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Mapping Auditory Synaptic Circuits with Photostimulation of Caged Glutamate

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Abstract

Photostimulation of neurons with caged glutamate is a viable tool for mapping the strength and spatial distribution of synaptic networks in living brain slices. In photostimulation experiments, synaptic connectivity is assessed by eliciting action potentials in putative presynaptic neurons via focal photolysis of caged glutamate, while measuring postsynaptic responses via intracellular recordings. Two approaches are commonly used for delivering light to small, defined areas in the slice preparation; an optical fiber-based method and a laser-scanning-based method. In this chapter, we outline the technical bases for using photostimulation of caged glutamate to map synaptic circuits, and discuss the advantages and disadvantages of using fiber-based vs. laser-based systems.

Keywords

Photostimulation; Fiber-based uncaging; Laser scanning; Glutamate uncaging; Synaptic input mapping; Auditory circuit connectivity

1 Introduction

Photostimulation with caged glutamate or “glutamate uncaging” has emerged as a robust and reliable tool for mapping functional synaptic connectivity in a variety of systems [1–6]. In the auditory system, glutamate uncaging has been used to reveal the organization of functional connectivity in the superior olivary complex [2, 7, 8], the ventral cochlear nucleus [9], the inferior colliculus [10], and the auditory cortex [11–15]. In glutamate uncaging studies, the origin and strength of synaptic inputs received by an individual neuron are determined by obtaining a whole-cell patch clamp recording from a neuron, exciting potential presynaptic neurons with glutamate uncaging, and assessing the resulting postsynaptic responses. Glutamate uncaging is achieved by brief (1–100 ms) pulses of light delivered to small, defined areas in the slice, either by an optical fiber [2, 6] or by a scanning laser [1, 4]. By systematically exciting many (100–1000s) discrete locations in the slice, the topographic distribution of stimulation sites that elicit postsynaptic responses in the recorded neuron is used to generate synaptic input maps of excitatory and/or inhibitory connections.

In this chapter, we outline the basic conceptual and technical principles of using fiber- and laser-based glutamate uncaging to map local synaptic circuits in the auditory system. To assist the reader in determining which type of system will be most appropriate for his/her

research questions and laboratory environment, we compare the advantageous and disadvantageous associated with fiber-based and laser-based uncaging. Although we focus on synaptic input mapping in the auditory brainstem and midbrain, the methods described here will be applicable to other brain regions as well.

2 Materials

Fiber-based and laser-based uncaging systems can each be incorporated into standard electrophysiological setups for visualized whole-cell recording in living brain slices (Fig. 1). A variety of caged compounds are commercially available from several vendors (Calbiochem, Temecula, CA; HelloBio, Bristol, UK; Life Technologies, Grand Island, NY; Molecular Technologies, Inc., Tocris, Bristol, UK).

2.1 Fiber-Based Uncaging

1. Electronic shutter: Uniblitz Model LSG with AlMgF₂ coating (Vincent Associates, Rochester, NY).
2. Mercury arc Lamp: 100-W lamp with Series Q lamp housing and UV grade fused silica condenser (Oriel, Irvine, CA or Newport Corporation, Irvine, CA).
3. Optical fibers: 5–50 μ m fibers (CeramOptec Industries Inc, Bonn, Germany or Polymicro Technologies, Phoenix, AZ).
4. Power supply (Opti Quip Inc., Highland Mills, NY).
5. Pulse generator (e.g., Master 8; A.M.P.I., Jerusalem, Israel).

2.2 Laser-Based Uncaging System

1. UV laser, 355 nm, 2 W (e.g., Model 3510–30; DPSS Lasers, Santa Clara, CA).
2. Polarizing beamsplitter for power attenuation (e.g., GT5-A, Thorlabs, Newton, NJ).
3. Mechanical shutter (e.g., Model LST200, nmLaser Products, San Jose, CA).
4. Galvo-driven mirrors (e.g., Model 6210H, Cambridge Technology, Bedford, MA).
5. Optical lenses: $f=10$ cm and $f=15$ cm (e.g., LA4380-UV and LA4874-UV, respectively, Thorlabs).
6. Dichroic beamsplitter (e.g., FF484-FDi01–25x36, Semrock, Rochester, NY).

