

Video Article

Retrograde Fluorescent Labeling Allows for Targeted Extracellular Single-unit Recording from Identified Neurons *In vivo*

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Abstract

The overall goal of this method is to record single-unit responses from an identified population of neurons. *In vivo* electrophysiological recordings from individual neurons are critical for understanding how neural circuits function under natural conditions. Traditionally, these recordings have been performed 'blind', meaning the identity of the recorded cell is unknown at the start of the recording. Cellular identity can be subsequently determined via intracellular¹, juxtacellular² or loose-patch³ iontophoresis of dye, but these recordings cannot be pre-targeted to specific neurons in regions with functionally heterogeneous cell types. Fluorescent proteins can be expressed in a cell-type specific manner permitting visually-guided single-cell electrophysiology⁴⁻⁶. However, there are many model systems for which these genetic tools are not available. Even in genetically accessible model systems, the desired promoter may be unknown or genetically homogenous neurons may have varying projection patterns. Similarly, viral vectors have been used to label specific subgroups of projection neurons⁷, but use of this method is limited by toxicity and lack of trans-synaptic specificity. Thus, additional techniques that offer specific pre-visualization to record from identified single neurons *in vivo* are needed. Pre-visualization of the target neuron is particularly useful for challenging recording conditions, for which classical single-cell recordings are often prohibitively difficult⁸⁻¹¹. The novel technique described in this paper uses retrograde transport of a fluorescent dye applied using tungsten needles to rapidly and selectively label a specific subset of cells within a particular brain region based on their unique axonal projections, thereby providing a visual cue to obtain targeted electrophysiological recordings from identified neurons in an intact circuit within a vertebrate CNS.

The most significant novel advancement of our method is the use of fluorescent labeling to target specific cell types in a non-genetically accessible model system. Weakly electric fish are an excellent model system for studying neural circuits in awake, behaving animals¹². We utilized this technique to study sensory processing by "small cells" in the anterior exterolateral nucleus (ELa) of weakly electric mormyrid fish. "Small cells" are hypothesized to be time comparator neurons important for detecting submillisecond differences in the arrival times of presynaptic spikes¹³. However, anatomical features such as dense myelin, engulfing synapses, and small cell bodies have made it extremely difficult to record from these cells using traditional methods^{11, 14}. Here we demonstrate that our novel method selectively labels these cells in 28% of preparations, allowing for reliable, robust recordings and characterization of responses to electrosensory stimulation.

Video Link

The video component of this article can be found at <https://www.jove.com/video/3921/>

Protocol

1. Prepare Dye-coated Needles

1. Electrolytically sharpen a 160 μm diameter tungsten wire¹⁵. Final needle tip diameters should range from 5-50 μm . The number of needles needed depends on the size of the region being labeled. We prepared 5 needles for 3-5 injections into the posterior exterolateral nucleus (ELp).
2. The night before the experiment, place a drop (<0.25 μl) of 2 mM dextran-conjugated Alexa Fluor 10,000 MW dye onto the distal 100 μm of each needle.
3. Allow the needles to air dry at room temperature, leaving concentrated dye at the tip. Store the needles at 4 °C in a dark container to protect them from light.

2. Prepare Animal for Surgery

1. Induce general anesthesia by placing the fish in a solution of 300 mg/L MS-222 in tank water.
2. Weigh the fish and measure fork length (tip of snout to fork of caudal fin) and body depth (maximal dorso-ventral distance in the transverse plane). These measurements should fall within the ranges indicated in **Table 1** so that the fish fits inside a recording chamber small enough to place under a water-immersion microscope objective (**Figure 1**).
3. Immobilize and electrically silence the fish by injecting 100 μ l of 3 mg/ml flaxedil into the dorsal body musculature.
4. Fill the recording chamber (**Figure 1A**) with tank water. Place the fish ventral-side down on the platform in the center of the chamber (**Figure 1**). Deliver an aerated solution of 100 mg/L MS-222 using a pipette tip placed in the fish's mouth (1-2 ml/min). Stabilize the fish with rods fixed in wax placed on both sides of the body (**Figure 1C**). Monitor the fish's health by checking for continuous blood flow in the ocular vessels and a normal body color.
5. Rotate the platform along its long axis and lower the back end of the platform so that one side of the dorsal surface of the fish's head is exposed above the water while the rest of the fish's body remains submerged. A small piece of Kimwipe should be placed on any non-submerged portion of the skin to prevent drying.

