

Date of Hipp Expt	
Animal	
Number of pups/embryos	
Animal Company Name	
Rat Strain	
Age	
Euthansia method	
Operator	
Dissection	
Hippocampal Dissection Start Time	
Storage medium at RT	
Macro-dissection Operator	
Hippocampal pairs number obtained	
Micro-dissection Hippocampal Operator	
Dissociation	
Operator	
type of dissociation media	
DM Lot	
DM Volume in ml	
Protease type	
Protease Unique ID	
Protease conc	
Protease Volum in ml	
DNase Unique ID	
DNase Conc	
DNase Volume in ml	
Temperature in C	
Incubation Duration	
Rinse Media	
Approximate Rinse Volume in ml	
Rinse Number	
Rinse Duration	
Trituration Media	
Approximate Trituration Volume in ml	
Approximate # of trituration	
Cell Counting	
Live Cell Number(10^6 /ml)	
Dead Cell Number(10^6 /ml)	
Total Cell Number(10^6 /ml)	
Total Volume (ml)	
Total live Cells number (10^6)	
%Alive	
Time of Counting - Finish time	

Cells Distributed to following Receiver(s)	
Receiver 1	
Receiver 2	
Receiver 3	
Receiver 4	
Receiver 5	
Receiver 6	
Receiver 7	
Receiver 8	
Receiver 9	

Preparation of Dissociated Hippocampal Neurons

Get the rats and sacrifice them by CO2.

- Dissect the pup's brains and collect Hippocampi in 15ml conical tube in **Storage Medium** at RT;
- Make the **dissociation media**;
- Remove medium from the hippocampi (from step1) and add the **dissociation media** from step 2;
- Add **Trysin** and **DNase** and incubate the hippocampi at 37°C for 15'.
Flip the tube with fingers during the incubation several times so all hippocampi float freely;
- Use cotton plugged Pasteur pipette to remove the supernatant and rinse w **Rinse Media**, make the suspension float and settle down;
- While rinsing, prepare 1 narrow-bore fire-polished (for smoothing the sharp edges) cotton plugged Pasteur pipette and pre-wet with rinsing medium;
- Remove **Rinse Media** from cells;
- With pipette prepared in step 6, add 1 pipette-full of **Rinse Media**;
- Triturate forcefully until all cells are dissociated and no cell clusters left. Try to triturate less than 10 times, if chunks of tissue are still present, do more. Remember the excessive trituration will reduce cell viability;
- Bring volume to 8-12ml to dilute cells, then count cells.

Add Note if any