## bamji lab chemical ltp protocol

## ecs (+TTX/Str) recipe for cltp

**Recipe for stock ECS**

NaCl 140 mM

KCl 5 mM

CaCl2 1.3 mM

HEPES 25 mM

D-glucose 33 mM

*pH 7.35 with NaOH, filter sterilize and store in fridge*

**To make ECS (+TTX/Str) add the following to ECS**

TTX 0.0005 mM (add on day of exp)

Strychnine 0.001 mM (add on day of exp)

Bicuculline

## important notes

Stock ECS solution should be sterilized (vacuum filtration) and stored in fridge.

* Supplements/drugs (TTX, strychnine, Gly) should always be added fresh to the stock ECS solution on day of exp.

Solutions should be pre-warmed before use (37°C).

## Solutions for cLtp protocol

**(-) Mg2+ ECS** = ECS (+TTX/Str) no further additives

**(+) Gly ECS** = ECS (+TTX/Str) + 200 μM Glycine (made fresh, add on day of exp)

**(+) Mg2+ ECS** = ECS (+TTX/Str) + 2 mM Mg2+

## getting started

* To make ECS (+TTX/Str), start by calculating how much of each of the above solutions you will need.
* Measure out the total volume of ECS forall solutions and add TTX and strychnine.
* Aliquot ECS (+TTX/Str) into 3 falcons, one for each of the 3 solutions.
* Add Gly to the **(+) Gly ECS** (from 200 mM stock made fresh from powder).
* Add Mg2+ to the **(+) Mg2+ ECS** (from 2M aliquot in freezer).
* Pre-warm all solutions to 37’C.

## cLtp protocol

1. Pipette off the media and save in a labelled falcon tube and store in the incubator.
2. Wash the neurons with 1 ml of pre warmed (-)Mg2+ solution **2 times,** leaving

12-well plate in the incubator for 10 minutes after second wash.

1. Aspirate the (-)Mg2+ solution and add 1ml (+)Gly solution. Incubate the neurons with (+)Gly solution for **exactly 3 minutes** (in 37’C incubator).
2. Wash the neurons with 1 ml of (+)Mg2+ solution **twice** and put back the saved media, until appropriate timepoint post cLTP.
3. Fix or lyse the cells as needed.

How does glycine cLTP work?

Glycine cLTP enhances post-synaptic NMDAR Ca2+ influx. Glycine is a co-agonist at the NMDAR (along with glutamate), and when present at high concentrations increases NMDAR open probability. The resulting increased NMDAR Ca2+ influx triggers post-synaptic cLTP, including insertion of AMPARs. Previous studies have measured mEPSCs after glycine cLTP, and observed an increase in the frequency and amplitude of mEPSCs.



Images from Lu et al., 2001

*Why do we include TTX in the solutions?* TTX is an Na+ channel antagonist that blocks action potential firing. With action potentials blocked, neuronal activity is greatly reduced, and the only glutamate release onto neurons will be due to small spontaneous pre-synaptic vesicle fusion events at the synapses. When we add glycine, any increased NMDAR activity/calcium influx will be highly localized to synapses, making this a highly synapse specific stimulation protocol.

*Why do we include strychnine in the solutions?* The high concentrations of glycine used to stimulate NMDARs will also activate *inhibitory* glycine receptors (GlyRs). Strychnine is a GlyR antagonist, so will prevent simultaneous neuronal inhibition caused by GlyR activation.

*Rationale for Step 1: Wash in Mg2+ free solution.* This is to alleviate the Mg2+ block from NMDARs, which are normally blocked at resting membrane potential by physiological extracellular concentrations of Mg2+ (~1mM). Because we’re adding TTX which blocks action potentials, there is no way the Mg2+ block can be relieved, as neuronal depolarization is essentially blocked, so we have to wash out *ALL* of the Mg2+ to properly activate NMDARs during cLTP.

*Rationale for Step 2: Wash in (+)Gly.* 200 μM glycine should saturate all NMDAR glycine binding sites. When spontaneous glutamate release occurs, glycine will boost the NMDAR Ca2+ influx and induce cLTP.

*Rationale for Step 3: Wash in (+)Mg.* This will immediately block NMDARs and end the cLTP stimulation. This step is important to ensure that all cells receive the exact same duration of cLTP treatment (3 mins).

Read this paper for more info! [https://doi.org/10.1016/S0896-6273(01)00194-5](https://doi.org/10.1016/S0896-6273%2801%2900194-5https%3A/doi.org/10.1016/S0896-6273%2801%2900194-5)

How much of each solution do I need?

**(-) Mg2+ ECS:** Standard ECS

 12 well: 1 ml wash/well for 3 washes = 3 ml/well

 10cm dish: 4ml wash/dish for 3 washes = 12 ml/dish

**(+) Gly ECS:** Standard ECS + 200 μM Glycine (made fresh, add on day of exp)

 12 well: 1 ml wash/well for 1 incubation = 1 ml/well

 10cm dish: 4ml wash/dish for 1 incubation = 4 ml/dish

**(+) Mg2+ ECS:** Standard ECS + 2 mM Mg2+

 12 well: 1 ml wash/well for 2 washes = 2 ml/well

 10cm dish: 4ml wash/dish for 2 washes = 8 ml/dish

For alternative cLTP and cLTD protocols see: <https://bio-protocol.org/prep410>