3. Surgery (Figure 2)

The basic surgical procedure described here is well established and reliably used for blind *in vivo* recordings in mormyrids¹⁶. For other applications, expose the desired regions for labeling and recording. The region containing axon terminals of the cells of interest must be reachable by a dye-coated needle. The region containing more proximal segments of those same axons must have sufficient space above the tissue to accommodate the working distance of the water-immersion lens (2 mm in our case).

1. Apply a 0.4% solution of lidocaine to the exposed surface of the head using a Q-tip.
2. Using a scalpel blade, cut the perimeter of a rectangular piece of skin. Remove the rectangle using a pair of forceps. The size of the rectangle will scale with fish size, but should be approximately 3 mm X 5 mm for a 6.2 cm fish (**Figure 2A**). The lateral edge of the rectangle should align with the center of the eye, the anterior edge of the rectangle should be just posterior to the eye, and the medial edge of the rectangle should be just lateral of the fish's midline.
3. Expand the exposed skull region anteromedially to expose an additional 2.5 mm square non-overlapping area (**Figure 2B**).
4. Completely clear and dry the exposed surface of the skull using the scalpel blade to scrape away any excess tissue and Kimwipes and forced air to dry the surface (**Figure 2C**).
5. Glue a metal post to the anteromedial exposed skull region using Super Glue. Wait until the glue is completely dry (**Figure 2D**).
6. Remove a rectangle of skull, approximately 2 mm X 4 mm for a 6.2 cm fish. Use a dental drill with a ~0.5 mm diameter ball mill carbide tip to thin the perimeter of the rectangle. Then, using a scalpel and forceps, cut the perimeter of the rectangle and peel it away to expose the underlying brain. Additional drilling or cutting with small scissors may be necessary to fully expose EL (**Figure 2E**). If muscle bleeding occurs, an electrocautery unit may be used.
7. Cut away both the dura mater (pigmented) and the pia mater (clear) using spring scissors or a needle and remove the cut portions with a pair of forceps. The anterior and posterior portions of the extero-lateral nucleus (EL) are now visible, ELa and ELp, respectively (**Figure 3A**).

4. Retrograde Labeling of Axons of Interest

1. Position a manipulator with a dye-coated needle (made in Step 1) above the target region containing axons of interest, in our case ELp.
2. Swiftly insert the needle approximately 25 μ m into the tissue. Wait 15-30 seconds, until all the dye has come off, and then retract the needle.
3. Repeat with additional fresh needles as needed, placing each one in a different location so that the dye is distributed throughout the target region. We used 3-5 needles per preparation.
4. Rinse excess dye from the cavity with Hickman's ringer solution.
5. Wait at least 2 hours for dye uptake and transport.

5. Visualization of Axons of Interest

1. Place the recording chamber, along with the fish, underneath the objective of an upright, fixed-stage epifluorescent microscope. As the body of the fish occludes light penetration, both white and fluorescent light sources must come from above. Careful placement of a fiber optic light source above the skull cavity allows satisfactory brightfield images. For epifluorescence viewing, fluorescence filter specifications should match the absorption/emission spectrum of the dye.
2. Switch respiration to fresh tank water and maintain the same flow rate. Place a ground wire in the exposed brain cavity and connect to the ground of the recording headstage (see 6.3).
3. Place a pair of recording electrodes next to the base of the tail and connect to a differential amplifier and recording device (e.g. audio monitor, oscilloscope, or computer) to monitor the electric organ discharge command (EODC). After the fish recovers from anesthesia, the EODC can be used as an indicator of the fish's condition.
4. Prepare a scaled sketch of the brain region viewed at low magnification. Include major blood vessels as landmarks (may vary from fish to fish) to identify the exact location of labeled axons visible only under high magnification (**Figure 3D**).
5. Confirm dye placement. First view entire tissue with brightfield illumination for orientation (**Figure 3A**). Then view with fluorescent illumination (**Figure 3B**). ELp will have diffuse labeling (**Figures 3B and 3C**). Minimize fluorescence excitation to limit the photodynamic and phototoxic effects of the dye.
6. Use the vessels as landmarks to locate ELa under high magnification. Illuminate with fluorescent light while searching for a labeled axon near the surface. (**Figure 4**).

