Video Article

Improved Preparation and Preservation of Hippocampal Mouse Slices for a Very Stable and Reproducible Recording of Long-term Potentiation

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Abstract

Long-term potentiation (LTP) is a type of synaptic plasticity characterized by an increase in synaptic strength and believed to be involved in memory encoding. LTP elicited in the CA1 region of acute hippocampal slices has been extensively studied. However the molecular mechanisms underlying the maintenance phase of this phenomenon are still poorly understood. This could be partly due to the various experimental conditions used by different laboratories. Indeed, the maintenance phase of LTP is strongly dependent on external parameters like oxygenation, temperature and humidity. It is also dependent on internal parameters like orientation of the slicing plane and slice viability after dissection.

The optimization of all these parameters enables the induction of a very reproducible and very stable long-term potentiation. This methodology offers the possibility to further explore the molecular mechanisms involved in the stable increase in synaptic strength in hippocampal slices. It also highlights the importance of experimental conditions in *in vitro* investigation of neurophysiological phenomena.

Video Link

The video component of this article can be found at https://www.jove.com/video/50483/

Introduction

Nowadays, there is limited understanding of how complex memories are stored and recalled at the neuronal circuit level. However, a unifying hypothesis of memory storage is available and broadly accepted: memories are stored as changes in the strength of synaptic connections between neurons in the central nervous system. On its own, research on synaptic plasticity has largely benefited from two breakthrough discoveries. (1) In a seminal experiment, Bliss and Lomo [1], using the intact anesthetized rabbit, found that delivery of a brief high-frequency (1 sec, 100 Hz) stimulation to the perforant path of the hippocampus caused a long-lasting (several hours) increase in the related synaptic connections. This fascinating phenomenon was called "Long-Term Potentiation" or LTP by Douglas and Goddard in 1975 [2]. (2) Later on, it was found that a similar phenomenon could be triggered in brain slices (0.4 mm) artificially maintained alive *in vitro*. The most widely studied LTP was observed *in vitro* by delivering one or several tetani to a bundle of axons (the so-called Schaffer collaterals) while recording the resulting field excitatory synaptic potential evoked in the pyramidal neurons of the so-called CA1 region. The mechanisms of LTP induction have largely been revealed. Basically, a Ca^{2+} influx through the NMDA receptors activates enzymes with two consequences: a phosphorylation of AMPA receptors (which increases their efficiency) and an incorporation of extra AMPA receptors in the postsynaptic membrane [3]. By contrast, the mechanisms of the maintenance phase of LTP are largely unknown, notably because it is experimentally much more difficult to maintain a slice healthy for many hours than for 30 to 60 min.

A lot of studies have been dedicated to the understanding of LTP mechanisms and interesting theories have been elaborated over the years [4-11]. But until now, the precise molecular mechanisms underlying the stable increase in synaptic strength have not been elucidated. This could be partly due to the difficulty to reproduce previous results in different laboratories using different techniques for the preparation and the maintenance of hippocampal slices. In their methodology paper, Sajikumar *et al.* [12] stressed the importance of experimental conditions for the preparation of rat hippocampal slices and the recording of stable LTP. In this video we present all the optimization steps developed in our laboratory over the years to be able to record a very stable LTP in mouse hippocampal slices.

This optimization has been made from protocols developed and successfully used by other laboratories which study LTP mechanisms in mice [13] and rats [11]. It allows experienced researchers to induce and record a very long lasting LTP in adult mice with a high rate of success. The physiological basis of the induced LTP was carefully checked and demonstrated [14]. In this methodology paper, we show that any modifications of experimental conditions, like temperature or oxygenation can have a profound impact on LTP maintenance while the dissection procedure can deeply modify slices excitability. It must also be emphasized that the precise control of all these parameters requires a training of several months for novice students.

Protocol

All animal procedures were carried out in accordance with National Institutes of Health regulations for the care and use of animals in research and with the agreement of the local ethics committee.

1. Preparation of Artificial Cerebro-spinal Fluid

The same media is used to dissect, cut and perfuse slices (1 ml/min) during the resting period and the electrophysiological recordings. This media is composed of 124 mM NaCl, 4.4 mM KCl, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$ and 10 mM D-glucose.

