, we discuss how the gradient index (GRIN) lens can be implanted and used to achieve these criteria.

1.2 *In Vivo* Recording

Researchers have combined drug self-administration (SA) with *in vivo* recording technologies to correlate neural activity with behavior. Different techniques are useful for different circumstances and have pros and cons (Table 1). For instance, electrophysiology uses wires (steel, tungsten, platinum-iridium, etc.) to measure voltage changes in the extracellular environment. These act as a method to determine single-cell 'spikes' at high temporal resolution in deep brain regions [12, 13]. Neuronal subtypes are identified by distinct waveforms [14]. Drawbacks include data acquisition limited by channels, delicate microwires, and inability to differentiate between electrochemically similar neurons without optical manipulations [15]. Expression of immediate early gene C-*fos* labels large active neuronal populations and can be combined with transgenic animals [16, 17] at high spatial resolution but low temporal resolution [18] and activity does not always trigger *fos* expression [19]. Fast-scan cyclic voltammetry detects neurotransmission based on voltage oxidation at high temporal resolution in deep brain regions for specific neurotransmission studies [20, 21]. However, it cannot distinguish single neurons and can be clouded by high background current [22]. Fiber photometry quickly detects changes in population activity [23] using different types of sensors from deep brain regions [24] at axon terminals [25] and differentiates genetics at low cost [26] but lacks single-cell resolution [27]. The GRIN lens assists *in vivo* imaging in deep brain regions [28], using transgenic animals [29], and cost-effective open-source devices like miniaturized microscopes (miniscopes) [30–33] to record hundreds of individual neurons *in vivo* (Figure 1A) over months [34] which can be difficult to analyze. Neuron activity can be correlated with deep learning behavioral analyses [35], and optically manipulated with another LED without additional fibers [36]. However, care must be taken because physically damaging the lens or photobleaching neurons obscures activity.

1.3 GRIN lens overview

Light of a particular wavelength is transmitted from a source (e.g., LED) through a filter and dichroic mirror downward through the GRIN lens towards samples [37]). Active cells emit visible light in response to specific wavelengths and relay though the same GRIN lens towards a sensor/objective (Figure 1A) [38]. Traditional microscopy enhances signal visibility by refracting light through glass and transmits signals from glass to air by focusing light to a point [39]. The GRIN lens is crafted to refract light within one continuous glass tube to a single focal point outside of the skull (Figure 1B) [40, 41].

Without the GRIN lens light cannot pass deep tissue, and visualization > ~1mm [42] is restricted to *ex vivo*. Combining GRIN lens with 1-photon [34, 35, 43, 44] and 2-photon [45] excitation techniques helps reach target areas. Additionally, commercial GRIN lenses made from thallium-containing glass and salt melts can leach toxins but bio-compatible coatings reduce toxic-effects without dropping image quality [41, 46].

1.4 Samples of GRIN lens use in the field

1.4.1 GRIN Lens Combined with Miniscope Technology

Miniscope development revolutionized *in vivo* neuronal detection [34, 47, 48]. Originally, miniscopes used fibers to transmit light away from the organism [49] but have compartmentalized [50]. This decreased cost for behavioral microscopy and coincided with open-source miniscopes [31, 33, 50]. Miniscope and GRIN lens combination allows for single-cell visualization in deep regions such as NAc [34], and ventral tegmental area [43]. Potential GRIN lens limitations include out-of-focus fluorescence and poor optical sectioning (i.e., the ability to resolve samples embedded in tissue from noise [51], but are attenuated using an electrowetting lens to adjust the focal point [52, 53]. Motion blur can be adjusted in post-collection processes [34, 47, 48]. Major GRIN lens benefits involve targeting populations while preserving individualistic neural data. To target specific cell types transgenic animals can be bred to express cre-recombinase [54] in a multitude of cell types [55] and receptors such as μ-opioid [56], DRD1-[57], DRD2-[58], and other receptors [59].