3 Methods

3.1 Photostimulation with Caged Glutamate

3.1.1 Caged Neurotransmitters—Synaptic input mapping with glutamate uncaging involves exciting small groups of presynaptic neurons by locally increasing the glutamate concentration with brief pulses of high intensity light and measuring postsynaptic responses in individual neurons via whole-cell patch-clamp recordings. Presynaptic stimulation is

achieved via rapid photolysis of photo-labile caged glutamate, which is biologically inert under ambient lighting conditions, but which releases free glutamate in response to light (Fig. 2a). A variety of caged glutamate compounds have been synthesized (e.g. [16–18]) and many are commercially available (*see* Subheading 2). Caged glutamate consists of a glutamate molecule covalently linked to a “caging” compound, which renders the glutamate biologically inactive. These covalent linkages undergo photolysis in response to light, at which point glutamate is “uncaged” and is able to activate glutamate receptors, thus eliciting action potentials in nearby neurons (Fig. 2a).

Different caging compounds exhibit distinct biophysical (e.g., photolytic efficiency and kinetics, stability against spontaneous hydrolysis in the absence of light) and biological properties (agonist or antagonist action and potential toxicity). Most caged glutamate compounds are photolysed by light within the ultraviolet spectrum (300–400 nm), but a number of caging compounds have also been developed with sensitivity to wavelengths within the visible range (400–700 nm) (for example, RuBi-Glutamate, Tocris; [19]). Visible light allows greater tissue penetration and reduced phototoxicity compared to UV light [20, 21], but caged compounds that are sensitive to visible light are also sensitive to ambient light, which requires careful light shielding during their usage. While the properties of most commercial caged compounds usually have been well characterized, the experimenter is advised to verify in their specific preparation that the chosen caged compound does not interact with their biological system, while in its supposedly inactive state. For example, the addition of caged glutamate to the external solution should have no effect on membrane potentials or synaptic transmission. Finally, the experimenter should also confirm that the UV light exposure necessary to elicit reliable action potential generation via uncaging does not itself interfere with the physiological properties of the neurons to be stimulated [22, 23].

3.1.2 Mapping Locations of Presynaptic Neurons—In a typical synaptic input mapping study, a whole cell recording is first obtained from an individual neuron in a living brain slice (Fig. 2b) immersed in a bath of recirculating artificial cerebrospinal fluid (ACSF). Caged glutamate is then added to the ACSF and allowed to reach a stable concentration (5–10 min) within the slice. Most studies use a working concentration of caged glutamate between 80 μ M and 3 mM [2, 3, 5, 7, 10–15], but the optimal concentration needs to be adjusted based upon the caged compound, the neurons to be stimulated, the uncaging system used, and the particular experimental conditions. An appropriate glutamate concentration will be high enough that uncaging reliably elicits action potentials, but low enough to both minimize buildup of free glutamate due to spontaneous hydrolysis and restrict the area of excitation (*see* Subheading 3.1.4). For uncaging, brief, focal UV light pulses (1–100 ms) are delivered to locations containing potential presynaptic neurons. Different stimulation sites are targeted either by moving an optical fiber above the slice (*see* Subheading 3.2) or by steering a laser light spot using computer-controlled galvanometers (*see* Subheading 3.3). If a photostimulation site contains neurons presynaptic to the recorded cell, a postsynaptic response will be recorded (*see* Note 1). At stimulation sites close to the recorded neuron, uncaged glutamate can also directly excite the recorded cell itself, giving rise to short

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¹If you are having trouble detecting postsynaptic responses, first check to make sure that sufficient uncaging light is indeed being delivered to the slice (if necessary, verify with photoactivatable fluorescent dyes). Next, ensure that the light is being delivered to the

latency (1–5 ms post-stimulus) membrane currents (“direct responses”). These direct responses can be distinguished from synaptic responses by their shorter onset latencies and by their insensitivity to blocking axonal spike propagation with tetrodotoxin (TTX), which abolishes synaptic but not direct responses (Fig. 2d, *right*). By systematically testing hundreds to thousands of stimulation sites, the positions of neurons are revealed that make functional synaptic connections with the recorded neuron in the slice.

Excitatory and inhibitory presynaptic inputs can be distinguished by recording synaptic currents at different membrane voltages of the recorded neuron in voltage-clamp mode (Fig. 2c, *from* [10]). To isolate glutamatergic excitatory inputs, the membrane potential of the recorded cell is held at the reversal potential for chloride, which eliminates membrane currents through glycine and GABA_A receptors (Fig. 2c, e, *left*). Conversely, to isolate inhibitory synaptic inputs, the membrane potential of the recorded cell is held near the reversal potential for glutamatergic neurotransmission (Fig. 2c, e, *right*) (*see* Note 2). The contribution of GABAergic and glycinergic connections to inhibitory input maps may also be isolated pharmacologically using specific antagonists for GABA and glycine receptors [10] (*see* Note 3).