6. Record Extracellular Activity

1. Pull suction recording electrodes using 1 mm OD, 0.58 mm ID borosilicate capillary glass with filament. Ideal tip size will depend on the diameter of the target axons, which in our case is 0.1-0.2 μm ¹⁷. For our application, electrode tip diameters were $1.5 \pm 0.4 \mu\text{m}$ (range: 1.0-2.4 μm) with a 5 mm long, narrow shank in order to approach labeled axons without moving the surrounding densely packed tissue.
2. Fill electrodes with filtered Hickman's ringer solution. Final tip resistance is $45.2 \pm 38.0 \text{ M}\Omega$ (range: 16 to 155 $\text{M}\Omega$).
3. Place the electrode in an electrode holder with a pressure port and connect it to an amplifier headstage mounted on a manipulator. Run a pressure line from the pressure port to a T-junction ending in a manometer and a syringe for monitoring and controlling pressure, respectively.
4. Connect the headstage to an amplifier and an analog-to-digital acquisition device.
5. With 30 mbar outward pressure in the electrode line, place an electrode next to a labeled axon. A low-light level camera interfaced with imaging software is used to visualize pipette placement. Start near the tissue surface and advance the electrode towards the axon. As you approach the axon, the outward pressure should cause a slight, but noticeable movement of the axon.
6. While the electrode is next to the axon, (**Figure 4A**, top) record the potential at the electrode while presenting test stimuli (in our case, we used 100 msec monophasic positive and negative transverse pulses at an intensity of 20 mV/cm; **Figure 1A**). Action potentials should not be observed, although an electrical artifact confirms proper recording/stimulation (**Figure 4A**, bottom).
7. Release the outward pressure in the electrode and repeat stimulation/recording. Action potentials should still not be observed (**Figure 4B**).
8. Apply slight ($125 \pm 25 \text{ mbar}$) suction to the electrode and repeat stimulation/recording. Action potentials should now be observed in response to stimulation (**Figure 4C**). Spontaneous activity may also occur. If action potentials are not observed, release the suction, clear the electrode with slight pressure, move the electrode slightly, and attempt suction again. Once action potentials are visible, close the pressure line.
9. Stimulate and record as desired.

7. Termination and Disposal

1. Once all desired recordings are complete, switch to respiration with 100 mg/L MS-222 until the EODC has stopped. No EODC should be detected for at least 10 minutes.
2. Dispose of the fish according to institutional guidelines and approved animal care protocols.

8. Representative Results

For our particular application, we are interested in studying stimulus coding by central sensory neurons. Successful recordings from labeled axons allow analysis of single-unit responses to sensory stimulation¹⁸. **Figure 5A** shows representative action potentials evoked by transverse electrosensory stimulation using bipolar electrodes located on the insides of the left and right walls of the recording chamber. Spike times can be presented as a spike raster plot (**Figure 5B**). A 25 msec pre-stimulus recording window shows the low level of spontaneous activity. This particular ELa "small cell" is long-pass tuned to stimulus duration at a stimulus intensity of 6 mV/cm, increasing the number of spikes per repetition as stimulus duration increases (**Figure 5C**). The mean first spike latency is $4.28 \pm 0.16 \text{ msec}$, consistent with the expected latency for small cells in ELa¹¹.