1. Weigh the different components for 2 L of ACSF except $MgSO_4$ which is already in solution (1 M). Using a standard solution allows to be sure of the exact concentration of Mg^{2+} because $MgSO_4$ in powder is highly hygroscopic. Ca^{2+}:Mg^{2+} ratio greatly influences LTP induction and maintenance [14] and thus their proportions must always be respected.
2. Put all the components except $CaCl_2$ in a glass graduated cylinder and bring to 2 L with distilled H_2O.
3. Stir vigorously. When everything is dissolved, add carbogen through an aquarium bubbler and tubing attached to the tank (95% O_2, 5% CO_2). Wait a few minutes and add calcium chloride when pH is fixed at 7.4 by the action of bicarbonate buffer.
4. Keep 600 ml in a refrigerated rectangular glass dish and cool down to 4 °C with ice around the dish. This media will be used for hippocampus dissection.
5. Filter the remaining 1,400 ml and keep in an Erlenmeyer flask.

2. Preparation of the Electrophysiological Rig and the Brain Slice Recording Chamber

The perfusion circuit is made of 2 peristaltic pumps, one pumping fresh fluid from a reserve tank and the other pumping used fluid from the recording chamber to a bin. Fresh fluid is added to a home-made perfusion system where it is re-oxygenated and warmed before reaching the interface recording chamber by gravity (**Figure 1A**). The interface recording chamber was designed by FST (Fine Science Tools, Vancouver, Canada, **Figure 1B**). Tissue slices are placed on woven net filter attached to a removable well ring and can be continuously maintained under interface conditions by adjusting the height of the suction well needle. A home-made plastic tube blocked at its end and perforated laterally (0.5 mm) is fixed to the suction needle to increase level stability in the chamber. The recording head is mounted over a water bath containing a gas dispersion stone which helps maintain a humid environment by passing moist air through deflection ports in the recording head (to decrease water droplet formation). Bath and medium temperature are controlled by continuously varying the level of DC current through a silicone rubber-embedded heater element in the water bath. Thermistors in the water bath and recording wells allow the chamber temperature to be set and precisely monitored at these sites.

The heating system of the chamber is completed by a global heating system controlling the temperature of the entire rig. The software developed by researchers at the University of Edinburgh (www.etcsystem.com) monitors and equalizes the temperature of the oxygenated air, the brain slice, the electrodes and the remaining rig providing a stable environment for long-term recordings (**Figure 1C**). The temperature used for mouse hippocampal slices is 28 °C.

1. Rinse all the perfusion circuit with distilled H_2O for a minimum of 20 min and start the heating systems.
2. Start the carbogen bubbling in the circuit. Carbogen arrives in the home-made perfusing tubes through filter candles and in the water bath below the recording chamber. The flow rate in the water bath is controlled by a flow meter. If the flow rate is too slow, tissue could become hypoxic. Conversely, if the flow rate is too high, the gas might not be sufficiently warmed and moisturized leading to drying of the tissue. High gas flow rate could also increase the chance of water spraying over the tissue from the water bath below leading to osmotic shock. This can be prevented by adding bits of nylon mesh (100 µm) at the outlet of the deflection ports.
3. Drain the circuit and fill up with filtered ACSF.
4. Put the ring which will support the slice in the holding chamber. A woven net filter with mesh openings ranging from 500 to 750 µm is stretched and attached to the ring with two-part resin epoxy glue. The woven net filter is made of 87% polyamide and 13% elastane (panty 15 den). Glue must be applied carefully to avoid the formation of reliefs at the surface of the ring.
5. Carefully remove all the air bubbles from the circuit.
6. Adjust the level of ACSF in the recording chamber with the screw of the suction needle. The speed of the peristaltic pumps is adjusted to 1 ml/min for the inlet pump and to 5 ml/min for the outlet pump.

The perfusion system is placed on a rigid vibration-resistant table and surrounded by a Faraday cage.

3. Preparation of the Dissection Area

Surgical instruments: a scalpel with a #11 blade, small standard dissecting scissors, spring scissors, curved forceps, two Heidemann spatulae and a spoon.

Supplementary material: a guillotine, an underpad, a McIlwain tissue chopper with a razor blade, filter paper, a glass Pasteur pipette with a rubber teat, 2 plastic Pasteur pipettes one of which with a wide mouth, a Petri dish, a metallic cylinder of 7 mm diameter (**Figure 2A**).

1. In the dissecting dish filled with cold ACSF (prepared in Step 1), immerse a refrigerated glass lid from a staining dish, wrapped in filter paper. Carefully remove air bubbles and oxygenate the ACSF through an aquarium bubbler (**Figure 2A**).
2. Lay out instruments in order of use. Add three layers of filter paper on the tissue chopper plate and a new razor blade cleaned with distilled water and ether (**Figure 2B**). The razor blade must be horizontal when it touches the paper. Blade force is adjusted to half of maximal value and speed to approximately one third of maximal value.

