Targeted single-cell electroporation of mammalian neurons *in vivo*

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In order to link our knowledge of single neurons with theories of network function, it has been a long-standing goal to manipulate the activity and gene expression of identified subsets of mammalian neurons within the intact brain *in vivo*. This protocol describes a method for delivering plasmid DNA into single identified mammalian neurons *in vivo*, by combining two-photon imaging with single-cell electroporation. Surgery, mounting of a chronic recording chamber and targeted electroporation of identified neurons can be performed within 1–2 h. Stable transgene expression can reliably be induced with high success rates both in single neurons as well as in small, spatially defined networks of neurons in the cerebral cortex of rodents.

INTRODUCTION

A central question in neuroscience is how specific molecular and cellular mechanisms within neurons can lead to complex brain functions, such as perception, learning and behavior. To address this question, it is crucial to be able to perturb and modify neural circuits on the single-cell level by regulating expression of genes in identified cells. The last few decades have witnessed a revolution in the use of genetics in neuroscience, driven by the identification and cloning of the major genes coding for signaling molecules in the brain[1]. These advances have been harnessed to produce transgenic animals to examine the functional role of particular genes[2,3].

Although these techniques have reached an increasing level of sophistication, involving region-specific, cell-type-specific and inducible expression or suppression of genes[4,5], the widespread nature of the genetic modification often results in compensation for the modification and/or downstream alterations of network function (see e.g., refs. 6,7), making it difficult to make direct causal links between single genes and brain function. To some extent, these problems have been overcome by viral transfection approaches, which allow expression to be limited to small groups of neurons in circumscribed regions of the brain[5,8,9]. *In utero* electroporation techniques have also been exploited to restrict expression to specific cell types[10,11].

However, both of these approaches are associated with several major limitations: (1) the number of cells cannot be tightly controlled; (2) single cells cannot be targeted; (3) not all cell types can be perfectly selected for using a genetic approach, especially when cell types are functionally defined (i.e., defined by their responses to stimuli); and (4) the spatial arrangement of the transgenic neurons cannot be rigorously defined. Solving these problems is becoming increasingly important, given the growing appreciation for the role of single neurons in neural networks. This has built on the pioneering work of Barlow about the role of single neurons in sensory perception[12], together with evidence for strong correlations between single-cell responses and psychophysical performance in behaving animals[13–15]. This work has more recently been further supported by the demonstration that activation of single neurons or small numbers of neurons can produce motor output and behavioral responses[16–18]. It is thus becoming clear that it is crucial to manipulate the genetic properties of single neurons not only to mitigate the downstream and/or compensatory effects on the network but also to better probe the causal links between genes, neurons and behavior.

One approach that can provide the desired single-cell specificity of genetic modification is single-cell electroporation. This approach has been applied in *Xenopus* tadpoles *in vivo*[19] and in mammalian neurons *in vitro*[20]. Although electroporation of calcium-sensitive dyes has been shown *in vivo*[21], it has been difficult to achieve reliable *in vivo* single-cell electroporation of DNA in mammalian brains. Here we present a protocol for visually guided single-cell electroporation of DNA in mammalian neurons *in vivo* based on our earlier published work[22]. Compared to other methods, our approach takes advantage of the *in vivo* two-photon microscopy[23,24] to visualize and identify the target neuron(s) and to directly monitor the process of electroporation[22] (**Fig. 1**). High success rates and reliable single-cell specificity can be achieved using this approach in different brain areas. We describe the approach using rodents, but it is equally applicable in any other species where neurons can be visualized using two-photon microscopy.

This approach has a number of advantages over existing techniques. First, it allows successful electroporation of DNA to be performed in any species for virtually any cell type that can be imaged with two-photon microscopy. This includes cell types for which no transgenic animals exist due to lack of selective promoters and cell types which cannot be infected by currently available viral vectors[25]. Second, cells can be selected for electroporation on the basis of their functional or anatomical profile. Third, the number of electroporated cells, and their spatial arrangement can be tightly controlled; the number of cells can be increased and their spatial arrangement modified by subsequent electroporation in the same network. Fourth, the method should be equally applicable for electroporation of RNA[26]. Finally, in principle, the method should allow electroporation not only at the soma but also into processes such as dendrites or axons (e.g., when the soma is not visible).

