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Monitoring local synaptic activity with astrocytic patch pipettes

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Abstract

Rapid signal exchange between astroglia and neurons has emerged as a key player in neural communication in the brain. To understand the mechanisms involved, it is often important to have access to individual astrocytes while monitoring the activity of nearby synapses. Achieving this with standard electrophysiological tools is not always feasible. The protocol presented here enables the monitoring of synaptic activity using whole-cell current-clamp recordings from a local astrocyte. This approach takes advantage of the fact that the low input resistance of electrically passive astroglia allows extracellular currents to pass through the astrocytic membrane with relatively little attenuation. Once the slice preparation is ready, it takes ~30 min to several hours to implement this protocol, depending on the experimental design, which is similar to other patch-clamp techniques. The technique presented here can be used to directly access the intracellular medium of individual astrocytes while examining synapses functioning in their immediate proximity.

INTRODUCTION

Background

A growing body of experimental evidence points to the molecular signal exchange between synapses and the surrounding astroglia as an important contributor to the activity of neural circuits in the brain, in health and disease¹⁻³. Over the past decade, knowledge about the versatile role of astroglia in neural function has expanded markedly, with multiple cellular mechanisms implicated in functional interactions between astroglial and neural networks⁴⁻⁷. Astrocytes *in situ* have been found to receive and process excitatory neurotransmitter signals via glutamate receptors expressed in their plasma membrane. These include Ca²⁺-permeable α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA_Rs), *N*-methyl-D-aspartate receptors and group I metabotropic glutamate receptors, with the latter cascade being associated with the robust induction of prominent and therefore best-documented astroglial Ca²⁺ signals (reviewed in ref. 8). The inhibitory neurotransmitter γ -aminobutyric acid (GABA) activates metabotropic GABA_B receptors in astrocytes, resulting in Ca²⁺ transients, which subsequently contribute to the regulation of astrocytic release of glutamate⁹ or ATP^{10,11}. Depolarization-dependent discharge of endocannabinoids from principal neurons activates astrocytic CB1 receptors also affecting downstream mechanisms of astrocytic glutamate release^{12,13}. Although the active signal transduction route from synaptic circuits to astroglia has been well established and almost universally accepted, the

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active role of astrocytes in shaping neural activity has been under debate^{8,14,15}. To provide a better understanding of the reported versatile effects of astroglia on neural communication, there is a growing need for experimental approaches that would enable causal association between the functioning of individual astrocytes and adjacent synaptic connections.

Other techniques available to monitor signal exchange between neurons and astroglia

A better understanding of the origins and physiological implications of molecular signals generated by cellular networks requires experimental probing of individual signaling cells. However, progress in deciphering astrocyte-synapse relations is often hampered by the difficulties of establishing a direct relationship between signals generated by individual astrocytes and their synaptic targets. To overcome this difficulty, some reported experimental designs feature sampling arrangements that aim to narrow down the group of synaptic connections associated with a particular astrocytic action. This aim, however, has to meet the standard electrophysiology requirement for having a relatively homogenous group of synapses that would faithfully represent the synaptic population under study.

In the context of neuron-to-neuron signal exchange, the most common and reliable method to probe the synaptic population of interest is associated with patch-clamp whole-cell recordings in individual cells. This approach, however, is rarely suitable for studying astroglia-neuron signaling, because individual protoplasmic astrocytes tend to occupy nonoverlapping, 70- to 80- μm -wide neuropil domains, whereas the spatial extent of neuronal dendrites often exceeds 500 μm . Therefore, synaptic activity sampled by the neuronal pipette is unlikely to faithfully represent synapses associated with the astrocyte in question (Fig. 1a), although successful attempts to restrict stimulation of presynaptic afferents to the astrocyte domain have been reported¹⁶. A more robust approach lies in sampling synaptic activity directly within the astrocytic tissue domain using an extracellular microelectrode (Fig. 1b). This method, however, has at least two potentially crucial disadvantages. First, the sampling volume of the extracellular electrode will be inevitably skewed with respect to the astrocyte soma, thus probably extending the catchment area of synaptic currents away from the astrocytic arbor. Second, it requires two microelectrodes (glass pipettes) to be positioned only 20–40 μm apart, which could be technically unfeasible if more than one astrocyte has to be tested (Fig. 1c). The latter arrangement, however, could be crucial in experimental designs in which a comparison between control and test cells *in situ* is required. To address these issues, we thus sought to find a robust approach to recording from more than one individual astrocyte while monitoring synaptic activity in local circuits.

