

Unit II: LTP in acute hippocampal slice preparation

Long-term potentiation (LTP) is a form of synaptic plasticity whereby activity in neurons gives rise to a change in synaptic strength. It can persist for many minutes, to hours, and even days in the mammalian brain. LTP can be recorded in many parts of the nervous system, but is very widely studied in the hippocampus. Using a small slice of the hippocampus, a little less than half a millimeter thick, an electrode can be placed on the axons thus stimulating them, and another electrode can be placed on the dendrites for recording the synaptic response. One can electrically activate the axons to produce action potentials, which then produce postsynaptic responses on the dendrites. It is the stimulation of the axons with different patterns and frequencies that can lead to long-lasting changes in the efficiency of synaptic transmission. This is LTP.

In NEUR 200 and possibly other courses, you have read extensively about LTP, but now you have an opportunity to see a real LTP experiment in action, and go through all the steps necessary to conduct an LTP experiment. Despite how simple the protocols may seem in papers, this is a very technically challenging set of experiments. During the LTP Prep lab (Lab 6A), you will prepare many of the materials used in an actual LTP experiment, and you will have the opportunity to make acute hippocampal slices from mouse brain. In the LTP Recording lab (Lab 6B) you will conduct an LTP experiment. Lastly, during the Data Analysis session you will analyze your data for LTP in preparation for your lab write-up.

Useful Resources:

<http://www.jove.com/video/50483/improved-preparation-preservation-hippocampal-mouse-slices-for-very>

<https://www.jove.com/video/2330/preparation-acute-hippocampal-slices-from-rats-transgenic-mice-for>

Core Bibliography:

Bliss and Lomo (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol* 232:331-356.

Barrionuevo and Brown (1983) Associative long term potentiation in hippocampal slices. *PNAS* 80:7343-7351.

Lab 6A: LTP Prep Lab

Station 1: Solutions

Careful solution preparation (weighing, measuring, and mixing) is critical for LTP experiments. It is important to store the brain slices in an environment that replicates the physiology of the brain in the body. The solutions contain the necessary salts and glucose to simulate these conditions. It's also necessary to provide the proper gasses to maintain slice health. Typically, solutions are saturated with mixture of 95% oxygen and 5% carbon dioxide (carbogen). Neurons need oxygen to breathe and carbon dioxide to maintain proper acidity levels.

It is common for a lab to use 10X stock solutions (Table 1) for the preparation of cutting and recording solutions (Table 2). The glucose (omitted from stock solution to prevent bacterial growth) and some ions (calcium chloride and magnesium chloride) must be added the day of recording. Recording solution, or artificial cerebral spinal fluid (ACSF) is used during LTP recording. Cutting solution is used for brain sectioning and is similar to recording solution except the Ca^{2+} is omitted to prevent the firing of action potentials while the hippocampal slices 'recover'.

Table 1. Stock Base Solutions

Standard Base (10X)	Conc. (M)
NaCl	1.19
KCl	0.025
NaH_2PO_4	0.01
NaHCO_3	0.262
Stock ions	
CaCl_2	2
MgCl_2	2

Today you will be making a standard recording solution (ACSF) from a 10X base stock solution containing the chemical compounds (sodium chloride, potassium chloride, monosodium phosphate, glucose, and sodium bicarbonate). The ACSF must first be bubbled with carbogen until the solution is saturated (~10-15 mins), before slowly adding the remaining ions (CaCl_2 & MgCl_2). If the solution turns cloudy or forms a film on top, the ions have not incorporated properly, or have 'fallen' out of solution.

Prepare 1 liter of ACSF (recording solution):

Note: Check all calculations with your instructor as you proceed:

1. Calculate & weigh glucose; record mass in Table 2 (below)
2. In a 500 mL beaker dissolve glucose in ~200 mL deionized H₂O
3. Transfer glucose solution to 1000 mL graduated cylinder
4. Add 100 mL 10X stock base solution
5. Bring level up to 1000 mL with deionized H₂O
6. Transfer solution to 1 L bottle (clearly label with solution, date & name(s))
7. Bubble ACSF (recording) solution with carbogen (record time)
8. Calculate and record volume needed of MgCl₂ stock solution (Table 2)
9. Calculate and record volume needed of CaCl₂ stock solution (Table 2)
10. Calculate the mass of NaCl, KCl, NaH₂PO₄, and NaHCO₃ for the liter of 1X ACSF you just made and record in Table 2 (verify calculations with your instructor)
11. After ACSF has bubbled for ~10-15 mins, slowly pipette MgCl₂ and CaCl₂ into bubbling ACSF; continue to bubble for an additional 10-15 mins.
12. Remember to come back and check your bubbling ACSF again to see if the ions stayed in solution (solution should remain clear, without a film on top)

