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Ex Vivo Electrophysiology

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We use this protocol and it's working

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Abstract

This protocol describes the preparation of brain slices, setup of the electrophysiology rig, and solutions for collecting whole-cell recordings.

Troubleshooting

Setup

1 First prepare a 10x stock aCSF solution by fist add about 200 mL of ddH20 water into a clean 2L flask. Then add each of the following powders:
§NaCl: **1250 mM**
§KCl: **25 mM**
§NaHCO3: **250 mM**
§NaH2PO4: **12.5 mM**

2 Next, fill the flask approximately three quarters with ddH2O and using a magnetic stirrer, allow the solution to be thoroughly dissolved. The mixed solution should then be brought to a final 2L volume and stored in a 2L glass bottle and refrigerated at 4°C.

Note: the 10x stock aCSF can be used for up to a month and should be remade fresh after that time.

3 Prepare 1 liter of 1X aCSF (to be used as both perfusate and cutting solution):
-Fill a 2L flask with about **450 mL** of ddH20.
-Add **4 mL** of $CaCl_2$solution to the bottle.
-Add **4 mL** of $MgCl_2$solution to the bottle.
-Add **5.02 g** of glucose (final concentration of 13.93 mM)
-Add **200 mL** of a 10X stock aCSF solution.
-Swirl mixture around well by hand for a few seconds.
-Fill remainder of flask to **2 L** with ddH20.
-Carbogenate solution for at least 10 minutes before using.

4 Using continuously carbogenated 1x aCSF: §Fill a small slice holding chamber with the 1x aCSF and add **5 mM** of L-glutathione at approximately **1:1000x** and **1 mM of** Na-Pyruvate at **1:300x**.
-Set the chamber aside near a **37 °C** heated water bath while the solution inside the holding chamber is continuously carbogenated.
-Additionally, decant approximately **100 mL** of 1X aCSF into a small glass beaker kept cold on ice while carbogenated (to be use for perfusate)

5 Next, decant approximately **100 mL** of the 1X solution into a **300 mL** L plastic bucket and place the bucket in a **−20 °C**freezer for **70—80 minutes**. This solution is to be used for cutting brain tissues and should be frozen over but not frozen solid. After taking the bucket out from freezer:
-Using a large spatula, break up ice and stir into a slurry.
-Add approximately **100 mL** of 1X aCSF kept at room temperature.
-Mix solution with a handheld blender until forming an easily flowing slush.
-Keep the bucket of the 1x aCSF slush cutting solution on ice while being carbogenated.

-The slush solution should consist of approximately one fifths of liquid solution and be settled to the bottom of the bucket.

6 Overdose the mouse with a **1 mL**intraperitoneallyinjection of a **150 mg/kg ketamine and 30 mg/kg xylamzine** mix. While mouse is overdosing: place the slice chamber in a heated water bath set to **37 °C** (water in the bath should come up the side of the chamber to approximately the same height as the aCSF). Next, after mouse is completely anesthetized:

-Transcardially perfuse mouse with the ice-cold 1x aCSF.
-Rapidly decapitate and extract brain in 1x aCSF slush.
-Using razor blade, cut brain down the midline.
-Glue brain medial side down on cutting block and place block quickly into cutting chamber of a Vibratome.
-Fill cutting chamber with aCSF slush solution, kept continuously carbogenated in chamber.
-Cut slices at **240 µm**thick
-Quickly transfer each cut slice into hold chamber kept in heated bath.
-Remove holding chamber from bath 30 minutes after last slice transfer is made.
-Allow holding chamber to equilibrate to room temperature (approximately 20 minutes) before transferring slices to recording chamber of electrophysiological rig.

Whole-cell Recordings

7 Using a pipette puller, glass pipettes should be pulled to ensure a pipette resistance of **3.2—3.8 MΩ**. For whole-cell voltage clamp recordings, a KMeSO4 solution is used containing the following:
-KMeSO4: **135 mM**
-KCl: **5 mM**
-CaCl2: **0.5 mM**
-HEPES: **5 mM**
-EGTA-K: **5 mM**
-ATP-Mg: **2 mM**
-GTP-Na: **0.5 mM**
-Biocytin: 0.20% (w/v in grams)

8 Transferred slices in recording chamber are continuously perfused with 1x aCSF that is kept at room temperature and continuously carbogenated. Neurons for recording are identified and recorded as follows:
-Pipettes are backfilled with KMeSO4 solution and inserted into headstage of amplifier.
-Pipettes are then pressurized to approximately **56 millibars**.
-Pipettes area offset before cell attachment.
-Neurons are clamped at **—80 mV** before whole-cell access is achieved.

9 Stimulus generation and data acquisition are performed using an amplifier (Molecular Devices), a digitizer (Molecular Devices), and pClamp (Molecular Devices).

10 *For current-clamp recordings*
Adjust the amplifier bridge circuit to compensate for electrode resistance and subsequently monitor it. Filter the signals at 1 kHz and digitize them at 10 kHz.
$KMeSO_4$and Na_2-GTP were from ICN Biomedicals and Roche, respectively. All other reagents were obtained from Sigma-Aldrich.

Excitability of SPNs:

11 Examine the frequency-current (F-I) relationship of each cell with current-clamp recordings as follows.
-Apply a series of 500 ms current steps of n beginning at −150 pA and incremented at 25 pA for each consecutive sweep.
-A **30 second** intertrial interval is used.
-The current steps are continued until depolarization block is reached.
-Monitor resting membrane potential was monitored for stability, and exclude cells that varied 20% from mean baseline from the analysis.

Corticostriatal responses recorded in voltage-clamp

12 -Perform electrical stimulation using parallel bipolar tungsten electrodes (FHC) placed in layer 5 of the cortex.
-Adjust stimulus width and intensity via a constant current stimulator (Digitimer) to evoke a first excitatory postsynaptic current (EPSC) with an amplitude of 200–400 pA in the presence of the $GABA_A$receptor antagonist SR95531 (10 µM) and CGP55845 (1 µM).
-Monitor whole-cell access was monitored with a −5 mV pulse throughout the recording. Determine off line the membrane capacitance (Cm) as $Cm = Q_t * V_{test}$, where Qt was calculated as the integral of the transient current elicited by V_{test}, a 10 mV voltage step.
-Take the average of the ratios of the second EPSC amplitude to the first EPSC amplitude for each recording sweep to calculate the paired-pulse ratio (PPR) for a given cell
-Exclude data if the series resistance of the patch pipette differed by > 20% between the two recordings.