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Ex Vivo Electrophysiology

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ABSTRACT

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

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Preparing Brain Slices

1 Setup

1.1 Decant about 150 mL of 1X Glycerol-based ACSF solution into a small bottle or beaker and place in the freezer (see recipes for all solutions at bottom).

1.2 Prepare 1 liter of 1X ACSF by

- Combine **100 mL** of a 10X stock solution, **900 mL** of ddH2O, and **2.25 g** of glucose in a 1 liter bottle.
- Place a bubbler in the bottle to carbogenate the solution.
- After a few minutes, add **2 mL** of $CaCl_2$ solution to the bottle.

1.3 Once mixed, decant some of the 1X ACSF into your slice storage chamber.

- Place the chamber in a heat bath at **35 °C** (water in heat bath should come up the side of the chamber about to where the ACSF level is)
- Attach to carbogen

1.4 Obtain a small bucket of ice, and place the clean chuck (block upon which the brain will be mounted for slicing) on the ice to pre-cool it.

1.5 Set up perfusion and dissection tools:

- forceps
- small scissors
- large scissors
- hemostat
- razor blade
- metal spatula

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- small glass petri dish

The perfusion kit (a 10 mL syringe, stopcock, about 20 cm of tubing, and a fitting) should be fit with a new 32G needle. Place the tools needed for the perfusion on a Styrofoam base with several napkins.
Draw up and log 1 mL of ketamine/xylazine mixture, which will be used to sacrifice the mouse.

1.6 Set up vibratome (Leica)
- Load a fresh blade
- Turn on the vibratome and make sure the settings are correct (default 275 micron thickness, 0.12-0.18 mm/sec slicing speed)

1.7 Right before anesthetizing the mouse, remove the bottle/beaker of cold 1X Glycerol-based ACSF from the freezer (it preferably has some ice crystals) and shake or stir with a spatula to make uniform icy mixture. Put this container into the ice bucket and place a bubbler in it.

2 Anesthetizing mouse.

2.1 Inject the adult mouse with 1 mL of ketamine/xylazine mixture (a terminal dose) and place in home cage (if singly housed) or a clean cage. It will take 5+ minutes to take full effect.

2.2 When all responses are absent, fill the 10 mL syringe (in the perfusion kit) with the 1X Glycerol-based ACSF and attach to the stopcock. Advance the plunger until the ACSF comes out the end of the needle (primed).

3 Transcardial perfusion with ice-cold Glycerol-based ACSF.

3.1 Place the mouse on the napkins and set up for a transcardial perfusion.

3.2 Locate the heart
- Open the chest cavity with the small scissors
- Cut under the rib cage on both left and right, down to the sides
- Using the hemostat, grab the bottom of the sternum (the xiphoid process) and pull this back to get a better view
- Use the small scissors to cut the thin membrane (diaphragm) separating the abdominal cavity (below) from the thoracic cavity (above). **Be sure not**

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to nick the heart in this process.

3.3 When the heart is visible, grab the forceps in your left hand and the back of the needle (on the tip of the perfusion kit) in your right. You may use the forceps to grab the side of the heart.

3.4 Insert the needle in the apex of the heart to hit the left ventricle (to your right, the mouse's left).

3.5 While holding your right hand very steady (with the needle in the ventricle) put down the forceps and grab the small scissors. Use the tips of these to cut open the right ventricle (to your left, the darkest red part of the heart). You should see a small leak of blood.

3.6 Use your left hand to slowly advance the plunger of the perfusion kit over about 30 seconds. This will perfuse the mouse's circulatory system with cold, carbogenated Glycerol-based ACSF, removing all the blood from the brain and cooling it.

3.7 Set the perfusion kit aside.

4 Dissection.

4.1 Decapitate the mouse and remove excess scalp over the top.

4.2 Use small scissors to cut along the curvature of the skull behind the cerebellum.

4.3 Use the same scissors to cut through the eye sockets from left to right.

4.4 Very gently, with upwards pressure to keep the scissors adjacent to the skull, insert the scissors into the skull cavity from the back of the cerebellum and cut through the superior surface of the skull along the midline.

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4.5 Using forceps, peel back the skull on either side of the midline to reveal the brain.

4.6 Use the spatula to scoop the brain out of the skull cavity and into a small glass petri dish. Cover this with some of the 1X Glycerol ACSF in the bottle. The brain can be dissected further either in this dish or placed on a piece of filter paper (saturated with Glycerol ACSF) on an inverted petri dish.

