Dendritic patch-clamp recording

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Published online 21 September; corrected online 30 November 2006 (details online); doi:10.1038/nprot.2006.164

The patch-clamp technique allows investigation of the electrical excitability of neurons and the functional properties and densities of ion channels. Most patch-clamp recordings from neurons have been made from the soma, the largest structure of individual neurons, while their dendrites, which form the majority of the surface area and receive most of the synaptic input, have been relatively neglected. This protocol describes techniques for recording from the dendrites of neurons in brain slices under direct visual control. Although the basic technique is similar to that used for somatic patching, we describe refinements and optimizations of slice quality, microscope optics, setup stability and electrode approach that are required for maximizing the success rate for dendritic recordings. Using this approach, all configurations of the patch-clamp technique (cell-attached, inside-out, whole-cell, outside-out and perforated patch) can be achieved, even for relatively distal dendrites, and simultaneous multiple-electrode dendritic recordings are also possible. The protocol—from the beginning of slice preparation to the end of the first successful recording—can be completed in 3 h.

INTRODUCTION

Dendrites are indisputably important for neuronal function. They represent the vast majority of the surface area of most neurons, receive the majority of a neuron's synaptic input, and display a range of excitable properties, which are critical for shaping synaptic plasticity and information flow in neural networks. Unfortunately, dendrites are also extremely thin, and as a result have for decades resisted investigation by neurophysiologists, particularly those wielding sharp microelectrodes. Although some key advances were made using intracellular sharp microelectrode recordings[1–3], this approach is extremely difficult and also does not permit direct biophysical investigation of dendritic channels. The advent of patch-clamp recordings from dendrites in brain slices[4] has finally rendered the dendrites of most neurons accessible to in-depth study using all configurations of the patch-clamp technique (cell-attached, whole-cell, outside-out, inside-out and perforated patch). These recordings have been essential for directly demonstrating the distribution and functional properties of voltage-gated ion channels in dendrites, and for investigating how the spread and interaction of electrical signals—action potentials and synaptic potentials—contribute to synaptic integration and the computational properties of single neurons. In particular, dendritic patching has been used to map channel densities[5–12], investigate differences in channel properties in somatic and dendritic membrane[12–15], define the passive electrotonic structure of neurons using compartmental models[16–18] and to explore the consequences of dendritic properties for electrical excitability and synaptic integration in dendrites (for recent examples, see refs.[19–22]). These studies, which have been covered in a series of recent reviews[23–27], have renewed and deepened our appreciation of dendritic function.

The ability to routinely make patch-clamp recordings from dendrites has resulted from several key advances in patch-clamp methodology and microscopy. Essentially, it evolved through continual improvements in methodology for making patch-clamp recordings from visualized neuronal somata in slices[28]. Although some 'early, 'laborious' efforts' were made to patch dendrites in slices[29] as well as in dissociated neurons[30], the key advance came with techniques for high-resolution visualization of dendrites in brain slices using infrared DIC videomicroscopy[31]. This allowed dendrites to be recorded from with high reliability[4]. After a decade of continual development of the technique, reflected in the protocol described below, it is currently possible to make recordings from very small, distal dendrites, even with multiple electrodes[19, 20, 32].

There is no fundamental difference between successful somatic and dendritic patching. The secret to dendritic patching is simply to ensure that the dendrite to be patched is healthy and clearly visible, and that both the slicing procedure and the patch-clamp setup have been optimized. The necessary techniques can be divided as follows: well-adjusted optics (to ensure optimal visualization of dendrites); stable recording conditions (so that a good quality recording can be maintained, and so that pipettes can be changed without disturbing an existing recording); and excellent slice quality (a prerequisite for both good optics and stable recording conditions).

Here we detail a procedure for patching dendrites in brain slices from most brain areas, which we have used successfully for recording from dendrites in cerebellar cortex, neocortex, substantia nigra, thalamus and hippocampus. There are, however, many parameters (e.g., slicer settings, tissue dissection, solution composition, patch-pipette tip size, pipette pressure, approach to the dendrite with the pipette) that may require careful adjustment until the optimal experimental conditions for each individual experiment are achieved. Equally, although we stress that high quality equipment (particularly slicer, manipulators, microscope and camera) is a prerequisite to success, a variety of brands and models have been used to perform successful dendritic patching experiments around the world.

Dendritic patching is not without its limitations. One clear limit is the size of structures that can be patched. To date, dendritic recording has been applied most successfully in cells with large dendrites, such as pyramidal neurons and Purkinje cells. Even in

these cells, however, the finer branches remain inaccessible to patch electrodes. Dendrites of smaller, multipolar neurons have been patched, but only at relatively proximal locations[33–36]. Neurons with very fine dendrites, such as stellate cells, have not yet been studied using dendritic recording, although this may someday be possible. The same limitation applies to structures smaller than dendrites: although it has proved possible to patch small structures such as axon initial segments[5,6] and presynaptic terminals[37–39], it seems unlikely that recordings will ever be obtained directly from dendritic spines using the patch-clamp technique. A related caveat is that, because of the small size of pipette tips required to patch dendrites, access resistance is often high (>20 MΩ). This compromises whole-cell voltage and current recordings as it filters the recording and adds an offset during current injection, which (if not accurately compensated for) makes the measurement of absolute values of membrane potential unreliable. This limitation can be sidestepped in some cases by making two adjacent dendritic recordings (one to measure voltage, and one to inject current[20,32]). Finally, as with all whole-cell patch-clamp experiments, dialysis of the neuron during whole-cell recording can disrupt its physiology, such as the biochemical pathways responsible for synaptic plasticity. This is especially relevant for dendritic recordings where the site of the biochemical process in question is likely to be closer to the recording pipette. This limitation can be overcome by using perforated-patch recordings[40], but again this is technically very challenging. Overall, however, dendritic patching remains the technique of choice for investigating electrical signaling in dendrites, complemented by imaging approaches and modeling, and we look forward to many exciting new findings using this method.

MATERIALS

REAGENTS

- Experimental animals: rats, mice **! CAUTION** All animal experiments must comply with national regulations.
- Artificial cerebrospinal fluid (ACSF; see REAGENT SETUP)
- Anesthetic (e.g., isoflurane)
- Biocytin
- $CaCl_2$
- Carbogen (95% oxygen, 5% carbon dioxide)
- D-glucose
- EGTA
- Fluorescent dyes (e.g. Alexa 594; Molecular Probes)
- HEPES
- KCl
- K-methanesulfate
- KOH
- MgATP
- $MgCl_2$
- Na_2ATP
- Na_2GTP
- NaCl
- NaH_2PO_4
- $NaHCO_3$