3. Attentively check if everything is ready (instruments, temperature ...) because you will not have time afterwards.
4. Ask someone to spray the dissection with a Pasteur pipette filled with cold ACSF.
5. Anesthetize the mouse with Nembutal IP (100 mg/kg) before decapitation. Halothane anesthesia can also be used. We have compared both methods and obtained same results. Decapitation must be performed under anesthesia but cervical dislocation can also be used. However cervical dislocation needs a very good practice to avoid animal suffering.
6. Dissect on the underpad as quickly as possible under cold ACSF continuous shower.

4. Brain Removal

1. While holding still the head with the index finger and the thumb of one hand on the muzzle, make an incision with the dissecting scissors along the middle of the top of the head starting at the edge of the guillotine cut and running rostrally to the frontal bone.
2. Cut through the cutaneous muscle on each side of the head to fully expose the skull plates and remove the muscles at the caudal side of the head.
3. With the dissecting scissors, cut the temporalis muscle on each side, along the temporalis plate, then cut the frontal plates in the middle, transversally. Then, make a little cut on the occipital bone, between the two plates.
4. For each side, cut at the caudal base of the occipital plates.
5. Cut along the sagittal suture with spring scissors.
6. With forceps, remove the skull halves by spreading them away from each other.
7. With the scalpel, cut transversally just after the olfactory bulb and just before the cerebellum then plunge the extracted brain into the dissecting dish with cold ACSF.

5. Hippocampal Dissection

1. Once the brain is immerged in the dissecting dish, sever the two hemispheres from each other with a scalpel inserted in the middle.
2. The dissection of hippocampus is performed with spatulae under visual control through a binocular surgical microscope (X25, **Figure 2A**). On one hemisphere, carefully spread the structures to see the lateral ventricle. Remove the brain stem and diencephalon. This is done by applying the spatulae on the frontal cortex on one side and on the diencephalon on the other side. Take care to not touch the hippocampus with the spatulae and to not stretch it during tissue sectioning.
3. Sever the fornix then gently push the hippocampus out of the cortex (roll away), by inserting a spatula in the ventricle.
4. When the hippocampus is extracted, remove excess cortex tissue and remaining blood vessels.

6. Cutting of the Slices

1. With a wide mouth plastic Pasteur pipette, transfer the hippocampus in a spoon with its alvear surface upwards (convex side).
2. Remove excess fluid with a standard plastic Pasteur pipette.
3. Tip up the spoon vertically nearly touching the filter paper of the chopper (**Figure 2B**) and drop off the hippocampus from the spoon, by rapidly touching the filter paper then move back the spoon.
4. Orientate the hippocampus and slice it transversally to 400 µm with the chopper (see **Figure 2C** for orientation of the hippocampus relative to the razor blade). Act as quickly as possible.
5. Remove the filter paper with the sliced hippocampus and wrap it around the metallic cylinder to spread the slices a little. Then, free the slices with sprays of ACSF using a standard plastic Pasteur pipette and collect them in a Petri dish filled with cold ACSF.

7. Incubation of Slices in Interface

1. Select slices with a standard plastic Pasteur pipette and rapidly place them in the recording chamber. Slices recover from the dissection trauma directly in the recording chamber, in interface at 28 °C.
2. The slice will most likely sink. Lower the fluid level to reach the slice level, and then raise it to make it float.
3. Turn the slice in the correct position while lowering the level. Slices are always orientated in the same way to facilitate the positioning of electrodes (2 stimulating and one recording electrodes) in the CA1 region.
4. Stop when the fluid is in interface. A "meniscus" of media around the slice is indicative of a sufficient level of media. The net filter must be saturated with media but not completely submerged.
5. Keep the chamber covered with filter papers placed over the perforated lid.
6. Let the slice rest for at least 1.5 hr at 28 °C.