- For most brain regions, we prepare coronal sections
- For striatal sections, make a coronal cut around the midbrain (between the visible colliculi on the dorsal surface) using a razor blade, and then another coronal cut approximately 3-4 mm from the frontal edge of the brain. This cut should be close to, but anterior to the most anterior portion of the striatum.
- For subthalamic nucleus (STN) and some other regions, we prepare sagittal sections. To prepare sagittal sections that contain areas closer to the midline (eg STN), when the brain has been removed from the skull, use a razor blade to make a vertical cut about 2-3 mm from the lateral side of the brain on either side, and then down the midline. Now spread the two hemispheres like you are opening a book, and glue the lateral face of the brain (on both left and right sides) onto the block in ste[4.9

4.7 Remove the chuck from the ice and wipe its surface dry.

4.8 Using a brush, paint a small area (5-6 mm in diameter) of Krazy Glue onto the center of the ice-cold chuck.

4.9 Scoop up the brain with the rounded (not pointy) end of the spatula, and place the posterior surface of the brain (midbrain) onto the chuck, so that the frontal face points up.

5 Slicing on the Vibratome.

5.1 Once the brain is glued to the chuck:

- place it in the slicing chamber on the vibratome (the dorsal side facing the blade, ventral side facing you for coronal sections)
- pour carbogenated ice-cold Glycerol-based ACSF into the chamber until the brain is just submerged

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- if you wish to keep track of the side of the brain for subsequent recordings, use a razor blade to make a small nick on the ventral surface, on the left side of the brain (which will now be on your right as you look down at the chuck).

5.2 Lower the vibratome blade until it is just above the brain, and just behind the dorsal surface of the brain.
- Push the button on the vibratome controller to mark the posterior edge of the slice window.

Advance the vibratome manually until you are just past the ventral edge of the slice window.
- Push the button on the vibratome controller to mark the ventral edge of the slice window.

Set the vibratome speed to 0.12 mm/sec.

5.3 Push “run”.
- The blade should be retracted to the dorsal edge of the slice window and begin vibrating and moving ventrally

Hit pause before it hits the brain.
Using the up/down controls, move until the blade is around the top of the brain, hopefully close to where the anterior part of the striatum begins.
Hit pause a second time to get the vibratome going again.

5.4 Watch as the vibratome cuts through the tissue, making sure it does not get caught on anything. After it passes through the ventral surface, a slice should float away from the rest of the brain. Likely the first slice or two will not be needed, and can be sucked up with a transfer pipet with the tip snipped off, and discarded in the necropsy bag.

5.5 When the first striatal slice has been cut, suck it up with the snipped transfer pipet and transfer to your warmed slice storage chamber. Repeat this process until all striatal slices of interest (typically 4-6 slices, which can be hemisectioned as desired) have been collected.

6 Cleanup.
- Stop the vibratome and retract the blade away from the slice.
- Rotate up and remove the blade, using the hex wrench.
- Remove the chuck from the vibratome, and scrape the remaining tissue and glue off the chuck and place in the necropsy bag.
- Put the rest of the mouse carcass, napkins into the necropsy bag and take this to the necropsy freezer.
- Place the bubbler in a 250 mL beaker of fresh ddH2O to clean it.
- After placing all sharps in the sharps container, take the rest of your instruments to the sink.
- Flush the perfusion kit with water, then air until there is no residual water in the tubing.
- Rinse and wipe instruments carefully with dI water and set aside to dry.

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- **Do not use any detergents.**

Recovery

7 Recovery.
After slicing, let the slices incubate at **35 °C** for 30-60 minutes, then move the chamber to **Room temperature** .
From this point forward you can transfer slices one at a time to the rig for slice recordings.

Rig Set Up

8 Rig set-up.

8.1 Open carbogen line to the rig.

8.2 Set the remainder of the 1X ACSF by the rig and place a bubbler in the bottle. Make sure the ACSF outflow bucket has been emptied into a waste container.

8.3 Turn on the devices:
- Multiclamp amplifier
- Peristaltic pump
- Sutter manipulator
- Light source
- LED

Open the video camera program and set to collect live images from the microscope.
Turn on the light to illuminate the recording chamber.
Open the appropriate template file in Igor and save the experiment as the name of your first cell.

8.4 Electrodes
- Pull electrodes on electrode puller (P97 Sutter Instruments).
- Use air can to clean out the tip fill container and the syringe for filling electrodes.
- Use this syringe to pull up about 0.4 mL of tip fill solution (stored in -4°C refrigerator), then place a syringe filter on the tip of the syringe, and push through into the tip fill container until there is a nice bead of tip fill.
- Remove an aliquot of internal solution (see recipes below) from the -80C freezer and place by the rig to thaw.

