Chapter 10

Patch clamp recording from cells in sliced tissues

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1. Introduction

Before the introduction of gigohm seal patch clamp techniques (Hamill *et al*. 1981) the best resolution that could be achieved when measuring ionic currents in cell membranes was of the order of 100 pA. The combination of the gigohm seal and the placement of the current-voltage converter clamping amplifier in the headstage gave a dramatic improvement in signal resolution allowing currents of around a pA or less to be resolved (Neher, 1992; Sakmann, 1992). This great improvement in technique and instrumentation was followed by a tremendous expansion in the variety of cell types accessible to electrophysiological investigation. This was particularly true in relation to experiments on small cells (e.g. adrenal chromaffin cells: Fenwick *et al.* 1983; and central neurones in tissue culture: Nowak *et al.* 1984; Cull-Candy & Ogden, 1985; Bormann *et al.* 1987) and should also have been true for neurones in brain slices. However, the problem of obtaining a clean access for the patch electrode to the surface of neurones in brain slices seemed insoluble and so the potential advantages of applying patch clamping to brain slices were not immediately achieved.

Signal resolution in patch clamp recordings

The signal resolution achieved in any particular experiment depends on several related factors but the basic point in this relates to the noise inherent in any resistor (e.g. Neher, 1992; see also Chapters 4 and 16). The rms thermal noise in a simple resistor is given by $\sigma_n = (4kT\Delta f/R)^{0.5}$ where σ_n is the rms of the current through the resistor, k is Boltzmann's constant, T the absolute temperature, Δf is the bandwidth and R is the resistance. Basically, what this equation says is that the current noise in the recording is inversely proportional to the source resistance. In order to measure currents of the order of a pA at a bandwidth of 1 kHz, the resistance of the source needs to be of the order of 2 GΩ (2×10^9 Ω). The 'source resistance' in this case is mainly the

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combination of the feedback resistor in the amplifier, the seal resistance, and the resistance of the preparation itself (cell or isolated patch). In addition, noise associated with the electrode and cell or patch capacitance add to the total (see Rae & Levis, 1992 for a discussion of noise sources). The noise contributions from each source add together as the square root of the sum of the squared rms noise for each component.

Some background to patching cells in slices

In principle the small cell size and small membrane currents of cells in the central nervous system make them ideal for patch clamp recording. However, in the years after patch clamping was first described, cells in intact central nervous system tissue were thought to be inaccessible for patch clamping because there was no apparent way to maintain the intact structure of brain tissue and yet achieve the clean cell membrane that is essential for forming a gigohm seal between glass pipette and cell (Hamill *et al*. 1981). In the meantime neurones and glia were studied in primary culture. Unfortunately, primary cultures have the disadvantage that changes in gene expression and synaptic connections, and even the identity of cells all become unknown factors. An alternative was to study neurones and glia after acute dissociation using enzymes such as papain or trypsin (Numann & Wong, 1984; Gray & Johnston, 1986; Kay & Wong, 1986; Barres *et al*. 1990). Although these preparations allowed the study of receptors and ion channels on adult central neurones (e.g. Kay & Wong, 1987; Huguenard *et al*. 1988; Sah *et al*. 1988; Gibb & Colquhoun, 1992), the possibility remained that the receptors or ion channels of interest could be altered by the enzymes used and synaptic transmission, of course, could not be studied.

Meanwhile, during the 1980s the techniques for recording from brain slices with intracellular electrodes were greatly improving, aided by the development of the discontinuous single electrode voltage clamp (Finkel & Redman, 1985). Several studies were published where simultaneous recordings from pairs of synaptically connected neurones in hippocampal slices were achieved (e.g. Knowles & Schwartzkroin, 1981; Miles & Wong, 1984; Scharfman *et al*. 1990; Sayer *et al*. 1990; Mason *et al*. 1991). However, these recordings were still limited by the lower resolution of intracellular microelectrode recording.

The problems of cell isolation and signal resolution were overcome by the introduction of techniques to allow patch clamp recordings to be made from brain slices (Edwards *et al*. 1989, adapted by Blanton *et al*. 1989) and even from the *in vivo* brain (Xing Pei *et al*. 1991; Ferster & Jagadeesh, 1992). The tremendous significance of this advance is seen by the fact that these techniques have rapidly been applied to many fundamental questions in neurobiology such as the mechanism of synaptic transmission at inhibitory (Edwards *et al*. 1990; Takahashi, 1992) and excitatory synapses (Hestrin *et al*. 1990; Silver *et al*. 1992; Stern *et al*. 1992, Hestrin, 1992a), as well as allowing single channel recording of the properties of receptors and ion channels in identified neurones from the brain (e.g. NMDA receptors, Gibb & Colquhoun, 1991; $GABA_A$ receptors, Edwards *et al*. 1990; glycine receptors, Takahashi & Momiyama, 1991).

2. Preparation of brain slices for patch clamp recording

Brain slices have been widely used for both biochemical and electrophysiological studies (for review see Alger *et al*. 1984), and the methods used to prepare brain slices are widely documented (e.g. Langmoen & Andersen, 1981; Cuello & Carson, 1983; Alger *et al.* 1984; Madison, 1991). Although different labs have their own individual variants, below is a brief description of the procedures that we use, which seem to give good results with a variety of brain areas (see also Edwards *et al*. 1989; Konnerth, 1990; Edwards & Konnerth, 1992).

(i) The animal is decapitated and the brain removed and placed in ice-cold physiological solution within 60 seconds of decapitation (the solution should be so cold that it contains a few ice crystals and to maintain this temperature the container should be sitting on ice).

(ii) Pause for 3-5 minutes while the tissue cools down.

(iii) Trim or block off the tissue using clean cuts with a sharp scalpel blade in preparation for gluing to the stage of the tissue slicer. Avoid squeezing or otherwise deforming the tissue at this stage.