These unique advantages of the electroporation approach should permit a wide range of new applications, elucidating the function of identified neurons within intact networks *in vivo*.

Experimental design

Choice of brain region. Targeted single-cell electroporation can theoretically be performed in any brain area accessible to two-photon imaging. We have successfully performed single cell-electroporation in the parietal cortex, the barrel cortex, the visual cortex and the cerebellar vermis. Depending on the spatial and/or experimental constraints, it may be advisable to choose an area with fewer blood vessels for better imaging quality and pipette access.

Recording chamber. We use a custom designed stainless-steel recording chamber that can also be used to attach the animal to the imaging setup (see **Fig. 2** and **Supplementary Fig. 1** online). Alternatively, the skull can be immobilized at a separate point on the skull, e.g., by implanting a screw nut or a metal bar.

Choice of plasmids. Multiple different plasmids can be electroporated simultaneously. For example, if the DNA of interest encodes a non-fluorescent gene product, add plasmid DNA encoding a fluorescent protein (e.g., green fluorescent protein; GFP) to the pipette solution in order to confirm successful electroporation and to identify the cells on subsequent days. We have not yet encountered a plasmid size limit for electroporation and have thus far successfully electroporated plasmids of up to 10 kb.

Approach of cells under visual control. Cells can be targeted in two different ways. Using transgenic animals specifically expressing fluorescent proteins in the cells of interest, they can be directly targeted using two-photon targeted patching[27,28]. Alternatively, they can be targeted using 'shadow imaging', a method in which the extracellular space is labeled using a membrane-impermeable dye, thereby creating a negative 'shadow' of the cell somata[22] (**Fig. 3**).

Electroporation parameters. For rodent cortical neurons, 50 square −12 V pulses of 0.5 ms duration at a frequency of 50 Hz are effective. The electroporation parameters should be optimized for each cell type and experimental condition by changing the amplitude and number of the pulses, as required. The success rate in our hands is around 75% (measured as the fraction of electroporated cells expressing the gene of interest after recovery).

Electroporation of multiple cells. Single cell electroporation can be used to sequentially electroporate up to 20 cells with the same pipette within 10–15 min. When targeting multiple cells, it is important to minimize lateral or vertical pipette movements within the neuropil. In our experiments, we generally avoid moving the pipette by more than 200 µm laterally or vertically within the brain. To electroporate cells further apart, use multiple pipettes.

Assessing expression of the gene of interest. Expression (or suppression) of the gene of interest can be assessed by co-expression of GFP or other reporters, or by using a direct functional assay. Depending on the experimental design, re-anaesthetize the animal and image and/or record from the same neuron(s) that were electroporated (see **Box 1**). This can be repeated multiple times in order to provide time series data, e.g., about changes in neuronal morphology or morphological dynamics[29,30].

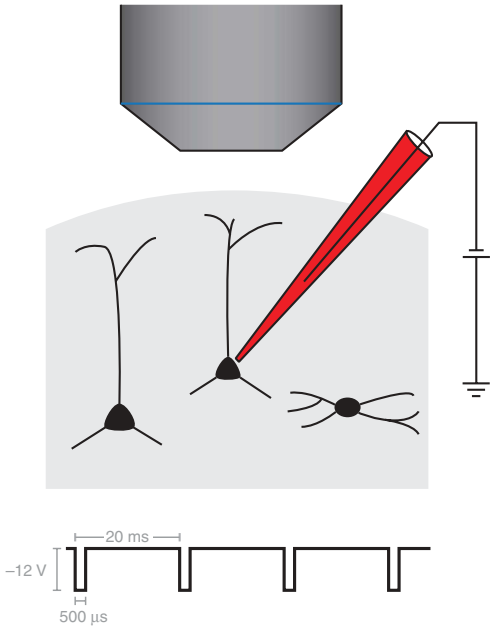

Figure 1 | Schematic diagram of targeted electroporation. Pipettes containing internal solution, Alexa 594 and a DNA plasmid (e.g., encoding enhanced green fluorescent protein (EGFP)) are placed in close proximity to the somata of neocortical neurons. Trains of negative voltage pulses are applied to electroporate the dye and DNA into the cells.