3.1.3 Synaptic Input Map Analysis—If synaptic response amplitudes are small and difficult to detect, or if there is a high level of spontaneous synaptic activity in the slice, which can make it difficult to categorize a synaptic event as an elicited response, mapping should be repeated several times to create averaged input maps (Fig. 3). These input maps can then be analyzed with respect to the amplitudes and the latencies of responses as well as the distribution of excitatory and inhibitory synaptic connections. For instance, investigators may wish to calculate quantities such as synaptic input area, total input charge (charge per map), or input charge density (charge per stimulation site). Such analyses have been used to characterize the processes of synaptic elimination and strengthening that occur during early development in the primary sound localization circuit formed by the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO) (Fig. 4) [2, 7, 8], in the inferior colliculus (IC) [10] and in the auditory cortex [3, 5, 13, 14]. Spatial analyses can relate the shape of synaptic input maps to the anatomical or functional organization of the circuit, such as tonotopy. Comparing the excitatory and inhibitory input maps can quantify the degree of spatial overlap between synaptic excitation and inhibition, in order to estimate local synaptic excitation: inhibition balance. In the IC, for example, excitatory and inhibitory input maps were found to exhibit a high degree of spatial overlap, and this overlap was subject to significant developmental regulation [10].

3.1.4 Interpretation of Input Maps—The accurate interpretation of synaptic input maps to reveal underlying synaptic circuitry rests upon a number of important considerations.

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desired location, if not, system calibration will be necessary. If postsynaptic responses are still undetectable, increase the concentration of caged glutamate and/or increase the duration and power of the uncaging light pulse. To confirm that photostimulation is leading to robust action potential generation in presynaptic neurons, perform excitability mapping (*see* Subheading 3.2).

²If you are having trouble visualizing inhibitory synaptic inputs, it may be worth increasing the driving force for chloride by changing to an internal solution with a higher chloride concentration to boost inhibitory synaptic inputs.

³Caution should be taken when blocking glutamate receptors, because this interferes with the ability of uncaged glutamate to activate presynaptic neurons.

First, in addition to depending upon the kinetics and concentration of the caged compound being utilized and the power of the UV photostimulus, the effective resolution of input mapping is also influenced by morphological and physiological properties of the pre-synaptic neurons being stimulated [2, 4]. The dendritic geometry and the dendrosomatic distribution of glutamate receptors influence what uncaging positions will generate action potential in the neuron, thus influencing the spatial resolution of presynaptic input maps. For example, if presynaptic neurons have large dendritic fields with a high density of glutamate receptors, uncaging anywhere over the dendritic tree may be able to elicit spikes, making it difficult to infer the location of the pre-synaptic cell body from those uncaging sites. This uncertainty may increase if there exists heterogeneity (in terms of dendritic arbor size and sensitivity to glutamate) in the population of presynaptic neurons being stimulated.

Differences in animal age and associated degrees in axon myelination, tissue density, and neuron physiology affect light penetration into the slice and the sensitivity of presynaptic neurons to glutamate uncaging [2, 4, 7]. Therefore, it is necessary to calibrate the uncaging efficiency for differences in the experimental preparation [2, 7, 10]. This is best done by generating “excitability profiles,” which involves recording from putative presynaptic neurons while uncaging glutamate in their vicinity to determine the area over which glutamate uncaging leads to the generation of action potentials (Fig. 3b) [2, 4, 10]. If excitability profiles are similar for neurons across experimental conditions, then variations in the excitatory and inhibitory input maps between experimental conditions can be attributed to differences in connectivity rather than to differences in the ability of uncaged glutamate to drive presynaptic neurons.