Overview and validation of our technique

While developing our method, we took advantage of previous work reporting composition of whole-cell astrocytic responses^{17–20} and a simple analysis of a standard whole-cell voltage-clamp configuration in a brain slice experiment (Fig. 1d). In this mode of recording, the currents registered by the amplifier directly depend on the relationship between (i) the patch pipette impedance Z_{pip} , (ii) the cell membrane impedance Z_c , which varies with the synaptic conductance, and (iii) the apparent extracellular medium volume-conductor impedance $\{Z_{\text{ex}}\}$, which itself depends on the recording probe configuration (such as the pipette tip diameter and its immediate environment) and varies with the density of extracellular current sinks and sources (Fig. 1d). As a simple approximation, Ohm's law relates $Z_m(t)$, Z_{pip} and $Z_{\text{ex}}(t)$ to the overall impedance Z_{tot} sampled by the amplifier²¹:

$$Z_{\text{tot}}(t)^{-1} = (Z_m(t) + Z_{\text{pip}} + \{Z_{\text{ex}}(t)\})^{-1}$$

In the case of neuronal recordings, $Z_m(t) \sim 100\text{--}300\text{ M}\Omega$ (characteristic input resistance range), whereas the remaining $Z_{\text{pip}} + \{Z_{\text{ex}}(t)\}$ value is in the several-MO Ω range, implying that $Z_m(t) \gg Z_{\text{ex}}(t)$. Therefore, by reporting the $Z_{\text{tot}}(t)$ value, the amplifier detects predominantly synaptic conductance changes affecting $Z_m(t)$. Passive astrocytes, however, have an input resistance that is more than an order of magnitude lower than that of principal neurons— $Z_m \sim 5\text{--}6\text{ M}\Omega$ in our experiments; the values we observed were slightly lower than the values classically reported in younger (9–12-d-old) rodents²². In this case, therefore, $Z_{\text{tot}}(t)$ is as much sensitive to $Z_{\text{pip}} + \{Z_{\text{ex}}(t)\}$ as to $Z_m(t)$. In other words, the astrocytic whole-cell pipette can be used, at least in theory, to sample local extracellular current sinks contributing to $Z_{\text{pip}} + \{Z_{\text{ex}}(t)\}$ (Fig. 1e). However, in baseline conditions, the excitatory synaptic activity can induce significant inward glutamate uptake currents and longer-lasting outward potassium currents in astrocytic membranes^{17,19}. Because these electrical signals are superimposed with any signals of extracellular origin, it was important to validate this method empirically *in situ*.

In our studies, we use the experimental preparation of acute hippocampal slices. We have validated our new recording technique in CA1-area astrocytes by monitoring responses of CA3-CA1 excitatory synapses evoked by electrical stimulation of Schaffer collaterals, as described earlier²³. In brief, in each experiment, we compared classical field postsynaptic excitatory potentials (fEPSPs), recorded using a standard extracellular electrode, with voltage responses recorded via a whole-cell astrocytic pipette (positioned in the immediate vicinity of an astrocyte; Fig. 2a) in current-clamp mode. We found that in astrocyte responses the voltage difference measured between the downward peak and the upward deflection ($\sim 30\text{ ms}$ after stimulus, Fig. 2b top), termed the astrocytic fEPSP (a-fEPSP) amplitude hereafter, scaled linearly with the classical measure of the fEPSP slope reported by the extracellular pipette with no apparent bias from glutamate transporter or potassium currents across the range of stimulation strengths (Fig. 2c)²³.

To understand the electrophysiological mechanisms underlying this robust relationship, we analyzed the composition of evoked astrocyte responses in some detail. It has long been established that astrocyte currents evoked by afferent stimulation and recorded in voltage-clamp mode consist of the fast field response, a 30- to 50-ms inward glutamate transporter current and a longer-lasting inward K^+ current contributed to by presynaptic and postsynaptic sources^{17,19,20,24}. Because relative contributions and kinetics of all these components could be different in current-clamp configuration¹⁷, we carried out their pharmacological dissection while measuring the a-fEPSP amplitude as the net voltage difference between the two time points described above: these are shown as ΔV_{Peak} and $\Delta V_{30\text{ ms}}$ in Figure 3. First, we found that blockade of postsynaptic AMPARs with $10\text{ }\mu\text{M}$ 1,2,3,4-tetrahydro-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) completely abolished the a-fEPSP amplitude signal (Fig. 3a,d). This is because the residual presynaptic signal left after the AMPAR blockade (orange, Fig. 3a), which contributes to the values of ΔV_{Peak} (Fig. 3b) and $\Delta V_{30\text{ ms}}$ (Fig. 3c), is canceled out when reporting the $(\Delta V_{\text{Peak}} - \Delta V_{30\text{ ms}})$ value (Fig. 3d). Second, we found that (3S)-3-[[3-[[4-(trifluoromethyl)benzoyl]amino]phenyl]methoxy]-L-aspartic acid (TFB-TBOA)-sensitive glutamate uptake had negligible contribution to the a-fEPSP amplitude (Fig. 3d). The observation that the blockade of postsynaptic activity completely suppresses the a-fEPSP amplitude, with little influence of glutamate transporters, explains the excellent linear relationship between a-fEPSP and fEPSP measures (Fig. 2c)²³.