Repeated recording from the same neuron following electroporation. For some experimental designs, particularly after genetic manipulations that change the functional properties of the cells[4,31,32], it is useful to be able to image and make electrophysiological recordings from neurons that have been electroporated once the gene of interest has been expressed. This can be achieved by making targeted recordings from the same cells by visualizing them using two-photon microscopy[27,28] (**Box 1**, **Fig. 4**).

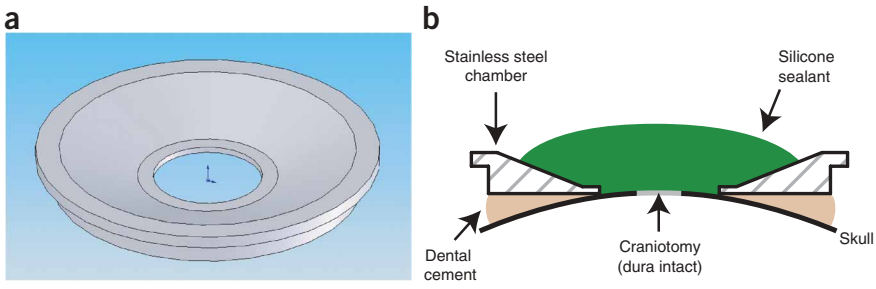

Figure 2 | Recording chamber for combining chronic imaging with chronic electrophysiology. A 3D rendering of the recording chamber design (diameter 11 mm) (**a**). Schematic diagram illustrating how to mount the recording chamber onto the skull (**b**).