An example experiment using cre-dependent viruses (e.g., GCaMP) injected into NAc (Figure 1A). In a DRD1-iCre rat only D1- expressing cells emit fluorophores when active and illuminated with a specific frequency [34]. Multiple genetic experiments using dual-color imaging through one GRIN lens [60] inject two spectrally distinct viruses: green-emitting cre-dependent GCaMP labels D1 cells and non-cre dependent red-emitting TdTomato [61] labels all cells. Intermittently shining distinct lights onto cells induces distinct fluorescence. Post-processing distinguishes D1 cells from remaining population. Deep learning analyses correlate cell-type specific activity associated with behavioral sequences [35] and can be theoretically applied to drug use during miniscope imaging [44, 62].

Optogenetic tools for excitation/inhibition can be integrated with the GRIN lens [36, 63]. Optogenetics use viruses containing proteins for excitation/inhibition of cells in response to specific wavelengths [64, 65]. Light optically manipulates neurons and resulting activity changes pass back to visualize activity *in vivo*. GRIN lens-coupled techniques can be applied in future studies to visualize neuronal progression throughout drug SA, abstinence, and relapse which have been pioneered using other in vivo techniques but can be adapted using GRIN lenses to record more neurons over longer periods of time.

1.4.2 GRIN lens in tandem with fMRI and fiberscope

An alternative GRIN lens approach is in conjunction with functional magnetic resonance imaging (fMRI) and a fiberscope. fMRI measures whole brain activity based on changes in

cerebral blood flow [66]. A fiberscope uses calcium signaling to visualize neurons through an implanted GRIN lens, but relays towards CMOS-sensor [67, 68]. Researchers can use fiberscopes as a control for fMRI signal to understand effects of drug-associated cues across brain regions. For example, NAc and PrL are interconnected [69] and implicated in SUD [70, 71]. Following lens implants, animals would be trained for drug SA paired with a specific cue (e.g., a tone or odor). Animals would be placed in an fMRI (whole-brain) while asleep or head-fixed but awake as the animal is re-exposed to drug cues and fiberscope imaging (single-cell) would simultaneously carry signals outside of the fMRI [72]. Variations in drug-associated cue signals between the imaging techniques, PrL vs. NAc, awake vs. asleep, and correlations with relevant limbic brain regions are also possible.

1.5 Future Directions

The GRIN lens enables a wide range of possibilities for future deep-brain microscopy. New techniques and tools can extract precise neurophysiological information such as sensors which detect changes in endocannabinoid [73], serotonin [74], dopamine [75], or voltage [76]. A novel “clear optically matched panoramic access channel technique (COMPACT) works by inserting a GRIN lens into an implanted tube which can be adjusted dorsoventrally to capture refracted light [77]. This method reduces GRIN lens scar tissue and allows for multiple within-subject mesolimbic targets implicated in SUD (i.e., PrL and NAc [78]). Overall, the GRIN lens provides powerful research and technological developments to ultimately help people suffering from SUD.

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Abbreviations:

SA	Self-Administration
SUD	Substance Use Disorder
NAc	Nucleus Accumbens
PFC	Prefrontal Cortex
PrL	Prelimbic Cortex
GRIN	Gradient Index
fMRI	functional magnetic resonance imaging