(iv) Apply a thin layer of cyanoacrylate glue (Super-glue^{R}) to the stage of the tissue slicer and then gently place the tissue at the correct orientation onto the glue (the most common orientation is to have the region of interest near to the blade, or at least try to avoid cutting through white matter before reaching the area to be sliced). Immediately, pour ice-cold solution over the tissue until it is submerged.

(v) Cut slices (100-300 μm thick) with vibrating slicer. Fine dissecting scissors or two hypodermic needles can be used to dissect out an area of brain from each whole brain slice.

(vi) Using a Pasteur pipette cut and fire-polished to an opening of 3-5 mm across, transfer each slice as it is produced to the holding chamber which should be in a water bath at 32-35°C with a good steady flow of O_2/CO_2 bubbling through the solution.

(vii) Incubate the slices at 32-35°C for at least 30 minutes before beginning recordings.

Equipment check-list

About 250 ml of ice-cold ‘slicing Krebs’ sitting on crushed ice

Large scissors for decapitation

Small scissors to cut open the skull

Curved, blunt forceps to remove top of skull

No. 11 scalpel to hemisect the brain

Small spatula to remove brain halves from skull

Large weighing boat or similar shallow container of ice-cold Krebs sitting on crushed ice to cool the brain halves

Cyanoacrylate glue

Large spatula to lift blocked-off piece of brain onto tissue block

Two fine hypodermic needles or fine dissecting scissors for dissecting small regions from the brain slice

Broken and fire-polished pasteur pipette (opening 3-5 mm)

3. Notes on making slices

The time factor

It is critical that the time from decapitation till immersion of the brain in cold solution is kept short (<1 min). Partly because of this, it seems easier to make healthy slices from younger animals (e.g. less than 3 weeks) where the skull is soft and can be removed more rapidly. In addition, the smaller brain of younger animals will cool more rapidly than a larger adult brain and may be more resistant to anoxia. To improve cooling some people remove the skull with the whole head submerged in ice-cold Krebs. Bubbling the Krebs during the cooling period may also improve cooling.

The whole process of making slices should preferably not take more that about 30 minutes. However, we have observed that tissue kept ice-cold for half an hour (e.g. the second half of the brain when making hippocampal slices) can still be glued to the slicer and good slices prepared from it. This can be useful if two people wish to slice different parts of the brain or as a backup if something goes wrong during slicing such as the tissue block coming off the slicer stage during slicing (this may happen occasionally although less often with practice: perhaps the block was moist before the glue was applied, or too thick a layer of glue was used, or the glue itself was too thick in consistency, or the surface of the tissue block is not flat).

Tissue slicers

The tissue slicer should be able to vibrate at sufficient frequency (around 10 Hz) and with a long enough stroke (1-2 mm) to cut cleanly through the tissue. Most commercially available slicers will do this when set at their maximum settings. Care must be taken that there is an absolute minimum of play or vibration in the mechanism driving the cutting blade. Use of a rotating blade for cutting slices, rather than an oscillating blade, has recently been described and a rotating blade slicer is now marketed by Dosaka (Model DTY 8700). However, we have no personal experience of rotating blade slicers as compared to oscillating blade slicers.

Depending on the brain area being sliced, it may be useful to view the slicing using a low-magnification dissecting microscope or large magnifying glass (some slicers come fitted with a magnifying glass). It is always useful to have a good light source available to illuminate the tissue block (e.g. using fibre optic light guides).

The simplest slicers have a manual movement of the tissue block towards the oscillating blade (e.g. Cambden Vibroslice, UK) and in our experience these work very well for a variety of different brain areas. Some slicers have a Peltier-cooled stage to maintain the tissue close to 0°C during slicing. However, it is perfectly adequate to have frozen Krebs in the bottom of the slicing chamber (or make Krebs ice-cubes) to ensure the tissue stays cool during slicing.

Some slicers have a motor drive to advance the blade or tissue during cutting (e.g. Dosaka 1500E, Japan; Camden Vibroslice, UK; FTB Vibracut, Germany; Technical Products Inc. Vibratome 1000, USA). An annoying feature is that some slicers

automatically reverse at the end of the cut, when what is often needed is to stop the blade in that position until a piece of tissue of interest is dissected free from the whole brain slice. The Vibracut has a useful innovation in that the tissue bath mounts on a magnet allowing it to be rotated to any angle, which avoids the difficulty of placing the tissue on the glue at exactly the right angle.

There are quite a variety of tissue slicers available with the tissue block inside a tissue bath and so suitable for cutting living slices. These slicers vary in sophistication and price. However we find that a simple slicer such as the Camden Vibroslice works very well. If a more sophisticated slicer is preferred, we recommend the vibrating Dosaka slicer or the Vibracut.

Slicer blades

The blades used for slicing should be as sharp as possible. High-carbon steel blades are preferable to stainless-steel (a high-carbon steel is magnetic and brittle and will break with a sharp snap). Stainless-steel razor blades are probably not as sharp.

Slicing different brain areas

Different brain areas are more or less difficult to slice and in the original description of the technique a large variety of different brain regions were successfully recorded from (Edwards *et al*. 1989). As well as taking the age of the animal into account, a high degree of myelination and vascularization of a particular area tends to make slicing more difficult (areas like this seem to require a particularly slow forward speed during cutting). The spinal cord and brain stem are regarded as difficult to slice, particularly in older animals, but in the last few years successful patch clamp experiments have been made with both young (e.g. Takahashi, 1992) and adult spinal cord (Yoshimura & Nishi, 1993) and with several parts of the brain stem (e.g. Forsythe & Barnes-Davies, 1993; Kobayashi & Takahashi, 1993).