This relationship remained robust during the induction of long-term potentiation (LTP) in the same synaptic population, over a wide range of LTP expression levels (Fig. 4). This was consistent with the mainly postsynaptic expression of LTP in this circuitry^{19,20}. We note that, for several minutes after tetanus, before the LTP level is established, the a-fEPSP

amplitude can deviate from the fEPSP slope (Fig. 4a), probably because of abnormal accumulation of extracellular K^+ , which can temporarily distort a-fEPSPs. We also found that the a-fEPSP measure was more accurate in representing extracellular fEPSPs than was the $V_{30\text{ ms}}$ value alone (Fig. 4b). Furthermore, our experiments showed that the initial slope of a-fEPSP traces (which is the standard measure in extracellular fEPSPs) was not fully eliminated by blocking AMPARs (Fig. 5a), in contrast with the complete suppression of the a-fEPSP amplitude (Fig. 3d). Correspondingly, the a-fEPSP slope showed a significant erroneous (false-positive) response after AMPAR blockade (Fig. 5b), probably because the remaining components of the transporter and K^+ signal contributed to the slope value. Finally, we tested whether the a-fEPSP amplitude could substitute for the fEPSP measure in gauging paired-pulse ratios and therefore changes in release probability of evoked responses. Our data indicated that the correlation between a-fEPSP and fEPSP measures was significant but showed a substantial data scatter for the paired-pulse ratio (Fig. 5c). Again, the increased data scatter was probably due to the evoked K^+ signals, which may last for >100 ms after a pulse and thus introduce a bias in the a-fEPSP values measured for the second response. Taken together, these control experiments provided evidence that the amplitude of a-fEPSPs recorded with an astrocyte pipette can faithfully represent the magnitude of evoked synaptic responses, at least in common conditions of single-stimulus trials.

Experimental design

A number of factors, such as pharmacological manipulations, location and method of stimulation, or the age of animal subjects, may change K^+ homeostasis or glutamate transporter kinetics and thus affect the waveform of a-fEPSPs. In such cases, control experiments may be required to understand the sensitivity of individual a-fEPSP components to the factors in questions. It should normally suffice to test how a-fEPSPs change upon blockade of postsynaptic activity (e.g., with AMPAR blockers such as NBQX) and glutamate uptake (e.g., using specific blockers such as TFB-TBOA²⁵). This dissection will help establish which particular measure of a-fEPSP is most representative of the postsynaptic response (Fig. 3a). Once the measure is selected on the basis of its pharmacological properties, one has to establish that it scales linearly with the slope of extracellular fEPSPs, the classical readout of postsynaptic current sinks (Figs. 2c and 4b).

When comparing synaptic responses (and their use-dependent changes) in two or more independent afferent pathways using two or more astrocytic recordings (Figs. 1e and 6), one has to bear in mind that physiological and morphological features of the chosen pathways may differ in a consistent way. To avoid any related measurement bias, such experiments have to be repeated while shuffling test and control pathways randomly. Alternatively, additional tests might be required to establish that all pathways in question are undistinguishable in terms of their morphological or functional features, including the properties of recorded astrocytes.

Advantages and limitations

The principal advantage of this method is that it allows recording, in whole-cell mode, from two neighboring astrocytes while comparing evoked synaptic activity in the immediate proximity of either cell (Fig. 6a). Because this protocol enables the manipulation of the intracellular medium independently in either astrocyte, we were able to show that clamping Ca^{2+} in the test astrocyte prevented the maintenance of synaptic LTP at nearby synapses, whereas LTP was at the baseline level at synapses near the control astrocyte (filled with a control solution, Fig. 6b). Another potential advantage of this approach is that whereas in normal conditions the a-fEPSP amplitude is insensitive to glutamate uptake and, similarly to classical fEPSPs, it cannot distinguish between presynaptic or postsynaptic origins of

amplitude changes, its pharmacological dissection (Fig. 2d) could unmask transporter currents and therefore enable monitoring release probability^{17,19,20} using the same astrocyte pipette.