Table 2. Recording Solution

Standard ACSF (1X)	Conc. (mM)	g/L
NaCl	119.0	
KCl	2.5	
NaH ₂ PO ₄	1.0	
NaHCO ₃	26.2	
Day of recording addition		
Glucose	11.0	
		mL/L
CaCl ₂ (2 M stock)	2.0	
MgCl ₂ (2 M stock)	2.0	

Station 2: Brain Removal

Note: this procedure will be demonstrated for you first

For the actual LTP experiment, the animal is deeply anesthetized with isoflurane (a general inhalation anesthetic agent) and confirmed by the absence of reflex activity following a toe pinch. For the greatest tissue health, we try to complete the brain removal procedure (from decapitation to brain submersion in ACSF) in one minute. This procedure becomes faster with practice. Take your time and don't worry about how long the procedure takes. For the purposes of this lab, the mouse will be sacrificed prior to the following procedure:

1. The mouse will be euthanized according to methods approved by the Institutional Animal Care and Use Committee (IACUC). In this case, the mice will be sacrificed prior to student dissection.
2. With the large scissors, decapitate the mouse rostral to the first cervical vertebra and place the head on paper towel.
3. Using the small scissors, make an incision in the middle of the scalp starting caudally to the occipital bone, to the nasal bone. Pull back scalp and cut the skull between the eyes.
4. Using the spring scissors, angled up & away from brain, cut through the skull plates along the sagittal suture (posterior to anterior) beginning at the base of the skull at the cerebellum through to the frontal skull plates.
5. Using spatula or forceps; open 2 skull flaps.
6. Turn brain into bubbling cutting solution severing optic nerves to release the brain from skull.
7. Incubate brain in ice-cold Ca^{2+} -free ASCF (cutting solution) for 1 minute.

Station 3: Preparation of Acute Hippocampal Slices

Note: this procedure will be demonstrated for you first

1. Place filter paper on the lid of the ice-cold Petri dish dampened with ACSF. Retrieve the brain from the ACSF using a spoon and place on the dampened filter paper.
2. Using the scalpel blade, remove the cerebellum and ~ 2mm of the rostral frontal lobes. Run the scalpel blade through the intrahemispheric fissure to completely separate the two hemispheres. Place one hemisphere back into the bubbling ACSF.
3. Stand hemisphere on its rostral end in a dissection dish containing cutting solution. Locate the midbrain and using the surgical scissors, gently hold the midbrain in place and slide spatula in the gap between the midbrain and the neocortex. Very gently, continue to slide the spatula down and separate the brainstem/midbrain/thalamus away revealing the inside of the lateral ventricle and the medial surface of the hippocampus. Use the sharp edge of the spatula to sever the fornix.
4. Using the scissors and the spatula, gently continue to pull the brainstem/midbrain/thalamus away from the cortex without completely severing the brain.
5. Gently slide the spatula into this gap, such that the long side of the spatula runs parallel with the long axis of the hippocampus. Once the spatula is completely under the hippocampus, hold down the brainstem/midbrain/thalamus firmly with the scissors and roll the spatula away from the scissors to physically separate the hippocampus from the rest of the brain.
6. Gently position the hippocampus on ice-cold tissue chopper platform with plenty of ACSF; section hippocampus (transverse plane) at 400- μ m intervals.
7. Transfer the slices to slice keeper and incubate at 37°C in carbogenated (95% O₂/5% CO₂) ACSF for at least 30 mins prior to transfer to the recording chamber.

Station 4: Electrodes

Here, you will learn to pull recording electrodes used in LTP studies. Your recording electrodes should have a resistance of 1-3 MΩ. A quick and easy shortcut to check the resistance of your electrodes is to use the ‘bubble test’.

1. Set the puller to Program 0 and press enter. Insert the glass capillary into the guide. You will need to press the release plates on both sides to allow the metal parts to move together. Center the capillary, tighten the tension screws to secure, and press ‘Pull’. The filament in the middle will heat up a few times, and the electrodes will eventually split into two with a pop.
2. Bubble test one of the electrodes. Attach the electrode to a 10cc syringe set to 10cc. Carefully place the electrode in a vial of 70% ethanol and hold up so you can see it against a dark backdrop. Depress the syringe slowly until you see bubbles escaping the electrode. Note the number of ccs it took (10cc minus the number you read). Bubble points between 1 and 3cc will give you electrodes with the proper resistance for field recordings.
3. If the electrode tests outside of the resistance range, discard the electrodes and alter the puller settings using the guidelines in table below. Adjust the Heat setting in increments of 5 as follows:
Reset >> Program 0 >> Enter >> Adjust Heat setting (leave all other parameter the same)
4. Pull another pair of electrodes and test again for resistance. Repeat (if necessary) by adjusting the Heat setting once again. Once you achieve a good electrode, confirm resistance of paired electrode. Store & label good electrodes in Petri dish lined with putty.
5. Reset all parameter to original Program 0 settings:
Heat: 535 Pull: [blank] Vel: 25 Del: 200
Press ‘Reset’ button one last time before moving on.

