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Electrophysiological investigation of microglia

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Running Head: Patch-clamp recording of microglia

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Abstract

Although microglial cells are not electrically excitable, they express a large repertoire of ion channels that are activated by voltage, stretch, extracellular ligands or intracellular pathways (*e.g.* Ca^{2+} , G-proteins). The patch clamp technique is the electrophysiological method of choice to study these channels whose expression varies largely in pathological conditions but also during normal development and aging. This chapter focuses on protocols allowing the recording and the analysis of these channels in acute brain slices, with a particular emphasis on the study of channels activated by extracellular ligands.

Key words: microglia, patch recording, acute slice, purinergic receptor, uncaging, $\text{CX}_3\text{CR1}$

1 Introduction

Microglial cells are probably the most plastic cells of central nervous system, remodeling their phenotype upon rupture of brain homeostasis in pathological conditions, but also during normal development and aging in reaction to more subtle changes of their environment. Changes in the expression of membrane channels and of G-protein coupled receptors (GPCRs) that lead to the activation of membrane channels constitute an important component of this remodeling and regulate key functions of these immune cells, including proliferation, migration, cytokine release and reactive oxygen species production [1–3].

Pioneer studies of microglia electrophysiology were conducted in primary cultures and in cell lines. They revealed, in culture resting conditions, the expression of inwardly rectifying potassium currents activated by hyperpolarization. An additional outward potassium current activated by depolarization was found upon treatment of the cultures with the bacterial endotoxin lipopolysaccharide (LPS) or interferon-gamma [4–7]. We now know that cultured microglial cells are in a particular reactive state even in the absence of pathological stimulus (such as LPS), and subsequent studies conducted *ex vivo*, in acute slices showed that non-reactive microglia in the adult brain do not express these potassium channels. However, in pathological conditions, or at different steps of the development, the expression of these channels is indeed up-regulated [8–11]. Moreover, surveying (i.e. non-reactive) microglial cells also express several ligand-gated ion channels and G-protein coupled receptors (GPCRs), among which purinergic receptors are central in regulating specific functions (e.g. chemotaxis, cell and process motility, phagocytosis, release of mediators). The expression of these receptors is also modulated according to the pathophysiological environment of the cells, thereby impacting on their functional properties and contributing to the diversity of microglia reactive phenotypes [12, 13], see also Hirbec et al., in this volume).

Schilling and Eder have previously reviewed in details the methods to record different types of voltage- and G-protein-activated membrane channels expressed by microglia in culture and in acute slices [14]. In the present chapter, we will focus on the approaches aiming at recording membrane currents induced by the activation of ligand receptors in acute slices of the CNS.

The reactivity of microglia cells is a rapid process that constitutes a limitation to the electrophysiological investigation of their properties. Even when using the best slicing procedures, cell damage at the surface of the slices cannot be avoided and will be a trigger of microglia reactivity. As a general rule, we usually record microglia during the first 0.5 to 3 - 4 hours following slicing. The best known electrophysiological markers of microglia reactivity, the inwardly and outwardly rectifying potassium channels, are not up-regulated during this time windows and it takes usually 12 to 24 h *in vivo* to induce their expression in response to a pathological stimulus [15]. However, this does not exclude that the expression of other channels and receptors could occur more rapidly, and this should be kept in mind when designing and interpreting slice experiments.

2 Materials

2.1 Equipment for identification and patch-clamp recordings of microglia in acute slices

1. Basic equipment for patch-clamp recordings of microglia is similar to that used for other cell types and includes an anti-vibration table, one or more micromanipulators (if possible with remote control), a patch-clamp amplifier, an analog-to-digital converter.
2. Slices should be maintained in a recording chamber, superfused with an artificial cerebrospinal fluid (see extracellular solution below), with a glass coverslip forming the bottom of the chamber, and should be placed on the optical path of an upright microscope (*see Note 1*).