There are several potential limitations to this protocol. First, it is not always possible to gauge precisely the extent of the neuropil volume contributing to the synaptic activity sampled by the recording astrocytic pipette. This limitation is, however, common for most extracellular recording techniques. Our experiments in which two neighboring astrocytes were patched (Fig. 6) suggest that the two corresponding synaptic pools can be readily distinguished by the two astrocytic pipettes. Second, some pharmacological manipulations can specifically alter glutamate uptake or potassium current kinetics recorded in astrocytes. Because this effect could potentially bias the a-fEPSP amplitude measure, control experiments have to be carried out (Fig. 3) to establish whether any adjustment is required. By the same token, because evoked K^+ currents can last for >100 ms after stimulus, the a-fEPSP amplitude measure could be biased during paired or repetitive stimuli (Fig. 5c), or for some period after tetanus (Fig. 4b). Again, such cases should be investigated using pharmacological dissection (Fig. 3) and direct comparison with extracellular fEPSP recordings (Fig. 2c). Finally, a-fEPSP recordings may be sensitive to the physiological condition of the recorded astrocyte. As with standard patch-clamp experiments, cell condition needs to be monitored using the resting membrane potential, the input resistance and, if available for measurements, the basal Ca^{2+} level.

Below we describe the protocol for recording synaptic field responses from within astrocytes. Standard procedures and equipment in electrophysiology, such as preparation of hippocampal slices, are not discussed unless they are crucial to the method or to its troubleshooting; detailed guidance on this can be found in refs. 26-30. We note that the present protocol requires some hands-on experience in patch-clamp electrophysiology of neurons and astroglia, in particular the standard skills to identify healthy cells and maintain them in stable conditions in whole-cell mode.

MATERIALS

▲ **CRITICAL** All reagents and equipment mentioned can be obtained from alternative suppliers/manufacturers.

REAGENTS

- $CaCl_2$, glucose, sucrose, HEPES, KCH_3O_3S , KCl, KOH, $MgCl_2$, $MgSO_4$, Na_2 -ATP, NaCl, Na-GTP, $NaHCO_3$, NaH_2PO_4 and Na_2 -phosphocreatine (all from Sigma-Aldrich)
- (Optional) Alexa Fluor 594 hydrazide, Oregon Green 488 BAPTA-1 (Invitrogen)
- Deionized water, at least 15 MΩ cm at 25 °C ▲ **CRITICAL** Water quality is especially important for intracellular solutions. If the quality of water produced by the available purifier is too low, an alternative is to buy ultrapure water, at least for intracellular solutions.
- Four-week-old rats (e.g., Sprague-Dawley or Wistar; male or female mice may be used, depending on research objectives) ! **CAUTION** All animal studies must be reviewed and approved by the institutional bodies overseeing animal care and use. All relevant ethics regulations have to be strictly followed. ▲ **CRITICAL** The age of the animals needs to be carefully chosen, because astrocytes and astrocyte networks may mature differently from neuronal networks.

EQUIPMENT

- An electrophysiology setup for submerged slices equipped with $\times 40$ to $\times 60$ infrared differential interference contrast (DIC) visualization, a patch-clamp amplifier capable of current and voltage clamp (e.g., MultiClamp 700B) and a constant current computer-driven stimulus isolator (e.g., DS3, Digitimer). An example of the working setup coupled with the two-photon excitation and two-photon uncaging system was described earlier³¹ ▲ **CRITICAL** The use of optimized infrared DIC optics with a $\times 40$ to $\times 60$ objective, with further $\times 2$ to $\times 4$ magnification, is advantageous for visualizing astrocytes ($\sim 10\text{-}\mu\text{m}$ soma size) up to $100\text{--}150\text{ }\mu\text{m}$ deep into the brain slice, where the tissue structure and cell physiology are least perturbed.
- Recording electrodes to record both classic field potentials and astrocyte field potentials pulled from borosilicate glass (e.g., GF150F-3, Warner Instruments). Pipettes with short taper and a resistance of $3\text{--}4\text{ M}\Omega$ appear to be well suited for recording of astrocyte field potentials (a-fEPSPs)
- Bipolar or monopolar stimulation electrodes (e.g., self-assembled from UESMGGSEFNNM or CBARC75, both from FHC) to be used for extracellular stimulation of axonal afferents

REAGENT SETUP

Slicing solution—Slicing solution is 80 mM NaCl , 65 mM sucrose , 2.5 mM KCl , 7 mM MgCl_2 , $1.25\text{ mM NaH}_2\text{PO}_4$, 0.5 mM CaCl_2 , 26 mM NaHCO_3 and 10 mM glucose (osmolarity, $300\text{--}310\text{ mOsm}$). Slicing solution should be freshly prepared on the day of the experiment.