EQUIPMENT

- Slicing equipment: standard equipment including various surgical tools, various beakers and dishes (these can be pre-filled with ACSF and frozen in order to keep the cooled ACSF poured into them ice-cold), a suitable cutting surface (e.g., a Sylgard filled Petri dish) and cyanoacrylate glue can be used for slice preparation, as described in numerous previous studies[4,28,41,42].
- Vibrating slicer: since great care should be taken to optimize the quality of the slices, a high-quality vibrating slicer is essential[43] (the Leica VT1000S and the Microm HM 650V are suitable). In particular, it is highly recommended to check the *z*-axis vibration of the slicer blade regularly, either using a custom-made apparatus[43] or via the manufacturer of the slicer.
- Incubation chamber: a submerged (or an interface) chamber optimized to ensure sufficient oxygenation of the tissue.
- Vibration isolation table (if a pneumatic system is used, an accompanying supply of compressed air or nitrogen will be required)
- Upright microscope: this must be equipped with IR-DIC or Dodt contrast optics (see **Box 1**). A fluorescence microscope equipped with a CCD camera can also be used, or alternatively a two-photon microscope equipped with scanning Dodt contrast optics.
- XY stage for microscope: this allows the microscope to be moved independently of the slice chamber and/or recording pipettes.
- Water-immersion objective: 40× or 60×, high numerical aperture (≥0.75), long working distance (>2 mm normally allows electrodes to be inserted at a sufficiently steep angle, typically >25°).
- Magnifier: this is placed between the objective and the camera and should offer between 2× and 4× magnification. Adjustable magnifiers with a choice of fixed positions are optically superior to variable-magnification devices.
- Video camera: CCD or Vidicon camera and controller for gain and offset adjustment (e.g., Hamamatsu C2400-07; Dage 1000; Optonis VX45)
- Video monitor: black and white (analog) is sufficient. Monitors with 1,000 lines of resolution are somewhat superior to standard monitors.
- Patch-clamp amplifier (e.g., Multiclamp 700B, Molecular Devices)
- Recording equipment (oscilloscope, AD/DA converter, computer with data acquisition software, etc.)
- Micromanipulators: The manipulators should allow continuous sub-micron movements, and must be motorized and remotely controlled, in order to make fine pipette movements as smooth and as stable as possible. If multiple electrodes are to be used, arrange them such that the pipette on one can be removed and changed without disturbing the pipette on the other(s).
- Pipette holders: The pipette holders should be stable (it is particularly important that the pipette is held so that it doesn't move when strong pulses of suction are applied, e.g. at break-in for whole-cell patching) and able to hold positive pressure for prolonged periods.
- Manometer: for monitoring pressure and suction changes
- Pressure valves: for holding pressure in the lines behind the patch pipette. A three-way tap may be used, but a switchable valve (e.g., Kuhnke) is preferable, as it allows much more rapid control of pressure.
- Pipette puller: should allow pipettes with a range of tip sizes and geometries to be pulled reproducibly.
- Solution filters (0.2 μm pore): to filter the patch-pipette solution.
- Patch-pipette fillers: either disposable fillers (e.g. made by pulling the melted tip of a Gilson pipette tip into a fine capillary) or well-cleaned reusable capillaries.

REAGENT SETUP

Standard ACSF 125 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.25 mM NaH_2PO_4 and 25 mM D-glucose. Final osmolarity should be ~310 mmol/kg. At all points it is essential that the ACSF in contact with the brain tissue is saturated with carbogen (which provides both oxygenation and pH buffering).

Slicing solution Depending on the brain area (i.e., prevalence of NMDA receptors and susceptibility to hypoxia), high quality brain slices can be cut in normal ACSF, saturated with carbogen. However, several procedures can be used to reduce activity levels during slicing in order to minimize excitotoxicity. These include thorough and rapid cooling of the preparation (ideally with cardiac perfusion of ice-cold slicing solution), replacing from 50% to 100% of the NaCl with iso-osmolar sucrose[44], changing the Ca:Mg ratio (e.g., 6 $MgCl_2$, 1 $CaCl_2$) or adding 2–10 mM kynurenic acid to the slicing solution, to eliminate ionotropic glutamatergic transmission.

Patch-pipette solution for whole-cell recording Standard intracellular solution contains 130 mM K-methanesulfate, 10 mM HEPES, 7 mM KCl, 0.05 mM EGTA, 2 mM Na_2ATP, 2 mM MgATP and 0.5 mM Na_2GTP, titrated with KOH to pH 7.2. Alternative principal anions are gluconate and methanesulfonate; note that all internal solutions are associated with washout of intracellular factors and some may also have pharmacological effects[45] (also see Kaczorowski C.C., Disterhoft, J.F. & Spruston, N. *Soc. Neurosci. Abst.* **31**, 737.17, 2005).

BOX 1 | OPTIMIZING THE OPTICS

As a general rule, it can be said: "if you can see it, you can patch it". Yet neurons in brain slices lack contrast. Optical methods are therefore required to increase contrast to allow visualization of small structures such as dendrites so that one can patch them. The two main methods used in light microscopy to increase contrast are phase contrast and differential interference contrast (DIC). Phase contrast works well in cell culture, but does not work well in brain slices. The preferred method is therefore DIC, which is available from the main microscope manufactures (Leica, Nikon, Olympus and Zeiss). An alternative is to use some form of oblique illumination. This can be achieved via an oblique condenser (e.g., Olympus) or via the Dodt-contrast method (Dodt Gradient Contrast; Luigs & Neumann, Germany[31,49]). The advantage of oblique illumination versus DIC is that it does not require optical components above the objective, which is advantageous for fluorescent microscopy. The disadvantage is that the image quality is not quite as good as DIC, although it can be sufficient for many applications. In addition to these methods of increasing image contrast, there is an advantage in illuminating the brain slice with near infrared light ($\sim$770 nm $\pm$ 45 nm) and detecting the image using an infrared-sensitive camera projecting onto a video screen. The longer wavelength of near infrared light results in it being scattered less as it passes though the brain slice, allowing visualization deeper into the slice with greater clarity. A full description of methods to patch dendrites of neurons using infrared light combined with DIC (IR-DIC) is described in ref. 4 based on the original description of IR-DIC by Dodt & Zieglgänsberger[50]. Below we describe several approaches for optimizing microscope optics for visualization of dendrites.

Achieving Köhler illumination

Köhler illumination such that light is focused onto the object plane, is critical for good resolution of the object of interest. To achieve this:
(i) Focus onto the surface of the brain slice, and then move the field of view to a clear region of the recording chamber.
(ii) Fully close down the microscope field diaphragm and focus the condenser so that the image of the field diaphragm is in focus. The condenser will now be focused to the same focal plane as the object of interest. Make sure the condenser is centered correctly. Do not close the condenser diaphragm, this will increase contrast, but at the cost of decreasing the numerical aperture of the condenser (and hence the optical resolution of the image). The best image quality is obtained by using a condensor with a numerical aperture as high as possible and using DIC optics to obtain contrast. This means the condenser diaphragm should be fully open for optimal image quality.
(iii) Move the infrared filter into the light path.
(iv) View image with an infrared camera transmitting to a black and white video monitor (LCD computer screens with video input can also be used but need to be of high quality). The choice of camera is critical. To obtain fast image refresh rates a video camera should be used, there are also a number of CCD cameras available that can display full-frame images at close to video rates. Make sure the camera you use is sensitive in the near infrared range and has the capacity for control of the gain and offset, either via a control box or through software. In addition, we recommend using a camera with as large a detector ("chip") and/or spatial resolution as possible.

