

Video Article

Single Cell Electroporation in vivo within the Intact Developing Brain

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Abstract

Single-cell electroporation (SCE) is a specialized technique allowing the delivery of DNA or other macromolecules into individual cells within intact tissue, including in vivo preparations. The distinct advantage of this technique is that experimental manipulations may be performed on individual cells while leaving the surrounding tissue unaltered, thereby distinguishing cell-autonomous effects from those resulting from global treatments. When combined with advanced in vivo imaging techniques, SCE of fluorescent markers permits direct visualization of cellular morphology, cell growth, and intracellular events over timescales ranging from seconds to days. While this technique is used in a variety of in vivo and ex vivo preparations, we have optimized this technique for use in *Xenopus laevis* tadpoles. In this video article, we detail the procedure for SCE of a fluorescent dye or plasmid DNA into neurons within the intact brain of the albino *Xenopus* tadpole. We also discuss methods to optimize yield, and show examples of live two-photon fluorescence imaging of neurons fluorescently labeled by SCE.

Video Link

The video component of this article can be found at <http://www.jove.com/video/705/>

Protocol

Equipment set-up:

1. The electrical equipment required for this technique are a stimulator, an oscilloscope, and a micropipette holder fitted with a silver wire to be inserted into the micropipette. We typically use an Axon instruments Axoporation 800A stimulator, however other common stimulators such as the Grass instruments SD9 stimulators are also suitable for single-cell electroporation. The stimulator is connected to the headstage, which interfaces with the silver wire electrode to be placed inside the glass micropipette containing an internal conducting solution. The other lead from the stimulator is connected to the oscilloscope input, which also receives input from the tadpole external ground. The tadpole external ground is simply a silver wire that remains in electrotonic contact with the tadpole during electroporation. The circuit is complete once the micropipette is placed in contact with the tadpole.
2. Single cell electroporation requires a microscope with a good working distance (at least 5cm), since this gives room to maneuver and allows the micropipette to be brought in at an angle of about 30-45 degrees.

Fabrication of micropipettes:

Preparation of appropriate micropipettes is a critical step in this protocol. The difficulty lies in balancing a narrow tip (less than 1µm in diameter) with one that will not easily break when puncturing the tissue. We have also found that tips with a taper angle of greater than 10 degrees are preferable.

1. Prepare a pulled glass micropipette with a tip diameter of less than 1µm, and a tip taper angle of greater than 10 degrees.
 1. Borosilicate glass with an internal filament with outer diameter of 1.5mm and inner diameter of 0.75mm is well suited for this purpose.
 1. The relatively thick glass provides a degree of rigidity to the tip.
 2. The internal filament is important in drawing back-filled fluid towards the micropipette tip.
2. Fabrication of suitable tips will often require several modifications based on observed results. Different tissues may require a tip with a slightly different shape or tip diameter.

Single-cell electroporation protocol:

For new users, it is advisable to begin with fluorescent dextran dyes since, unlike genetically encoded fluorescent proteins, they can be immediately seen under epifluorescence. Use of fluorescent dyes enables the user to directly see the location of the micropipette tip within the

tissue, and upon electroporation gives instant feedback as to whether the equipment has been properly set up and whether the micropipette tip is appropriate.