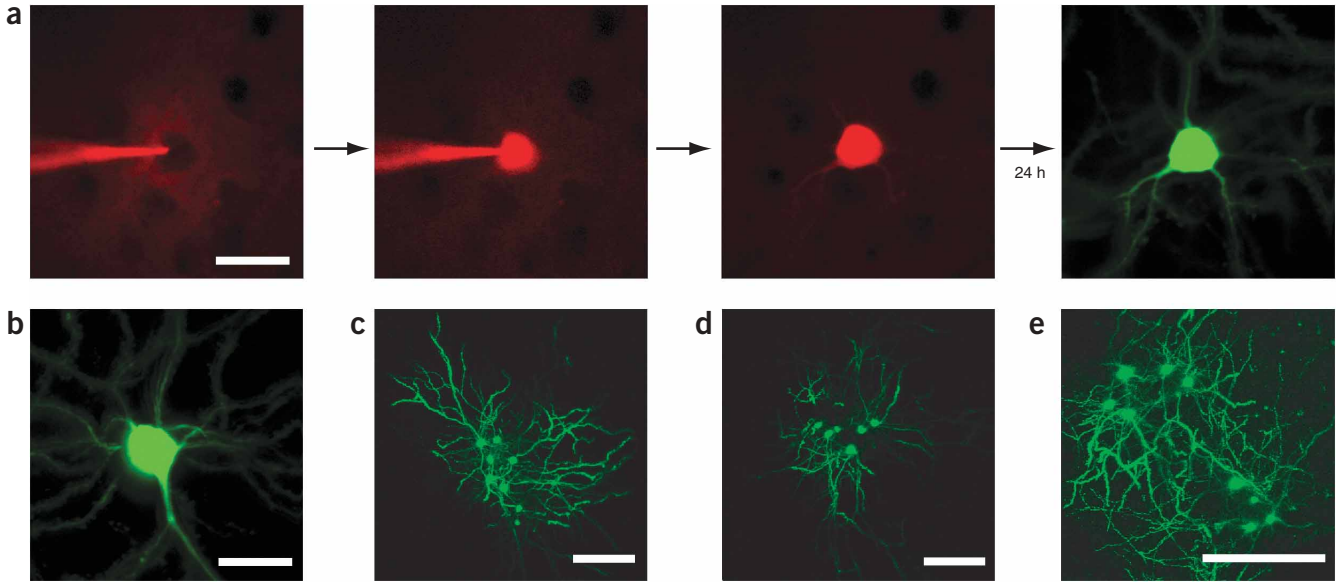

Figure 3 | Electroporation of single neurons and groups of neurons. Negative contrast image of a neocortical layer 2/3 pyramidal neuron (**a**) from left to right: before electroporation, during electroporation and a few minutes after electroporation, filled with Alexa 594. Right: image of the same neuron 24 h later, showing bright enhanced green fluorescent protein (EGFP) fluorescence throughout the dendritic tree. Another layer 2/3 pyramidal cell 24 h after electroporation (**b**), examples of groups of electroporated neocortical layer 2/3 neurons (**c**–**e**). Scale bars: 20 μm (**a**,**b**), 100 μm (**c**–**e**).

MATERIALS

REAGENTS

- Experimental animals (e.g., rats or mice) ! CAUTION All animal experiments must comply with the relevant institutional and national animal care guidelines.
- Fluorescent dye (Alexa Fluor 594 hydrazide, Invitrogen, cat. no. A10438)
- Plasmid DNA at a concentration of ~5 μg μl^{-1} in H_2O (prepared with the Qiagen MaxiPrep Kit, Qiagen). For expression of enhanced green fluorescent protein (EGFP), we use the plasmid pCAG-GFP (Addgene plasmid 11150, Addgene)
- Anaesthetic (Ketamine/Xylazine, Pfizer Animal Health/Millpledge Veterinary)
- Analgesic (Buprenorphine, Reckitt Benckiser Healthcare)
- Non-steroidal anti-inflammatory analgesic (Carprofen, Pfizer Animal Health)
- Eye lubricant (Allergan Lacri-Lube, Allergen Pharmaceuticals)
- KWIK-seal silicone sealant (World Precision Instruments)
- Methyl Methacrylate cement (Lang Dental)
- Cyanoacrylate glue (RS components, cat. no. 159-3963)
- NaCl (Sigma, cat. no. S9888)
- KCl (Sigma, cat. no. P9333)
- HEPES (VWR International, cat. no. 441474J)
- $CaCl_2$ (VWR International, cat. no. 190464K)
- $MgCl_2$ (Sigma, cat. no. 63069)
- $KMeSO_4$ (Sigma, cat. no. 83000)
- Mg-ATP (Sigma, cat. no. A9187)
- Na_2-ATP (Sigma, cat. no. A7699)
- Na_2-GTP (Sigma, cat. no. G8877)
- Ethylene glycol tetra acetic acid (EGTA) (Sigma, cat. no. E4378)

EQUIPMENT

- Two-photon microscope for *in vivo* imaging (Sutter Instruments, Prairie Technologies or custom-built[33]) ▲ CRITICAL The microscopy system must be kept as clean as possible for recovery experiments.
- Water-immersion objective (e.g., Olympus Japan 40× 0.80w LUMPlanFl/IR or Nikon Japan 16× 0.80w LWD) ▲ CRITICAL Objectives should have a large pipette access angle.
- Emission filters (Chroma Technologies ET520-65 and ET610-60)
- Tunable Ti:Sapphire laser (SpectraPhysics USA MaiTai, SpectraPhysics)
- Image acquisition software (ScanImage[34])
- Amplifier or stimulus isolation unit for electroporation (e.g., Molecular Devices, Axoporator 800B; for further discussion, see ref. 35)
- Motorized micromanipulators (Sutter Instruments MP-285)
- Borosilicate glass capillaries (Harvard Apparatus GC150F-7.5)
- Pipette puller (e.g., Narishige PC-10)
- Sterile dissecting environment (such as a laminar flow hood)
- Dissection instruments and glass bead sterilizer (Fine Science Tools)
- High-speed dental drill (NSK)
- Custom recording chamber (see **Fig. 2**)
- Stereotaxic apparatus (or other head-fixation device)
- Animal heating pad (FHC)
- Surgical microscope (for dissection, Zeiss)
- Hair trimmer (Surrey Pet Supplies)
- Centrifugal Filter (Millipore Ultrafree-MC)

REAGENT SETUP

Artificial cerebrospinal fluid (ACSF) 150 mM NaCl, 2.5 mM KCl, 10 mM HEPES, 2 mM $CaCl_2$, 1 mM $MgCl_2$; pH 7.3 (adjusted with NaOH), 300 mOsm; Store at 4 °C, use within 2 weeks.