3. Upright microscopes for patch-clamp experiments are usually equipped with optics allowing the observation and the identification of the cells within the slices (*e.g.* differential interference contrast, Dodt contrast). To get a better image deeper in the slices, the source of the incident light should be filtered in the near infra-red range (700 - 800 nm). A system for epifluorescence illumination and observation is necessary to identify fluorescent microglia, either in transgenic mice or after acute labeling (*see Note 2*, Fig. 1A). Combining the use of transmitted light with visualization of the fluorescence helps for the placement of the pipette and the formation of the seal. This can be achieved easily by removing the emission filter from the fluorescence cube or by using a long path filter allowing the transmission of the infra-red light.
4. Two-photon microscope is often used to study microglial motility. Protocols for studying microglia by means of two-photon microscopy are beyond the aim of the present chapter (but see **chapter XX** in this volume). However, if a two-photon microscope is available, it could be used to take a general image of a relative large volume to image several microglial cells deep in the tissue with a high spatial resolution, thus helping the identification of the cells to patch (Fig. 1B, C; see also below).

2.2 Animals

There are now several lines of transgenic mice that express a genetically encoded fluorescent protein in microglia. The most popular one is the CX₃CR1^{+eGFP} mouse [16]. This is a knock-in mouse in which an exon of the *Cx3cr1* gene has been replaced by an eGFP cassette. This gives extremely bright microglial cells that are easy to identify in acute slices and with no real problem of bleaching (Fig. 1A - C). Heterozygous animals should be used as homozygous mice are knockout for CX₃CR1 (*see Note 3*), the receptor of the chemokine fractalkine, which is an important signaling pathway between microglia and neurons [17, 18]. Other mouse models with tagged microglia include Iba1-GFP [19] and Csf1R-eGFP [20].

2.3 Slicing and recording solutions

1. Brain slice preparation for microglia recordings is similar to methods used for neuronal recordings. However, tissue preservation is even more crucial when working on microglia, since it limits the activation of microglia. Several methods are proposed in the literature, all of them aiming at reducing neuronal depolarization and activation of excitatory receptors during and/or after the slicing procedure. Addition of glutamatergic antagonists to the slicing solution, replacement of extracellular NaCl by sucrose or N-methyl-D-glucamine (NMDG), increase of the extracellular magnesium concentration while decreasing that of calcium have been used.
2. The pH of all solutions should be adjusted to ~7.3 and osmolality measured and adjusted to 315 - 330 mOsm/kg (*see Note 4*).
3. Extracellular solution 1: Suitable for neonatal animals, up to postnatal day (P) 15. This is a high magnesium artificial cerebral spinal cord fluid (aCSF) of the following composition (in mM): 120 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 10 glucose, 1 CaCl₂, and 7 MgCl, pH 7.3 when bubbling with carbogene (95% O₂, 5% CO₂).
4. Extracellular solution 2: Suitable for juvenile and young adult animals (up to P30). This is a sucrose-based solution, containing (in mM): 215 sucrose, 2.5 KCl, 1.3 NaH₂PO₄, 26 NaHCO₃, 20 D-glucose, 5 sodium pyruvate, 7 MgCl₂, and 1 CaCl₂ (pH 7.3, 330 mOsm).
5. Extracellular solution 3: Suitable for adult animals (> P30). This is a NMDG-based solution, containing (in mM): 93 NMDG, 2.5 KCl, 1.2 NaH₂PO₄, 30 NaHCO₃, 20 HEPES, 2 thiourea, 25 D-glucose, 5 sodium ascorbate, 3 sodium pyruvate, 10 MgCl₂, and 0.5 CaCl₂ (titrate pH to 7.3 with hydroxylchloride before addition of the divalent cations to avoid precipitation). In all cutting solutions, kynurenate (1mM) can be added to block ionotropic glutamate receptors.