8. Recording of Synaptic Responses

1. Recording electrode: glass capillaries are pulled to obtain a tip resistance of 2-5 MΩ when filled with ACSF. A small piece of filter paper is placed around the tip of the electrode and bone wax is applied at the extremity to reduce condensation. Stimulating electrodes: platinum-iridium bipolar cluster electrodes with a 12.5 µm diameter are purchased from FHC (USA). With proper care, each electrode can be used at least 30 times (**Figure 1B**).
2. Position the electrodes in the *stratum radiatum* of the CA1 region, all the electrodes lined up (**Figure 3A**). Electrodes are lowered 75 to 150 µm under the surface of the slice with Narishige micromanipulators. The two stimulating electrodes are placed to stimulate two distinct bundles of Schaffer collaterals. When the electrodes are lowered onto the slice, filter papers are carefully placed all around to close the chamber.
3. Biphasic stimulation (0.08 msec pulse duration per half-wave) is performed at constant voltage with a Grass stimulator connected to SIU-V isolation units. Maximal response is checked by increasing stimulus intensity from 2 V to maximum 12 V. Field EPSPs are amplified 1,000 times with a WPI ISO-80 amplifier and filtered at 10 Hz and 10 kHz. The signal is then sent to a PC through a National Instrument A/D

converter. Stimulation, data acquisition and analysis are performed using the WinLTP program (www.winltp.com). Field EPSPs are recorded at 40% of the maximum amplitude obtained in an input-output curve. For each slice, the fEPSP slopes are normalized against the average slope over the 30 min preceding LTP induction.

4. LTP is triggered by applying a single train of stimulation (100 Hz) at test strength on one pathway while the second pathway serves as a control.

9. Cleaning of the Setup

1. Rinse all the circuit with a 3% solution of hydrogen peroxide (H_2O_2) for at least 10 min, then drain. The circuit will be carefully rinsed with distilled water before starting any experiment. The use of H_2O_2 is not absolutely required as other labs use only distilled water for cleaning but we have noticed the deposition of dark residues in the tubing system when using only water.
2. Replace water in the water bath beneath the recording chamber.
3. Bath the stimulating electrodes tips in alcohol for 5 min.

Representative Results

This methodology has been used to analyze the properties of long-lasting long-term potentiation induced in acute hippocampal slices from adult C57Bl/6J mice (JANVIER SAS, France) [14]. Surprisingly, improvement of the experimental conditions has led to a new way of looking at LTP. We showed that long-lasting increase in synaptic strength did not require the synthesis of new proteins.

Here, we show that LTP induction depends on slices viability and excitability. When dissection of the hippocampus was too slow or too harmful, slices excitability increased and polysynaptic responses could be observed after LTP induction (**Figure 3B**). In this case, LTP induction was much less effective and potentiation was not maintained.

We would like to emphasize the fact that, even in slices apparently healthy, technical conditions have a great influence on the duration of the LTP induced by a single train of stimulation. In previous publications, our group showed that a short-lasting LTP can be transformed into a long-lasting one by modifying the recovery conditions of the slice [15]. Over years of daily practice, we observed that successive improvements in our technique have led to a progressive increase in the duration of the LTP induced by a single train in standard conditions. Indeed, recordings made in 2005 showed a short-lasting LTP going back to baseline within 3 hr (**Figure 3C**, filled circles). Modifications in the dissection procedure reducing tissue stretching have made it possible to obtain an LTP sustained for 6 hr [16] (**Figure 3C**, filled squares). And finally, improvements in the interface oxygenation and the temperature control, combined with electrode standardization have led to an LTP stable for more than 8 hr (**Figure 3C**, open circles). The difference between the three groups is significant (one-way ANOVA, $F(2,20) = 49,5$, $P < 0.001$).

Moreover, any modification of temperature or oxygenation induced an increase or a decrease of the synaptic response (**Figure 4**). The control of these parameters was checked by always using two stimulating electrodes. LTP was induced on one bundle of Schaffer collaterals while another bundle was used as an internal control of synaptic strength stability.