Adjusting conventional DIC optics

DIC optics requires four components: two polarizers, one below the condenser and one above the objective (the polarizer above the objective is commonly called the "analyzer", and can not usually be adjusted) and two DIC (Nomarski or Wollaston) prisms. These are located above the objective and below the object (usually in the condenser; this also can not usually be adjusted), in between the two polarizers. Light enters from below, passes through the first polarizer and then the first DIC prism. The DIC prism splits this polarized light into two slightly offset beams of oppositely polarized light, which then enter the slice. Structures in the slice of differing refractive index (e.g., the edges of cells) cause different parts of these two beams to undergo phase shifts. After the light is focused up through the objective, the second DIC prism recombines the two beams of (initially oppositely) polarized light into one. Light that has not been phase shifted emerges with the same polarization as it entered the slice. Phase shifted light beams are recombined to give light with a different polarization. This light then passes through the upper polarizer (the analyzer), which is orientated at 90 degrees to the lower polarizer ("crossed"). As a consequence, any light that is not modified by the structures in the slice (so that it emerges from the upper DIC prism with an unaltered polarization) does not pass. Only light that has been altered by structures in the slice, and therefore contains information about these structures, can pass through the analyzer and be detected. In addition, the upper DIC prism can also be offset, biasing the polarization of the whole image, so that the background can appear light, and refractive structures can appear brighter or darker than the background. This increases the range of resulting light intensities, and so improves the signal-to-noise ratio of the image.
There are three key requirements for correctly adjusted DIC optics:
(i) The polarizer and analyzer must be "crossed" (at 90 degrees to one another). The easiest way to adjust this is to rotate the polarizer while viewing the image with the camera until the image is darkest (make sure that both the bias of the upper DIC prism is zero at this point, and that any "automatic gain control" (AGC) of the image is switched off, if your camera/software has this facility). As indicated above, the position of the analyzer is usually fixed.
(ii) The DIC prism above the objective should be adjusted (in or out) to obtain an image that appears three-dimensional. Objects typically have a light side, with a "shadow" on the opposite side, as if the sun is shining from one side of the image. Adjustment in the opposite direction can "flip" the image so that convex structures like cell bodies look concave (like holes, **Fig. 2b,c**).
(iii) The light intensity and offset and gain control of the camera should be adjusted to use the full dynamic range of contrast available. The image on the video monitor should have a smooth range of contrasts, with a few very bright or very dark areas. Cell membranes should stand out clearly.
A more detailed explanation and illustration of DIC illumination can be found in ref. 51, and an interactive tutorial is available here: http://microscopy.fsu.edu/primer/java/dic/wavefrontrelationships/index.html

Adjusting de Sénarmont DIC optics

In order to introduce bias to the polarization, the conventional DIC technique uses a moveable DIC prism above the objective. An alternative and recently quite popular solution (named in honor of Hureau de Sénarmont), is the use of a fixed 1/4 wave plate inserted between the

BOX 1 | CONTINUED

polarizer and first DIC prism prior to the condenser. All other components are the same, although now rather than adjusting the offset of the DIC prism above the objective one adjusts the rotation of the polarizer. Image quality when adjusted correctly is the same as with conventional DIC. Microscope manufactures argue that this system has the advantage over conventional DIC that it eliminates the risk of bumping the stage, specimen, manipulators or nosepiece when adjusting the DIC prism above the objective. In practice, this is not an issue, as the position of this DIC prism is usually not changed during an experiment. In our experience, correct adjustment of de Sénarmont DIC optics is slightly more burdensome, as it involves the removal of the lower DIC prism. To correctly adjust de Sénarmont DIC optics, first remove the lower DIC prism from the light path (this usually needs the dismounting of the condenser). Then, remove one eyepiece and look down into the microscope. By rotating the polarizer, a dark fringe should appear on the image. By rotating the 1/4 wave plate, center the fringe. At this stage, the polarizer is crossed with respect to the analyzer and the 1/4 wave plate does not introduce any bias. Fix the orientation of the 1/4 wave plate and reinsert the lower DIC prism into the light path. Now focusing on the specimen, slightly adjust the polarizer to get the best image quality. Depending on the direction of this rotation one can flip the appearance of objects from concave to convex as described above (**Fig. 2b,c**).

For a thorough description and illustration of the de Sénarmont method, see this tutorial: http://microscopy.fsu.edu/primer/java/dic/desenarmont/index.html

Using fluorescence to aid visualization of dendrites

In some cases, even optimal adjustment of DIC optics cannot resolve finer dendrites sufficiently for routine patch-clamp recording. This problem can be solved for example by using the visualization of a fluorescent dye (loaded through the somatic electrode or genetically expressed) as a complement to DIC optics[52]. However, to use DIC optics alongside fluorescent microscopy the analyzer must be repositioned, so that it does not compromise the fluorescence excitation beam. This is usually done by inserting a dichroic mirror between the upper DIC prism and the analyzer, which will reflect the excitation beam but let transmitted and emitted light through. An alternative is to use Dodt contrast (or oblique illumination) instead of DIC, as in these cases there is no need for extra optical elements in the light path above the specimen. It is possible to use one camera for imaging both the transmitted and emitted light, with the added advantage of being able to observe the IR and fluorescence image at the same time. By flashing the excitation beam on and off, one can enhance visual identification of the target dendrite. It is also possible to use a confocal or two-photon microscope to visualize fluorescent dendrites. Again, the light path must be designed to also allow subsequent visualization of the identified dendrite using contrast-enhanced optics for making the patch-clamp recording.

A fluorescent dye (e.g., 1–25 μM Alexa 594) can be included to visualize dendrites during the experiment, and biocytin (0.1–0.4%) can also be added for subsequent recovery of cell morphology. Final osmolarity should be ~285 mmol/kg.

Patch-pipette solution for cell-attached recording As with somatic cell-attached recording, the patch-pipette solution is based on the bath ACSF. Often a HEPES-buffered version is used to avoid potential changes in pH in the absence of carbogenation. A typical recipe is the following: 125 mM NaCl, 10 mM HEPES, 3 mM KCl, 2 mM $CaCl_2$, pH 7.4 with NaOH. Changes in solution composition can be made to pharmacologically isolate conductances of interest[12], to make ionic substitutions, or to increase ion concentration gradients across the patch membrane in order to increase the size of currents recorded[8].

EQUIPMENT SETUP

An illustration of a typical setup used for dendritic patch-clamp recording is shown in **Figure 1**.

Pipettes Preferably thick-walled, borosilicate glass; can be filamented to ease filling with internal solution. The tip size depends on the size of the structure to be patched. Typical resistances range from 6 to 15 MΩ. Smaller tips can improve the chances of successful seal formation or recording stability, but can lower the chances of breaking in and will result in higher access resistance.

Experimental slice chamber A glass-bottomed chamber must be used with IR-DIC optics. The use of thin coverslip glass allows appropriate focusing of condensers with high numerical aperture and short working distance.

Perfusion system This consists of a reservoir of ACSF, bubbled to saturation with carbogen via a sintered glass bubbler (Robu), siphoned into the recording chamber via oxygen-impermeable tubing (e.g., Teflon), and removed into a collection reservoir via outflow tubing connected to suction. A 'dripper' interrupting the inflow tubing breaks the continuous flow of solution, reducing the electrical noise transmitted to the recording chamber via the ACSF column