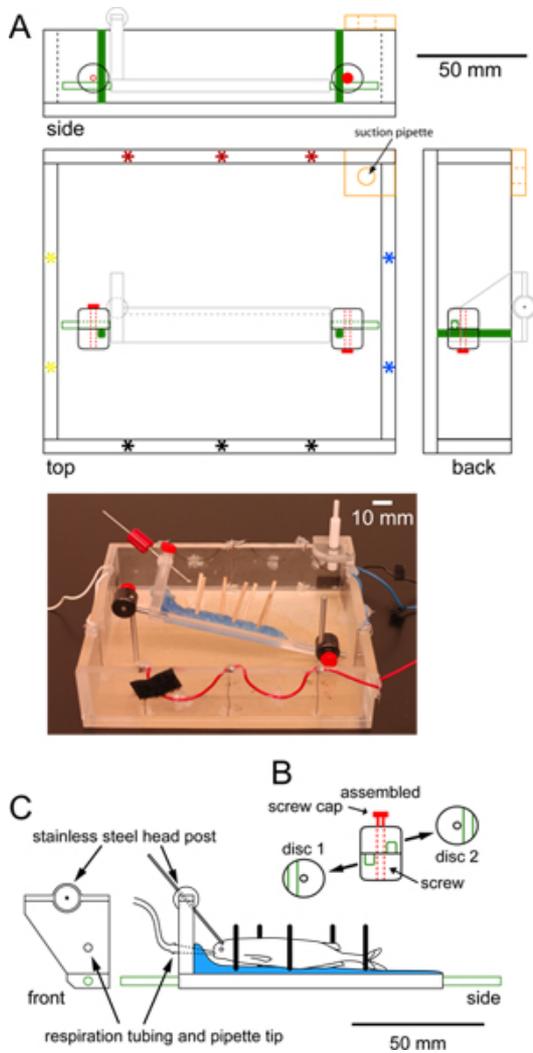


Figure 1. Specifications for a recording chamber that can fit underneath the objective of a fixed-stage epifluorescent microscope. (A) To-scale square recording chamber made from plexiglass showing top, side and back views. Paired sets of stimulating electrodes (asterisks) at the periphery allow for either transverse (red-black) or longitudinal (blue-yellow) stimulation. An additional piece of plexiglass in the corner, with a rubber-lined hole in the center (orange), holds a suction pipette that maintains a constant water level. Two vertical stainless steel posts screwed into the bottom of the chamber (solid green) connect to stainless steel posts (green outline) attached to the platform supporting the fish (light grey, detailed in **C**) via adjustable disc clamps. A photograph of the chamber is shown below the to-scale drawing. **(B)** Individual and assembled views of the circular plastic disc clamps used to secure the platform to the vertical posts. Each disc clamp has a groove (green) for a post and a center hole for the tightening screw. Disc clamps are rotated so the grooves are perpendicular to each other. Tightening the screw (red) clamps the posts in place to prevent further vertical and rotational movement of the platform. **(C)** Front and side to-scale views of the plexiglass platform used to hold the fish in place. The platform is coated with a layer of paraffin wax (blue) that holds wooden dowels (black bars) in place to support the fish. Tubing for respirating the fish passes through a hole in the 'headboard' of the platform and ends in a pipette tip placed in the fish's mouth. A stainless steel head post (gray bar) connects to the platform via a ball joint allowing 360 degree rotation. Stainless steel horizontal posts (green) are screwed into either end of the platform. [Click here to view larger figure.](#)