2.4 Recording solutions

- 3 Extracellular solution 4: this is the standard aCSF for long-term storage and recording of the slices, containing (in mM): 124 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 10 glucose, 2 CaCl₂, and 1 MgCl, pH 7.3 when bubbling with carbogene (95% O₂, 5% CO₂), osmolarity ~320 mOsm.
- 4 The intracellular solution has to be adapted to the type of currents to be recorded. A series of intracellular solutions adapted to different currents are listed in [14]. Two typical solutions are given here for recordings of basic characteristics and purinergic currents:
- 5 Intracellular solution 1: this is a potassium gluconate-based solution to record potassium and non-selective cationic currents, containing (in mM): 132 K-gluconate, 10 N-[2-hydroxyethyl]piperazine-N'[2-ethanesulfonic acid] (HEPES), 10 ethylene glycol-bis(β-aminoethyl ether)-tetraacetate (EGTA), 1 CaCl₂ and 1 MgCl₂ (pH 7.35 adjusted with KOH, osmolarity ~300 mOsm).
- 6 Intracellular solution 2: this is a cesium gluconate-based solution allowing to block potassium currents and to record non-selective cationic currents, containing (in mM): 125 Cs-gluconate, 10 HEPES, 10 EGTA, and 1 CaCl₂ and 1 MgCl₂ (pH 7.35 adjusted with CsOH, osmolarity ~300 mOsm).
- 7 Notice that nucleotides, such as ATP and GTP, have been omitted (*see Note 5*).

3 Methods

3.1 Slice preparation

The procedure should be quick but accurate in order to minimize possible tissue damage. The quick step concerns the time window between animal death and cooling down the brain. With small animals such as young rats and mice, once the brain is put in an extracellular solution, especially with protective extracellular solutions 2 or 3, kept at 4°C, cell metabolism is much reduced and the tissue will not suffer from lack of oxygen (*see Note 6*). The accurate phase concerns the dissection of the brain area to be sliced, the slicing itself and the manipulation of the slices.

1. Animals can be killed by cervical dislocation or anesthetized with isoflurane. Anesthetics may have an impact on the preparation, so it is advisable to keep the procedure constant (which may or not include anesthesia). The animal is decapitated and the head is placed in a large Petri dish containing ice-cold cutting solution for few seconds. The brain is then dissected out quickly and placed in another dish still containing the cold cutting solution bubbled with 95% CO₂ / 5% O₂. A block of brain containing the structure of interest is prepared and glued with the appropriate orientation on the vibratome support. To maintain low temperature, all the above procedures can be done on ice.
2. Vibratome settings should be adjusted to minimize vertical oscillations of the blade that will damage the surface of the slices. The speed of advance of the blade must be slow. Bubbling of the solution is not necessary at this stage, if the cutting solution is kept low (4 - 8°C). Bubbling can induce turbulence in the solution that could also damage the slice surface while cutting.
3. After slicing, slices need to recover before they can be used for experiments. When slices are cut with extracellular solution 3 for older animals, recovery is done at 32 - 36°C, first in the same protective solution for 5 - 7 minutes and then for 20 - 30 minutes in extracellular solution 1. Otherwise, when slicing is done with extracellular solutions 1 or 2, cut slices are transferred (*see Note 7*) in the recovery chamber at 32 - 36 °C for 30 - 60

minutes in extracellular solution 1. Slices are then kept at room temperature in extracellular solutions 1 or 4 until recordings. Readers can refer to [25] for more details on the NMDG protective cutting procedure.

4. Slicing procedure will inevitably produce some damages to the slices, in particular at the surface. This will activate microglial cells that will move toward the damage cells at the surface. If the slicing procedure is done correctly, during the first 3 - 4 hours following slicing microglial cells below 30 - 50 μm from the surface do not show signs of activation, such as changes in morphology or expression of inward or outward potassium currents (*see Note 8*).
5. Recently, microglial cell recordings were performed in acute slices of human temporal lobe tissue obtained from surgical resection of patients diagnosed with pharmacoresistant medial temporal lobe epilepsies associated with a hippocampal sclerosis [26]. Tissue blocks were transported from the operating room to the laboratory in a extracellular solution 2 equilibrated with 95% O_2 and 5% CO_2 , at 2 - 10 $^\circ\text{C}$. Slices of 300 μm thickness were cut in the same solution under sterile conditions with a vibrating tissue slicer. Before imaging and electrophysiology, slices were transferred to an interface chamber at 32 - 36 $^\circ\text{C}$ in extracellular solution 1. Experiments started after 1.5 hours of recovery in these conditions.