1. Fill micropipette with solution of compound to be electroporated either by using a loading syringe or by back-filling.
 1. If using plasmid DNA, ensure that samples are endotoxin-free, and at a concentration between 1-2 μ g/ml, prepared in water.
 2. The concentration of fluorescent dextran dyes should be empirically determined. We often use fluorescent dyes diluted in water to approximately 300 μ M.
 3. Typically, volumes of 0.5-1 μ l are sufficient.
 4. Prior to loading, briefly centrifuge solutions at high speed to reduce the amount of particulate debris drawn into the micropipette, which may lead to clogging.
 5. Air bubbles within the tip often affect current flow and should be dislodged by vigorously flicking the micropipette prior to mounting on the headstage.
2. Insert micropipette into holder or headstage mounted on a 3-axis micromanipulator. Ensure that silver wire electrode is inserted into micropipette, and is in electrotonic contact with the internal solution.
3. Anesthetize tadpoles by immersion in 0.02% MS-222 (3-aminobenzoic acid ethyl ester) prepared in tadpole rearing medium (Steinberg's solution, pH 7.4).
 1. We normally use stage 44-48 albino *Xenopus laevis* tadpoles in our experiments.
 2. Tadpoles are usually fully anesthetized after approximately 5 minutes.
 3. Several tadpoles may be anesthetized simultaneously to reduce wait-time, however, avoid exposure to MS-222 for periods beyond 1 hour.
4. Transfer a single anesthetized tadpole to electroporation chamber using a plastic transfer micropipette.
 1. The electroporation chamber can be easily made in-house by carving a tadpole-shaped cavity in a small Sylgard® silicone block, and inserting a silver ground wire into the cavity such that it will be in electrotonic contact with the tadpole in the chamber.
5. Position tadpole dorsal side up using a moistened paintbrush.
6. Lower the micropipette until it is in contact with the skin, directly adjacent to the region of interest.
 1. Targeting regions with densely packed cell bodies will greatly increase chances of successful electroporation. We typically target the tadpole optic tectum.
 2. Bring the micropipette in at an angle of 30 to 45 degrees. Shallower trajectories make skin puncture more difficult, while steeper angles often obstruct one's view.
7. Continued lowering of the micropipette will initially dimple the skin, and then pass through into the underlying tissue.
 1. Superficial cells are preferred for *in vivo* imaging, and can be targeted by ensuring that the micropipette tip is lowered slowly upon dimpling of skin.
8. One should constantly monitor the micropipette resistance once placed within the tissue. If using an Axoporation 800A (Axon Instruments), resistances of approximately 10-40M Ω are optimal. Resistance is a good indicator of whether the tip diameter is appropriate for single-cell electroporation.
 1. High micropipette resistances (>100M Ω) are indicative of clogged tips or micropipettes with tips that are too narrow.
 2. Low micropipette resistances (<10M Ω) are indicative of a tip diameter that is too large, often a result of a broken tip.
 3. If using a stimulator that does not provide a direct resistance measure, micropipette resistance can indirectly measured by observing the amplitude of the voltage pulses measured by the oscilloscope (discussed below).
9. Apply voltage train.
 1. For plasmid DNA, pulse trains found to be most effective consist of negative square wave pulses 1ms in duration, delivered at 300Hz with pulse train duration of 500ms.
 1. The pulse voltage is determined empirically such that the amplitude of pulses measured on the oscilloscope is between 0.75 and 1.5 μ A.
 2. For fluorescent dextran dyes, positive square wave pulses of 300 μ s duration are delivered at a frequency of 300Hz with pulse train duration of 10ms.
 1. As with the protocol for DNA, the pulse voltage is determined empirically such that the amplitude of pulses measured on the oscilloscope is between 0.75 and 1.5 μ A.
 2. Additional adjustments to the stimulus parameters can be made based on observing the results of electroporation under epifluorescence.
 1. If electroporated cells appear dim, pulse parameters should be gradually increased until cells appear brighter.
 2. On the other hand, if clusters of cells are labeled, pulse parameters should be reduced until single cells are labeled.
10. Retract micropipette, re-insert into a different site within the tissue, and apply voltage train.
 1. To improve yield, we typically electroporate multiple sites in each hemisphere of the tadpole optic tectum with sufficient spacing to ensure that labeled cells do not overlap.
11. Transfer the electroporated tadpole into a container with Steinberg's rearing solution.
 1. Tadpoles will rapidly recover from anesthesia within a few minutes.
12. The same micropipette can be used for numerous tadpoles. It is important to constantly observe the amplitude of the pulses observed on the oscilloscope.

1. If the pulse amplitude remains low after significantly increasing the pulse intensity this is likely a result of the tip being clogged, often seen when electroporating many tadpoles in one sitting.
 1. A method to dislodge a minor tip clog is to apply a single positive pulse after inserting the micropipette into the tissue.
 2. If the clog cannot be dislodged, or clogging frequently recurs, replace the micropipette.
2. If the pulse amplitude remains very large after significantly decreasing the pulse parameters, this is a sign that the tip has broken and needs to be replaced.

Screening for successfully electroporated cells:

1. After electroporation, tadpoles are screened for labeled cells using an upright epifluorescence microscope.
2. In the case of fluorescent dyes, tadpoles may be screened after as little as 30 minutes post-electroporation.
 1. We have found, however, that longer intervals are associated with lower background fluorescence levels.
3. For genetically encoded fluorescent proteins, screening is usually conducted at least 12 hours post-electroporation.
 1. Cells expressing fluorescent proteins will continue to get brighter with time, therefore dim cells may be re-screened after an additional interval of 12-24 hours.
4. Tadpoles are anesthetized as described above, placed in a Sylgard® chamber and coverslipped. Individual tadpoles are then examined under epifluorescence.
 1. Note that excessive exposure to epifluorescence light will result in phototoxicity that may kill the labeled cell. Therefore, minimize the amount of time that cells are exposed to the fluorescent light.
5. Return tadpoles to container with Steinberg's rearing solution.

Discussion

Single-cell electroporation (SCE) is a powerful tool for determining gene function and performing targeted genetic manipulation. The transparency of the albino tadpole and the accessibility of the brain make this model system ideally suited for visualizing neuronal growth and intracellular events within a live organism. SCE allows the visualization of the growth of a single neuron, and to perform cell-autonomous manipulations within an otherwise unaltered brain. While this video article demonstrates the procedure for single-cell electroporation in *Xenopus laevis* tadpoles, this technique has been used in other organisms, and has also been used in hippocampal slices and dissociated cell cultures.

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References

1. Bestman JE, Ewald RC, Chiu SL, Cline HT. In vivo single-cell electroporation for transfer of DNA and macromolecules. *Nature Protocols*. 2006;1(3):1267-72.
2. Dunfield D, Haas K. Single cell electroporation. In: *Encyclopedia of Neuroscience* (4th Ed.) Elsevier, Amsterdam. (in press)
3. Haas K, Jensen K, Sin WC, Foa L, Cline HT. Targeted electroporation in *Xenopus* tadpoles in vivo—from single cells to the entire brain. *Differentiation*. 2002 Jun;70(4-5):148-54.
4. Haas K, Sin WC, Javaherian A, Li Z, Cline HT. Single-cell electroporation for gene transfer in vivo. *Neuron*. 2001 Mar;29(3):583-91.