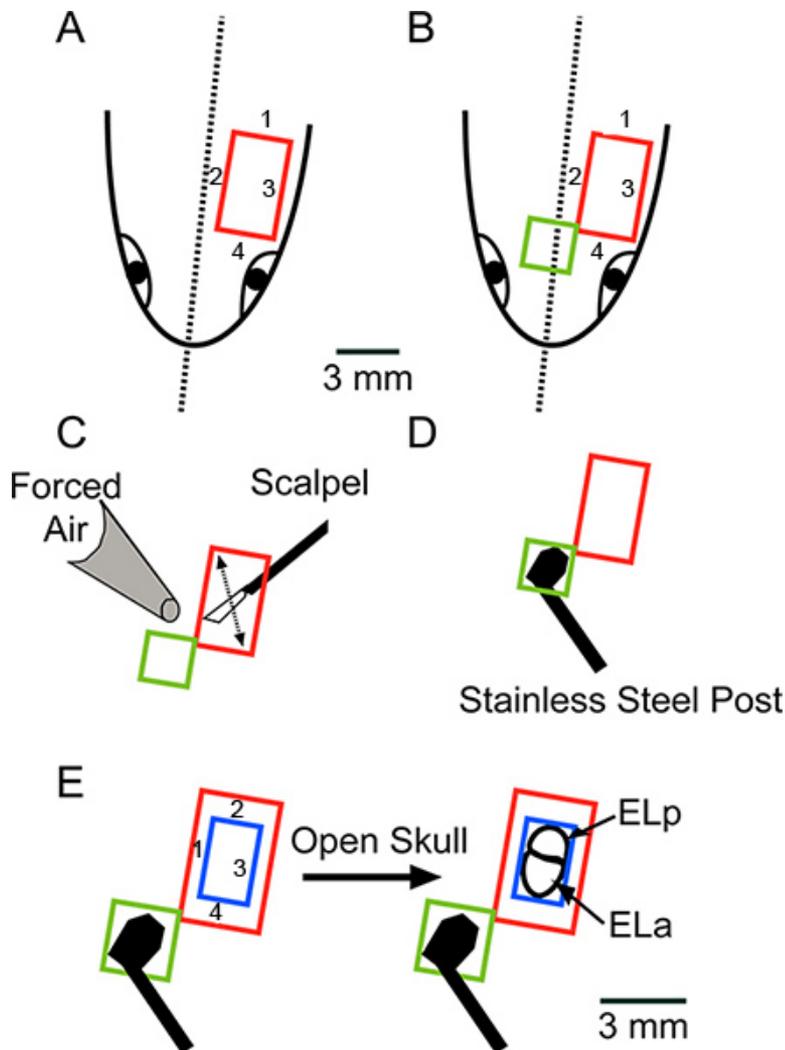


Figure 2. Schematic overview of surgery looking down at the dorsal surface of the head. (A) Make four cuts, in the order indicated, to remove a rectangular piece of skin (red). **(B)** Extend the opening anteromedially to remove an additional rectangular piece of skin (green). **(C)** Scrape off any remaining fat or ligaments by moving the scalpel blade as indicated by the arrow and dry the surface completely with Kimwipes and forced air. **(D)** Glue a stainless steel post to the skull using Super Glue. Scale bar in **A** applies to **A-D**. **(E)** Use a dental drill to make four cuts, in the order indicated, to remove a rectangular piece of bone (blue), exposing the anterior and posterior extrolateral nuclei (ELa and ELp, respectively). [Click here to view larger figure.](#)

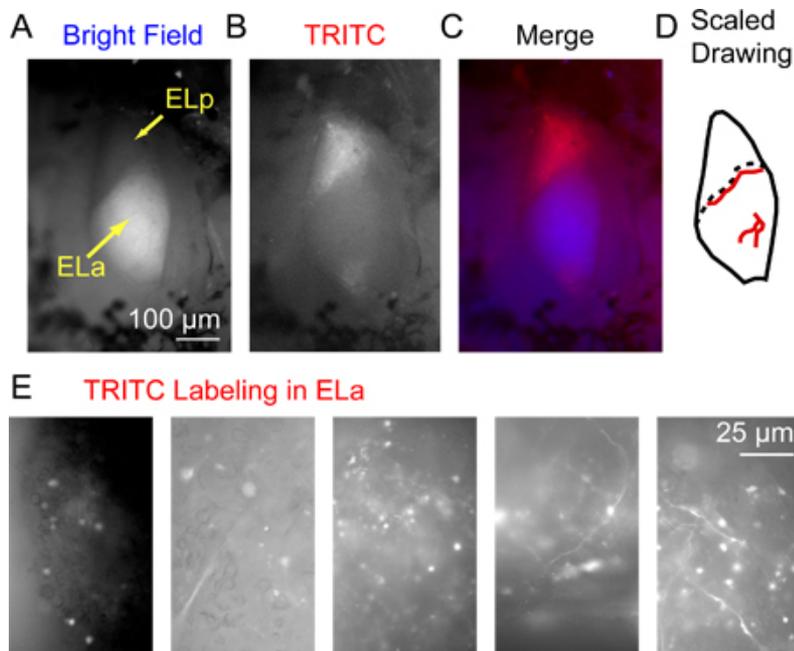


Figure 3. Fluorescent labeling in the posterior extero-lateral nucleus 3 hours after injection of dextran-conjugated Alexa Fluor 568. (A) The anterior and posterior extero-lateral nuclei (ELA and ELp, respectively) visualized with bright field illumination from above. Note that the extensive myelination within ELa gives it a relatively bright appearance that distinguishes it from ELp. (B) The same area visualized using epifluorescence viewed through a TRITC filter. (C) A merged image using blue for A (brightfield) and red for B (TRITC). (D) Example of a scaled drawing of ELa and ELp including major blood vessels (red lines) that can be used as landmarks to identify the exact location of labeled axons visible only under high magnification (the exact location of blood vessels varies from fish to fish). Dotted line indicates the border between ELa and ELp. (E) Sample images acquired using a TRITC filter from 5 different preparations illustrating a range of successful labeling patterns of small cell axons and somas in ELa. [Click here to view larger figure.](#)