Pipette solution 133 mM $KMeSO_4$, 7 mM KCl, 10 mM HEPES, 2 mM Mg-ATP, 2 mM Na_2-ATP, 0.5 mM Na_2-GTP, 0.05 mM EGTA; pH 7.2 (adjusted with KOH), 280–290 mOsm; filtered using a 0.45 μm syringe filter. Store at −20 °C, use within 6 months.

PROCEDURE

Surgery for chronic imaging and recording ● TIMING 45–60 min

1| Anaesthetize the animal and place it on a heating blanket (set to 37 °C) in a sterile surgery environment. We anaesthetize adult mice with an intraperitoneal injection of 100–120 mg kg^{-1} ketamine and 15–18 mg kg^{-1} xylazine.

! CAUTION All animal experiments must comply with the relevant institutional and national animal care guidelines.

▲ CRITICAL STEP Ensure that a surgical level of anaesthesia has been reached before continuing. Continuously monitor the depth of anaesthesia by testing the animal's reflexes and top up anaesthesia when necessary.

BOX 1 | STRATEGY FOR REPEATED RECORDING FROM THE SAME NEURON FOLLOWING ELECTROPORATION

1. Re-anaesthetize the animal, remove the silicone plug and place the animal on a heating mat. Carefully remove the dura with fine forceps after making a small cut using an injection needle and cover the brain with around 200 µl of 1.5% (wt/vol) agarose.
2. Under the two-photon microscope, image the target cell of interest and place it in the center of the field of view (**Fig. 4a**; if necessary, switch to a higher magnification to ensure that the cell is within less than 50 µm from the center of the field of view). Use your previously made sketch (or image) of the vasculature to help you find the cell (see Step 21).
3. Knowing the pipette angle, move the motorized microscope stage parallel to the pipette axis to a point above the tissue (**Fig. 4b**). Although this can be achieved by using trigonometric calculations to set the XYZ position accordingly, we recommend using a microscope stage with a diagonal axis, the angle of which can be set to the pipette angle.
4. Outside the tissue, move the pipette tip to the center of the field of view of the microscope (**Fig. 4c**). This ensures that the targeted cell and pipette axis are along the same line.
5. Move the pipette along its axis into the brain tissue (**Fig. 4d**). Once the pipette tip is in the vicinity of the target cells, proceed with established targeted patching protocols (refs. 27,28).

2| Shave the skin above the brain area of interest with hair trimmers, and apply local anaesthetic such as EMLA (eutetic mixture of local anaesthetics) cream (around 200 µl) to the skin. Lubricate both eyes with a drop of eye lubricant. Wait for 2–5 min for the local anaesthetic to take effect.

3| Remove the skin (and if necessary, muscle tissue) above the area of interest. Apply cyanoacrylate glue to the edge of the skin to fix it to the skull and reduce the risk of inflammation.

4| Using a fresh scalpel blade, remove the connective tissue on top of the skull by carefully scraping it away.
▲ CRITICAL STEP Too much pressure can damage the skull and the brain.

5| Using stereotaxic coordinates and brain vasculature as references, mark the area of interest with a tissue marker. If necessary a thin layer of cyanoacrylate glue can be applied to the skull and left to dry, to improve subsequent adhesion of the recording chamber to the skull.

6| Fix the recording chamber above the area of interest using sparing amounts of cyanoacrylate glue.

7| Using a razor blade, shorten the large end of a 200 µl pipette tip so that it can be attached to a 1 ml syringe and cut the small end at a 45° angle using a sterile scalpel blade. Prepare dental cement by mixing the components, as described by the manufacturer. Use the syringe with the modified pipette tip to apply the dental cement underneath and around the recording chamber (enough to fill the gap between the chamber and the skull). The rest of the exposed skull should be covered with dental cement. Wait for 5–10 min to allow the dental cement to dry.
▲ CRITICAL STEP Handle the recording chamber with care since the dental cement will not be completely dry until hours after the procedure.