A second issue in interpreting synaptic input maps is discerning whether responses are due to activation of monosynaptic or polysynaptic connections. One method for distinguishing monosynaptic responses from polysynaptic responses is to decrease neuronal excitability by raising the concentration of divalent cations in the ACSF and/or pharmacologically blocking NMDA receptor activation [4]. Decreasing circuit excitability reduces the likelihood that uncaging-driven spiking in presynaptic neurons will elicit action potentials in postsynaptic neurons, restricting the activation of polysynaptic pathways. If NMDA receptor currents are critical for presynaptic spike generation, activation of polysynaptic inputs can be decreased without NMDA receptor blockade by reducing the concentration of caged glutamate and/or the uncaging light intensity, in order to elicit only one or very few action potentials in presynaptic neurons. Finally, another method to evaluate whether input maps reflect monosynaptic connections is to disinhibit the brain slice with antagonists of inhibitory neurotransmission in order to favor the recruitment of polysynaptic responses. If excitatory input maps remain unchanged in the disinhibited slice, then this can be taken as further indication that they consist of monosynaptic connections [10].

3.2 Optical Fiber- Based Input Mapping

3.2.1 System Composition—A relatively simple and affordable method for creating movable localized UV light illumination in slices is the use of an optical fiber. A basic fiber-based photostimulation system is composed of a mercury arc lamp as a UV source, a condenser to focus the light into the optical fiber, an electronic shutter to control light pulse

duration and an optical fiber for light delivery (Fig. 1a). The electronic shutter needs to allow short and precise opening/closing times (<10 ms) and withstand the high light and heat output from a mercury arc lamp. Additionally, to achieve a desired illumination spot size it is important to select an optical fiber with UV transmittance and an appropriate diameter (we have used fibers with diameters ranging from 5 to 200 μm). Constructing a fiber-based uncaging system is relatively simple and can be constructed in a few days; a detailed description of the necessary components and their assembly is given elsewhere (see [6]).

3.2.2 Advantages of Uncaging with Optical Fibers—The major advantages of using a fiber-based uncaging system over a laser-based uncaging system include its lower cost, reduced time for assembly, and the ability to use multiple fibers for simultaneous stimulation of several sites [24]. The cost of a fiber-based system, including the light source, electronic shutter, optical fiber and accessory equipment, is approximately \$5000, which is an order of magnitude lower than the cost of a laser-based system. A fiber-based system can be quickly added to an existing electrophysiology setup, and the location of the optical fiber above the brain slice, and hence the stimulation area, can be monitored and recorded via the existing video camera of a visualized whole-cell patch clamping setup. If multiple optical fibers are needed, they can be added using the additional ports on the mercury lamp housing. Additional fibers may also allow the sharing of one lamp housing between several electrophysiological setups. Using multiple optical fibers enables the simultaneous stimulation of distinct sources of presynaptic input, in order to examine how different input sources are integrated.

3.2.3 Limitations of Uncaging with Optical Fibers—The major limitations of using a fiber-based uncaging system, compared to laser-based systems, include generally poorer spatial resolution and longer time for manually changing uncaging positions. The spatial resolution of a fiber-based system, as defined as the spike-eliciting area, will depend upon a number of factors including UV light power, UV flash duration, optical fiber diameter. In general, this area should be experimentally determined for the specific preparation. In our experience, the spatial resolution of an optical fiber with a 20 μm diameter light-conducting core (Polymicro Technologies, Phoenix, Arizona) is approximately 50 μm in the MNTB [2]. In general, fiber-based systems are less suitable for experiments that require very small, subcellular stimulation areas. Because the optical fiber is moved manually between uncaging positions, input mapping is slow, requiring that stable recordings for 1–3 h depending on the number of stimulation sites. In our hands, it takes about 2 h to stimulate 100–150 sites in the MNTB [2, 25]. Thus, fiber-based systems are less well-suited for uncaging experiments that requiring large numbers (>200) of stimulation positions or if long stable recordings cannot be obtained. In summary, fiber-based uncaging systems are inexpensive, flexible, and relatively easy to assemble, but their drawback is limited spatial resolution and slow scanning speed due to manual positioning (Table 1).

3.3 Laser Scanning- Based Input Mapping

3.3.1 System Composition—A typical laser-scanning photostimulation (LSPS) system consists of a UV laser for light generation, an attenuator to regulate light intensity, a

mechanical shutter to control UV light pulse width, voltage-controlled galvo-driven mirrors to steer the location of the laser spot, lenses to focus the UV light, and a dichroic beam splitter to reflect laser light down through the microscope to the sample, while letting visible light from the sample pass through to the camera for visualization of the brain slice (Fig. 1b). Software programs control the mirror galvanometers (and thus the location of the laser spot in the field of view) to generate a series of laser targets that can be scanned through in random or defined sequences ScanImage & Ephus, are freely available software packages offered through openwiki.janelia.org. Stimulation/acquisition software, we have been using in ref. [10], is available up request from nguy-ena@tcnj.edu.