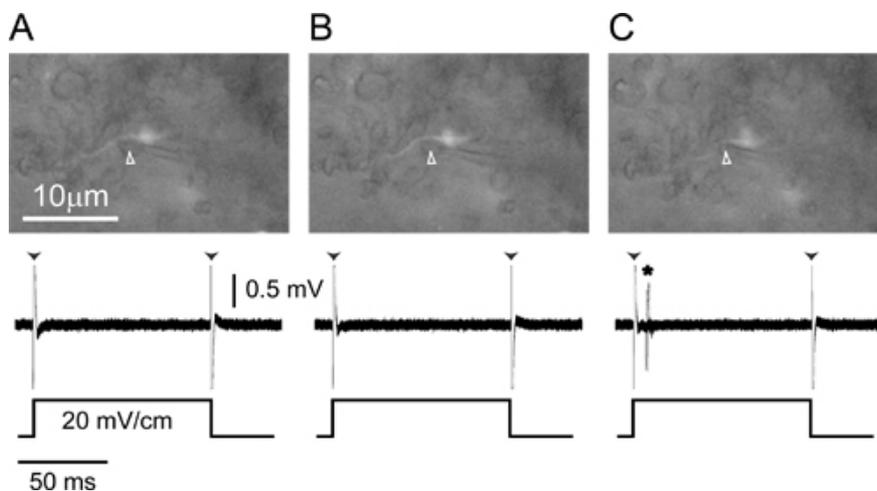


Figure 4. Single-unit extracellular recording from a labeled axon. (A) Recording electrode (arrow head) under positive pressure placed adjacent to a labeled small cell axon in ELa (top) records only edge artifact (arrow heads) in response to a 100 msec 20 mV/cm monophasic, contralateral-positive, transverse square pulse (bottom). (B) Releasing outward pressure from the electrode (arrow head) causes the axon to move slightly towards the electrode (top) but there are still no responses to the stimulus (bottom). (C) Slight negative pressure pulls the axon into the electrode (top, arrow head) and action potentials in response to stimulus onset are now visible (bottom, asterisk). Bottom portions of all three panels are overlaid responses to 20 repetitions of the stimulus. [Click here to view larger figure.](#)

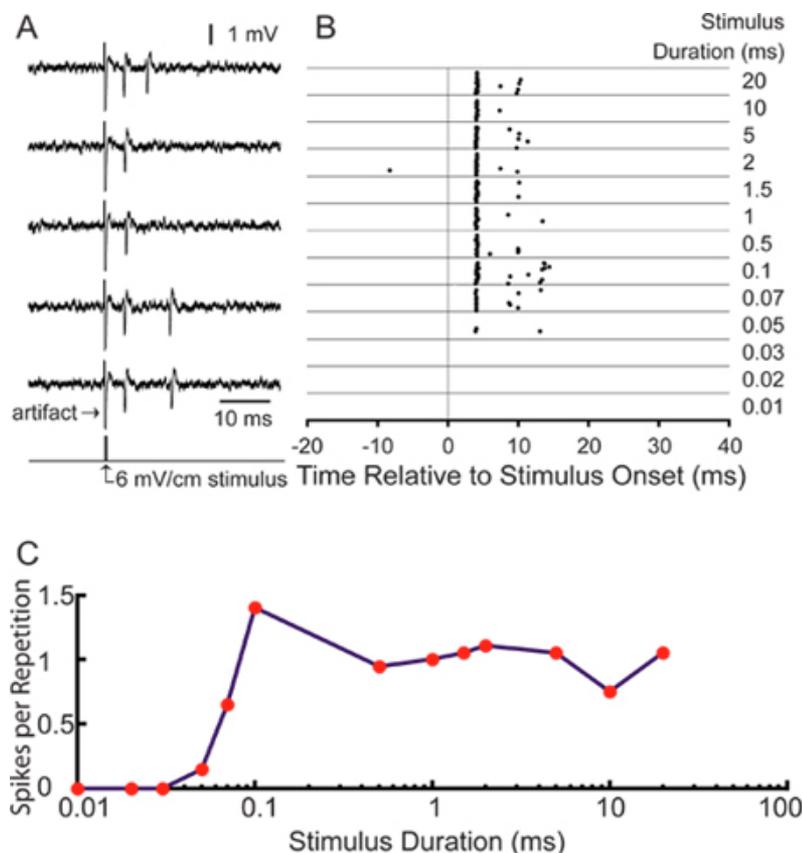


Figure 5. Representative results using this technique. (A) 5 sample traces showing action potentials evoked by a 0.1 msec 6 mV/cm monophasic, contralateral-positive, transverse square pulse stimulus. (B) Raster plot showing spike times during 20 repetitions of a 75 msec recording window for the same unit stimulated at time 0 with 6 mV/cm stimuli at the range of durations listed on the right. (C) Duration tuning curve quantifying the responses displayed in the raster as spikes per stimulus repetition. [Click here to view larger figure.](#)

	Mass (g)	Fork Length (cm)	Body Depth (cm)
Mean	2.42	6.20	1.14
Standard Deviation	0.64	0.52	0.18
Range	1.2-4.0	5.5-8.4	0.9-1.6

Table 1. Optimal weight, length and body depth ranges for fish. Optimal weight, fork length (tip of snout to fork of caudal fin) and body depth (maximal dorso-ventral distance in the transverse plane) ranges allowing fish to fit in the recording chamber illustrated in Figure 1. Fish that are too small may be less likely to survive the surgery and will have a small ELp, making dye placement challenging. Fish that are too large will have a large, over-reaching cerebellum that will reduce access to ELa and ELp and may prevent lowering of the high power, water-immersion objective close enough to focus on ELa and ELp.