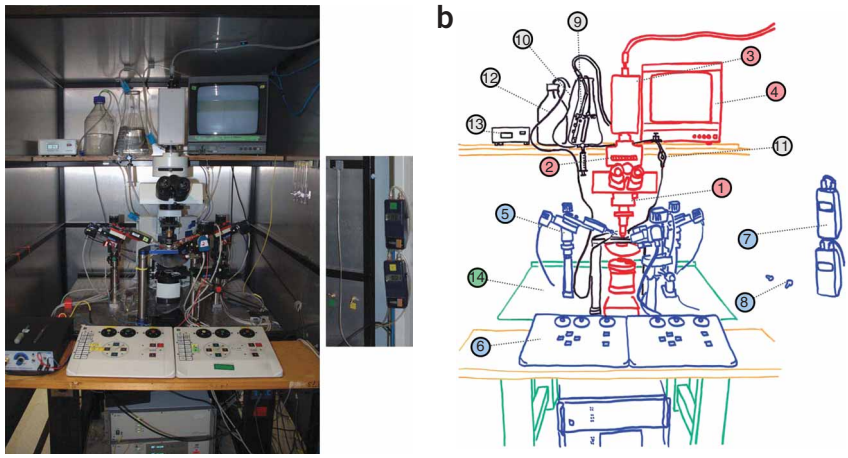

Figure 1 | Patch-clamp setup. (**a**) Composite photograph of a typical patch-clamp setup (in a Faraday cage) used for dendritic recording. (**b**) Schematic sketch of the image in **a**. Red, imaging equipment; blue, electrode manipulators and pressure controlling equipment; black, perfusion system; green, vibration isolation table. (1) an upright microscope equipped with a 40× objective and IR-DIC optics, mounted on an XY stage; (2) magnifier; (3) video camera; (4) black and white video monitor; (5) three micromanipulators (oriented so that each pipette can be changed independently); (6) micromanipulator remote control panels, mounted on a bench which is well separated from the vibration isolation table (boxes containing micromanipulator controller electronics are below the vibration isolation table); (7) manometers; (8) switchable pressure valves; (9) reservoir of carbogen-bubbled ACSF; (10) oxygen-impermeable Teflon tubing providing inflow to the recording chamber (heating jacket prior to chamber inflow not visible); (11) dripper, interrupting solution inflow; (12) outflow from chamber, connected to suction via a collection reservoir; (13) temperature monitor (connected to a thermocouple element placed in the recording chamber, not illustrated); (14) vibration isolation table.

acting as an aerial. It also reduces the number of physically disruptive bubbles that flow into the chamber, and allows monitoring of the rate of ACSF inflow to the recording chamber.

Temperature controller Solution can be heated prior to entry to the experimental slice chamber using a self-made or commercial temperature-regulated heating jacket around the inflow; a small temperature probe close to the slice can be used to accurately monitor recording temperature.

'Harp' A U-shaped piece of flattened platinum with nylon threads (pulled from nylon tights) glued across it, used to hold down the brain slice in the recording chamber.

PROCEDURE

Slice preparation ● TIMING Approximately 90–120 min

1| Prepare slicing and recording solutions by bubbling to saturation with carbogen (takes at least 0.5 h). Cool the slicing solution on ice. Prepare the incubation chamber; fill with carbogen-bubbled ACSF and warm to 35 °C.

■ **PAUSE POINT** These materials can be prepared in advance.

2| Anesthetize the animal (e.g., using isoflurane).

! **CAUTION** Appropriate guidelines and regulations for animal experiments must be followed.

Younger animals (e.g., <P25 rats) generally yield slices of higher quality.

3| Kill the animal by decapitation. With the head immersed in ice-cold slicing solution, lift off the skull and carefully remove the brain, transferring it rapidly to ice-cold slicing solution.

! **CAUTION** Appropriate guidelines and regulations for animal experiments must be followed.

▲ **CRITICAL STEP** There must be a balance between care and speed for this and the next step in order to minimize physical damage and hypoxia of the tissue.

? **TROUBLESHOOTING**

4| Place the brain on a suitable cutting surface (e.g., a Petri dish with a Sylgard bottom; the brain can be secured with dissecting needles if necessary) and submerge it in ice-cold, carbogen saturated slicing solution. If required, gently remove the meninges above the area of interest. Deftly cut a block of the tissue of interest. Cut a base parallel to the plane of the dendrites to be patched. (See also **Table 1**.)

▲ **CRITICAL STEP** The orientation of the slice should be parallel to the plane of the dendritic tree under investigation. This may involve using an angled slicing stage to ensure the correct orientation, as well as optimizing the orientation of the tissue block prior to slicing. **Table 1** details optimal cut orientations used to prepare brain tissue blocks from several commonly studied brain areas.

? **TROUBLESHOOTING**

5| Slide the base of the tissue block onto a thin film of cyanoacrylate glue on the cooled slicing stage. Fill the chamber with ice-cold carbogen-saturated ACSF.

? **TROUBLESHOOTING**

TABLE 1 | Preparation of tissue blocks for slices from various brain regions.

BRAIN AREA	BLOCK PREPARATION
Cerebellar vermis	For parasagittal slices, cut coronally in front of the cerebellum to separate it from the forebrain. Cut transversely below the cerebellum to separate it from the brainstem. Cut sagittally, as parallel to the midline of the cerebellum as possible, on either side of the cerebellar vermis. Glue either surface to a flat slicing stage (the first cut is usually more parallel to the midline).
Hippocampus	Make a longitudinal cut to separate the brain into two hemispheres. Place the brain on the cut (medial) surface and use a scalpel to cut a few millimeters (adult rat) off the dorsal surface of the cortex. This cut surface should then be glued down for slicing. The first few slices must be discarded before reaching the ventral hippocampus. As the vibratome blade is moved downward, each slice is obtained from a progressively more dorsal region of the hippocampus. With this procedure, optimal orientation of dendrites occurs in the dorsal hippocampus. To optimize dendritic orientation in the mid or ventral hippocampus, the angle at which the dorsal surface of the cortex is cut must be adjusted to tilt the hippocampus accordingly.
Neocortex	For slices from the S1/M1 border, cut the cerebral cortices apart sagittally, along the midline. Glue the medial surface to a slicing stage angled at 10-15° (sloping towards the slicer blade) with the dorsal cortex facing the blade. Slice off a 3000 μm section and discard it. Cut a 1000 μm thick slice half way through the tissue; separate this with a scalpel cut across the slicer's blade. Cut ~1800 μm worth of 300 μm slices, detaching each slice from the brain block with a scalpel cut. Lay them out in order in the slice incubation chamber. Each slice should be inspected for dendrites that run parallel to the surface of the slice; these dendrites are typically found in slices number 3 and/or 4 (where slice surfaces face each other).
Substantia nigra	For coronal slices, make two coronal cuts in front of the cerebellum and behind the somatosensory cortex. Glue the block onto its caudal surface with the ventral surface of the brain facing the blade. Cut slices down to the subthalamic nucleus, and then carefully select only slices containing substantia nigra.

6| Cut thin slices (150–300 μm) of tissue, advancing the slicer blade at a speed, vibration amplitude and frequency that ensures the tissue is cleanly cut, without being compressed or displaced. Between slices, raise the blade/lower the tissue by 100–200 μm in order to avoid dragging the blade across the surface of the tissue as it is moved back to the start of the slicing cycle. Keep the slicing chamber cool throughout the entire procedure (using ice or a Peltier element).
▲ CRITICAL STEP High slicing speeds will squash the tissue rather than cut cleanly through it, while overly slow speeds increase the duration of time the tissue remains as a block, and thus unexposed to oxygenated ACSF. Overly high vibration frequencies or amplitudes are associated with larger *z*-displacements of the slicing blade, while overly low frequencies prevent clean slicing of the tissue. Optimal settings on different slicers differ, but the following parameters might be used as an initial guide: blade advance speed, 0.1–0.25 mm/s; blade vibration frequency, 45–85 Hz; blade vibration amplitude, >1 mm.
? TROUBLESHOOTING

7| Place slices in incubation chamber filled with ACSF, store at 35 °C for 30–60 min, then keep at room temperature until use. Saturate the ACSF with carbogen before slicing, and maintain carbogen bubbling throughout. Slices will exhibit the best optics and most healthy cells in the first hour following the warm incubation period. They are generally usable for 3–5 h after incubation; after this, cell death and swelling usually make it progressively more difficult to visualize healthy dendrites, thus making the chance of successful dendritic patching very poor.