3.3.2 Advantages of Uncaging with LSPS—The major advantages of a laser-based uncaging system over a fiber-based uncaging system are its superior spatial and temporal resolution. Because light for uncaging is focused through the microscope objective, the uncaging spot can be very small when using high-magnification objectives. If required, spatial resolution (including in the *Z*-axis) can be improved further with two-photon uncaging, to the extent that individual dendritic spines may be stimulated [26, 27]. Because of the use of software-controlled mirror galvanometers, LSPS systems can rapidly scan through a very large number of stimulation sites, with the minimum inter-stimulus interval between sites effectively being determined by the duration of synaptic responses, rather than by the time required to move between targets. This allows for the mapping and examination of large input areas. For example, in our mapping studies in the IC, we usually scan about 500 discrete sites at 2 Hz, completing one map iteration in about 5–10 min. The speed of automated laser scanning also makes it feasible to repeat mapping protocols multiple times over relatively short periods, thereby, increasing input map reliability and investigating changes in input map organization after pharmacological or other experimental manipulations. Compared to fiber-based experiments, which require that neurons be held stable from 1 to 3 h, even extensive LSPS experiments can generally be completed in 30 min or less.

3.3.3 Limitations of Uncaging with LSPS—The major limitations of using an LSPS uncaging system compared to fiber-based systems include its higher cost, and the greater level of technical skill required to assemble the system. The most expensive item is the UV laser. We use a 355 nm solid-state UV laser with 2 W of power (DPPS lasers, Inc) costing approximately \$30,000. Additionally, while detailed instructions are available (*see* [4]), compared to a fiber-based system, assembly and trouble shooting of an LSPS system requires more time (at least several months) and a higher level of technical skill. In summary, compared to fiber-based systems, LSPS-based uncaging systems afford superior spatial and temporal resolution, but they are more expensive and require greater technical expertise to operate (Table 1).

3.4 Choosing the Right Approach

Deciding which uncaging system is most appropriate for your laboratory will depend upon your specific research question and the available financial resources. In cases where synaptic input maps are relatively small and where the general locations of these inputs can be predicted from previous anatomical studies, a fiber-based system will likely prove effective.

Additionally, investigators with little prior knowledge of optics, who aim to begin collecting data as soon as possible, may wish to opt for a fiber-based system. However, in cases where either the location or the number of synaptic inputs are unknown, or where the synaptic input maps are large, the higher mapping speed of a computer controlled, semi-automated LSPS-based system will be preferable.