8| Make a circular 500 µm diameter craniotomy over the marked area using a dental drill. Carefully and evenly thin the edge of the craniotomy until the bone bends when gently touched with tweezers. Fill the recording chamber with artificial cerebrospinal fluid (ACSF) (pre-warmed to 37 °C) and wait for 5–10 min for the bone to get soft. Then, remove the skull cap with small tweezers[36].
▲ CRITICAL STEP It is very important not to damage the dura with the tweezers. This could cause subdural bleeding and edema, which can have severe effects on tissue health, especially for the chronic preparation.

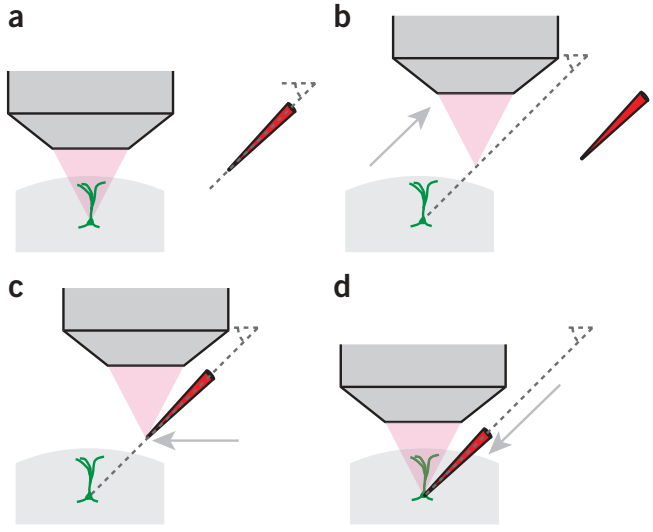

Figure 4 | Strategy for targeted recording from an earlier electroporated neuron. Image of the cell of interest (**a**). Move the field of view diagonally outside the brain along the pipette axis of movement (**b**). Move the pipette tip to the center of the field of view (**c**). Move the pipette along its axis into the brain tissue (**d**). (See **Box 1** for detailed description).

9| In some cases, there can be minor bleeding on top of the dura after removing the skull cap. If this occurs, briefly re-perfuse the ACSF inside the recording chamber using a Pasteur pipette until the bleeding stops.

10| Transfer the animal to the microscope setup.

Two-photon targeted single-cell electroporation ● TIMING 15–45 min

11| Fill a high-resistance (12–15 MΩ) patch pipette with 5–10 µl pipette solution (at room temperature: 18–22 °C) including 100–200 ng μl^{-1} plasmid DNA and 50 µM fluorescent dye (e.g., Alexa 594 hydrazide).

12| Applying a pressure of 10–15 mbar, lower the pipette into the meniscus and use the microscope in bright-field imaging mode at a low magnification to place the pipette above the craniotomy, and center the pipette in the field of view.

13| Switch to two-photon imaging. Increase the pressure to >100 mbar, and lower the pipette until it touches the dura. This can be monitored either visually or on the basis of a pipette resistance increase.

14| Push the pipette axially through the dura.
▲ **CRITICAL STEP** Insertion through the dura often clogs the pipette.
? TROUBLESHOOTING

15| Lower the pipette pressure to 50 mbar and approach the cells of interest (**Fig. 1**). This can be done either by targeting of cells using transgenic markers[27] or by targeting of unlabeled cells using 'shadow imaging'[22].

16| When the pipette is 50–100 µm within the area of interest, lower the pressure to 20 mbar and approach the cells of interest.

17| Once the pipette is barely in contact with the cell membrane of the target cell, slowly move the pipette towards the center of the cell until the tip resistance increases by about 30%.

18| Release the positive pressure on the pipette and apply a train of voltage pulses by the electroporation device (see **Supplementary Video 1** online).
▲ **CRITICAL STEP** Monitor the electroporation under visual control: the signature of successful electroporation is immediate filling of the cell soma with fluorescent dye.
? TROUBLESHOOTING

19| Once electroporation is successful, carefully retract the pipette by at least 50 µm before reapplying positive pressure.