Parameter	Increase	Decrease
Heat	Longer Taper Higher Resistance	Shorter Taper Lower Resistance
Pull	Smaller Tips Longer Taper	Larger Tips Shorter Taper
Velocity	Smaller Tips	Larger Tips
Time/Delay	Shorter Taper	Longer Taper

Lab 6B: LTP Recordings

I. Configure Recording Instrumentation

1. Check that the gravity fed ACSF is bubbling with 95/5% O₂/CO₂
2. Set flow rate to 2-3 mL per minute
3. Position recording and stimulating electrodes in micromanipulators near the chamber
4. Position light for proper illumination; position the scope over the recording chamber slice stage and focus
5. Turn on amplifier, stimulus isolator, and Powerlab digitizer
6. Check that stimulus isolator box is set to deliver a single, biphasic pulse, 0.1 or 0.2 ms long and up to 1 mA in amplitude. For now, set the amplitude to 500 uA.



7. Open the Chart settings file '**LTP single stim**'. (This file will stimulate the slice 20 times at 8 s intervals. We will use this protocol to determine if our electrodes are correctly placed, perform a baseline response, perform an input-output (I-O) curve, and obtain post-tetanic responses.)
8. Lower stimulating and recording electrodes into ACSF in recording chamber and press 'Start' in Chart. Make observations of your recordings in Chart. Remove one electrode and compare recording with previous trace. Repeat by replacing the electrode and removing the other electrode.

II. Record Field Potentials

1. Place a slice in the chamber using a transfer pipette and position as illustrated on the whiteboard
2. Carefully place the twisted bipolar stimulating electrode on the stratum radiatum of CA1 (you can lower stimulating electrode more later, if necessary)
3. Carefully position the glass recording electrode downstream in the stratum radiatum of CA1 just barely touching the slice
4. Hit start and look for field potentials. If you do not see a response, lower electrodes ever so slightly (~10-20uM)! Repeat as necessary. If you do not see field potentials, reposition electrodes closer to each other.
5. Once you successfully obtain fEPSP move to perform an I-O curve:
 - a. Set stim to 200 uA, and after each stim increase the strength by 25 uA every 8 secs. Following this procedure, when you get to the 20th stimulus you will be delivering a whopping 700 uA stimulus. Using the I-O curve, calculate the half maximal stimulus you will use to measure baseline responses and induce LTP. **'Save as'** and **Name** this file. Set stimulus strength using the "Pulse Amplitude" knob on A-M Systems box, shown above. Make sure to write down what strength you used.
6. Measure baseline response: Load the LTP baseline protocol file **'LTP single stim'** and run! **'Save as'** and **Name** your file.
7. Deliver tetanus: Load the tetanic stimulation protocol **'tetanus_settings'** file and run! **'Save as'** and **Name** this file. (You won't perform analysis on this file, but it may be helpful to look at how the slice responds to the tetanus.)
8. Measure post-tetanic responses using the baseline protocol at immediate, 10-minute and 30-minute time points. Reload the baseline protocol **'LTP single stim'** file for each post-tetanus time point and run! **'Save as'** and **Name** your files.

LTP Data Analysis

You will be analyzing your LTP data using three chart data files: baseline, 10 minutes, and 30 minutes after tetanus.

Below is a relatively simple method for analyzing your data. Please feel free to play around with the Chart software and devise other methods for analyzing your data if you prefer.

1. Open the Chart file and click analysis when prompted.
2. The display should show two traces: the top trace will be the potentials you recorded, and the bottom trace will be the derivative of the top trace.
3. Use the crosshairs on the bottom trace to find the maximum (negative) slope of the fEPSP. This should be the lowest point on the bottom trace that aligns with the fEPSP on the top trace. Sometimes noise can create an artifact that disrupts the slope. Inspect these points with your lab partner(s) to make sure your slopes are not disrupted by artifact.
4. Record all 20 slopes for each of the 3 files.
5. Enter your data into Excel and calculate the means and standard errors for each condition.
6. Perform an appropriate statistical test comparing baseline, 10 minutes, and 30 minutes post tetanus. This will tell you if you got potentiation. Did you get long-term potentiation?
7. Make a figure/s summarizing your data for you lab write-up.