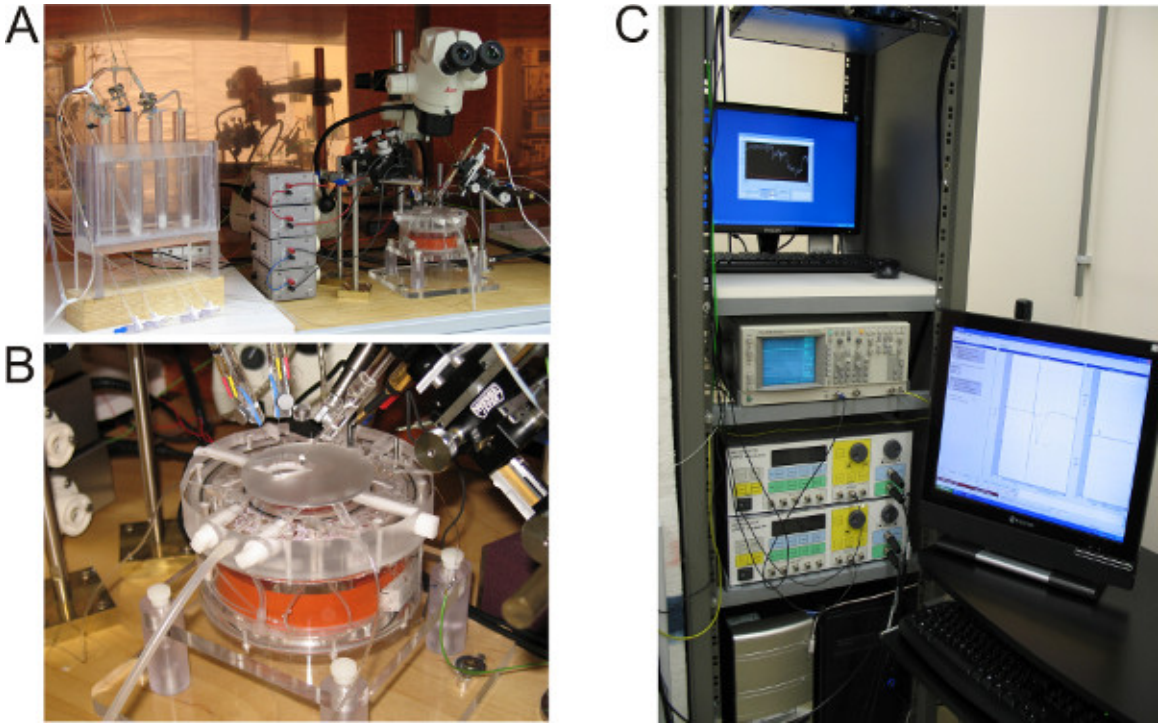

Figure 1. Electrophysiological rig and brain slice recording chamber. (A) Perfusion circuit with home-made gravity perfusion system alimented by a peristaltic pump, isolated stimulation units (two per electrode), surgical microscope and recording chamber. **(B)** Interface recording chamber with stimulating and recording electrodes. Filter papers have been removed from the lid to see the ring supporting the slice. **(C)** Control unit with 2 stimulators, oscilloscope, A/D converter, temperature control software and WinLTP software.

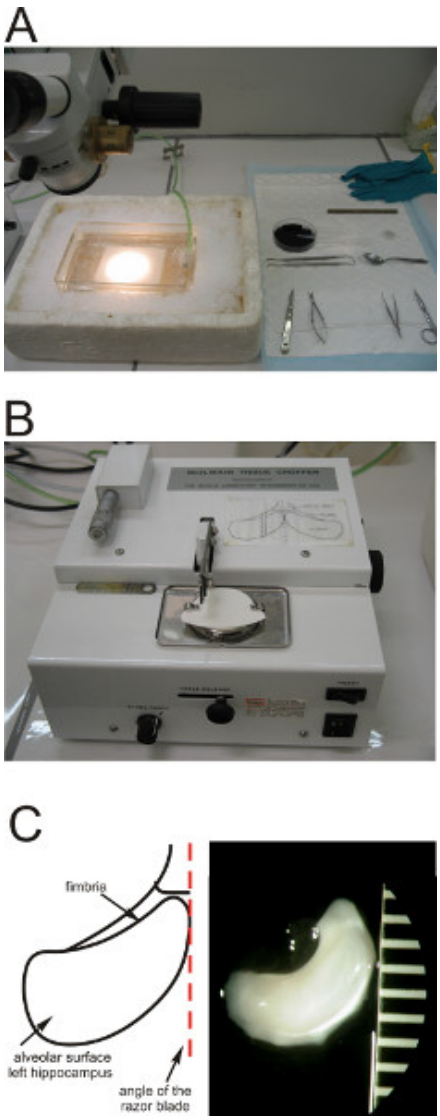

Figure 2. Tools and material used for hippocampus slicing. (A) Dissection dish containing refrigerated ACSF and surgical microscope. Scalpel mounted with a #11 blade, small standard dissecting scissors, spring scissors, curved forceps, two Heidemann spatulae, spoon, 2 plastic Pasteur pipettes one of which with a wide mouth, Petri dish, metallic cylinder of 7 mm diameter and ring which supports the slice in the recording chamber. **(B)** McIlwain tissue chopper. **(C)** Drawing and photograph of the left hippocampus in position for cutting. The orientation of the razor blade is indicated by the red dashed line. Scale of the reticle: 1 mm. Click here to view larger figure.