Getting the right angle

Many neurones have their dendritic tree angled in a particular orientation or plane. A big improvement in cell survival is obtained if care is taken to cut the slices at an angle that will preserve the dendrites (e.g. transverse slices of hippocampus to maintain the pyramidal cell apical dendrites). Of course, the angle of slicing could also be important in maintaining synaptic connections. Obviously, if a particular input is to be stimulated then the angle of slicing must be arranged to avoid cutting the incoming axons. Alternatively, it may be necessary to stimulate locally (e.g. with a patch electrode pushed into the slice) if the desire is to stimulate a local interneurone.

In general it is harder to obtain healthy, large neurones in slices compared to obtaining healthy small neurones, probably because of the problem of neuronal death if some of the dendrites are cut, but perhaps also due to differences in resistance to anoxia between different cell types. Thus, in hippocampal slices, even when CA1 and CA3 cells look poor, it is often possible to see healthy granule cells. Likewise in cerebellar slices it is more difficult to obtain healthy Purkinje cells than healthy granule cells.

Slicing solutions

Slices are made with a standard extracellular Krebs solution. For example we use (in mM) NaCl 125, KCl 2.5, $CaCl_2$ 2, $NaHCO_3$ 26, NaH_2PO_4 1.25, $MgCl_2$ 1, Glucose 25, of pH 7.4 when bubbled with 95% O_2 and 5% CO_2. Although the exact composition of the Krebs solution varies between laboratories, particularly in the concentrations of Ca^{2+}, $NaHCO_3$ and glucose, it is generally considered important that the K^+ concentration is less than 3 mM (to avoid epileptiform activity in the slice) and that a high glucose concentration is used.

Efforts to improve cell survival during slicing include the substitution of sucrose for 50% of the NaCl in the Krebs (Aghajanian & Rasmussen, 1989), inclusion of Hepes buffer as well as HCO_3^- buffer in the Krebs, raised extracellular Mg^{2+}, use of NMDA channel blockers and excitatory amino-acid antagonists.

Slice incubation chamber

A good incubation chamber must be able to provide a good circulation of freshly oxygenated solution since the slices must be kept in good condition in the chamber for the whole of the experimental day (10-12 hours). It must be stable not only to prevent mechanical disturbance of the slices but also so that slices can be placed in or removed from the chamber without disturbing any of the other slices. Preferably it should be simple to make and clean. Different laboratories use different types of incubation chamber. Here we describe a simple construction illustrated in Fig. 1 which we find easy to make and use.

This incubation chamber uses a standard 100 ml beaker. It contains a piece of light cotton clamped across two rings made for example using the base and lid of a 35 mm Petri dish which have had the top and bottom broken out (Falcon dishes seem to work best). This makes a tight net of cotton on which the slices will rest. The cotton clamp is then wedged halfway down the beaker using a piece of stiff plastic tube about 3-4 cm long. This plastic tube should reach from almost the bottom of the beaker to about 5 mm below the surface of the Krebs. A gas bubbler is inserted into the tube to near the bottom and generates a stream of bubbles which by rising to the top of the tube draws the Krebs from the bottom of the beaker, so generating a circulation of Krebs which will act to hold the slices down on the net. The incubation chamber is placed in a heated water bath (a large water tank, 5-10 litres, heated with a standard aquarium heater is sufficient) and covered (e.g. with a Petri dish lid) to prevent evaporation.

The incubation chamber can be dismantled every night and reassembled next day with a new piece of cotton (standard white muslin is cheap enough that a meter of material bought at the local drapers shop will last for years!). However, if the chamber is rinsed with distilled water and then left to soak overnight in distilled water acidified with a few drops of HCl, then the same chamber can be used for several days at a time.

Immobilizing the slice in the recording chamber

It is necessary to immobilize the slice during recording so that no movements occur as a result of the solution flowing through the bath. Typical flow rates would be

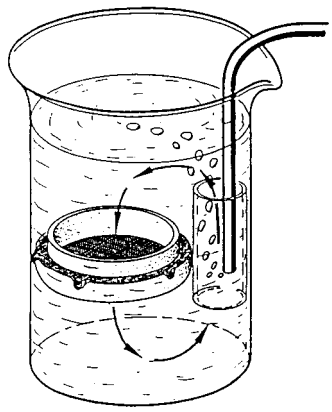

Fig. 1. A simple incubation chamber for maintaining brain slices. The chamber is made using a 100 ml beaker containing a cotton support which allows the slices to be held in a gentle circulation of oxygenated Krebs solution. The cotton support is made from standard cotton muslin stretched across two rings made from the top and bottom of a 35 mm Petri dish. This is then wedged halfway down the beaker using a stiff plastic tube which extends well below and a little above the cotton support. The tube should reach from near the bottom of the beaker to about 5 mm below the surface of the Krebs. When a bubbler is placed near the bottom of the tube the bubbles rise up the tube drawing solution with them and generating a current which flows down over the slices. (From Edwards & Konnerth, 1992).

between 1 ml and 3 ml per minute for bath volumes of less than 1 ml and stability is improved if the inflow and outflow are in separate chambers connected to the recording chamber by small, submerged passages. This has the disadvantage of tending to slow solution exchange around the slice so a good compromise is to have the outflow in a separate chamber and place the inflow on a ramp running directly into the recording chamber.

Several methods have been described for immobilizing the slice including the use of fibrin clots (Takahashi, 1978; Blanton *et al*. 1989) and pieces of netting. However, many people find that a grid (described in Edwards *et al*. 1989) made of flattened platinum wire with single nylon strands glued across it with cyanoacrylate glue works well for holding the slice firmly on the bottom of the recording chamber.

4. Visualizing cells in living brain slices

How healthy is the slice?

In the past the health of the slice was generally determined from physiological parameters such as resting membrane potential of impaled cells, size of the action potential, population spike etc. Comparison of these parameters measured *in vitro* with the same parameters measured *in vivo* suggests that healthy sliced brain tissue behaves in a remarkably similar way to the *in vivo* state. However, in the past only a few studies used high-resolution optics to allow cells in slices to be visualized

(Yamamoto, 1975; Takahashi, 1978; Llinas & Sugimori, 1980) so that a direct visual assessment could be made of the health of the sliced tissue.