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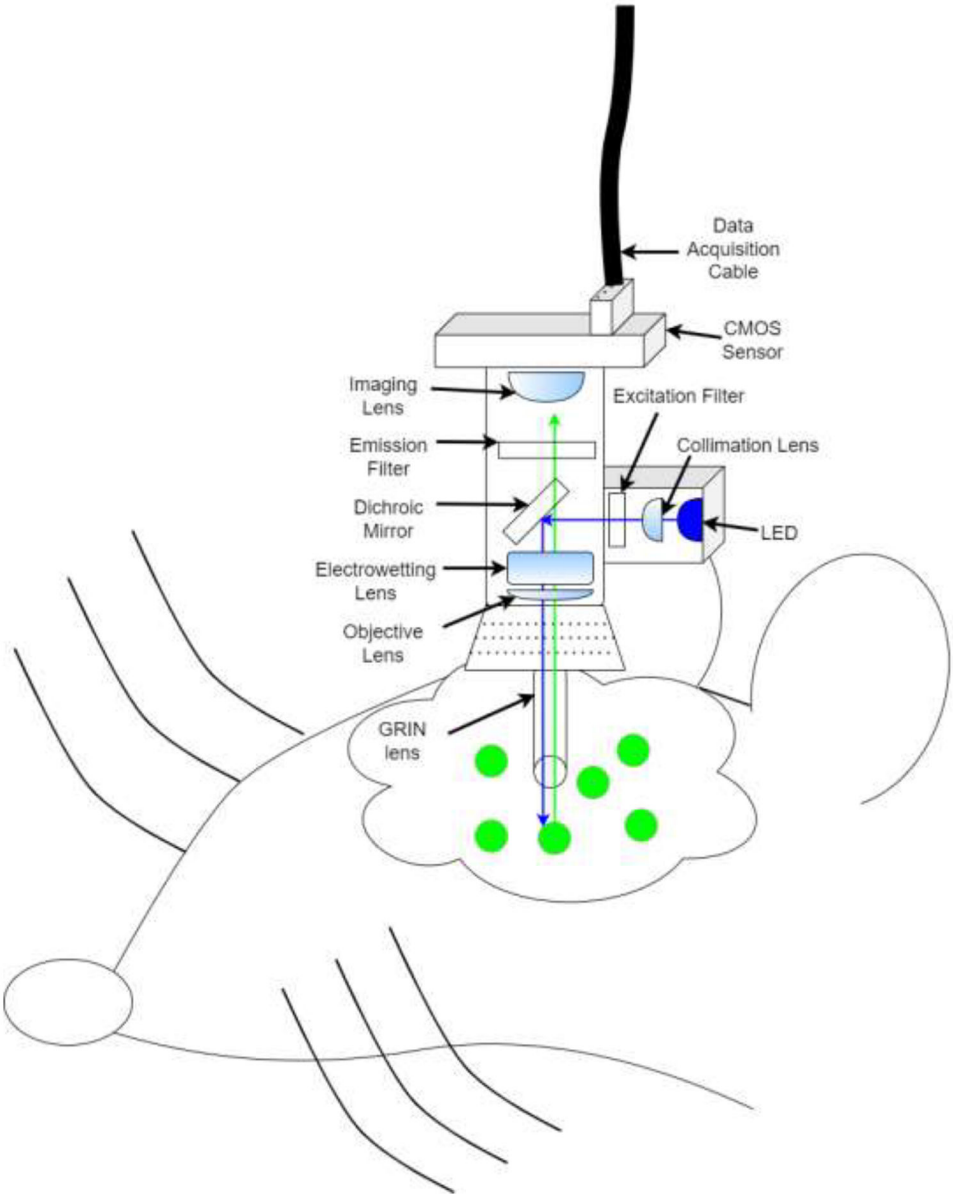

Figure 1A:

Depiction GRIN lens+ miniscope *in vivo* imaging. Miniscope is head mounted to a baseplate and allows for freely animal movement. A blue LED is triggered and light is reflected by a dichroic mirror into brain via the GRIN lens (see also, Figure 1b). Neurons infected with GCaMP emit green fluorescence while active in response to blue light. Green light (i.e., neural activity) is relayed back through the GRIN lens, past the dichroic mirror towards an imaging sensor and relayed offsite for analysis