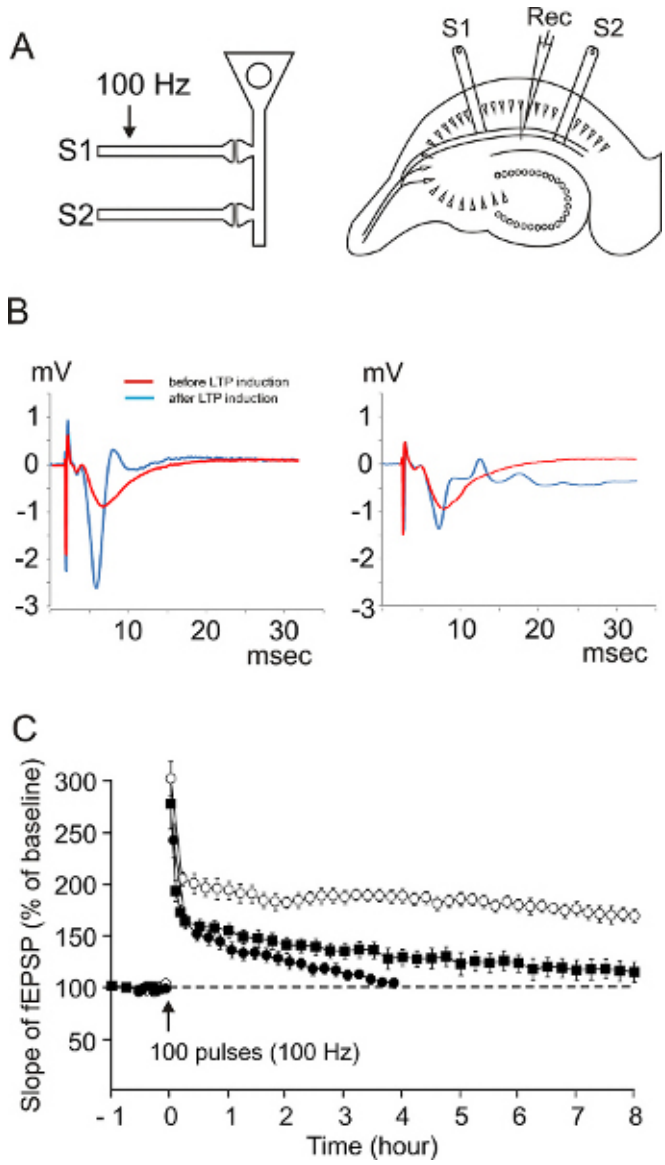

Figure 3. Induction of LTP in hippocampal slices. (A) Sketch showing the two independent synaptic inputs S1 and S2 to the same neuronal population. In each slice, two stimulating electrodes (S1 and S2) were put in place. S1 pathway was used to induce LTP, while S2 pathway acted as a control. **(B)** Sample fEPSP traces from individual experiments in a perfectly healthy slice (left) and in a slice presenting a high level of excitability (right). They were recorded just before the LTP induction (red traces) and one hour after LTP induction (blue traces). When slices are not perfectly healthy (right), polysynaptic responses are observed and fEPSP slope potentiation is reduced. **(C)** Comparison of the time courses of the fEPSP slope after LTP induced by a single train of high-frequency stimulation (100 Hz, 1 sec) recorded in 2005, 2010 and 2011 in our laboratory. First experiments were recorded in 2005 (filled circles, n = 11). Subsequent recordings (Villers, *et al.* 2010) benefited from an improved dissection procedure (filled squares, n = 6). Current results arise from optimization of interface oxygenation and temperature control along with electrode standardization (open circles, n = 6). Click here to view larger figure.