Intracellular solution—Prepare a solution containing $135\text{ mM KCH}_3\text{O}_3\text{S}$, 10 mM HEPES , $10\text{ mM Na}_2\text{-phosphocreatine}$, 4 mM MgCl_2 , $4\text{ mM Na}_2\text{-ATP}$ and 0.4 mM Na-GTP . Adjust the pH to 7.2 using KOH; the resulting solution, osmolarity has to be in a narrow range of $290\text{--}295\text{ mOsm}$ per liter: if it is outside this range, the salts have to be checked for contamination and the solutions freshly prepared. The solution is normally prepared in advance and stored at $-20\text{ }^\circ\text{C}$ in $\sim 1\text{-ml}$ aliquots (use one or two aliquots per experiment). We routinely add the membrane-impermeable dyes Oregon Green 488 BAPTA-1 (OGB1, $200\text{ }\mu\text{M}$) and Alexa Fluor 594 hydrazide ($40\text{ }\mu\text{M}$) to the intracellular solution. The morphological fluorescent dye is advantageous for cell identification if an imaging system is available. Although adding OGB1 is not directly relevant to the current protocol, it was used in the original study²³. If, however, Ca^{2+} -sensitive indicators are not used, alternative Ca^{2+} buffers should be considered, in accordance with standard electrophysiological protocols. ! **CAUTION** Because the composition of intracellular solution can affect transporter currents¹⁷, it should be checked against published recommendations with respect to specific pharmacological designs.

Extracellular solution—Prepare a solution containing 119 mM NaCl , 2.5 mM KCl , 1.3 mM MgSO_4 , $1\text{ mM NaH}_2\text{PO}_4$, 26 mM NaHCO_3 , 2 mM CaCl_2 and 10 mM glucose . This should be freshly prepared on the day of the experiment or from premade concentrated stocks (normally stored in a refrigerator at $5\text{ }^\circ\text{C}$ for up to $1\text{--}2\text{ d}$), except for NaHCO_3 , CaCl_2 and glucose, which need to be added on the day of the experiment. ▲ **CRITICAL** Before adding CaCl_2 , the extracellular solution needs to be continuously bubbled with $95\%\text{ O}_2/5\%\text{ CO}_2$ for at least 10 min . The osmolarity of the extracellular solution should be $295\text{--}305\text{ mOsm}$ per liter. The pH of the bubbled solution should be 7.4.

PROCEDURE

1| Prepare acute, 350- μ m-thick transverse slices from isolated rat or mouse hippocampi using an appropriate standard, long-established technique²⁸⁻³⁰ optimized to preserve the main hippocampal circuitry. For example, we cut acute slices using ice-cold slicing solution (see Reagent Setup) and store the slices in the same solution at 34 °C for 15 min. All solutions must be continuously bubbled with 95% O₂/5% CO₂.

? TROUBLESHOOTING

2| Place the slices in an interface-type storage chamber in extracellular solution in a humidified atmosphere containing 95% O₂ and 5% CO₂. Slices need to rest for at least 1 h before an experiment. While the slices are resting, prepare the electrophysiology setup for the recording. Ensure that the well-bubbled extracellular solution has been circulating for at least 10 min before the start of recording.

3| Place and fix the slice in the recording chamber. To isolate excitatory CA3-CA1 connections, we routinely block inhibitory GABAergic transmission (e.g., by 50 μ M picrotoxin added to the extracellular solution). In this case, it is advisable to make a cut between CA3 and CA1: this will suppress epileptiform activity in the slice.

4| Place the stimulation electrode into the slice near the border between the two connected regions being studied (for example, the border of CA1 and CA3) under visual control, using low magnification.

▲ **CRITICAL STEP** The stimulation electrode should be placed far away from the site of recording (e.g., the middle of the stratum radiatum of the CA1 region) to predominantly stimulate axons synapsing in the recording region and to prevent direct current injections into postsynaptic neurons and astrocytes in the recording region.

5| Find and patch an astrocyte in the whole-cell patch-clamp configuration. To find an astrocyte in the CA1, switch to high-magnification infrared DIC visualization and look for small cell bodies in the CA1 stratum radiatum at a depth beyond ~40 μ m into the slice. Although astrocytes usually have a few main processes originating from the soma, in contrast to neurons they are often barely visible. After establishing the whole-cell configuration, the recorded cell must be identified as an astrocyte. This can be done by confirming that it has a lower membrane potential than –85 mV, symmetric responses in both the voltage- and current-clamp modes, no action potentials in response to strong depolarization and an input resistance well below 10 M Ω .

▲ **CRITICAL STEP** Because the cell input resistance is often comparable to or lower than the access resistance, it is advantageous to calculate the input resistance from the voltage responses to current injections in current-clamp mode, with careful adjustment of the bridge balance.

▲ **CRITICAL STEP** The use of fluorescence microscopy or two-photon excitation imaging, if available, helps to better characterize the cellular identity of the patched cell in acute slices. Astrocytes patched with an Alexa 594-containing intracellular solution should show the typical ‘bushy’ appearance (Fig. 2a), and after a few minutes of dialysis, the neighboring gap junction-coupled astrocytes should be observed (Fig. 6a)²³.

? TROUBLESHOOTING

6| Record astrocyte field potentials. Evoke voltage deflections of the astrocyte membrane potential in current clamp by using short (100 μ s) rectangular current pulses delivered through a standard extracellular stimulating electrode placed in the Schaffer collateral area (Figs. 2 and 3).