3.2 Selecting microglial cells for recordings

1. As previously mentioned, slicing damages the slice surface and this may lead to microglia activation. However, microglial cells deeper than 50 μm are viable until 4 - 5 hours after slicing procedure. If a two-photon microscope is available, it could be useful to take an image of the area of interest to help choosing the cells to record. In a control adult animal, the choice can be dictated by the ramified morphology of the cells, with

processes located symmetrically around the cell body (*see Note 9*). A microglia with processes oriented towards the surface of the slice is probably reacting to damages induced by slicing.

2. In case transgenic animals are not available or cannot be used, living microglia can be labeled by incubating slices with lectins conjugated with different fluorochromes. The original method used Texas Red-conjugated tomato lectin from *Lycopersicon esculentum* (Sigma) at 100 µg/mL, with a 45 minutes of incubation at 37 °C under 5% CO₂ [8]. More recently, microglial cells were also labeled by incubating acute slices with 25 µg/mL Alexa (568 or 594) - conjugated isolectin B4 (Thermo Fisher) for 30 minutes in darkness at room temperature (see for instance [27]). This approach works usually better on slices of young animals (second postnatal week).
3. Alternatively, microglial cells can be labeled by ejecting the lectin from a glass pipette [28]. This method has been used recently to label microglia in acute slices of human temporal lobe tissue obtained from surgical resection of patients diagnosed with pharmaco-resistant medial temporal lobe epilepsies associated with hippocampal sclerosis [26]. The pipette (2 - 3 µm at the tip) containing 50 µg/ml of the tomato lectin conjugated to Dylight 594 dissolved in the following solution 150 NaCl, 150; KCl 2.5 and 10 HEPES 10 mM (pH 7.4) is placed at 100 - 120 µm under the slice surface where microglia are less affected by slice preparation. Multiple pressure ejections (1 minute application) provide a good staining of human microglia after 5 - 10 minutes (Fig. 1D, E). Fluorescent signals are maintained up to 2 - 3 hours.

3.3 Establishment of the gigaseal and of the whole cell configuration

Mechanical injury may attract microglia processes [29]. Therefore, the insertion of recording electrodes into the slices should be done very gently to minimize the attraction of microglia process toward the pipette. In particular, the positive pressure applied through the pipette to keep the tip clear when moving the pipette in the tissue should be kept as low as possible to minimize mechanical damage. However, the tip resistance of pipettes used for recording microglia is usually higher than that used for somatic neuronal recordings, typically between 5 - 8 M Ω when using intracellular solutions 1 or 2. If a two-photon system is available, the effect of pipette insertion can be easily monitored. It can be also observed with a classical epifluorescence microscope if a major damage is induced. Once the pipette is close to microglia membrane, simply releasing the pressure is often sufficient to establish a gigaseal. If not, a mild negative pressure can be applied. A voltage around -60 mV should be imposed and after few minutes of stable gigaseal the membrane can be broken with a stronger but shorter negative pressure to establish the whole cell configuration.

3.4 Recording intrinsic properties

An electrophysiological characterization of intrinsic membrane properties can be done at the beginning of the recording, before extensive intracellular dialysis, and if a potassium-based intracellular solution (e.g. intracellular solution 1) is used. Hyperpolarizing and depolarizing steps of 50 ms can be used to determine I/V relationship of each recorded cell. To identify potentially inward and outward rectification currents, voltage steps can be applied from -140 to +40 mV, with steps of 20 mV.

Resting membrane potential and input membrane resistance can be obtained from the linear fit of the I/V curve around -60 mV. For accurate measurements only small steps which do not activate voltage dependent potassium channels should be considered. The membrane capacitance and the access resistance can be determined from the fit of the capacitance

transient of the current in response to small voltage step from -60 mV. Microglial cells have a very high input resistance (around 3 - 4 G Ω in non-activated cells), and a capacitance around 20 - 30 pF. These values change in activated microglia as a consequence of morphological changes and additional expression of potassium channels. Thus a decrease in the resistance and an increase in the capacitance are usually measured in activated microglia [15].