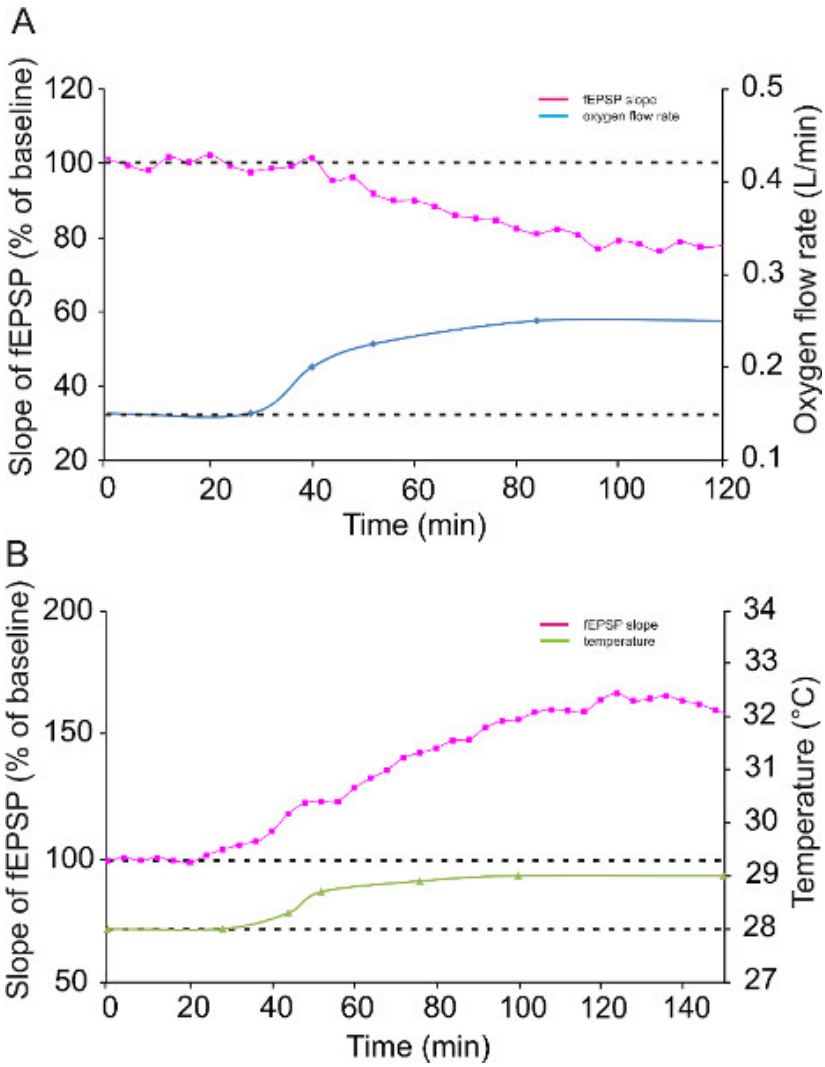

Figure 4. Variation of fEPSP slope as a function of temperature and oxygen flow rate. (A) Increasing oxygen flow rate in water bath beneath the recording chamber from 0.15 to 0.25 L/min (blue curve) induces a decrease in fEPSP slope of 20% (pink curve). **(B)** Increasing the temperature from 28 to 29 °C (green curve) induces a more than 50% increase in fEPSP slope (pink curve). Click here to view larger figure.

Discussion

We have developed in our laboratory a protocol resulting from the combination of methods developed and used by other laboratories having a big expertise in LTP recordings [11,17]. This protocol is adapted to adult mouse hippocampus and can be used in animals of any age and any background genotype. It also allows the analysis of LTP in transgenic mice developing neurodegenerative diseases like Alzheimer's disease [18,19].

Utilization of this protocol for rat hippocampal slices could necessitate some adaptations. For example, most of the studies performed in rats use a temperature of 32 °C instead of 28 °C. Mathis *et al.* [20] proposed another method for the preparation of hippocampal slices from aging mice or rats.

Material and methods

C57BL/6J mice come from JANVIER SAS (France) but same results are obtained with C57BL/6J mice from Charles River France.

The hippocampus is rapidly isolated while the tissue is submerged in cold ACSF to reduce cell damage. This allows a reduction in excitotoxicity but is known to induce synapse proliferation [21,22] which can mask further structural synaptic plasticity.

Then we use a tissue chopper for slicing instead of vibratome. A tissue chopper is particularly well adapted to the sectioning of small pieces of tissue like mouse hippocampus. On the tissue chopper, the hippocampus is always orientated in the same way according to the procedure of Alger *et al.* [23]. They placed the striations on the alvear surface, visible with oblique lighting, parallel to the razor blade. This method provides hippocampal slices of the dorsal part cut with an angle of 15° from the transverse axis (See **Figure 2C**). Subsequently, slices are manipulated with a minimum of direct contact.

Slices are maintained in interface at 28 °C directly after dissection. This method has been shown to reduce polysynaptic activity and epileptogenicity in mouse and rat slices [24].

External parameters are carefully controlled to obtain reproducible results. Bipolar cluster stimulating electrodes (FTC) are used instead of home-made electrodes in order to increase reproducibility in induction, seeing that the amplitude of LTP induction really depends on the electrodes quality.