▲ **CRITICAL STEP** Before beginning an actual experiment, the stimulus strength (injected current or applied voltage) needs to be optimized, usually at 20–50% of the strength at which responses saturate. In any case, stimulation intensities should be limited to a range well below values at which any visible damage to the tissue around the stimulation electrodes can be observed during high-frequency stimulation.

7| Store data on a hard disk in a standard file format. The typical data set collected in these experiments includes standard traces of electrophysiological (patch-clamp) recordings. These traces represent real-time electric current (or voltage) responses in individual recording trials, usually lasting up to 1,000 ms each; the total number of trials over one experiment varies depending on the experimental design, and may reach many hundreds. The choice of file format will depend on the software that is used to control and carry out experimental recordings, and the same or compatible software has to be used to read and analyze recorded traces. Standard patch-clamp techniques and conventions can be applied for trace storage, filtering and analyses²⁶. To obtain information about synaptic currents from astrocytic recordings, measure the traces as described in ref. 26 and illustrated in the present protocol (Fig. 3a).

? TROUBLESHOOTING

Step 1, preparation of acute brain slices

The preparation of acute brain slices has been extensively studied, and all relevant technical issues have been described in the literature^{28–30}.

Step 5, establishing the whole-cell patch-clamp configuration

This is relatively straightforward. We have found that passive astrocytes can be successfully probed in the whole-cell mode using a variety of intracellular solutions. In most cases, when establishing the giga-seal configuration was difficult, faulty intracellular solutions and brain slice quality were found to be mainly responsible. Increasing the pipette resistance beyond 5 M Ω simplifies the procedure but increases the access resistance, which needs to be low for recording a-fEPSPs and for efficient dialysis of the astrocyte.

Step 5, cell identification

Unfilled passive astrocytes do not have a highly distinct morphology when viewed with infrared DIC optics. If the patched cells spike (i.e., are excitable neurons), one should try to patch smaller cells with more round-shaped bodies. It is beneficial to get used to the astrocyte morphology by labeling them acutely with SR101 (ref. 32) and systematically comparing their fluorescence images with their appearance in DIC. However, the dye has been reported to have side effects³³, and therefore its use on a regular basis should be considered with caution. In our hands, good brain slice quality also improved the chances of patching passive astrocytes as opposed to other glial cells or neurons.

Low-amplitude or unstable a-fEPSPs usually result from the deteriorating slice health, even when patch-clamp conditions appear stable. This can be easily diagnosed using classical fEPSP recordings, which should show the hallmarks of the synaptic pathway under study. For instance, for Schaffer collaterals, indicators of suitable recording conditions include

robust paired-pulse facilitation and a relatively small fiber volley compared with a large fEPSP.

● TIMING

Step 1, slice preparation: 1 h

Step 2, rest period and preparation: 1 h

Steps 3 and 4, recording setup: 15 min

Step 5, find, patch and identify astrocyte: variable

Step 6, set up stimulus intensity: 5 min; sample experiment with dual astrocyte recordings: ~2 h, including setup of the recording configuration

Step 7, sample storage and analysis: variable, depending on the experimental design and required data modality

ANTICIPATED RESULTS

Typically, results are in the form of real-time current traces recorded from astrocytes and stored off-line as individual trials each lasting up to 1,000 ms (Figs. 2b and 3a; also example traces in Figs. 4a and 6b). The total number of such trials per experiment varies depending on experimental design and recording stability, but each experimental epoch (i.e., baseline condition or time during drug application) is normally represented by at least several dozens of individual trials. Individual trial traces, or their measured parameters (as in Fig. 3a), are often averaged over each experimental epoch, and the epoch averages are then analyzed statistically (Figs. 2b, 3b-d and 4b). Real-time pharmacological manipulations during cell recording enable paired statistical comparisons in the same cell (e.g., baseline versus drug response as in Fig. 3, or baseline versus LTP response as in Fig. 4). This provides a high-power statistical design. Normally, in acute slice patch-clamp experiments, one cell is recorded per slice and often per animal, in which case the standard statistical unit can be equally thought of as a cell, a slice or an animal. An additional advantageous feature of the present protocol is that it facilitates data collection both from test and control astrocytes simultaneously, thus allowing paired comparisons plus direct *in situ* control for the effect under study (Fig. 6).

One immediate application of the protocol is to monitor the effect of astrocytic intracellular Ca^{2+} signaling, or other signaling cascades, on excitatory transmission or its use-dependent plasticity, such as LTP, at nearby synapses. This can be achieved by using dual astrocyte whole-cell recordings in order (i) to block Ca^{2+} signaling (or to perturb other signaling cascades) independently inside the test astrocyte by loading it with an appropriately modified intracellular medium, and (ii) to compare local synaptic responses between the corresponding control and test pathways (Fig. 6). In the case of the classical LTP paradigm, after establishing the recording configuration, Schaffer collaterals are stimulated twice per min for at least 10 min of baseline recording before a high-frequency stimulus is delivered ($3 \times$, 100 pulses at 100 Hz). The ability of the high-frequency stimulus to induce plasticity can then be investigated by continuing stimulation twice per minute and recording the astrocyte field potential (Fig. 6).