One great advantage of visualizing the cells in slices is that it allows an immediate assessment of the health of the slice to be made and cells suitable for recording to be carefully picked. The first examination of a healthy slice under the microscope is a fantastic sight! In a hippocampal slice for example, lots of bright shiny cells should be visible with a variety of cell dendrites and different cell morphologies present. Compared to blindly inserting the electrode into the slice, a great deal of time can be saved by first picking out the good cells particularly, for example, if the cell of interest is a relatively rare interneurone. If the slice does not contain many bright cells, but instead is uniformly dark with many round opaque cells with clearly visible nuclei evident, then the slice should be discarded (see also Edwards & Konnerth, 1992 for a discussion of visually assessing the slice).

Labelling and identifying cells

It was partly as a result of the desire to record from identified cells (Takahashi, 1978) that the techniques for patch clamping visually identified cells in brain slices were developed (Edwards *et al*. 1989). Retrograde transport of fluorescent dyes or fluorescent beads (e.g. Takahashi, 1978; Katz *et al*. 1984; Gibb & Walmsley, 1987) has been used to allow subsequent identification of living cells in slices or following dissociation. However, these procedures can only be used where cells have a definite projection (e.g. motor neurones) and require expensive fluorescent optics on the microscope. Instead, cell bodies and parts of the dendritic tree can be easily observed using differential interference contrast (DIC) Nomarski or Hoffmann modulation optics. The identification of the cell to be studied then depends on the use of information about the local anatomy, and the size and morphology of the cells of interest. For most purposes this is sufficient to identify a cell clearly.

Microscope requirements

The particular brand of microscope used is not critical. Zeiss, Olympus and Nikon all make upright microscopes which can be used for visualizing cells in slices (Micro-Instruments in Oxford make a good customized microscope fitted with Nikon optics). Ideally the microscope should have a fixed stage so that focusing occurs without moving the preparation relative to the patch electrode. Although the standard Olympus BHS is not a fixed-stage microscope, it can easily be converted and Olympus will now do this conversion if requested. This is a cheap and satisfactory option. It is also important that the microscope is not mounted on rubber feet but instead is firmly fixed to the vibration isolation table or the electrode will crash into the slice every time the focus is adjusted! Use of a high numerical aperture (e.g. 0.75) ×40 water immersion objective on a standard upright microscope preferably fitted with Nomarski optics allows visualization of neurones and their dendrites with a resolution of about 1-2 μm, if the cell lies within 20 μm of the surface of the slice (looking from above). For Nomarski optics to be effective, however, the maximum

slice thickness is around 300 μm. The thicker the slice, the more the light is scattered passing through the slice and the dimmer and lower the resolution of the image. On the other hand, it is more difficult to obtain healthy thin slices: slices 200-300 μm are usually a good compromise.

The choice of objective is a compromise between the need for high resolution (high numerical aperture) and the need for a reasonable working distance (at least 1.5 mm) to allow access to the surface of the slice with a normal patch electrode. The Zeiss ×40 achromat (numerical aperture 0.75, working distance 1.9 mm) fitted to the Zeiss Axioskop is a good example. In most countries the Zeiss Axioskop is considerably more expensive than the Olympus BHS fitted with the newly released Olympus 40× water immersion objective (NA 0.7, WD 3 mm). Unlike the Axioskop, the Olympus does not have infinity-corrected optics and so the new Zeiss and Olympus objectives are not interchangeable. There is also a Nikon 40× water immersion objective (NA 0.55, WD 2 mm) but, although this is a little cheaper, the image resolution seems to be not as good presumably because of the lower numerical aperture. The Zeiss Axioskop microscope gives excellent image quality. This may be partly because infinity-corrected optics are superior to standard optics (at least in principle) but could also be the result of a very stable condenser and Nomarski system combined with a very good light source. For patch clamping very small cells it may be an advantage to fit the microscope either with an octovar giving variable intermediate (1.0×, 1.25× and 1.6×) magnification or 16× eyepieces to give an overall magnification of more than 600×.

In principle, it might be expected that, when the water-immersion objective is in contact with the bath solution, a ground loop will occur because the objective will also be in electrical continuity with the rest of the microscope which is usually earthed. In practice we know of varying experiences on this where some objectives did, and some did not need insulating from the microscope, perhaps because some objectives are coated, which effectively insulates them anyway. If necessary, a solution is to manufacture an insulating collar to insert between objective and nose-piece.

It should be noted that for best results the numerical aperture of the condenser lens should always be as high or higher (0.9 for example) than that of the objective. This generally means that the working distance of the condenser will allow only a thin glass cover slip or glass base for the recording chamber, if the light from the condenser is to be focused properly on the surface of the slice (plastic chambers, although fine for phase contrast optics, destroy Nomarski imaging).

Whatever the precise optical arrangement it is essential for best results that good microscopic practice is followed (see e.g. Bradbury, 1989). In particular, good Köhler illumination must be set up with the condenser adjusted to focus the light source diaphragm exactly in the plane of the cells of interest. For DIC optics, the polarizers should be 90° to each other and the analyzer adjusted for optimum image quality. Secondary diaphragms in the condenser are then used to cut down the light entering the tissue and so improve the sharpness of the image.

It is often less tiring to view the image using a CCD camera in combination with a

standard monitor. These are relatively cheap (e.g. from Radio Spares) and the smaller cameras (<300 g) are light enough to mount directly on top of the microscope trinocular head without applying too much weight to the microscope focusing mechanism. It is generally important to ensure that the camera is insulated from the microscope to avoid conducting interference into the patch clamp signal. When using a CCD camera, a better image may be achieved if the secondary diaphragms on the condenser are left open and the gain and contrast of the camera controller used to optimize the image, although this will tend to make the image down the eye pieces look very washed-out (Levis & Rae, 1992).