3.5 Activating ligand-gated channels and GPCRs

Microglial cells express a variety of ligand-gated channels and of ligand-activated G protein-coupled receptors, the functions of which have been best characterized for purinergic receptors [12, 30, 31]. When purinergic responses are tested, this can induce a change of microglia phenotype. It is thus important to design experiments in a manner favoring short-duration recordings, fast and local applications. Responses to drug are tested in voltage clamp configuration, to avoid contamination of voltage-dependent conductance.

Three methods can be used to study the response of microglia to several ligands: bath application, local application through a pipette, and uncaging.

3.5.1 Bath application

The drug can be applied through the perfusion system. The method is easy to implement but presents some disadvantages. Several seconds may be needed to reach the desired concentration in the bath and within the slice. This could be incompatible with studying channels that quickly desensitized (such as P2X₄ receptors). Furthermore, the entire slice is exposed to the drug. Therefore slice should be changed after each drug application. Pharmacological experiments for testing different concentrations of ligands, different agonists or antagonists (Fig. 2A) can be done, provided that the reproducibility of the responses has been tested.

When microglia are held at a holding potential around -60 mV, bath application of ATP triggers a biphasic response consisting of an inward current directly mediated by the opening of P2X receptor-channels, followed by an outward current consecutive to the activation of P2Y receptors. Since the outward current is mainly mediated by the secondary activation of potassium channels, it can be eliminated using a Cs-based intracellular solution (*e.g.* intracellular solution 2; Fig. 2A). Bath application of selective agonists can also allow the activation of specific types of receptors (*e.g.* Bz-ATP for P2X₇ and UDP for P2Y₆; Fig. 2C). The responses obtain by bath application provide several pieces of information, such as the peak amplitude of the response and the associated change in membrane conductance. An easy way to follow changes in this latter parameter consists in applying a short hyperpolarized pulse of fixed voltage every few seconds. An I/V curve of the induced response can also be obtained by stepping or ramping the voltage command through a range spanning values of possible reversal potentials. To this end, an I/V curve should be measured at least just before the drug application and at the peak or steady-state of the current response induced by the drug. The specific I/V relationship of the drug-induced current is obtained by subtracting the first from the second I/V curve (Fig. 2D). A third I/V curve can be performed after complete wash-out of the drug to verify that membrane properties have fully recovered.

3.5.2 Local application through a pipette

Drugs can also be applied locally through a pipette. This is the method of choice if you need to deliver several times the drug through brief applications and for instance test the effect of another drug, such as an antagonist, through bath perfusion. However, the actual concentration reaching the microglia is unknown (lower than the solution in the pipette). Local applications can be performed through iontophoresis, but in this case the kinetics of application remains relatively slow unless the pipette is almost in contact with recorded cell.

Direct effects of current and pH should be controlled for. Another method is pressure application. This is usually performed using pipettes of a similar size than recording patch pipettes. Parameters such as the size of the pipette, the concentration of the drug, the applied pressure, and the distance between the application pipette and the recorded cell must be empirically adjusted to favor rapid application without inducing artifacts (*see Note 10*). In theory this method is suitable for studying fast desensitizing responses. However, to achieve really fast applications, high pressure should be applied and this could lead to micro lesions in the slice and attract microglial processes. Thus, controls with pressure application of aCSF should be done to check that no response is detected in the absence of the drug. If really fast applications are required, outside-out patches should be pulled from the recorded cell and moved above the slice in front of fast application system (e.g. double-barrel pipette controlled by a piezoelectric device).

3.5.3 Uncaging

Caged compounds are light-sensitive probes that encapsulate biomolecules that remain inactive until irradiating light breaks the cage, leading to the liberation of the active form of the biomolecule. To avoid spontaneous uncaging it is better to work in the dark. The caged molecule can be applied through the perfusion solution to reach the vicinity of the recorded cells. Then light is shined onto the preparation to release the active biomolecule onto the recorded microglia. The spatial and the temporal resolution of the application can be precisely controlled, since the compound becomes active only after light exposure. This is the most suitable method to apply a drug locally (*see Note 11*), in particular to study fast desensitizing responses (Fig. 2B).