References

1. Callaway EM, Katz LC. Photostimulation using caged glutamate reveals functional circuitry in living brain slices. *Proc Natl Acad Sci U S A*. 1993; 90(16):7661–7665. [PubMed: 7689225]
2. Kim G, Kandler K. Elimination and strengthening of glycinergic/GABAergic connections during tonotopic map formation. *Nat Neurosci*. 2003; 6(3):282–290. [PubMed: 12577063]
3. Zhao C, Kao JP, Kanold PO. Functional excitatory microcircuits in neonatal cortex connect thalamus and layer 4. *J Neurosci*. 2009; 29(49):15479–15488. [PubMed: 20007472]
4. Shepherd GM. Circuit mapping by UV uncaging of glutamate. *Cold Spring Harb Protoc*. 2012; 9:998–1004.
5. Viswanathan S, Bandyopadhyay S, Kao JP, Kanold PO. Changing microcircuits in the subplate of the developing cortex. *J Neurosci*. 2012; 32(5):1589–1601. [PubMed: 22302801]
6. Kandler K, Nguyen T, Noh J, Givens RS. An optical fiber-based uncaging system. *Cold Spring Harb Protoc*. 2013; 2:118–121.
7. Hirtz JJ, Braun N, Griesemer D, Hannes C, Janz K, Löhrke S, Müller B, Friauf E. Synaptic refinement of an inhibitory topographic map in the auditory brainstem requires functional Cav1.3 calcium channels. *J Neurosci*. 2012; 32(42):14602–14616. [PubMed: 23077046]
8. Clause A, Kim G, Sonntag M, Weisz CJ, Vetter DE, Rübsamen R, Kandler K. The precise temporal pattern of prehearing spontaneous activity is necessary for tonotopic map refinement. *Neuron*. 2014; 82(4):822–835. [PubMed: 24853941]
9. Campagnola L, Manis PB. A map of functional synaptic connectivity in the mouse anteroventral cochlear nucleus. *J Neurosci*. 2014; 34(6):2214–2230. [PubMed: 24501361]
10. Sturm J, Nguyen T, Kandler K. Development of intrinsic connectivity in the central nucleus of the mouse inferior colliculus. *J Neurosci*. 2014; 34(45):15032–15046. [PubMed: 25378168]
11. Barbour DL, Callaway EM. Excitatory local connections of superficial neurons in rat auditory cortex. *J Neurosci*. 2008; 28(44):11174–11185. [PubMed: 18971460]
12. Lee CC, Imaizumi K. Functional convergence of thalamic and intrinsic projections to cortical layers 4 and 6. *Neurophysiology*. 2013; 45(5–6):396–406. [PubMed: 24563558]
13. Meng X, Kao JPY, Kanold PO. Differential signaling to subplate neurons by spatially specific silent synapses in developing auditory cortex. *J Neurosci*. 2014; 34(26):8855–8864. [PubMed: 24966385]
14. Watkins PV, Kao JPY, Kanold PO. Spatial pattern of intra-laminar connectivity in supragranular mouse auditory cortex. *Front Neural Circ*. 2014; 8:15.
15. Kratz MB, Manis PB. Spatial organization of excitatory synaptic inputs to layer 4 neurons in mouse primary auditory cortex. *Front Neural Circ*. 2015; 9:17.
16. Stensrud K, Noh J, Kandler K, Wirz J, Heger D, Givens RS. Competing pathways in the photo-Favorskii rearrangement and release of esters: studies on fluorinated p-hydroxyphenacyl-caged GABA and glutamate phototriggers. *J Org Chem*. 2009; 74(15):5219–5227. [PubMed: 19572582]
17. Conrad PG 2nd, Givens RS, Weber JF, Kandler K. New phototriggers: extending the p-hydroxyphenacyl pi-pi absorption range. *Org Lett*. 2000; 2(11):1545–1547. [PubMed: 10841475]
18. Givens RS, Rubina M, Wirz J. Applications of p-hydroxyphenacyl (pHP) and coumarin-4-ylmethyl photoremovable protecting groups. *Photochem Photobiol Sci*. 2012; 11(3):472–488. [PubMed: 22344608]
19. Shembekar VR, Chen Y, Carpenter BK, Hess GP. A protecting group for carboxylic acids that can be photolyzed by visible light. *Biochemistry*. 2005; 44(19):7107–7114. [PubMed: 15882049]

20. Rial Verde EM, Zayat L, Etchenique R, Yuste R. Photorelease of GABA with visible light using an inorganic caging group. *Front Neural Circ.* 2008; 2:2.
21. Fino E, Araya R, Peterka DS, Salierno M, Etchenique R, Yuste R. RuBi-glutamate: two-photon and visible-light photoactivation of neurons and dendritic spines. *Front Neural Circ.* 2009; 3:2.
22. Leszkiewicz DN, Kandler K, Aizenman E. Enhancement of NMDA receptor-mediated currents by light in rat neurones in vitro. *J Physiol.* 2000; 524(Pt 2):365–374. [PubMed: 10766918]
23. Leszkiewicz DN, Aizenman E. Reversible modulation of GABA(A) receptor-mediated currents by light is dependent on the redox state of the receptor. *Eur J Neurosci.* 2003; 17(10):2077–2083. [PubMed: 12786974]
24. Kandler K, Katz LC, Kauer JA. Focal photolysis of caged glutamate reveals an entirely postsynaptic form of long-term depression of hippocampal glutamate receptors. *Nat Neurosci.* 1998; 1:119–123. [PubMed: 10195126]
25. Noh J, Seal RP, Garver JA, Edwards RH, Kandler K. Glutamate co-release at GABA/glycinergic synapses is crucial for refinement of an inhibitory map. *Nat Neurosci.* 2010; 13(2):232–238. [PubMed: 20081852]
26. Matsuzaki M, Ellis-Davis GC, Nemoto T, Miyashita Y, Lino M, Kasai H. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci.* 2001; 4(11):1086–1092. [PubMed: 11687814]
27. Smith MA, Ellis-Davis GC, Magee JC. Mechanism of the distance-dependent scaling of Schaffer collateral synapses in rat CA1 pyramidal neurons. *J Physiol.* 2003; 548(Pt. 1):245–258. [PubMed: 12598591]

Fig. 1. Schematic illustration of fiber-based and laser-based UV photostimulation systems. **(a)** Optical fiber system. **(b)** Laser-scanning photostimulation system. The focal lengths and positions of the two lenses are chosen to ensure that the laser spot is parfocal with the image across the entire field-of-view. Light paths shown in *blue*. *CCD* camera

Fig. 2.