Dodt & Zieglgänsberger (1990) have described the use of Nomarski optics in combination with an infra-red filter placed in the normal light path to give infra-red DIC imaging of cells in brain slices. The infra-red image is then visualized with an infra-red-sensitive CCD camera (specialist infra-red CCD cameras are expensive but even an ordinary CCD camera is quite sensitive to infra-red light up to about 1000 nm wavelength) and the image can then be stored on video tape or on computer using a frame grabber. Analogue or digital image enhancement techniques can then be applied to the image. The improved resolution achieved with infra-red DIC may be partly due to reduced scattering of infra-red light during transmission through the slice. Although the infra-red imaging may add considerably to the cost of the microscope, it allows imaging much deeper in the slice and may be useful for specialist applications such as patching directly on to dendrites in slices (Stuart *et al.* 1993).

5. Recording from cells in slices

Electrodes

The electrodes used for patching cells in slices are fabricated in the normal way. Thick-walled glass and coating with Sylgard^{R} are useful in minimizing the noise associated with the fact that the electrode is immersed quite deep in solution under the objective. For clamping large or fast currents where it is important to minimize the series resistance, it may be better to use thin-walled glass. The choice of glass can make a big difference with a thick-walled Aluminosilicate glass (e.g. Clark Electromedical SM150F 7.5) having a much lower noise than a thin-walled borosilicate glass (e.g. Clark Electromedical GC150TF 7.5). Rae & Levis (1992) discuss in detail a wide range of glass types for patch clamping.

Selecting a healthy cell

Selecting the best cell for patch clamping requires experience of the particular brain slice in use under the conditions presented by the way the microscope is adjusted. A good guide, however, is that the cells should be smooth with a clear outline and have a ‘soft’ appearance (see also Edwards & Konnerth, 1992). Cells that appear very shiny or ‘hard’ in appearance do not make seals easily and if observed for some time, appear to die gradually. Dead cells are opaque with visible nuclei and are often

swollen and round. Although gigohm seals and single channel currents can be observed if a seal is made on a dead cell, these cells have no resting membrane potential and the recording is invariably lost on attempting to form the whole-cell configuration.

Patching cells under visual control

Cells are cleaned using a blunt patch pipette (tip diameter 3-10 μm according to the size of cell being cleaned). This pipette is inserted into the bath without filling and will fill with some Krebs by capillary action. The cleaning pipette is brought up close to the surface of the slice and then positive pressure used to produce a gentle stream of solution which will break up the surface of the slice over the cell of interest. After a few seconds of positive pressure, light suction can be used to remove the debris overlying the cell. The cleaning pipette is then discarded and a recording electrode filled and placed in the bath near the chosen cell. Finding the electrode under a water-immersion objective is often difficult at first: one method is to wind up the objective so that it is focused far above the slice, but still in solution. The electrode tip is then placed under the objective and moving from side to side, the point where the electrode cuts the light beam from the condenser is found. Looking down the objective, the electrode can then be found with only a small sideways movement of the electrode and then the electrode can be lowered vertically in full view until it is just above the slice.

Obtaining good seals on cells in slices involves essentially standard patch clamping procedures. All types of patch clamp configuration have been used in slices. The method is straight-forward. Positive pressure is applied to the back of the electrode. Then under visual control, the electrode is advanced until the tip is just in contact with the cell surface, detectable by a small increase in the electrode resistance. At this point the stream of solution from the electrode tip should be evident producing a dimple on the surface of the cell. On removal of the positive pressure the electrode resistance should increase a little more and then a small amount of suction applied to the back of the electrode should begin the sealing process. At this point it is often best to maintain gentle suction and wait. A gigohm seal may form immediately or may take some minutes. Sealing in slices seems to be generally much slower than with cultured or dissociated cells. Often, sealing is more successful if a negative voltage (around −50 mV) is applied to the electrode and this has the advantage that if the patch breaks through on seal formation then the cell membrane potential will be clamped near the resting potential (our usual procedure is to leave the pipette potential at 0 mV until a resistance of about 50-100 MΩ is achieved and then to depolarize the membrane by setting the holding potential to −50 mV).

Stimulating axons or cells in slices

Perhaps the single biggest advantage of being able to make patch clamp recordings from brain slices is that it allows synaptic transmission to be studied at synapses which are probably identical to those functioning *in vivo*. By using the patch clamp

whole-cell recording configuration, the resolution of synaptic currents is increased by one to two orders of magnitude compared to what was possible with conventional intracellular recordings. In any whole-cell recording it is often possible to observe spontaneously occurring synaptic currents; most commonly $GABA_A$ receptor-mediated. The spontaneously occurring currents are due to the on-going firing of cells in the slice and are largely abolished by the application of tetrodotoxin to the slice, leaving only spontaneous miniature synaptic currents ('minis'). Since the origin of the spontaneous synaptic activity is generally unknown it is often preferable to stimulate a presynaptic cell or axon directly (either a single cell or axon, or a whole bundle of axons).

In order to try to stimulate a single presynaptic cell or axon a stimulating electrode (usually a standard patch electrode filled with Krebs) is pushed gently into the slice near the cell being recorded from and then short rectangular voltage pulses (e.g. 5-50 V in amplitude, 50-500 μs in duration) are applied to the electrode and the trace observed to see if a synaptic current occurs immediately after the stimulus artifact. The stimulating electrode is moved slowly through the slice until a connection is found or, if no currents are evoked, a different place in the slice is selected and the process repeated. Generally this procedure is more successful than the much harder method of obtaining a whole-cell recording on both pre- and post-synaptic neurones, although this is probably the only way to be sure that a single cell is producing the recorded input (and even a single cell may make multiple synapses on the post-synaptic neurone). An alternative strategy is to place the stimulating electrode on the surface of a nearby cell. If the presynaptic neurone also has a synaptic connection with this cell then it is possible to generate antidromic action potentials from there which will produce synaptic currents at the cell under the recording electrode. This strategy worked well with both inhibitory currents in hippocampal granule cells (Edwards *et al*. 1990) and with excitatory currents in visual cortex interneurones (Stern *et al*. 1992).