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Figure 1.

Whole-cell recordings using an astrocyte patch pipette are sensitive to extracellular field potentials. **(a)** Depiction of whole-cell recordings from individual principal neurons and adjacent astrocytes. Note that the neuronal somatic pipette will normally report electrical activity at synapses across the entire dendritic tree (including space clamp-related attenuation; blue shadow), whereas synapses affected by the recorded astrocyte represent only a small proportion of synaptic inputs on the recorded neuron. **(b)** Illustration of the protocol for recording synaptic activity in the vicinity of a patched astrocyte using an extracellular recording pipette. Note that the populations of astrocyte-affected and monitored synapses overlap but are unlikely to coincide. **(c)** An extension of the experimental arrangement shown in **(b)** for testing the influence of two neighboring astrocytes on synaptic activity in the test (orange) and control (green) pathways using standard field recording techniques with extracellular pipettes. Note that, in addition to the incomplete overlap of astrocyte-affected and recorded synaptic populations, patching two close astrocytes and putting two more recording electrodes in the immediate vicinity of the patched cells is technically challenging. **(d)** Illustration of the relationship between cell membrane and extracellular currents recorded in a cell. Notations: Z_m , Z_{pip} and $\{Z_{ex}\}$ denote, respectively, the impedance of the pipette, of the cell membrane and of the apparent extracellular medium volume conductor (shown in brackets, because it is intricately linked to the probe pipette properties and therefore Z_{pip}). **(e)** A diagram of the experimental arrangement in which two pipettes are used to hold two neighboring astrocytes in the whole-cell mode while monitoring extracellular synaptic potentials in their immediate vicinity.

Figure 2.

The amplitude of field responses recorded through an astrocytic whole-cell pipette (a-fEPSPs) faithfully represents the initial slope of fEPSPs recorded with a standard extracellular electrode. **(a)** Experimental arrangement; area CA1 of acute hippocampal slices. A fluorescence image of a patched astrocyte (left, Alexa Fluor 594 channel, $\lambda_{\text{ex}}^{2\text{P}} = 800 \text{ nm}$, 50–60 μm deep in the slice, single optical section) and the same arrangement in the DIC channel (right). Electrical stimuli are applied to Schaffer collaterals through a bipolar extracellular stimulating electrode (not shown), and responses are recorded both through an extracellular glass electrode placed in the immediate proximity of the astrocyte (ep, extracellular pipette) and a whole-cell astrocytic patch pipette (ip, intracellular pipette), as depicted (For illustration clarity, gap junctions in this example were blocked with carbenoxolone; such blockade however, should be consistent with the research objectives.). **(b)** The voltage dynamics recorded in response to afferent stimulation. Top, electrical responses of a passive astrocyte held in current-clamp mode (a-fEPSP), and their respective stimulus strengths; arrow and dashed lines show the a-fEPSP amplitude measure. Bottom, simultaneously recorded local extracellular fEPSPs at the same stimulus strength values (color coded). **(c)** Statistical summary for $n = 4$ acute slices; dots and error bars are means $\pm$ s.e.m. for individual experiments. The data indicate that the a-fEPSP amplitude (as shown in traces) is tightly correlated with the fEPSP slope across the sample, with no detectable bias from the components representing glutamate transporter current or potassium conductance; dashed line, regression line; R , coefficient of correlation; NBQX, data under the AMPA receptor blockade with 10 μM NBQX. The data shown in this figure were obtained while performing the experiments described in ref. 23.

Figure 3.

The electrophysiological makeup of a-fEPSPs makes them suitable for representing extracellular fEPSPs. **(a)** a-fEPSPs in baseline conditions (black, average of ten trials), after AMPAR blockade with 10 μ M NBQX (orange), and glutamate transporters blockade with 500 nM TFB-TBOA (green). Voltage deflections are shown measured relative to pre-pulse baseline (horizontal dashed line) at the downward peak (ΔV_{Peak}) and 30 ms after the stimulus ($\Delta V_{30 ms}$), as indicated. Presynaptic excitation reflected by the fiber volley (FV, gray arrow) remains unchanged throughout. **(b)** The downward peak deflection was reversed upon AMPAR blockade ($n = 12$, $P < 0.001$), and was inhibited by the subsequent blockade of glutamate transporters ($n = 8$, decrease by 0.056 ± 0.009 mV, $P < 0.001$). **(c)** The longer-lasting increase of the membrane potential (upward deflection) after stimulus was reduced by NBQX (by 0.16 ± 0.061 mV, $P < 0.03$, $n = 12$), but remained highly significant above zero ($P < 0.001$). Subsequent blockade of glutamate uptake produced an insignificant reduction (by 0.037 ± 0.019 mV, $n = 8$, $P = 0.13$). **(d)** The a-fEPSP amplitude measure ($\Delta V_{30 ms} - \Delta V_{Peak}$) was reduced virtually to zero by the AMPAR antagonist NBQX ($P < 0.001$, $n = 12$; control (Ctrl): 0.78 ± 0.087 mV, NBQX: -0.0039 ± 0.0232 mV), with no significant effect of subsequently applied TBOA, as indicated. Error bars are s.e.m.