Irradiating light for uncaging is in the U.V. range and is usually provided by a flash lamp or a laser and can be guided to the sample either through an optic fiber or through the objective.

The latter option provides the possibility to shape the laser beam with a holographic system, thereby uncaging molecules in a specific region of the field of view (the soma, or a single process or two processes simultaneously)[32]. If the application has to be highly local, the two-photon laser is the best option, since uncaging will occur only in the focal plane. In these experiments, proper controls should be done to make sure that the observed effect is due to the uncaging of the compound and not a direct consequence of the light or the heat caused by the lamp/laser.

Several caged neurotransmitters are commercially available (caged ATP, glutamate, GABA, choline) but there are also caged molecules to study intracellular signaling pathways (caged IP₃, cAMP, etc.). However, in the latter cases the caged compound needs to be introduced into the microglia, most likely through the patch pipette.

4. Notes

1. As for neurons, many functions of microglial cells depend on the temperature. Thus unless required by specific experimental conditions, recording should be done close to physiological temperature (33 - 36°C).
2. Even with the best optics on this transmitted light path, identification of microglia is almost impossible and requires the use of fluorescent markers.
3. There were some contradictory reports on the role of fractalkine signaling in synaptic plasticity [21–23]. However, it is worth noting that impairment in hippocampal plasticity has been observed with mice heterozygous for CX₃CR1 [21].
4. It can be convenient to prepare a solution ten times concentrated (10x) with all salts without sucrose, glucose, MgCl₂ and CaCl₂ (which would precipitate in solution with NaHCO₃). The solution can be then daily prepared by adding water, sucrose and glucose. After bubbling 5 min with carbogene, add CaCl₂ and MgCl₂ from 1M stock solutions.

5. For neuron recordings, Mg-ATP and GTP in millimolar range are usually included in the patch pipette to avoid run-down of several currents during intracellular dialysis. However, microglial cells express a high density of purinergic receptors that are activated by these nucleotides. When approaching the cell with positive pressure before establishing the gigaohm seal activation of purinergic receptors can compromise subsequent analysis of these receptors and/or trigger secondary responses associated with these receptors (process motility, phagocytosis...). In our hands, whole-cell recordings of microglia are more stable when nucleotides are excluded from the intracellular solution. If nucleotides have to be added to the intracellular solution, it is recommended to use the backfilling technique, as usually used for gramicidin/nystatin perforated patch recordings [24]. The tip of the pipette should be loaded with a solution without nucleotides and back filled with the required recording intracellular solution.
6. In older (or bigger) animals, heart perfusion with extracellular solution 3 can be performed before dissecting the brain to get a better preservation of the tissue.
7. To manipulate and transfer slices, use a Pasteur pipette. Carefully break the thin part of the pipette and fire polish it. Mount the broken part on the support and use this tool to aspirate slices with the solution into the pipette. This method allows a better preservation of the slices than the classical brush used for example in immunohistochemistry.
8. Microglia react to bacteria. It is important that all surgery tools, chambers to keep slices, the perfusion system, and the recording chamber are routinely cleaned with HCl (or similar), and/or ethanol and rinsed with distilled water before experiments. Filtering all extracellular solutions through 0.2 μm filters also helps avoiding bacteria growth during the duration of experiments, especially when recovery and recording solutions are heated.