The alternative method of stimulation is to use a bipolar platinum stimulating electrode to stimulate a whole region of the slice or a whole fibre tract at once. This method may be much more convenient and reliable in some situations such as stimulating the Schaffer collaterals in a hippocampal slice. The advantages are that the electrodes probably don't need to be moved after placement on the slice and the stimulus artifact can be much smaller than when using a local electrode. The disadvantage is that the stimulus is not localized in any way and so many axons may contribute to the measured synaptic response, even in situations where the stimulus voltage has been adjusted to provide the smallest reliable input.

A problem can arise when stimulating if the stimulus artifact feeds into the clamp command voltage producing a voltage jump in the cell, but this can usually be avoided if the stimulus reference is connected to the common earth of the setup.

Blind patching

This method was originally developed in experiments on turtle cortex slices (Blanton

et al. 1989). In marked contrast to the method described above, no attempt is made to visualize individual cells. Instead the electrode is slowly advanced ('blindly') into the slice while positive pressure is applied to the back of the electrode. The first contact of the electrode with the slice produces a change in resistance which is ignored and the electrode is advanced further into the slice. A cell is detected by an increase in electrode resistance and then the positive pressure is removed, suction applied to the electrode and a seal attempted in the usual way. The amazing thing about this procedure is that it works very well! It is now widely used, particularly in hippocampal slices where it is viewed as easier than standard intracellular recording with sharp microelectrodes. Stuart *et al*. (1993) used a very similar procedure to the blind patching method (except the patch electrode was moved under visual control) to make recordings from the dendrites of cells in slices.

The advantages of the blind patching method are (a) it requires no sophisticated microscopy (a dissecting microscope is used to guide the electrode onto the right area of the slice) and so it is cheaper to set up, (b) no cleaning of the slice is necessary before recording, so saving time, (c) the cell is not disrupted by the cleaning procedure which could conceivably damage synapses on the cell body and (d) cells deep in the slice can be recorded from. The disadvantage of this method of course is that the actual cell is not visually identified. Blind patching may also tend to give lower seal resistances and less stable series resistance so that the quality of the final recording may be lower.

6. Applications of patch clamp recording from cells in slices

Clearly, the key to the success of this technique is that it brings all the power of patch clamp procedures to bear on the study of almost intact nervous system. The main advantages over previous approaches are increased signal resolution, absence of enzyme treatment, manipulation of the intracellular environment and the ability to record from identified cells which are essentially in their *in vivo* state.

The increased signal resolution obtainable results from the fact that, when using the whole-cell recording configuration, the resistance and capacitance of neurones are often high enough and low enough respectively to allow resolution of currents as small as a few pA at a bandwidth of 1 kHz. The electrode resistance (i.e. the series resistance in whole-cell recordings) and capacitance are the key places where the signal-to-noise ratio and recording bandwidth can be degraded. The electrode capacitance is often high because the electrode may be immersed several millimetres in the solution in order to reach under a water-immersion objective, so use of thick-walled glass and careful coating of the electrode with a hydrophobic coat is useful. In addition, blocking unwanted synaptic activity and other conductances in the cell either with drugs or by ion substitution can greatly reduce the background noise in a recording. When recording synaptic currents it may be an advantage to use a patch clamp fitted with a capacitor feedback headstage since this headstage design allows larger transient currents to be recorded at high

resolution than the traditional patch clamp with a resistor feedback in the headstage (see Levis & Rae, 1992 for discussion of the capacitor feedback headstage).

Using the whole-cell configuration, neurones can be rapidly filled with fluorescent dyes and their structure examined in living slices (Edwards *et al*. 1989). Access to the intracellular environment can be used to study second messenger pathways involved in signal transduction following synaptic transmission. Cells can be filled with Ca^{2+}-sensitive dyes and localized changes in intracellular Ca^{2+} concentration measured in response to synaptic transmission (Konnerth *et al.* 1992; Alford *et al.* 1993). It may also be possible to fill cells with 'caged' Ca^{2+} and use localized light flashes to produce discrete and localized changes in intracellular Ca^{2+}. Similar experiments are possible using other caged compounds such as caged ATP and caged IP_3 (see Chapter 15). In addition, patch clamping in slices is now being used to look at the properties of ion channels and receptors on identified cells at defined developmental stages of the nervous system (e.g. NMDA receptors: Hestrin, 1992b; Farrant *et al*. 1993).

In the future it is likely that more detailed and precise investigations of the mechanism of synaptic transmission will be made in which patch clamp techniques will be applied to recording from both pre- and post-synaptic neurones. For example, perfusing different fluorescent dyes into the pre- and post-synaptic neurones and using confocal laser-scanning microscopy (Edwards *et al*. 1989) may mean that the anatomy and function of synaptic connections can be studied together in a single experiment.

It is way beyond the scope of this article to mention even a small fraction of the present applications of patch clamp techniques to brain slice experiments and so here we will try to highlight a few situations where patch clamping in slices has been particularly useful.

Studies of synaptic transmission in slices

The advantage of studying synapses, receptors and ion channels in sliced brain tissue has been exploited in a variety of brain slice preparations but particularly, in the hippocampus (e.g. Edwards *et al*. 1990; Keller *et al*. 1991; Colquhoun *et al*. 1992), cerebellum (Konnerth, 1990; Farrant & Cull-Candy, 1991; Silver *et al*. 1992) and visual cortex (Stern *et al*. 1992; Hestrin, 1992a).