Slice transfer and visualization ● TIMING **Approximately 10–30 min**
8| Transfer a brain slice to the recording chamber and place the 'harp' on top of it to secure it. Make sure that the harp holds the slice firmly in place, as movement of the slice will make dendritic recording impossible. Perfuse the chamber at a fairly high rate (typically 1–2 ml/min, although 4–6 ml/min can be used in some cases; see Hajos, N. *et al. FENS Abstr.* **2**, A009.13, 2004) with carbogen-saturated (and heated, if relevant) ACSF to ensure adequate oxygenation.
▲ CRITICAL STEP Transfer of the slice is best accomplished using a broken-off glass Pasteur pipette with the broken end inserted into a small rubber bulb. Do not touch the surface of the slice with a brush or any other instrument when transferring slices. The harp should be oriented so that the fine nylon threads are parallel to the orientation of the dendrites to be patched. Note that adequate oxygenation of the slice is essential. This can be aided by improving solution flow in the chamber (e.g., by positioning the open end of the u-shaped harp so that it faces toward the ACSF inflow).
? TROUBLESHOOTING

9| Lower the water-immersion objective into the bath solution and, at high magnification, focus on the surface of the slice.
▲ CRITICAL STEP At high magnification, it often becomes apparent that vibrations (often too 'minor' to have been observed at lower magnification) are being transmitted to the set-up. Find and remove these sources of instability.
? TROUBLESHOOTING

10| Adjust the optics for the best resolution (see **Box 1**).
▲ CRITICAL STEP

11| Scan the slice for healthy looking somata; inspect these candidate cells for suitable (connected) dendrites. Center the chosen dendrite in the middle of the video monitor screen. (See also **Fig. 2**.)
▲ CRITICAL STEP Aim for a dendrite with a smooth, well-defined membrane surface (**Fig. 2**). For neurons with extensive branching, draw the dendrite-to-soma trajectory on the screen with a marker pen, for later distance measurement. Avoid dendrites where the membrane appears either dark, crisp and jagged, or faint and swollen (**Fig. 2e,g**). (Unfortunately, these dead dendrites/cells often are the structures most clearly visible in the slice. They can, however, serve the useful function of allowing one to check that the main axis of the dendrites of interest run roughly parallel to the cut surface of the slice, which is essential when aiming for distal dendrites).

Dendrites deeper than about 50 μm in the slice are very difficult to visualize and/or to approach while maintaining clean pipette tips. It is possible, however, to follow a dendrite deep into the slice from the soma to a distal, more superfical location. Even if the dendrite seems to disappear at points it is possible to spot the same dendrite again at a more distallocation. The diameter and general appearance and trajectory of the dendrite are good clues to its identity.
? TROUBLESHOOTING

Patch-clamp recording from dendrite ● TIMING **Approximately 10 min (until break-in)**
12| Fill a patch-pipette with enough solution to just cover the electrode wire and insert it securely into the pipette holder.
▲ CRITICAL STEP The solution should be filtered through a 0.2 μm pore filter, to remove debris that can interfere with sealing and block the pipette tip.

13| Apply positive pressure to the pipette holder (via tubing connected to pipette holder). Use a switchable valve or three-way tap to hold the pressure behind the pipette.

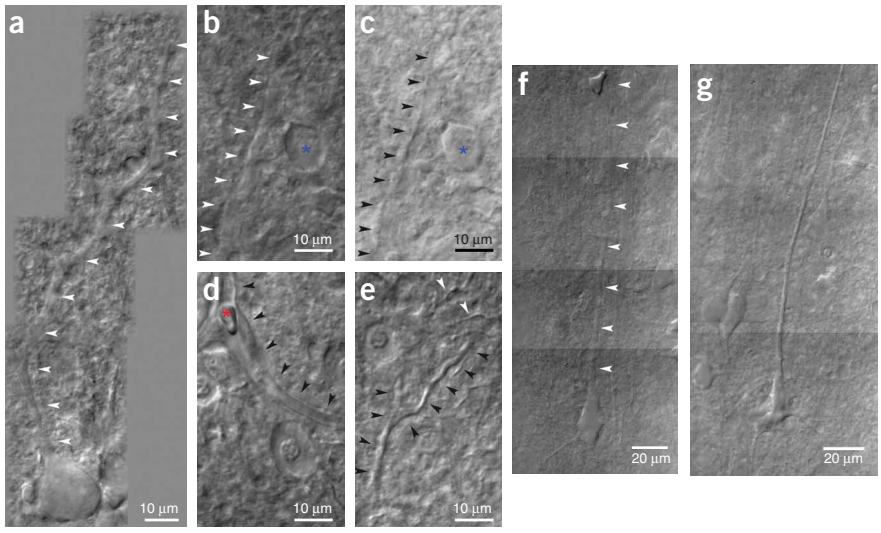

Figure 2 | Imaging dendrites using videomicroscopy. (**a**) Composite DIC image of a Purkinje cell with one of the main dendritic branches shown (white arrowheads). Same cell as in **Supplementary Video 1**, showing the process of dendritic patching. (**b**) Healthy dendritic branch of a Purkinje cell (white arrowheads). Also shown is the soma of a damaged interneuron (blue asterisk). Note that the healthy dendrite appears convex compared to the damaged interneuron, which has a concave, hole-like appearance. (**c**) The same image as in **b** but inverted digitally, reproducing the effect of rotating the 1/4 wave plate or biasing the upper DIC prism in the opposite direction. Note the inverted appearance of healthy and damaged structure. (**d**) Image of blood vessels, which sometimes can be mistaken for dendrites. Key identifying features are red blood cells in the lumen (red asterisk; these will be absent if cardiac perfusion has been used to cool the brain before slicing) and thicker edges. (**e**) Dead Purkinje cell dendrites. Compare the crisper, more contrasted appearance with **a** and **b**. White arrowheads show thin, presumably spiny dendrites, which are not usually visible when alive. (**f**) Example of an IR-DIC image of the soma and apical dendrite of a layer V pyramidal neuron in somatosensory cortex which has a high probability for sealing, with a low contrast, smooth appearance (white arrowheads). (**g**) An unhealthy L5 pyramidal cell in the same area with a low probability for high-resistance sealing. Note high contrast of dendrite and "collapsed", dimpled appearance of soma.

Pressure application is required before entering the bath solution in order to deflect any dirt floating on the surface of the bath, which might otherwise block or dirty the pipette tip. Increasing the pressure too much for extended periods of time, on the other hand, tends to drive any particles present in the intracellular solution into the pipette tip, thus clogging it. This is more critical during dendritic recording due to the use of small pipettes. It is useful (but not essential) to be able to monitor the amount of pressure in the pipette via a manometer. This allows the pressure used for patching to be adjusted, and will highlight any loss in pressure due to air leaks. The amount of pressure used will depend on tip size; typically 30 mbar is used.

14| Lower the pipette into the ACSF, and move it into position, in the center of the video monitor. Check that the tip of the pipette is not blocked.
Searching for cells and inserting pipettes is faster under low magnification (1× on the magnifier); switch to 2× or 4× to target dendrites.

15| In voltage clamp, apply a 'test pulse' (typically a 10 mV, 10 ms square pulse of voltage, applied at >10 Hz), and monitor the current required for this voltage deflection on an oscilloscope (or computer monitor). The resistance of the pipette tip can be calculated using Ohm's law (Resistance = Voltage/Current).
The tip resistance gives a useful indicator of the tip size (6-15 MΩ is typical for dendritic patching), and to verify that the pipette is unblocked (with block identifiable when the pipette resistance jumps to a higher value).