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8.5 Place the intake line into your ACSF container and turn on the peristaltic pump to circulate (clockwise arrow). Wait until the fluid has entered the recording chamber, then turn on the in-line heater (Warner Instruments). Make sure the outflow line is placed correctly at the back of the chamber and adjust as needed to keep level in the chamber.

Whole Cell Recordings

9 Whole-cell recordings.

9.1 Using a snipped transfer pipet, transfer a slice to the center of the recording chamber. Place the U-shaped slice holder (AKA “harp”, made of platinum and nylon thread) over the slice such that the slice is pinned down well, and the area of interest is between two threads of the harp.

9.2 Using the video and the stage X, Y knobs, move the slice until you see the area of interest at the center of the field under low magnification (4X).

9.3 Load a pipette with tip fill and back fill, and slide onto the wire/electrode holder (Warner) on the right side. Tighten the threaded plastic at the tip of the holder to hold the pipette in place. Now carefully swing the headstage/electrode holder into place over the recording chamber, and tighten the screw to hold in place.

9.4 Apply positive pressure to the back of the electrode by using a 10 mL syringe and the stopcock on the back of the tubing leading to the electrode holder. A good default choice is to inject air from about 2.4 to 2 mL on the side of the syringe, and then quickly close the stopcock.

9.5 Using the manipulator, lower the electrode until it is in the ACSF and over the area you wish to record from.

9.6 On the Multiclamp, zero the pipet offset and check the electrode resistance. It should be 2-5 MOhms (preferably about 3 MOhms for most whole-cell recordings).

9.7 Carefully lift the low-mag objective and swing in the high mag (40X water immersion) objective, and lower this until it is either just about touching the shank of your pipette, or the ACSF has wicked up onto it. If you have lowered it but the ACSF has not yet wicked onto it, you can use a transfer pipette to drip a small amount of ACSF onto the objective. Use the focus knobs to focus on

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the tip of your pipette, which should be above the slice. Using one hand to adjust the focus and the other to adjust the Z on the manipulator, slowly lower the electrode towards the slice (focus down, lower the electrode, repeat).

9.8 When the electrode is close to but still above the slice, move the electrode to the lower right hand corner of the screen, and now use the focus knobs to look at the slice. You can now pick a cell to patch, and using the controls, move it to the center of the screen.

9.9 Focus back up on your electrode, and use the manipulator to bring it just over the cell in the center of the screen, and then lower it down until you see a small dimple on the surface of the cell (if you are an expert you may not need to create a dimple) and quickly release the positive pressure on the electrode by opening the stopcock.

- Look at the oscilloscope: the pipette resistance will now probably be higher (e.g. 10 or 20 MOhms).

9.10 Using your mouth, apply gentle, continuous suction to the stopcock in order to form a gigaohm seal. When it hits several hundred MOhms or a gigaohm, you can set the holding potential to -70 mV, in preparation for breaking in.

9.11 Once you obtain a gigaohm seal, you can break in by applying stronger, brief suction to the stopcock. This may require several tries. Start gentle, then increase strength as needed until you break in.

- You will know you have broken in because the resistance will decrease to 100-250 MOhms (roughly the input resistance of the cell).

Hit the “break in” button in Igor to mark the time you broke in.

9.12 Note any particulars of the recording (e.g. mouse code name, any transgenes or constructs injected, brain region/side you are recording from, cell type, if known, the type of ACSF, any drugs in the bath, and type of internal solution).

9.13 After 5 minutes to allow the internal solution to dialyze the cell, you can start your recordings. The types of acquisition routines you will use in Igor will differ based on the type of recording.

9.14 When you have completed your recording of a particular cell, set the holding potential to 0 mV and remove the electrode, first slowly, then when you have pulled the high mag objective up, and switched to low mag, you can pull it up quickly. Then swing out the electrode holder, remove the electrode and discard in the sharps container. Save your Igor file, re-open a template and save as the next cell name. Repeat process above.

Cleanup

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10 10. Cleanup.

10.1 Remove the slice from the recording chamber and either place in a well plate with 4% PFA (if postmortem histology is needed) or in a necropsy bag. Repeat this process with any excess slices that remain in the slice storage chamber.

10.2 Replace the harp in the recording chamber.

10.3 Take a 250 mL beaker full of ddH2O and place the carbogen bubbler and intake tubing into this beaker.

10.4 Run water through the rig for a minimum of 10 minutes, preferably longer. You may use the high speed mode if you are able to stay in the room and make sure there is no overflow.

10.5 Discard any leftover ACSF, internal solution, and clean out your internal solution syringe with water and air.

10.6 Carefully clean the slice storage container (all three parts) with water and your fingers. **No detergents**.

10.7 Use a napkin to dry off as much as you can, especially the netting and the corners of the base of the chamber, to reduce the chance of bacterial or fungal growth. Leave it on the drying rack.