The properties of $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) were studied in hippocampal granule cells in order to investigate in detail the mechanism of inhibitory synaptic transmission (Edwards *et al*. 1990). The choice of this particular cell type was important in maximizing the resolution of the recordings. These cells have a small soma with long fine dendrites. The cell capacitance is therefore relatively small allowing wide-bandwidth voltage clamping and the cell input resistance is high (>1 GΩ) giving good signal resolution when care is taken to block as many of the cell ionic conductances as possible. Inhibitory synaptic connections are on, or close to the cell soma and so there is a good space-clamp of the synaptic currents.

Figure 2A shows examples of miniature and stimulus-evoked IPSCs recorded from a hippocampal granule cell. The inhibitory synaptic currents were found to have fast risetimes (0.5 ms) and were of relatively small amplitude: miniature currents were around 10 pA (equivalent to the activation of only about 10 $GABA_A$ receptor channels at the peak) and evoked currents were 10-100 pA. Distributions of the amplitude of miniature and evoked currents could be described by the sum of several Gaussian components (Fig. 2B) with the peak of each component being a multiple of the predominant component in the miniature IPSC amplitude distribution. These results are not consistent with the idea that the quantal size (size of miniature currents) is determined by the amount of transmitter in each transmitter packet (as seems to occur at the neuromuscular junction). Instead, the results support the hypothesis of a different mechanism for central synaptic transmission where the quantal size is determined by the number of receptors on the postsynaptic membrane (see Edwards *et al*. 1990 for discussion).

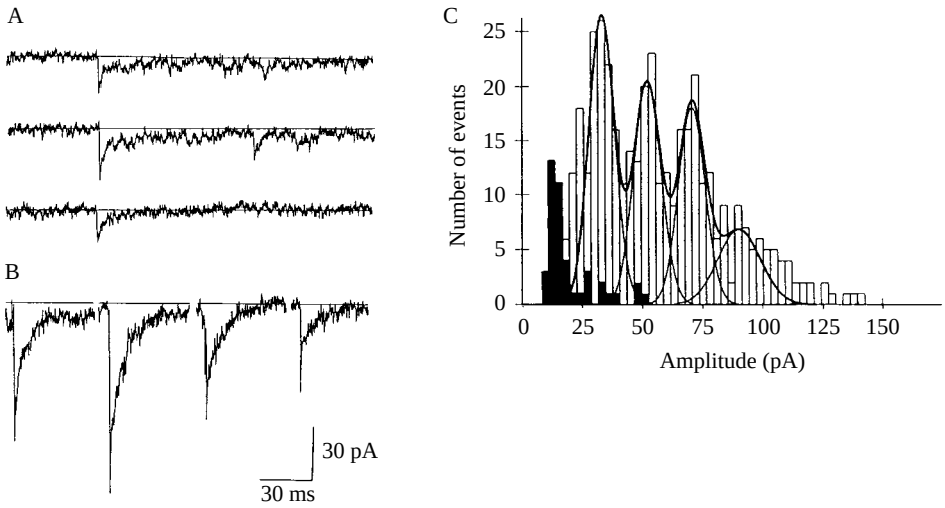

Fig. 2. Properties of miniature and stimulus-evoked IPSCs recorded from granule cells in hippocampal slices. (A) Three traces showing miniature IPSCs recorded in Krebs solution containing 1 μM tetrodotoxin. The traces are displayed with the peak of each current aligned. (B) Examples of four consecutive IPSCs evoked by stimulation of a nearby neurone in the slice. The stimulus was a rectangular voltage pulse of 5 V amplitude and 200 μs duration applied at a rate of 1 Hz. Traces were filtered at 2 kHz (−3 dB Bessel) and sampled at 10 kHz. Experimental details are given in Edwards *et al*. 1990. (C) Distributions of miniature and evoked IPSC amplitudes. The open bins show the distribution of the amplitudes of 428 evoked IPSCs in a cell voltage-clamped at −50 mV. The thick line superimposed on the histogram show the sum of 4 Gaussian functions which were fitted to the bins using a least-squares fitting routine. This was fitted over the amplitude range from 25-115 pA. The bin width is 3 pA. The Gaussian parameters fitted to the data were (mean±SD) 33±4.9 pA, 52±5.3 pA, 71±6.6 pA and 91±6.2 pA. The mean separation between the peaks was therefore 17.4 pA. The filled bins show the amplitude distribution of 43 miniature IPSCs recorded in the same cell in the presence of 0.5 μM TTX. The peak of the miniature IPSC distribution occurs at 13.8 pA. The background noise standard deviation was 2.8 pA. (Adapted from Edwards *et al*. 1990).

Single channel current recordings from cells in slices

A number of different labs now use patch clamping in brain slices as a means of obtaining single channel recordings (e.g. NMDA receptors, Gibb & Colquhoun, 1991; Edmonds & Colquhoun, 1992; Farrant *et al*. 1993; $GABA_A$ receptors, Edwards *et al*. 1990; glycine receptors, Takahashi & Momiyama, 1991). The rationale behind this approach is that the receptors and ion channels are presumably in the same location on the cell and in the same condition as they would be *in vivo*. This is potentially a powerful approach to elucidating the functional role of receptors and ion channels in central neurones since an understanding of the number, distribution and functional properties of receptors and ion channels is critical to understanding the processing of information at the neuronal level. At present, most of this information comes from recordings made from the cell body of neurones. However, it is quite likely that channel distribution and possibly functional properties are regulated according to location in the cell membrane and so techniques for recording from cell processes (Stuart *et al*. 1993) could be particularly useful in the future.

A second important reason for making single channel recordings from slices is that it allows information from *in situ* mRNA hybridization studies or antibody labelling studies to be used to pick cells expressing particular receptors or ion channel proteins. These results will contribute to determining the relationship between structure and function for different receptors.