Figure 4.

The a-fEPSP amplitude measured using an astrocyte pipette robustly represents extracellular fEPSPs during long-term potentiation. (a) One-cell example. Blue and red circles, the slope of fEPSP in stratum radiatum and the a-fEPSP amplitude, respectively. Red arrow, high-frequency stimuli (three trains of 100 pulses at 100 Hz, 60 s apart) applied at time point zero. Gray-shaded area, experimental epoch for measuring established (steady-state) LTP. The temporary departure of a-fEPSP data points from fEPSP data points after LTP induction (0–5 min) is likely to reflect transitory (abnormal) changes in the K^+ and transporter current induced by tetanus; these post-tetanus data were not used for LTP measurements.

Experimental arrangement as in Figure 2a. (b) Statistical summary of experiments depicted in a ($n = 37$ slices). Red dots, LTP expression in individual experiments relative to baseline, measured using the initial fEPSP slope (abscissa) and the a-fEPSP amplitude (left ordinate; all individual slices are shown, including those showing insignificant LTP). Gray dots, a subset of experiments ($n = 15$) in which the high frequency-induced changes in $\Delta V_{30 \text{ ms}}$ (Fig. 3a) were documented (right ordinate), showing relatively weak correlation with the fEPSP slope. The data shown in this figure were obtained while performing the experiments described in ref. 23.

Figure 5.

Testing the suitability of other a-fEPSP components to represent the classical fEPSP initial slope measure. **(a)** The initial slope of the a-fEPSP was measured, using the standard fEPSP measurement method, in baseline conditions. Application of $10 \mu\text{M}$ NBQX (orange; see Fig. 3) left a significant positive slope remaining ($0.042 \pm 0.010 \text{ V s}^{-1}$, $n = 12$, $P < 0.01$), and the addition of TBOA (green) had no significant further effect ($n = 8$, $P = 0.85$). **(b)** Relative errors of the a-fEPSP measures (amplitude and initial slope) due to astrocyte response components unrelated to synaptic AMPAR activation; the error was estimated as the residual value in NBQX relative to control (Ctrl; baseline) response. The a-fEPSP amplitude measure gave a negligible error ($-1.0 \pm 2.6\%$), whereas the a-fEPSP slope was significantly biased ($-19.7 \pm 4.8\%$, $P = 0.0024$, $n = 12$). **(c)** The paired-pulse ratios (PPR, 50 ms interpulse interval) for the fEPSP slope and a-fEPSP amplitude ($n = 13$ slices) are positively correlated, albeit with prominent data scatter. Error bars show s.e.m.

Figure 6.

Individual astrocytes influence LTP induction mainly at nearby synapses: an experiment enabled by the recording of local synaptic activity through an astrocytic patch pipette. **(a)** In acute hippocampal slices, two neighboring astrocytes (a1 and a2) are held in whole-cell mode, filled with two different intracellular solutions: control solution (see Reagent Setup) containing 200 μM OGB-1 and 40 μM Alexa Fluor 594 hydrazide, and ' Ca^{2+} clamp' solution containing, in addition to the above, 0.45 mM EGTA and 0.14 mM CaCl_2 to clamp intracellular free Ca^{2+} at a steady-state level of 50–80 nM (calculation by the web-based MaxChelator, <http://maxchelator.stanford.edu/>). Imaging: Alexa Fluor 594 channel; $\lambda_{\text{x}}^{2\text{P}} = 800 \text{ nm}$ ($\sim 150 \mu\text{m}$ z-stack; false colors). Further astrocytes filled via gap junctions can be seen. **(b)** Having a Ca^{2+} clamp solution inside the test astrocyte suppresses local synaptic LTP, but not LTP, near the neighboring control astrocyte. Graph, a-fEPSP amplitudes (mean $\pm$ s.e.m.) recorded from the control (blue) and test (red) astrocytes ($n = 9$). Traces, respective characteristic a-fEPSPs recorded before (color-coded as above) and after (gray) LTP induction. The data shown in this figure were obtained while performing the experiments described in ref. 23.