16| Lower the pipette to ~50 μm above the surface of the slice (**Fig. 3a**).
Note that the time spent with the pipette close to the neuron should be minimized when using physiological internal solution, as the stream of high potassium solution can lead to continuous excitation of neurons in the slice, potentially triggering plasticity and/or excitotoxicity. However, when making paired somatic and dendritic recordings, this excitation, seen at the soma, can be a helpful indication that the dendritic pipette is approaching a dendrite belonging to the same cell (this is especially useful when trying to record distally from long dendrites, where the path of the dendrite may be obscured or ambiguous). Once this excitation has been observed, it can be helpful to hyperpolarize the soma (or hold it in voltage clamp) while establishing the dendritic recording.
? TROUBLESHOOTING

17| On the patch-clamp amplifier, zero any voltage offset between the electrode and earth.

18| For the final approach towards the dendrite of interest (**Fig. 3b**), increase the positive pressure and move the pipette towards the dendrite of interest. The flow of intracellular solution from the pipette tip should clear away extracellular debris as you move towards the dendrite. This can also increase the visibility of the dendrite (see **Supplementary Videos 1** and **2**

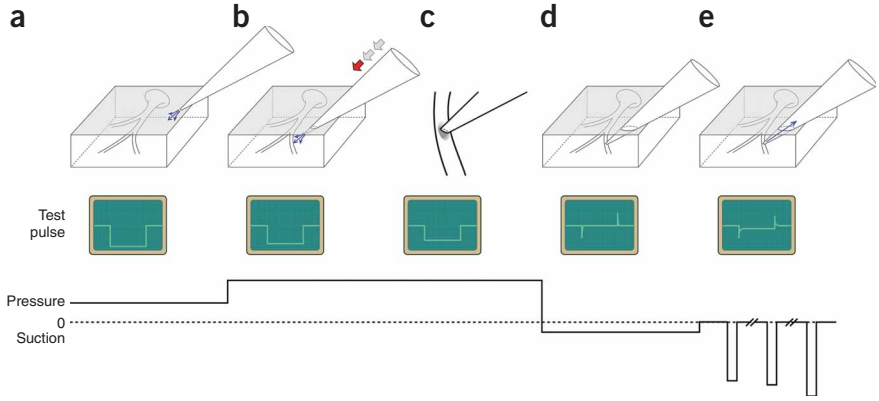

Figure 3 | Steps in dendritic patching. A schematic illustration showing the basic steps involved in making a dendritic patch-clamp recording. Top: a section of the slice, containing a healthy, connected dendrite and soma, together with a patch pipette. Middle: oscilloscope displaying the command current for a negative voltage test pulse (offset so as to be centered on the screen). Bottom: the pressure applied to the patch pipette (dotted line: 0 mbar). (**a**) A patch pipette, with ~30 mbar of pressure blowing external solution from its tip, is lowered into position above a clearly visible dendrite. (**b**) With increased pressure, such that slice debris is cleared from in front of the pipette tip, the pipette is moved in the diagonal 'in and out' direction (red arrow) through the slice and onto the dendrite. (**c**) When good contact is made with the surface of the dendrite, a 'dimple' starts to form in the dendritic membrane. (**d**) Immediately, the pressure behind the pipette is released, gentle suction applied and the holding potential lowered to ~−75 mV. The steady-state current required to clamp the patch at this potential (observable on the oscilloscope and on the patch-clamp amplifier display) indicates a giga-ohm seal has formed. If your patch-clamp amplifier allows it, compensate now for the charging of the pipette capacitance (remove the capacitive transients). (**e**) Brief pulses of suction are applied until the membrane under the pipette tip is ruptured, as seen by a change in the test-pulse current (now reflecting both the charging of the cell's membrane and the local input resistance).

online). The amount of positive pressure used will depend on pipette tip size and the type of dendrite being patched; e.g., for pyramidal neurons 120–180 mbar is typical (using ~8–10 MΩ pipettes), while for Purkinje neurons 30–50 mbar is typical (using ~7–8 MΩ pipettes).

You should endeavor to move the pipette cleanly through the slice towards the dendrite, without squashing the tissue (this can subsequently cause problems maintaining the contact between the pipette and the dendrite as the tissue slowly 'relaxes' back to its original position). One way of achieving this is by moving the pipette mainly in the diagonal 'in and out' axis.

? TROUBLESHOOTING

19| Aiming towards slightly off center (to the side closest to the pipette) of the dendrite, move the pipette diagonally onto, then slightly down into the dendrite (see **Supplementary Videos 1** and **2**). While doing this, focus continuously on the tip of the electrode.

▲ CRITICAL STEP The aim is to solidly connect with the dendrite, but not to deform it by more than a few micrometers. Actual movement of the dendrite by the pipette by a few micrometers is desirable, as it verifies that the pipette is in direct contact with the dendrite.

? TROUBLESHOOTING

20| When good contact is made, a dimple should start to form in the dendritic membrane (**Fig. 3c**, **Supplementary Videos 1** and **2**). When the dimple starts to appear, immediately release the pressure on the pipette, then apply light negative pressure to the pipette via gentle suction (<10 mbar), and finally hyperpolarize the voltage clamp command potential.

▲ CRITICAL STEP Hyperpolarization helps a giga-ohm seal to form between the pipette and cell membrane; holding potentials down to ~-75 mV can be used, but this should be readjusted to approximately the cell's resting potential before breaking into the cell.

? TROUBLESHOOTING

21| Continue light suction while monitoring the seal resistance by observing the size of the test-pulse current. If successful, a giga-ohm seal should form (**Fig. 3d**). The quality of the subsequent recording is normally directly correlated with the quality of the giga-ohm seal (at least 5 GΩ is desirable). In cases where the dendrite moves upon release of pressure (because the tissue expands again), it can be helpful to gently withdraw the pipette tip during seal formation, helping to insure continuous solid contact. If a giga-ohm seal doesn't form, reject the seal, change the patch pipette and try again, on another cell if necessary.

? TROUBLESHOOTING

22| Once a stable giga-seal has formed, withdraw the electrode slightly, so that it is not pressing into the dendrite.

Cell-attached, perforated or inside-out patch recordings can be made starting at this point: continue on to options A, B or C, respectively. For making whole-cell or outside-out recordings, continue on to the next steps.

A second dendritic recording could also be made at this point (before visibility of the structure has deteriorated with time or dialysis of the cell); alternatively it can be made after breaking into the first dendrite (as break-in can be difficult after a long period spent cell-attached).

(A) Cell-attached patch recording
Cell-attached patch recordings can be made using conventional techniques[4,46] as soon as the dendritic giga-ohm seal has formed. If current densities are being measured, care should be taken to keep the patch pipette tip size constant, reducing one possible source of variation of the membrane patch size. It is often useful to measure the dendritic resting potential at the end of the experiment in order to determine the transmembrane potential. This can be achieved by rupturing the patch and immediately recording the resting membrane potential (before significant wash-in of the patch pipette solution).

(B) Perforated patch recording
If a perforating agent has been included in the patch-pipette solution, such as nystatin or gramicidin[40], then a perforated-patch recording can be made simply by waiting for the perforating agent to form channels. Electrodes should be tip-filled with solution not containing the perforating agent to permit high-quality seals to form. Electrode stability is of particular importance when making perforated patch recordings from dendrites given the long time usually required for perforation (typically 10–20 min).

(C) Inside-out patch recording
Inside-out patches can be made using standard techniques[46] from the starting point of a cell-attached recording by slowly and gently removing the pipette from the dendrite. Care should be taken to detect vesicle formation (which can be rectified by briefly exposing the patch to air).