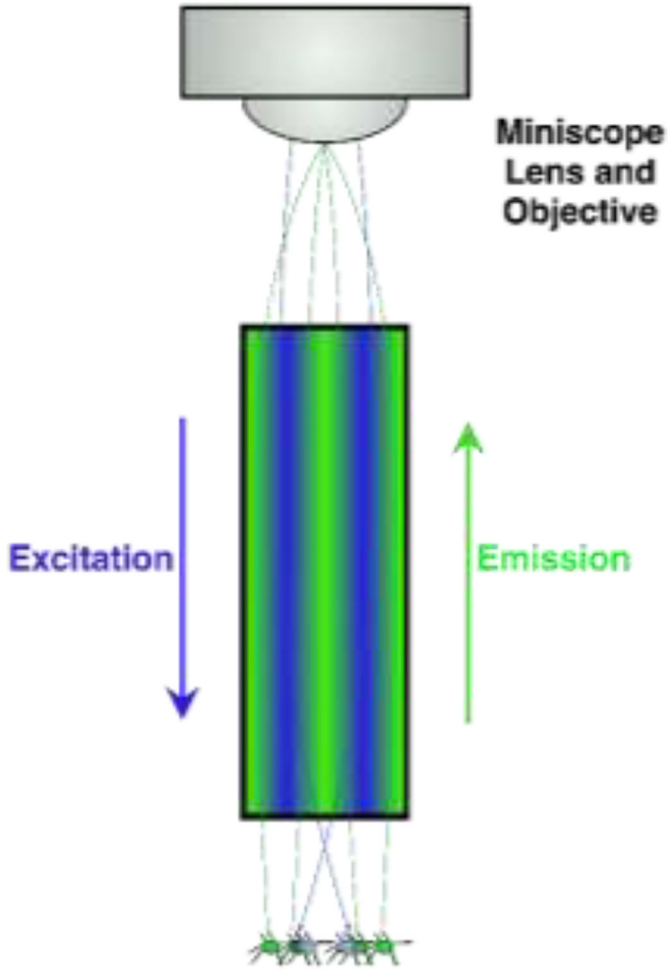

Figure 1B:

Schematic of an enlarged GRIN lens (seen in Figure 1A: Blue LED light is relayed through the GRIN lens. When neurons are active and simultaneously lit by the LED, these neurons fluoresce and produce light (green), some of which is gathered by the GRIN lens and relayed towards the objective for offsite analysis. Light is manipulated by the GRIN lens and meets at a specific focal point where the image is clear. Out-of-focus (i.e., focal length adjustment) can be via physical placement of the objectives or with an electrowetting lens (see Figure 1A) to resolve the image.

Table 1:

In vivo recording techniques benefits/drawbacks

In Vivo Techniques	Materials	Pros	Cons
Extracellular Electrophysiology	• Microelectrode (tungsten, steel, or platinum-iridium)	• Single neuron resolution • Can even detect multiple neurons per channel • High Temporal Resolution • Physiological identification of neurons by firing properties • Deep Brain Probing	• Number of neurons dependent on inserted channels • Unable to differentiate between cell types with similar electrophysiological properties • Microwires are delicate and can break
Protein c-Fos	• c-Fos protein (Activity Marker)	• Superior spatial resolution (whole brain analysis) • Cell-type and pathway specific genetic markers	• Slow result designation • Activity is not always triggered by c-Fos • No single neuron targeting
Fast-Scan Cyclic Voltammetry	• Carbon fiber Microelectrode	• High temporal resolution (~100ms) • Can detect different types of neurotransmission (dopamine, noradrenergic, etc.) • Deep Brain Probing	• Large background current • Requires background subtraction • Localized (not single neuron) targeting
Fiber Photometry	• Glass Cannula	• Extends beyond Calcium Indicators • Can use transgenic animals for genetic studies • Cost-efficient • Region specific population activity at axon terminals • Deep Brain Probing	• No individual cellular resolution (whole field analysis)
GRIN Lens	• Glass Lens with Biocompatible coating	• Can be combined with transgenic animals for genetic studies • Optogenetics do not require a separate channel • Sensor based longitudinal recordings over months • Single Neuron targeting and population targeting over months • Deep Brain Probing • Open-source miniscopes allow for cost effective application	• Lens requires biocompatible coating • Scratched lenses, photobleaching, and scar tissue can impede detection of neural activity • High analytical burden (100s of single neurons from a single subject) • Out-of-focus fluorescence must be attenuated • Post-processing must be taken to correct for motion blur