

Acute Cerebellar Slice Preparation Using a Tissue Chopper

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[Abstract] Acute cerebellar slices are widely used among neuroscientists to study the properties of excitatory and inhibitory synaptic transmission as well as intracellular signaling pathways involved in their regulation in cerebellum. The cerebellar cortex presents a well-organized circuitry, and several neuronal pathways can be stimulated and recorded reliably in acute cerebellar slices. A widely used acute cerebellar slice preparation technique was adapted from Edwards' thin slice preparation method published in 1989 (Edwards *et al.*, 1989). Most of the acute cerebellar slice preparation techniques use a vibrating microtome for slicing freshly dissected cerebellum from various animal species. Here we introduce a simpler method, which uses a tissue chopper to quickly prepare acute sagittal cerebellar slices from rodents. Cerebellum is dissected from the whole brain and sliced with a tissue chopper into 200-400 μ m thick slices. Slices are allowed to recover in oxygenated aCSF at 37 °C for 1-2 h. Slices can then be used for electrophysiology or other types of experimentation. This method can be used to prepare cerebellar slices from mouse or rat aged from postnatal day 7 to 2 years. The preparation is faster and easier than other methods and provides a more versatile diversity of applications.

Keywords: Acute cerebellar slice, Cerebellum, Mouse, Rat, Electrophysiology

[Background] Acute cerebellar slice preparations, like many other current brain slice preparation techniques, originated from Edwards' thin slice preparation method published in 1989 (Edwards *et al.*, 1989). In general, cerebellum was quickly dissected and immersed in Ca^{2+} -free aCSF and glued to the stage of a vibratome. After slicing, slices were recovered in regular aCSF for 1-2 h before use (Llano *et al.*, 1991a and 1991b; Kano and Konnerth, 1992). Here we introduce a method adapted from the acute hippocampal slice preparation method used in our laboratory. Freshly dissected cerebellum is rapidly sliced (around 10 s) on a McIlwain tissue chopper without the need for glue or oxygenation during slicing. The slices prepared using this method are as healthy as the ones prepared with a vibratome and can be used for electrophysiology or a variety of other manipulations.

Materials and Reagents

1. Disposable pipette (VWR, catalog number: 16001-180)
2. Parafilm (VWR, catalog number: 52858-000)
3. 60 mm and 100 mm Petri dishes (VWR, catalog numbers: 25373-085 and 25373-100)
4. Filter paper (VWR, catalog number: 28460-030)

5. Painting brushes
6. Gas dispersion tube (Ace Glass, catalog number: 9435-10)
7. Mice
 - a. Adult C57BL/6 (WT) mice
 - b. Adult calpain-1 KO mice
 - c. Adult calpain-1 PHLPP1 double-KO (DKO) mice
8. Isoflurane
9. NaCl
10. KCl
11. CaCl₂
12. MgSO₄
13. D-glucose
14. NaHCO₃
15. KH₂PO₄
16. Artificial cerebrospinal fluid (aCSF) (see Recipes)
17. Cutting solution (see Recipes)

Equipment

1. Chill dissection tools
 - a. Angled-tip dissector scissor (Fine Science Tools, catalog number: 14082-09)
 - b. Scissor (Fine Science Tools, catalog number: 91401-14)
 - c. Curved-tip forceps (Fine Science Tools, catalog number: 11051-10)
 - d. Surgical blade
 - e. Spoon
 - f. Dissecting spatula
2. Brain slice keeper (AutoMate Scientific, catalog number: BSK4)
3. Beakers
4. Ice box
5. McIlwain Tissue Chopper (The Mickle Laboratory Engineering, Brinkmann)
6. Water bath
7. 95% O₂/5% CO₂ air tank
8. -80 °C freezer

Procedure

1. Prepare 1 L of artificial cerebrospinal fluid (aCSF) (see Recipe 1).
2. Pour 500 ml of freshly prepared aCSF into a brain slice container. Put the brain slice container in a 37 °C water bath. Connect the brain slice container to a 95% O₂/5% CO₂ air tank and

oxygenate aCSF. Gas flow pressure is set at around 2 p.s.i.

3. Pour 100 ml of cutting solution into a 150 ml beaker. Cover the beaker with parafilm, and place it in a -80 °C freezer for 5-10 min, until the solution has a slushy consistency.
4. Place the ice-cold cutting solution on a large ice tray. Bubble the solution with a gas dispersion tube connected to a 95% O₂/5% CO₂ air tank for 5 min.
5. Place the lid of a 60 mm Petri dish on ice. Put a round filter paper onto the lid and wet the filter paper with the cutting solution. This is the brain dissection stage.
6. Chill dissection tools including angled-tip dissector scissors, large scissors, a curved-tip forceps, a surgical blade, a spoon, a disposable pipette with the tip cut off and two dissecting spatulas on ice. The whole dissection setup is shown in Figure 1.



Figure 1. Dissecting setup for acute cerebellar slice preparation. A beaker with cutting solution, a dissection stage, and dissection tools were placed on a large ice tray.

7. Anesthetize animal with isoflurane and decapitate with the appropriate tool (e.g., scissors). Immediately extract the whole brain with dissection tools and place it into the ice-cold cutting solution. This step should be done within 1 min.
8. Chill the brain in ice-cold cutting solution for 1 min.
9. Place the brain onto the cold dissection stage. First, cut off forebrain, midbrain, and medulla. Then, cut off pons to isolate the cerebellum (Figure 2).