20| The same pipette can be used to electroporate multiple cells: reapply positive pressure of 20 mbar, check whether fluorescent dye leaves the pipette tip and restart at Step 16 (**Fig. 3**; see **Supplementary Video 2**).

21| If necessary, record an image of the vasculature pattern close to the electroporated cell(s) using a CCD camera (or make a sketch) to provide a reference map[30,31], which can subsequently facilitate the finding of the electroporated cells.

Recovery ● TIMING >2 h

22| After electroporation, use sterile cotton buds or tissue to carefully remove the excess liquid.
▲ **CRITICAL STEP** Do not let the ACSF dry by evaporation, as this will leave a salt residue on the brain surface.

23| Cover the brain with silicone elastomer sealant.

24| Administer analgesic (e.g., by a subcutaneous injection of 0.05 mg kg^{-1} buprenorphine) and place the animal in a heated recovery chamber (set to 33 °C) until it regains consciousness (usually within 1–2 h).

25| Return the animal to its housing, and add non-steroidal anti-inflammatory analgesia to the drinking water (e.g., Carprofen 5% (wt/vol), further diluted to a final concentration of 1:1,500).
▲ **CRITICAL STEP** Monitor the animal's well being on a regular basis during the recovery period, according to local animal care guidelines.
■ **PAUSE POINT** Depending on the type of electroporated DNA, wait one or more days for the relevant genes to reach sufficient expression levels (e.g., one day for expression of EGFP).

26| Assess expression (or suppression) of the gene of interest.
? TROUBLESHOOTING

● TIMING

Steps 1–10: surgery for chronic imaging and recording: 45–60 min
Steps 11–21: two-photon targeted single-cell electroporation: 15–45 min
Steps 22–25: recovery: >2 h

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 1**.

TABLE 1 | Troubleshooting advice.

Step	Problem	Possible reason	Solution
14	No dye coming out of the pipette	Debris in the pipette solution	Apply a brief pulse of pressure (50 mbar, half a second) to unblock the pipette. If this does not help, replace the pipette: do not proceed if dye does not flow freely at pressures below 30 mbar. Check for any potential sources of debris in your pipette solution. Filter the solution on the day of use through a 0.45 μm centrifugal filter. Make sure the pipettes are polished and do not scratch the silver wire (which may release debris)
		Clogging of pipette during penetration of the dura	Insertion through the dura often clogs the pipette. This can be assessed by the amount of fluorescent dye leaving the pipette tip: fluorescent dye should be coming out of the pipette at pressures of 20 mbar and higher. As the pipette will usually be unclogged when retracted from the tissue, this process can be repeated until the pipette penetrates the dura without clogging. In some cases it may help to enter the brain through an already present hole in the dura for subsequent approaches. Note that the pipette tip resistance can be unaffected even when no dye can be ejected from the pipette. Always use both visual cues and pipette resistance to assess whether the pipette tip is clean
	Bad visibility (e.g., of pipette tip)	Too much background fluorescence	Avoid spilling too much fluorescent dye inside the brain. Reduce the pipette pressure as soon as the pipette breaks through the dura
		Poor craniotomy or bleeding on brain surface	A poor craniotomy can sometimes be indicated by edema or a darker than normal color of the brain tissue. Improve your surgery skills. If there is obvious bleeding on top of the dura, perfuse with fresh ACSF until the bleeding stops. To minimize bleeding during removal of the skull cap, apply ACSF onto the skull and wait at least 5–10 min before removing it. To reduce edema, inject dexamethasone (2–4 mg kg^{-1}) at least 30 min prior to removal of the skull
18	Cells don't take up fluorescent dye	Pipette clogged	See above
26	Scar tissue formation after recovery	Multiple (e.g., contaminated equipment)	Sterilize all surgery equipment before use and during the procedure using a glass-bead sterilizer. Ensure that the glue and cement linking recording chamber and brain form a complete seal. Dry the brain surface before applying silicone elastomer sealant. Administer anti-inflammatory drugs during recovery
	Low success rates	Too close to the cell: cell membranes fuse with the pipette causing cell death	If withdrawal of the pipette draws the cell body or the cell membrane with it, the membrane has fused with the pipette. Replace the pipette and try with another cell, reducing the mechanical pressure of the pipette on the cell before electroporating
		Too far from the cell: no DNA taken up	Target the center of the cell soma and increase the mechanical pressure onto the cell before electroporation. The tip resistance should increase by about 30%
		Sub-optimal electroporation parameters	Change the amplitude and/or number of voltage pulses while directly monitoring electroporation under visual control
		Not enough DNA in the pipette solution	Increase the DNA concentration within the pipette solution. In our hands, 200 ng μl^{-1} yields good results
		Pipette clogged	See above