Figure 3 shows examples of single NMDA receptor channel currents recorded from an outside-out patch excised from a CA1 cell in a hippocampal slice. The NMDA receptor is sensitive to nanomolar concentrations of glutamate and glycine and therefore when an outside-out patch containing NMDA receptors is isolated from a neurone in a slice, spontaneous channel activity is often evident due to background release of glutamate and glycine from the slice. In order to avoid this the patch is moved away from the slice towards the bath inflow and at the same time raised towards the surface of the bath reducing the depth of immersion of the electrode and so reducing the background noise in the recording.

Recordings like those shown in Fig. 3 were used to determine the properties of single NMDA receptor activations (Gibb & Colquhoun, 1991). Information from changes in the distribution of patch closed times (such as shown in Fig. 3B) with changes in glutamate concentration was used to identify which closed times and open times in the data record were likely to occur within a single receptor activation. Each activation was found to be composed of numerous openings and closings so that, although the distribution of open times (Fig. 3C) suggests a mean open time of around 3 ms, the length of each receptor activation will be several tens of ms. The results of these experiments led to the same conclusion as obtained from macroscopic experiments on NMDA receptor-mediated EPSCs and currents in outside-out patches: the time course of the NMDA receptor-mediated synaptic current is determined by the kinetics of the NMDA receptor activation (Hestrin *et al*. 1990; Lester *et al*. 1990).

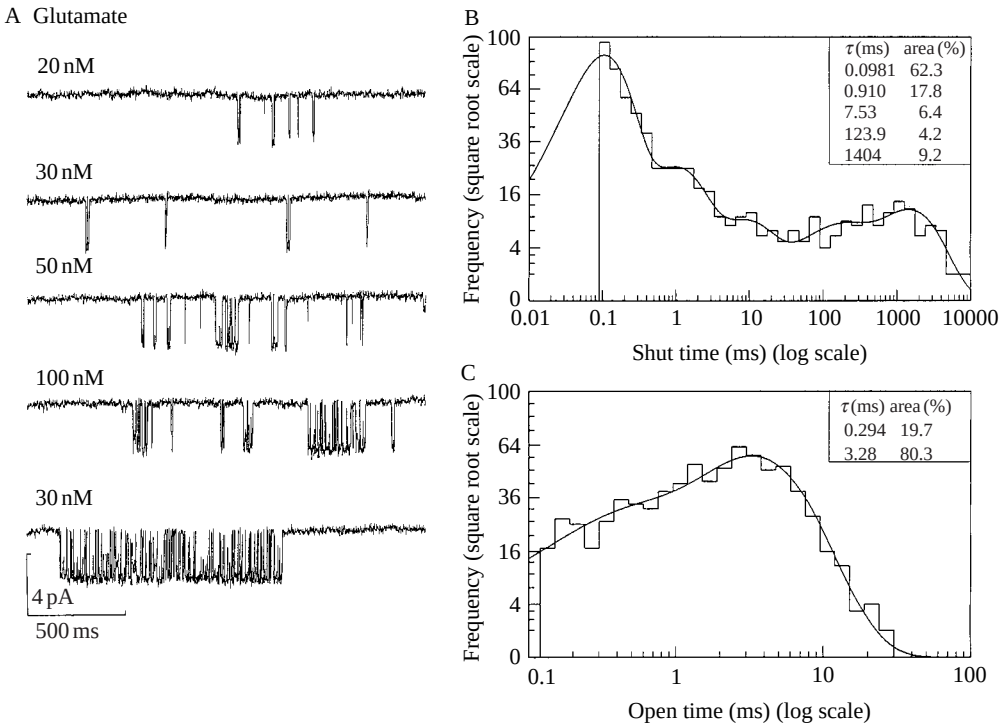

Fig. 3. (A) NMDA receptor channel. Examples of NMDA receptor channel openings recorded from a hippocampal CA1 cell outside-out patch in response to 4 different glutamate concentrations. Each trace shows a 2 second long period of activity. Each glutamate concentration was applied for between 120 and 200 seconds with 60 seconds wash between applications. The mean opening frequency was 3.48 s^{-1} at 20 nM and 19.9 s^{-1} at 100 nM. The lowest trace shows a clear example of the high activity periods which are occasionally observed at all glutamate concentrations. 1 μM glycine was present during all recordings. The patch membrane potential was −60 mV. (B) Distribution of 645 channel closed times recorded from an outside-out patch at a glutamate concentration of 30 nM. The mean measured closed time was 223 ms. The distribution is displayed using a log(t) transformation of the x-axis and a square root transformation of the bin frequencies (Sigworth & Sine, 1987) and fitted using the maximum likelihood method (Colquhoun & Sigworth, 1983; Colquhoun, Chapter 6) with 5 exponential components with time constants and relative amplitudes as shown. The fit predicts a total of 1061 gaps in the distribution with a distribution mean of 135 ms. The resolution for this record was 90 μs for closed times and 120 μs for open times. (C) The distribution of 767 openings from the same recording as analysed in B. The mean measured open time was 2.97 ms. The distribution was fitted with 2 exponential components and predicts that there were 847 openings in the distribution with a mean of 2.69 ms. (Adapted from Gibb & Colquhoun, 1991).

7. Conclusions

Although a relatively new technique, it is already clear that making patch clamp recordings from brain slices is an enormous advance in the study of brain function. The technique has allowed the application of all the patch clamp configurations to cells in brain slices and so many experimental approaches that are new to brain slices

are being used. Brain slices seem to be another major field of investigation that has been revolutionized by the application of patch clamp techniques. It is fun to speculate about which field patch clamping will be applied to next. As Fred Sigworth has commented (Sigworth, 1986), 'The patch clamp is more useful than anyone expected'!

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