Application Site	Dye Type	Fish attempted	Label in ELa	Label in ELp	Units attempted	Recordings
ELa	Alexa Fluor injection	32	26 (81.2%)	19 (59.4%)	50	4 (8.0%)
ELa	Alexa Fluor soaked filter paper	1	0	0	0	0
ELa	Di-I in DMSO	8	6 (75.0%)	0	0	0
ELa	Di-O crystals	5	3 (60.0%)	2 (40.0%)	9	0
ELp	Solid Alexa Fluor crystals	2	0	2 (100%)	0	0
ELp	Alexa Fluor coated tungsten wires	43	29 (67.4%)	41 (95.3%)	119	26 (21.8%)

Table 2. Success rates for each dye injection method. Success rates for each dye injection method. Methods are divided based on injection site and dye type. For each method, the total number of fish attempted and the percentage of these experiments that resulted in successful labeling in ELa and ELp is shown. Note that the targeted recording area is the opposite of the application site (bolded boxes). For the injection site, dye uptake was considered successful with labeling of both somas and axons. In contrast, at the recording site, only preparations with

labeled axons were counted as successful labeling experiments. The total number of attempted units and the percentage of these units that resulted in successful recordings are also shown.

	# of injection sites	Average volume per injection (µl)	Range of volumes per injection (µl)	Average total injection volume (µl)	Range of total injection volumes (µl)
Microinjector with Hamilton	3 or 4	0.144	0.091 - 0.91	0.516	0.378 - 0.669
Nanoinjector with glass pipette	2 - 4	0.069 (fixed)	Fixed volume injected 1-6 times	0.621	0.414 - 0.966
Microinjector with glass pipette	2 - 6	0.093	0.058 - 0.202	0.360	0.252 - 0.540

Table 3. Quantities of dye applied for each of the three methods used to inject Alexa Fluor into ELA Quantities of dye applied for each of the three methods used to inject Alexa Fluor into ELA (first method from **Table 2**). Dye injection into ELA was performed with both a nanoinjector and a microinjector using either a 33 gauge Hamilton syringe needle or a pulled glass capillary pipette. We varied the number of injection sites, the volume per injection and the total volume of dye injection.

Discussion

Once mastered, this technique will allow one to target identified neurons, including individual axons, for *in vivo* recordings in many model systems. In addition, this technique allows one to reliably record spike output from neurons with unique anatomical characteristics that make traditional *in vivo* recording methods challenging. We have utilized this technique to record from ELA "small cells" in mormyrid weakly electric fish. Previous attempts to study the tuning properties of "small cells" were unsuccessful due to challenging recording conditions^{11, 14}. Similar anatomical features create barriers to obtaining single-unit recordings from many different vertebrate auditory and electrosensory neurons⁸⁻¹⁰. To overcome these challenges in our system, we took advantage of the fact that "small cells" are the only cells in ELA that project to ELP. Thus, retrograde transport of dye placed in ELP limits labeling in ELA to "small cell" somas and axons. Fluorescent labeling of the axons allowed precise electrode placement next to labeled axons, making single-unit recordings from identified cells possible despite inaccessible somas. We attempted somatic recordings, but were unsuccessful, probably due to the surrounding engulfing synapses^{11, 17}. However, somatic labeling was clearly visible suggesting that this technique could be used to target somatic recordings in other cell types and other circuits. Fluorescent labeling of neurons through retrograde transport *in vivo* has been used for guiding targeted recordings *in vitro*¹⁹⁻²¹. A similar technique was used for targeted *in vivo* recordings from motor neurons in zebrafish spinal cord²². Our work represents a novel expansion of this approach, in which both labeling and recording are done *in vivo* within the brain. Our method demonstrates that *in vivo* labeling of CNS areas with a retrograde tracer can be expanded to the study of other intact circuits with similarly selective projection neurons. For example, in mammalian auditory processing, the inferior colliculus (IC) serves as an important relay center for inputs from multiple rhombencephalic structures²³. Dye injection into the IC would selectively label the projection cells from each of these nuclei. The superior colliculus (SC) serves a similar function for vision²⁴. Spinal cord preparations are particularly well suited for this technique, as the spinal cord is easily accessed, dye injection can occur far from the recording site, and it can be combined with intracellular recording and filling of select neurons to acquire more detailed anatomical information²⁵. Finally, tract-tracing is a well established technique used throughout the central nervous system to map complex circuitry²⁶. Our method can be utilized to add functional information to these studies as has been done with calcium-sensitive indicators in cat visual cortex²⁷.