ETC system from the University of Edinburgh [11] maintains a constant and uniform temperature inside the whole experimental rig and carbogen consumption is stabilized with a flow meter. Interface level is controlled visually with the help of a binocular surgical microscope and is maintained very stable with a peristaltic pump aspiration system.

Results

This protocol allows the induction of a very stable LTP which remains dependent on external conditions like temperature and oxygenation. We have previously demonstrated that LTP induced in these conditions was dependent on NMDA receptors, α-CaMKII autophosphorylation and PI3-kinase activation which are the classical molecular pathways involved in LTP [14]. By contrast, we were unable to reproduce the dependency of the maintenance phase of LTP on new protein synthesis. The ability of protein-synthesis inhibitors, and of anisomycin in particular, to prevent the development of the late phase of L-LTP when they were applied around the induction has been repeatedly reported [4,25]. This has led to the commonly held hypothesis that stabilizing synaptic plasticity for longer than about 2-3 hr required the triggering by the LTP-inductive stimulus of a transitory synthesis of new proteins [26]. However, recent experiments have suggested that things were more complicated [27-32]. LTP induced in our experimental conditions was maintained even in the presence of protein synthesis inhibitors suggesting that other mechanisms than new proteins synthesis could be involved in the lasting phase of LTP.

Nevertheless, *in vitro* experimentation always raises the question of the physiological relevance of the observed phenomenon. Slicing induces modifications in the phosphorylation state of proteins involved in activity-dependent forms of synaptic plasticity [33] and alteration in the metabolic state of the tissue [34]. The rate of protein synthesis is reduced to 10 to 15% of that observed *in vivo* [35] and balance between mRNA and proteins is disturbed [36]. For all these reasons, we must be cautious in the interpretation of the results.

By definition LTP is a rapidly induced long-lasting (days, weeks) enhancement of an excitatory synapse, which probably plays an important role in long-term memory. LTP, *in vivo*, can last for several weeks and similar changes in synaptic strength occur during learning [37,38]. In addition, most of the time, when long-term LTP is prevented by mutations, long-term memory is impaired [39].

More problematic is the parallel between short-term memory and short-lasting long-term potentiation. The first problem is that the duration of each phenomenon is unknown. Short-term memory has been studied 1 hr to 1 day after learning procedure whereas short-term LTP is supposed to last less than 5 hr. The second question is whether short-term memory (or short-lasting LTP) is merely a step forward long-term memory (or long-lasting LTP) or a separate entity with distinct molecular mechanisms [40]. Thirdly, we don't know if short-term LTP is induced by a weak stimulus that falls short of causing long-lasting LTP or if it is induced by the same stimulus but appears only when mechanisms underlying long-lasting LTP fail.

The long-lasting stability of LTP under our experimental conditions could be viewed as a short-term LTP equivalent to short-term memory lasting between 10 hr and 24 hr. In this form of synaptic plasticity, protein synthesis, which is highly reduced in slices, is not needed and LTP can be induced by a single train of stimulation. This long-lasting increase in synaptic strength could be due to a stable increase in the number of AMPA receptors in the post-synaptic membrane without spine remodelling.

According to this hypothesis, protein synthesis could be needed for spine enlargement and structural modifications of the synapses leading to long-term memory lasting several weeks. In this case, the decremental phase of LTP could only be observed 24 hr after induction. Unfortunately, until now, acute slices can only be maintained alive during approximately 16 hr.

This hypothesis could be tested by using Tc1 mouse model of Down syndrome. Morice *et al.* [41] have shown that, in these mice, short-term LTP *in vivo* and short-term memory are impaired whereas long-term synaptic plasticity and memory are preserved.

Discrepancies between different studies performed in different laboratories could be due to the duration of short-lasting LTP due to variation in the enzymatic activity, in the metabolic state of the slices or in the rate of protein degradation [42]. Protein synthesis could be viewed as a permissive replenishment process involving reposition of enzymes that have been used by the learning process, or of other constitutive cell elements [43].

These results highlight the importance of experimental conditions on LTP stability. A stable LTP independant of protein synthesis could help to study the role of long-lasting post-traductional modifications of synaptic proteins and the mechanisms underlying stable increase of the number of AMPA receptors in the post-synaptic membrane in spite of receptors turnover.

This video shows one detailed protocol which should hopefully help to obtain reproducible results in different laboratories.

Disclosures

No conflicts of interest declared.

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