9. In slices of CX₃CR1^{+eGFP} mice, two types of cells tagged with fluorescent indicator can be distinguished according to their capacitance. One type has a capacitance of about 30 μF and processes that extend from the cell body in all directions. A second type is located close to blood vessels, often at dividing branch points, has a smaller capacitance and a less “isotropic” morphology. These latter cells could be perivascular monocytes.
10. Mechanical artifacts may arise from too much pressure applied to the pipette to deliver the drug or from a pipette too close to the cell. Moreover the drug may leak out from pipette tip and freely diffuse between each application, inducing unwanted responses and/or desensitization. This artifact may occur if the pipette tip is too large and/or it is too close to the cell, and/or if the drug concentration is too high.
11. Caged compounds are relatively expensive and cannot be bath applied for hours through a regular perfusion. One alternative, easy to implement, is to use a closed perfusion system: A small volume of aCSF (e.g. 5 - 10 ml) containing the appropriate concentration of the caged compound is used to perfuse the slice and the solution sucked from the recording chamber is sent back into the vial containing the aCSF with the caged compound. The solution must be constantly bubbled with carbogene. Alternatively, the caged compound can be applied locally by pressure ejection from a glass pipette in the vicinity of the recorded microglia. In this latter case, the concentration of the cage that will reach the cell is less well controlled.

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Figures

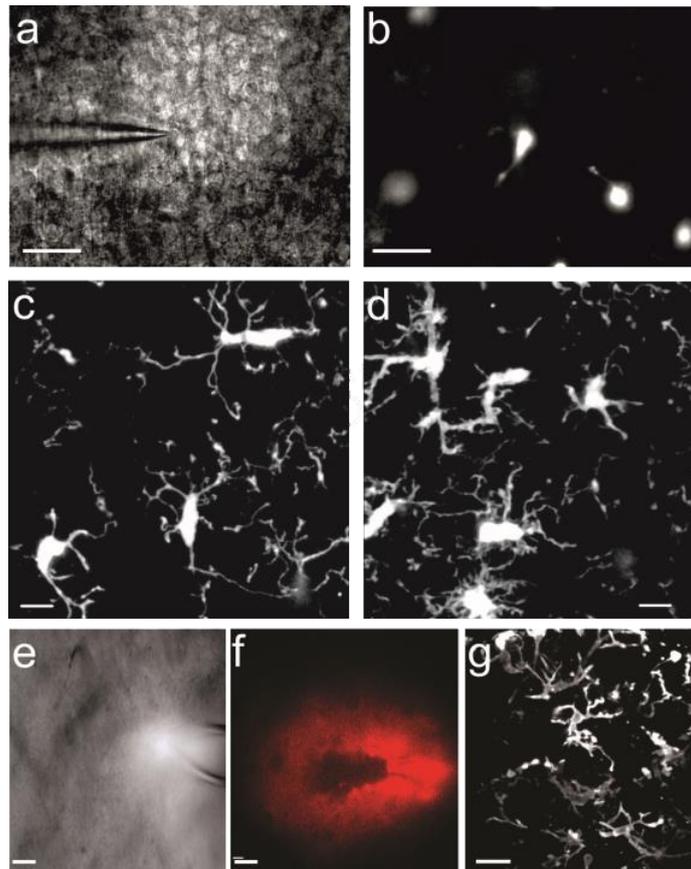


Fig. 1 Identifying microglia in acute brain slices. A, B, Differential interference contrast (A) and epifluorescence (B) images of a microglia recorded in layer 4 of the “barrel” somatosensory cortex of a $CX_3CR1^{+/eGFP}$ 7 days old mouse. C, D, Two-photon maximal intensity projections ($z = 19 \mu\text{m}$) images of $CX_3CR1^{+/eGFP}$ hippocampal microglial cells in acute slices obtained in a control mouse (C) and in a mouse two days after i.p. kainate injection (D). E, F, G, Tomato lectin labeling of microglia in acute human hippocampal slice. Bright field illumination (E) and two-photon microscopy illumination (F) of the same field of view during the ejection of the tomato lectin from a patch pipette (visible on the right). G, Two-photon imaging of microglia 10 minutes after the end of the tomato lectin ejection procedure. Scale bars, $30 \mu\text{m}$ in A and B, $10 \mu\text{m}$ in C and D, $20 \mu\text{m}$ in E, F and G.