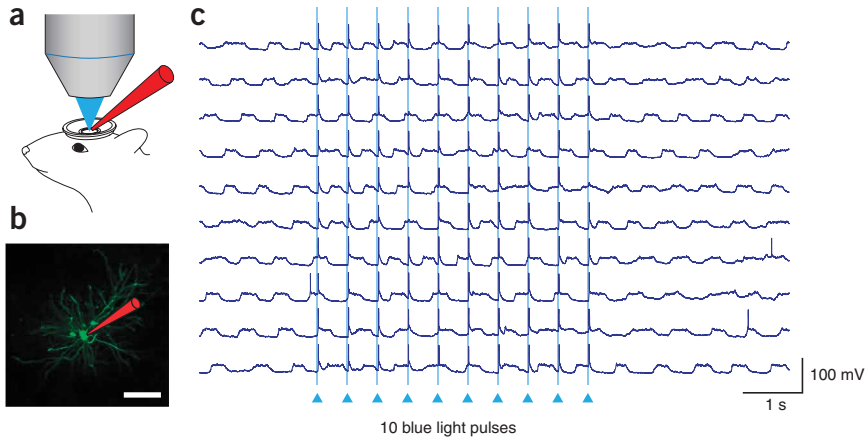

Figure 5 | Activation of cortical neurons with light following targeted electroporation of channelrhodopsin-2. Schematic illustration of the recording and imaging setup (**a**). Two-photon image of a small network of layer 2/3 parietal cortex neurons *in vivo* expressing channelrhodopsin-2 and enhanced green fluorescent protein (EGFP) 3 d after targeted co-electroporation of the respective plasmid DNA (**b**). Scale bar: 100 μm. Targeted patch-clamp recording from a single layer 2/3 neuron (indicated with the red electrode in b) exhibiting spontaneous up and downstates (**c**). Reliable and temporally precise spiking was triggered by illumination with brief pulses of blue light (5 ms; $\lambda = 473$ nm) to activate channelrhodopsin (ten consecutive traces are shown; 97% of pulses triggered a spike).

ANTICIPATED RESULTS

Under optimized conditions, an experienced experimenter can electroporate between 1 and 20 visually selected layer 2/3 neurons in a single session, with a ~75% success rate. Electroporated cells can subsequently be imaged and also targeted for whole-cell patch-clamp recordings. These neurons are viable and display normal electrophysiological properties[22], and stable transgene expression can be observed months after electroporation. In **Figure 5**, we demonstrate how this approach can be used for targeted expression of channelrhodopsin[31,37] in defined neurons in the cerebral cortex. Co-electroporation of plasmids for GFP and channelrhodopsin in a small population of neurons allowed these neurons to be subsequently targeted for *in vivo* patch-clamp recordings, confirming that brief pulses of blue light trigger action potentials in the electroporated neurons with very high reliability.

The specificity of the electroporation approach permits a wide range of new applications. By using transgenes influencing neuronal function[4,31], targeted single-cell electroporation enables the experimenter to specifically modify neuronal function with single cell precision, and allow the assessment of the function of single genes in intact, unaltered neural networks. Targeting identified cells also allows for sequential manipulation of cells with different DNA constructs on consecutive days (which is impossible with viral vectors because of variability in the infected populations). Finally, the functional impact of different numbers of neurons—or different spatial patterns of neurons—for network activity and behavior can now be explored by electroporating multiple neurons in a defined spatial arrangement. This approach therefore offers unprecedented precision in the ability to identify the functional role of individual genes and probe the functional architecture of neural circuits.

Note: Supplementary information is available via the HTML version of this article.

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