23| To enter the whole-cell recording mode, break into the cell by rupturing the membrane beneath the pipette tip using strong, brief suction (**Fig. 3e**).
▲ **CRITICAL STEP** Start gently; if 'break-in' isn't achieved, gradually increase the strength (but not duration) of the suction pulse. An alternative approach is to use a gentle "ramp" of suction, terminating immediately on break-in. If break-in using suction is unsuccessful, another method is to use strong hyperpolarization (e.g. below –120 mV), to cause dielectric breakdown of the membrane below the pipette. In this case, switch to current clamp immediately upon break in; otherwise the entire cell membrane will break down.
? TROUBLESHOOTING

24| After breaking into the cell, monitor the amplitude of the test-pulse current for a few minutes; if it starts to decrease (indicating an increase in the access resistance) gently try to clear the electrode tip using brief suction. A stable, low access resistance achieved at this point is more likely to remain low for a longer time.

25| At this point, you can make a whole-cell patch-clamp recording (A), or proceed with outside-out patch formation (B).

(A) Whole-cell patch recording
During whole-cell recordings, continuously monitor the position of the pipette relative to the dendrite, as well as the access resistance. It is possible to accommodate slight movements of the dendrite (e.g. due to swelling of the slice) by using the micromanipulator to gently maintain the position of the pipette on the dendrite.
Monitor and compensate for the access resistance of the pipette by applying a test pulse and adjusting your amplifier's bridge balance and capacitance compensation circuitry. Deteriorating access resistance can sometimes be improved by using brief, gentle suction or positive pressure (although this can also destroy the recording and even the dendrite). Note that the relatively high access resistance associated with dendritic recordings (typically >20 MΩ) means that bridge balance and capacitance compensation (in current clamp) must be scrupulously applied. In voltage clamp, expected series resistances are high, and likely beyond accurate compensation. This, together with the space clamp errors associated with recordings from neurons with dendrites, makes whole-cell dendritic voltage clamp imprudent for most circumstances, although offline compensation can help overcome many of these limitations[11].
If access resistance is sufficiently high (e.g., >50 MΩ) and cannot be improved, the recording should be abandoned.
Recordings should also be terminated if seal resistance deteriorates (as indicated by depolarization of the membrane potential towards 0 mV, together with a large decrease in apparent input resistance).

(B) Outside-out patch recording
An outside-out dendritic patch can be made using conventional techniques[4,46]. Briefly, this involves gently withdrawing the pipette from the dendrite, and monitoring the gradually increasing access resistance using a test pulse.
Continued access to the dendrite can also be detected by monitoring dendritic action currents (for dendritic locations with substantial backpropagating action potentials).
A sudden increase in access resistance while maintaining a high patch resistance indicates that the elongating tube of membrane connecting the pipette to the dendrite has broken and sealed over to form an outside-out patch.
? TROUBLESHOOTING

26| Simultaneous double dendritic (or double somatic-dendritic) recordings can be carried out from the same neuron by repeating the above process with a second electrode. Care must be taken to minimize vibrations in the setup when mounting

the second electrode and inserting it into the tissue. One way this can be achieved is by lowering both electrodes at the same time into the chamber until they are both above the neuron of interest, and then making seals in quick succession (this also minimizes problems of dendritic membranes becoming less visible with time spent whole cell, possibly due to dialysis of the cell with internal solution). Alternatively, the setup should be optimized to minimize vibrations when changing/inserting electrodes (e.g., transfer of vibrations during activation of mechanical manipulators).

● **TIMING**

Step 1, preparing equipment: 30–60 min
Steps 2–6, slice preparation: 20–30 min
Step 7, slice incubation: 30–60 min
Steps 8–11, slice transfer and dendrite visualization: 10–30 min
Steps 12–26, establishing giga-seal and relevant patch-clamp configuration: ca. 10 min (10–20 min for perforated patch-clamp)
Step 22/25, recording: 10–40 min (depending on conditions)
Repeat Steps 9–26 (and 8 as necessary) while healthy dendrites can be seen in the slices (3–5 h)

TABLE 2 | Troubleshooting table.

PROBLEM	POSSIBLE REASON	SOLUTION
Tissue does not come cleanly off the slicing blade, causing mechanical damage to the slices	Blade not cutting easily through the slice (especially through white matter)	Try cutting slightly thicker slices and advancing the cutting blade more slowly. Also, if the slicer allows for this, increase the horizontal displacement of the blade. If possible, orientate the slice so that the white matter is cut through last. Use younger animals, which may have less connective tissue and myelination. Avoid cyanoacrylate glue being splashed up the side of the tissue block: drop a small amount of ice cold ACSF over the block before filling the slicing chamber. Also, excessive amounts of glue are more likely to cause problems by creeping up onto the tissue. On the other hand, too little glue leads to instability of the brain and poor slices. Ensure the meninges are removed and the cutting blade is held at a suitable angle.
	Tissue block too small, so mechanically unstable	Embed in agar before slicing (2% agar dissolved in ACSF, cooled to 40 °C before embedding, and rapidly solidified with ice-cold ACSF).
Few healthy looking cells in slice, detected soon after slicing	Brain damaged mechanically or by ischemia while removing it from the skull, or during cutting of tissue block	Transfer the brain from the skull to ice-cold slicing solution as quickly as possible ($<$ 1 minute). Handle the brain with minimal mechanical disturbance (squashing, pulling, cutting), especially near the area of interest. For animals with larger brains (e.g. older animals) submerging in ice-cold slicing solution may not cool central brain areas quickly. Cardiac perfusion with ice-cold ACSF before decapitation can be used.
	Excitotoxicity due to neurotransmitters released by slicing damage, excess depolarization following excessive Na^+ influx, and/or excessive Ca^{2+} influx	Slice in sucrose ACSF (to reduce Na^+ influx), or in a high Mg^{2+}, low Ca^{2+} ACSF (to increase block of NMDA receptors, decrease excitability and reduce Ca^{2+} influx).
	Incorrect osmolarity or composition of ACSF	Check osmolarity & composition, make up ACSF (and stock solutions) again.
	Lack of oxygenated ACSF during preparation	Ensure ACSF for slicing, storage and recording is saturated with carbogen by starting the carbogen bubbling at least half an hour before starting the procedure.
Slices initially look good, but swell and/or deteriorate quickly	Lack of oxygenated ACSF in the recording chamber	Increase the flow rate of ACSF (up to 4-6 ml/min), ensuring that it is still heated to the correct temperature, and that it isn't moving the slice. Use oxygen impermeable tubing (e.g. Teflon) for the inflow to the recording chamber. Place the slice on a trampoline (a mesh covered platinum ring) in the chamber, to allow better access of ACSF to the bottom of the slice.
	Contamination of perfusion lines, the chamber, the harp, the objective	Thoroughly rinse perfusion line, chamber and harp after each experiment with 70 V/V% ethanol and then distilled water, and allow to dry. Replace perfusion lines and chamber frequently.

TABLE 2 | Troubleshooting table (Continued).