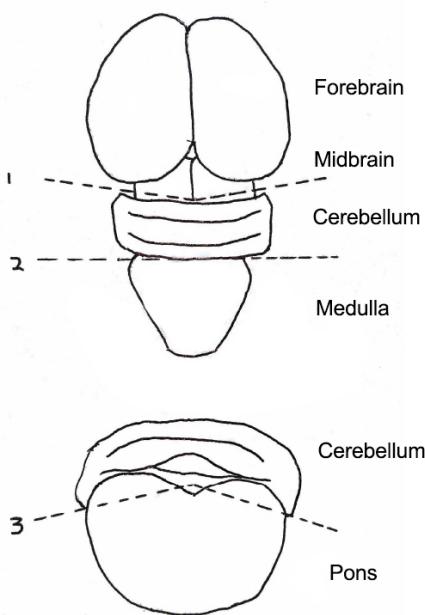


Figure 2. Steps to isolate cerebellum from whole mouse brain. Forebrain, midbrain, and medulla were cut off, followed by the removal of pons beneath the cerebellum. Top, the axial view of mouse brain. Bottom, the coronal view of Cerebellum and Pons. Numbers and dotted lines indicate steps and sites of the incision.

10. Immediately transfer the cerebellum onto the stage of a McIlwain Tissue Chopper. Drain the extra solution around the tissue. Slice the cerebellum into 200-400 μm thick sagittal slices (Figure 3).



Figure 3. McIlwain Tissue Chopper (The Mickle Laboratory Engineering, Brinkmann)

11. Move the cerebellum to a 100 mm Petri dish containing ice-cold cutting solution and carefully separate each slice without damaging them using two small painting brushes.
12. Transfer slices into warm aCSF in the brain slice container using a disposable pipette with the tip cut off.

13. Incubate slices in aCSF at 37 °C for 1-2 h with the 95% O₂/5% CO₂ bubbling on. Slices can now be used for electrophysiology or slice treatment.

Data analysis

We prepared acute cerebellar slices of 3 months old mice following the protocol we described here and recorded excitatory postsynaptic potentials (EPSPs) elicited in the Purkinje cell body layer by electrical stimulation of the parallel fibers at various stimulation intensities (Wang *et al.*, 2016). This stimulation elicited a typical P1-N1-P2-N2 waveform (Barnes *et al.*, 2011), where N1 corresponds to the presynaptic fiber volley and N2 to the postsynaptic population spike (Figure 4). Responses elicited by stimulation intensity below 120 mA were too unreliable to be analyzed, and only responses elicited by stimulation intensities above 120 mA were analyzed by calculating the ratio of N2 over N1, which reflects the efficiency of synaptic transmission. At all intensities, the N2/N1 ratio was smaller in calpain-1 KO mice as compared with WT mice, and the overall difference between the two genotypes was statistically significant (Figure 4). The N2/N1 ratios of the EPSPs recorded in cerebellar slices from calpain-1, and PHLPP1 double-KO (DKO) mice at all stimulation intensities were intermediate between those in WT and calpain-1 KO mice, indicating that synaptic transmission was at least partially restored in the cerebellum of DKO mice (Figure 4).

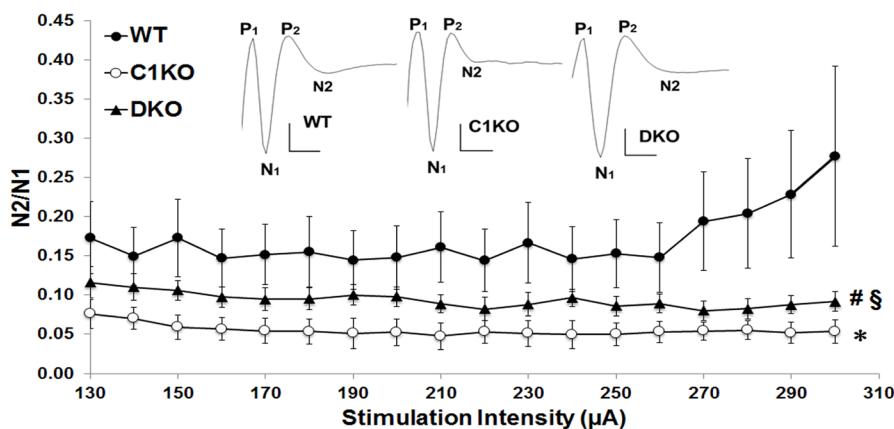


Figure 4. Parallel fibers to Purkinje cell EPSPs are reduced in adult calpain-1 KO mice, and partially restored in adult calpain-1 and PHLPP1 double-KO (DKO) mice. Acute cerebellar slices were prepared as described in Procedure, and field EPSPs were evoked by parallel fiber stimulation recorded in the Purkinje cell layer. Results were calculated as ratios of N2 over N1 and represent means \pm SEM of 10 to 11 slices from three to five mice. * $P < 0.001$, as compared with WT (univariate ANOVA followed by Bonferroni test); # $P < 0.001$, as compared with WT (univariate ANOVA followed by Bonferroni test); § $P < 0.001$, as compared with DKO (univariate ANOVA followed by Bonferroni test) (from Wang *et al.*, 2016).

Recipes

1. Artificial cerebrospinal fluid (aCSF)

110 mM NaCl

5 mM KCl

2.5 mM CaCl₂

1.5 mM MgSO₄

1.24 mM KH₂PO₄

10 mM D-glucose

27.4 mM NaHCO₃

2. Cutting solution

124 mM NaCl

26 mM NaHCO₃

10 mM glucose

3 mM KCl

1.25 mM KH₂PO₄

5 mM MgSO₄

3.4 mM CaCl₂

Cutting solution can be stored at 4 °C for 1 week

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Competing interests

There are no conflicts of interest.

Ethics

Animal use in all experiments followed NIH guidelines and all protocols were approved by the Institution Animal Care and Use Committee of Western University of Health Sciences.

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