10.8 Turn off the carbogen line at the wall, remove the bubbler and intake line from the water, and, leaving the peristaltic pump on, run air through the rig for 5-10 minutes, until the lines appear dry.

10.9 Discard the waste ACSF in the appropriate waste container (sink for regular ACSF, designated waste containers if any drugs have been added).

Remove the cleaned harp from the recording chamber and place in the small

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10.10 petri dish next to the rig for storage.

10.11 Use a kimwipe to make sure the recording chamber is clean and dry.

Recipes

11 Internal Solutions

11.1 $CsMeSO_3$ Internal Solution (for most voltage-clamp recordings)

A	B	C	D	E	F
Compound:		FW:	conc. (mM):	100 mL:	200 mL:
CsMeSO3		228	120	2.736 g	5.472 g
CsCl		168.4	15	0.2525 g	0.505 g
NaCl		58.44	8	0.047 g	0.094 g
EGTA		380.4	0.5	0.019 g	0.038 g
HEPES		238.3	10	0.2385 g	0.477 g
pH: 7.3 with CsOH					
mOsm: 285-290					

11.2 $KMeSO_3$ Internal (for most current-clamp recordings):

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A	B	C	D	E	F
Compound:		FW:	conc. (mM):	100 mL:	200 mL:
KMeSO3		134	130	1.742 g	3.484 g
NaCl		58.44	10	0.059 g	0.117 g
MgCl2		stock (1 M)	2	200 uL	400 uL (1 M stock)
CaCl2		stock (1 M)	0.16	16 uL	32 uL (1 M stock)
EGTA		380.4	0.5	0.019 g	0.038 g
HEPES		238.3	10	0.238 g	0.477 g
pH: 7.3 with KOH					
mOsm: 285					

11.3 High Cl Internal (for detection of IPSCs at -70 mV)

A	B	C	D	E	F
Compound:		FW:	conc. (mM):	100 mL:	200 mL:
CsCl		168.4	120	2.02 g	4.04 g
CsMeSO4		228	15	0.342 g	0.684 g
NaCl		58.44	8	0.047 g	0.094 g
EGTA		380.4	0.5	0.019 g	0.038 g
HEPES		238.3	10	0.2385 g	0.477 g
pH: 7.3 with CsOH					
mOsm: 285-290					

11.4 Cell-attached solution

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A	B	C	D	E	F	G
Compound:		FW:	conc. (mM):			
NaCl		58.44	150			
KCl		74.55	2.5			
CaCl2		stock	2			
MgCl2		stock	1			
HEPES		238.3	10			
D-glucose			10			
pH: 7.3 with NaOH						
Additions:					50 mL	20 mL:
lidocaine (QX314)		343.31	5		0.0888 g	.03 g
Mg-ATP		507.2	2		0.0507 g	.0203 g
Na-GTP		523.2	0.3		0.0078 g	.0031 g

12 External Solutions

12.1 1X Sucrose Cutting Solution

A	B	C	D	E
Compound:		Conc. (mM):	FW:	g/L:
NaCl	powder	79	58.44	4.62
NaHCO3	powder	23	84.01	1.91
Sucrose	powder	68	342.3	23.27
Glucose	powder	23	180.2	4.09
KCl	powder	2.3	74.55	0.17
NaPhosphate Monobasic Monohydrate	1.1	138	0.16	
MgCl2	1 M stock liquid		203.3	6 mL of stock
CaCl2	1 M stock liquid	0.5	147	0.5 mL of stock

12.2 1X Glycerol Cutting Solution

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A	B	C	D	E
Compound:		Conc. (mM):	FW:	g/L:
Glycerol	liquid	250	92.09	23.02
KCl	powder	2.5	74.55	0.1863
NaH2P04-H20	powder	1.2	119.98	0.14397
HEPES	powder	10	238.3	2.383
NaHCO3	powder	21	84.01	1.7642
Glucose	powder	5	180.16	0.9
MgCl2	1 M stock liquid	2		2 mL of liquid stock
CaCl2	1 M stock liquid	2		2 mL of liquid stock

12.3 10X ACSF

A	B	C	D	E
Compound:		Conc. (mM):	FW:	g/L:
NaCl	powder	125	58.44	73.05
NaHCO3	powder	26	84.01	21.84
NaH2PO4-H2O	1.25	138	1.73	
KCl	powder	2.5	74.55	1.86
MgCl2	1 M stock liquid	1	203.3	10 mL of stock
Add to 1x ACSF:				
CaCl2	1 M stock liquid	1M stock	147	2 mL of stock
Glucose	powder	powder	180.16	2.25

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