The surgery, which is well established, reliable, and regularly used for blind *in vivo* recordings¹⁶, must be completed with minimal bleeding and no damage to the surface of the brain to allow the animal and the tissue to survive. With practice, the surgery and dye application can be completed in 30-45 minutes. We successfully labeled "small cell" axons in 67% of the preparations. Most preparations have only 1 or 2 visible labeled axons, but some have as many as 8. Of the 119 labeled units attempted, we obtained single-unit recordings from 26 units distributed over 12 preparations (**Table 2**). Thus, data were collected from 41% of preparations with labeled axons for an overall success rate of 28%.

The critical aspect of dye application is labeling depth. Shallow insertion of the tungsten wire will result in the dye being washed away. However, if the penetration is too deep, labeled axons will not be visible for targeting. Further, some mechanical damage to the cell must occur for the dye to be adequately taken up^{25, 28}. However, too much damage will kill the cells. We attempted labeling with other dyes and other methods (**Table 2**), including anterograde labeling through dye injection into ELA paired with recording from labeled axons in ELP (**Table 3**). We hypothesize that anterograde labeling was not successful because labeling was limited to cells with somatic damage, making them unresponsive. Additionally, pre-synaptic terminals may have been disturbed. By contrast, retrograde labeling minimizes both of these concerns. The amount and location of dye application can be modified according to the particular circuit being studied. Maximal labeling occurs with the greatest dye concentration, which we achieved using coated tungsten wires. However, for labeling axons with deep projections, dye may come off a tungsten needle as it is advanced. In these cases, pressure injection would be more appropriate. Dye uptake and transport are rapid, with labeled axons in our preparation being visible as early as 2 hours post-injection and additional labeled axons appearing as late as 6 hours post-injection. Thus, labeling and recording can be accomplished in a single day, eliminating technical difficulties associated with survival surgery. Timing will vary for each application depending on the distance required for dye transport.

Another critical aspect is electrode placement. It is important to enter the tissue close to the site of the labeled axon to prevent clogging of the tip. For dense tissue such as in ELA, a long, thin shank on the recording electrode minimizes excess movement of surrounding tissue. If a successful recording is not achieved on the first attempt, repeat with fresh electrodes until a recording is obtained or the tissue is disrupted to the point at which the axon is no longer visible. However, it is also important to quickly place the electrode next to the axon to minimize the amount of fluorescent exposure, which can cause phototoxicity, bleaching and may affect physiological properties of the cell²⁹⁻³¹.

Once a segment of axon is successfully suctioned into the recording electrode, recordings can be obtained for several hours. If units are consistently lost in less than 1 hour, consider making smaller electrode tips to prevent the axon from slipping out. On the other hand, too small

of a tip may result in clogging, low signal-to-noise, or damage to the axon. A steady decrease in spike amplitude and the 'return' of a unit with additional suction is an indication that the tip is too large. Too much suction may cause irreparable damage to the axon. One solution is to allow a small leak in the air line so the pressure slowly returns to zero. Rapidly equalizing the pressure will result in a transient relative-outward 'push' which may expel the axon.

Although this technique represents a major advantage for obtaining targeted recordings from identified projection neurons, it will not be useful for distinguishing local interneurons, as dye would be taken up by all cell types at the injection site. Theoretically, the use of multiple fluorophores with separate injection sites may allow this method to be expanded. For example, comparison of single- versus double-labeling could be used to distinguish interneurons from projection neurons following dual injections at two points in the circuit³². Similarly, retrograde tracers can be combined with other advanced imaging techniques, such as two-photon imaging, as was done recently in zebra finch High Vocal center (HVC)³². Also, recording regions are limited to those near the surface when using epifluorescence microscopes, as we were only able to resolve 1 μ m structures within the first 30 μ m of tissue. However, this depth could be extended through the use of other microscopy techniques, such as two-photon microscopy³³ or objective-coupled planar illumination microscopy³⁴. Overall, this technique represents an important advancement in the study of neural circuits *in vivo* because it can be used to record from single neurons in many different circuits in a variety of model systems - including those that are relatively inaccessible.

Disclosures

No conflicts of interest declared.

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