Using laser scanning photo stimulation with caged glutamate to map synaptic connections in the inferior colliculus. **(a)** UV light hydrolyses the covalent linkage between glutamate (*black*) and the caging compound (*red*, caging group is 4-methoxy-7-nitroindolyl (MNI) group from MNI-caged-L-glutamate, Tocris) (*left*), thereby releasing free glutamate (*middle*) that drives nearby neurons to spiking (*right*). Spiking in presynaptic neurons leads to neurotransmitter release and synaptic currents in the recorded postsynaptic neuron. **(b)** Schematic illustration of input mapping in the inferior colliculus (IC) with whole-cell patch clamp recording electrode. Light pulses are delivered to a series of stimulation targets (stim grid) **(c)** Excitatory (*red* trace) and inhibitory (*blue* trace) synaptic inputs can be distinguished by holding the membrane potential of the recorded cell near the reversal potential for chloride (-65 mV) and glutamatergic neurotransmission (0 mV), respectively. Compared to direct stimulations (*black* trace), synaptic responses have longer onset latencies. **(d)** *Left*, Example of an excitatory synaptic input map obtained with laser-scanning photostimulation from an IC neuron in a P7 mouse. *Right*, Synaptic inputs are abolished by mapping in the presence of TTX, which blocks action potential generation leaving only direct responses intact. **(e)** Example of average excitatory (*left*, same map as 2D, *left*) and inhibitory (*right*) synaptic input maps. Direct response sites are shown in black. (Panel (c) modified from ref. [10])

Fig. 3. Reliability and spatial resolution of synaptic input mapping. **(a)** Repeated mapping of excitatory inputs (Iterations 1–3) reveals very similar maps. Responses are averaged to generate an “average map” for further analysis. Stimulation sites eliciting direct responses in average map are shown in black. Example is from the IC of a postnatal day (P)8 mouse. V_{hold} —membrane holding potential. **(b)** Spatial resolution of glutamate uncaging with a 20 μm diameter optical fiber in the medial nucleus of the trapezoid body (MNTB) of developing rats. **(Bi)** Uncaging elicited glutamate responses of individual MNTB neurons at P3 and P11. Stimulation sites that elicit action potentials (*filled red circles*) are found immediately around the cell body at the tip of the recording electrode (rec). Stimulation sites at greater distances elicit either subthreshold responses (*black filled circles*) or no responses (*open yellow circles*). *D* dorsal, *M* medial. Scale bar, 100 μm . **(Bii)** Age-dependent changes in the average spike-eliciting distance along the mediolateral and dorsoventral dimensions of the MNTB. (Panel (b) modified from ref. [2], Fig. 4)

Fig. 4.

Functional refinement of the developing MNTB-LSO pathway revealed by fiber-based glutamate uncaging. **(a)** Schematic illustration of the pathway from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) and the experimental configuration; functional synaptic connections from the MNTB to the LSO are revealed by stimulating presynaptic MNTB neurons with uncaged glutamate, and measuring postsynaptic responses in the recorded LSO neuron. Light stimulation for glutamate uncaging is delivered by an optical fiber. **(b)** Examples of MNTB input maps obtained at P3 and P14. **(c)** Developmental changes in normalized input map area and input map width **(d)** along the tonotopic axis. Map area is normalized to MNTB cross-sectional area and input map width is normalized to mediolateral MNTB length. (Panels **(b)**–**(d)** modified from ref. [2])

Table 1

Advantages and limitations of fiber-based and laser-based uncaging

Uncaging system	Advantages	Limitations
Optical fiber	Inexpensive	Spatial resolution
	Easy to set up and use	Temporal resolution
	Flexibility (multiple fibers and illumination size)	Manual control
UV laser	Spatial resolution	Cost
	Temporal resolution	Technical expertise required
	Automated control	