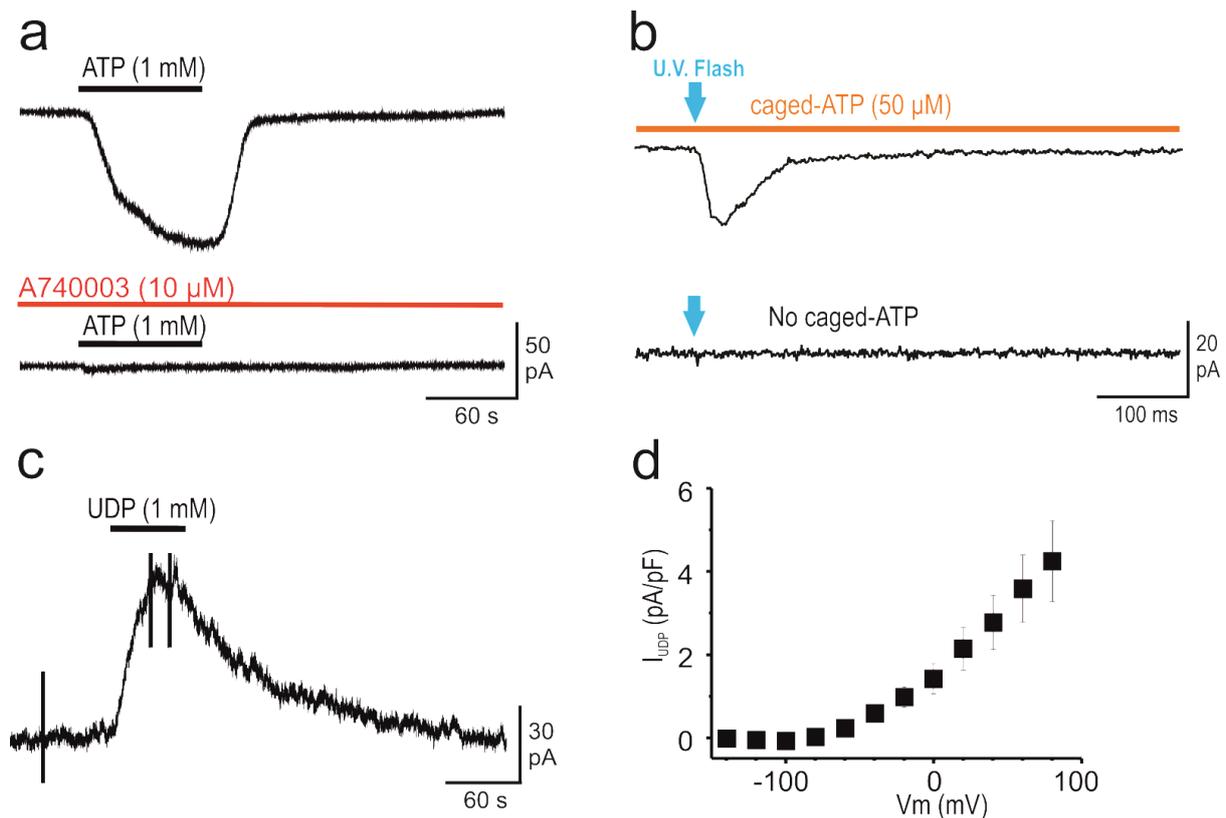


Fig. 2 Activation of purinergic receptors of microglia in mouse hippocampal slices. A, Inward current induced by bath application of ATP in a Mg^{2+} -free ACSF (upper trace) and blockade of the response by the selective P2X7 receptor antagonist A740003 (lower trace). B, Inward current induced by U.V. light (1 ms flash, blue arrow) uncaging of ATP (upper trace). No response was elicited by light in the absence of caged-ATP in the bathing ACSF (lower trace). In A and B, microglial cells were recorded with Cs-based intracellular solution (intracellular solution 2) and were held at potential of -70 mV. C, Outward current induced by bath application of the P2Y₆ receptor agonist UDP in a microglia recorded with a K-based intracellular solution (intracellular solution 1) and hold at -20 mV. D, Mean I/V curve of the UDP-induced responses of 7 microglia. I/V curves were established by subtracting current responses to brief hyperpolarizing and depolarizing voltage pulses elicited before and during the UDP application (vertical deflections visible in C). C and D are modified from reference [15].