Can image cell bodies but not dendrites	Optics not optimized	Re-align Köhler illumination, DIC illumination and camera image adjustments.
	Poor slice quality	Reslice, following the same advice for 'Few healthy looking cells in slice'.
		Making thinner slices may help to allow more unscattered light through to the top layer of dendrites; this must be traded off against increased physical damage to the slice.
		The surface of the slice (where the visible dendrites would be located) can be damaged by the large z-displacement of the slicer blade. This varies with the make of slicer and the blade position. It's also therefore useful to be able to measure the z-displacement so that it can be minimized (amplitudes of <2 μm can be achieved).
Dendrites not parallel to plane of slice	Tissue block cut at the wrong angle	Some tissue slicers can be adapted so that angle of the cutting block can be adjusted.
		If the brain structure of interest is curved (e.g. cerebral cortex or hippocampus), lay slices out in the incubation chamber in the order they are cut in (e.g. clockwise starting at a known point), all orientated with the same face upwards; work through the slices until the correct plane is found.
		If the dendrites are severed because their plane is projecting out of the slice, it is sometimes possible to find healthy dendrites on the flip side of the slice. To avoid problems (cell death) due to poor access to oxygenated ACSF to the side of the slice resting on the chamber bottom, make the decision to flip the slice quickly (within a few minutes of placing it in the recording chamber).
When preparing for dual recordings, can visualize healthy somata and dendrites, but cannot trace an unambiguous, continuous path between any two.	Long and/or convoluted path of dendrites visually obscured at some point	Approach the most distal part of a clearly connected dendrite with the patch pipette; the flow of solution from its tip should clear debris and make the path of the dendrite more apparent.
		Improve your optics, or use a different method to help visualize the dendrite (e.g. fluorescence – see **Box 1**).
Debris is not cleared as the pipette is moved within the slice or onto the dendrite	Patch-pipette tip blocked by particles in the intracellular solution	Try to shift small blockages with very high pressure (applied with a 50 ml syringe).
		Replace patch pipette. Try increasing the pressure behind the pipette to increase the flow of intracellular solution moving debris from in front of the tip.
		Pass the intracellular solution through a 0.2 μm filter. Ensure cleanliness of all materials coming into contact with pipette solution (e.g., pipette filling needles, hands).
	Insufficient pressure on the patch pipette, so inadequate flow of solution out of pipette tip	Ensure all connections in the pressure application system are airtight.
		Monitor pressure behind the patch-pipette using a manometer. Increase the pressure applied slightly.
When patching, giga-ohm seal fails to form	Patching 'unhealthy' dendrites	Select a (better) dendrite and try again.
	No solid contact between pipette and dendrite	Wait until you see a clear dimple on the dendrite before releasing pressure. Be sure to push the pipette into the dimple. Too little pressure can be insufficient to form a dimple and too much will push away the dendrite.
Dendrites are difficult to pin down, pinging away like a rubber band.	Aiming for a long stretch of unbranched dendrite	Branch points can help to anchor the dendrite, so areas close to them are easier to patch. Over-sampling of branch points should be done cautiously, as they may have specialized properties differing from the surrounding dendrites.
	Deterioration of the connective tissue of the slice	The 'rubber band effect' is more often seen in old slices that have deteriorated. Search for another healthy-looking dendrite, or try again on a fresh slice preparation.

TABLE 2 | Troubleshooting table (Continued).

	Pressure behind pipette too high, thus softening the tissue around the dendrite too much	Reduce pressure.
Patch lost while breaking in	Movement of the pipette when applying suction	Pipette movement affects dendritic more than somatic recordings because of the small size of dendrites. Ensure that pipette holder's o-rings are in good condition and tightened firmly. However, overtightening can cause the o-rings to break down over time.
Dendritic recording is lost unexpectedly	Movement of the pipette relative to the preparation	Gradual or rapid movement of the slice or the pipette will easily disrupt the connection between the small dendrite and the pipette. Minimize slice swelling (see above) and rig vibration, and make manipulators as smooth and stable as possible.

ANTICIPATED RESULTS

Making patch-clamp recordings from dendrites is fundamentally similar to—though considerably more difficult than—making patch-clamp recordings from the somata of neurons in brain slices. Therefore if high-quality long-lasting somatic patch-clamp recordings cannot be achieved using your preparation, dendritic recordings will be extremely challenging, if not impossible. A prerequisite before beginning dendritic recording, therefore, is optimization of conditions for obtaining good, stable somatic recordings.

Once the conditions for somatic recording have been optimized (in particular slice quality, optics and setup stability), then the success rate for obtaining dendritic recordings depends primarily on the diameter of the dendrite being patched, which is usually related to the distance of the intended recording site from the soma. Other factors can also play an important role, such as the light scattering properties of the local neuropil, which can impede visibility of the dendrite. Some experience is usually required in order to optimize the correct final approach to the dendrite; timing is also important, since (unlike for the soma) a dendrite can often slip away from underneath a pipette before the positive pressure can be released. Typically several months of continuous practice are necessary before satisfactory success rates can be achieved (typically 1–2 high-quality dendritic recordings per preparation).

When the experimenter has become proficient enough to reliably obtain high-quality seals in dendritic recordings, the main problem encountered is drift of the electrode position relative to the slice. This can terminate cell-attached recordings (by degrading the seal), and causes a progressive increase in access resistance in whole-cell recordings. Drift can be minimized by optimizing both slice quality (to minimize swelling of the slice) and the stability of the pipette. However, some degree of drift is unavoidable, and access resistance can also increase even if there is no drift. Therefore, access resistances in whole-cell recordings must be carefully monitored, documented and compensated electronically.

Despite these potential pitfalls, dendritic patching is now a routine and reliable technique. Under optimal conditions, cell-attached recordings of channel activity, and whole-cell recordings of membrane potential with relatively stable access resistances, can be maintained for over 30 min. This length of recording allows many different protocols to be applied to investigate dendritic channel function and dendritic excitability. It also permits the use of dendritic recordings to directly monitor synaptic efficacy and dendritic channel properties during long-term plasticity experiments[27,47,48].

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

ACKNOWLEDGMENTS We thank M. London, F. Pouille, M. Scanziani and J. Sjöström for their helpful comments on the manuscript. This work was supported by the Gatsby Foundation (M.H.), the Wellcome Trust (M.H., J.T.D, E.A.R.), the NH&MRC of Australia (G.J.S., M.H.P.K., J.J.L.) and the US National Institutes of Health (NS-35180, NS-46064 to N.S.).

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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Corrigendum: Dendritic patch-clamp recording

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Nat. Protocols doi:10.1038/nprot.2006.164; published online 21 September; corrected online 30 November 2006.

In the version of this article initially published online:

p. 1235, left column, last line: Quotation marks were misplaced. The sentence should begin: "Although some early, 'laborious' efforts..."

p. 1236, right column, last four lines, and p. 1238, first text line: Reference citations were inserted in the wrong place and misspelled. The sentences should read: "Alternative principal anions are gluconate and methanesulfonate; note that all internal solutions are associated with washout of intracellular factors and some may also have pharmacological effects[45] (also see Kaczorowski, C.C., Disterhoft, J.F. & Spruston, N. *Soc. Neurosci. Abst.* **31**, 737.17, 2005). A fluorescent dye (e.g., 1–25 μM Alexa 594) can be included..."

p. 1238, first line under EQUIPMENT SETUP: "Recording" was omitted. The sentence should read: "An illustration of a typical setup used for dendritic patch-clamp recording is shown in **Figure 1**."

p. 1239, Table 1, first item in right column: Mispunctuated. The sentence should read: "Cut sagittally, as parallel to the midline of the cerebellum as possible, on either side of the cerebellar vermis."

p. 1240, last paragraph in Step 11: Text was misplaced. The sentences should read: "It is possible, however, to follow a dendrite deep into the slice from the soma to a distal, more superficial location. Even if the dendrite seems to disappear at points it is possible to spot the same dendrite again at a more distal location.

p. 1246, Table 2, last item in right column: Punctuation was misplaced. The sentence should read: "Minimize slice swelling (see above) and rig vibration, and make manipulators as smooth and stable as possible."

These errors have been corrected in all versions of the article.