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Striatal Synaptosomes Preparation from Mouse Brain

Shiqi Ma and Alexander Sorkin*

Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, United States
*For correspondence: sorkin@pitt.edu

[Abstract] The striatum is located in the subcortical region of the forebrain, it contains medium spiny neurons, cholinergic interneurons and GABAergic interneurons, and receives dopaminergic projection in the nigrostriatal pathway. This protocol provides a method to collect synaptosomes from mouse brain dorsal stratum. The synaptosomes can be used to study dopamine uptake activity, dopaminergic terminal endocytosis/trafficking using biochemical and microscopy methods, and protein analysis (Sorkina *et al.*, 2018).

Keywords: Striatum, Synaptosomes, Dopamine, Dopamine transporter, Endocytosis

Materials and Reagents

1. Pipette tips
2. Eppendorf tubes
3. 10 cm glass plates
4. Two 8-week HA-DAT knock-in mice (Rao *et al.*, 2012) or wild type mice
5. Gey's balanced salt solution (Sigma-Aldrich, catalog number: G9779)
6. D-glucose (Sigma-Aldrich, catalog number: G8644)
7. HEPES (Sigma-Aldrich, catalog number: H0887)
8. Sucrose (Sigma-Aldrich, catalog number: 84097)
9. NaCl (Sigma-Aldrich, catalog number: S3014)
10. KCl (Sigma-Aldrich, catalog number: P5405)
11. $CaCl_2$ (Merck, catalog number: 208291)
12. $MgCl_2$ (Merck, catalog number: 442611)
13. Filter units (Thermo Fisher Scientific, catalog number: 124-0045)
14. Freshly-prepared sucrose buffer (see Recipes)
15. Krebs Ringer solution (see Recipes)

Note: Items 1-3 and 5-12 can be ordered from any qualified company.

Equipment

1. Gilson Pipettes
2. Iris dressing forceps (Kent Scientific, catalog number: INS650915)
3. Mayo-operating scissors (Kent Scientific, catalog number: INS700540)

4. Extra fine Bonn scissors (Fine Science Tools, catalog number: 14083-08)
5. Interchangeable blades (2x) and the handles (2x) (Fine Science Tools, catalog numbers: 10035-10; 10035-00)
6. Glass homogenizer (DWK Life Sciences, Kimble™, catalog number: 885300-0001)
7. Centrifuge (Eppendorf, model: 5424, 24-tube, Max 21,130 *x g*, refrigerated)

Note: Items 1-6 can be ordered from any qualified company.

Procedure

1. Prepare fresh sucrose buffer (Recipe 1). Keep the buffer on ice.
2. Add D-glucose to 20 ml Gey's balanced salt solution to reach a final concentration of 1.8 g/L. Put the buffer in a 10 cm glass plate on ice.
3. Euthanize two 8-week mice using carbon dioxide. Decapitate the mice using mayo-operating scissors.
4. Use extra fine Bonn scissors to break the skull and use iris dressing forceps to take the brain out. Quickly rinse the brains in ice-cold Gey's balanced salt solution with 1.8 g/L D-glucose. *Note: Keep the other brain in the buffer while dissecting one brain in Step 5. It is better not to exceed 30 min to keep brain in the buffer.*
5. Place the brain with the ventral side facing a 10 cm glass plate. Place the dressing forceps between the cortical lobes and the cerebellum and snap the cerebellum off (see Spijker, 2011). Gently use the forceps to open the cortex on one side, the striatum can be recognized by the surrounding white tissue. Striatum looks relatively transparent. Use the two interchangeable blades with the handles to collect the striatum. Get the striatum of the other side in the same way. Place the tissue in a glass homogenizer with ice-cold sucrose buffer (1 ml sucrose buffer for striatal tissue from one brain, the tissue usually weights 45-50 mg).
6. Homogenize the tissue 10-15 times, carefully avoid generating bubbles. Transfer the homogenization to a new 1.5 ml tube.
7. Centrifuge the homogenate at 1,000 *x g* for 10 min at 4 °C, and move the supernatant into a new 1.5 ml tube. Centrifuge at 12,500 *x g* for 20 min at 4 °C. Remove the supernatant using 1 ml tip, then a 200 µl tip. The pellet in the bottom that contains the synaptosomes is white.
8. Re-suspend the synaptosomes in 0.5-1 ml Krebs Ringer HEPES solution. Vortex for about 30 s or until the pellet is dissolved. Use the synaptosomes to perform future experiment (such as dopamine uptake assay) immediately.

Recipes

1. Freshly-prepared sucrose buffer
   Dilute 1 M stock-solution of sodium-HEPES buffer (pH 7.4) to 5 mM with H_2O, add sucrose to a final concentration of 0.32 M, check pH and adjust if not 7.4

Note: Keep the buffer on ice. There is no need for sterilization of this buffer.

2. Krebs Ringer HEPES (KRH) solution
   Prepare 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 5.5 mM HEPES solution in dH_2O, adjust pH to 7.4 if necessary
   Note: Filter the buffer using the disposable filter unit. Add D-glucose to the final concentration of 1.8 g/L before each use.

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