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# Synaptoneurosome Preparation from C57BL/6 Striata

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**[Abstract]** Activity-dependent local mRNA translation endows synapses to remodel their structure and function (Bramham and Wells, 2007). This process is tightly controlled by the state of phosphorylation of several components of the translational machinery including initiation factors and ribosomal proteins (Buffington *et al.*, 2014). The present protocol describes a method to prepare striatal synaptoneurosomes, from adult mice, containing both pre- and postsynaptic elements in which the level of synaptic phospho-proteins can be quantified (Biever *et al.*, 2015).

## Materials and Reagents

1. 1 ml dounce tissue grinder (Capitol scientific, Wheaton®, catalog number: 357538)
2. 5 ml and 60 ml syringes (BD, Plastipak, catalog number: 309647 and 300866, respectively)
3. Nylon net filters 100 µm (Merck Millipore Corporation, catalog number: NY1H02500)
4. Mitex membrane filter 10 µm (Merck Millipore Corporation, catalog number: LCWP02500)
5. 1.5 ml Eppendorf tubes (Eppendorf, catalog number: 0030120086)
6. C57BL/6 mice (≥8 weeks old, male or female) (Mus musculus)
7. Calcium chloride ($CaCl_2$) (Sigma-Aldrich, catalog number: C5670)
8. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S7653)
9. Potassium chloride (KCl) (Sigma-Aldrich, catalog number: P9333)
10. Potassium phosphate ($KH_2PO_4$) (Sigma-Aldrich, catalog number: P3786)
11. Sodium bicarbonate ($NaHCO_3$) (Sigma-Aldrich, catalog number: 71630)
12. Magnesium chloride ($MgCl_2$) (Sigma-Aldrich, catalog number: M8266)
13. D-(+)-Glucose (Sigma-Aldrich, catalog number: 67528/G6728)
14. HEPES (pH 7.4) (Sigma-Aldrich, catalog number: H3375)
15. Sodium orthovanadate (Sigma-Aldrich, catalog number: S6508)
16. Sodium fluorate (Sigma-Aldrich, catalog number: S7920)
17. Sodium pyrophosphate decahydrate (Sigma-Aldrich, catalog number: 221368)
18. Glycerol phosphate disodium salt hydrate (Sigma-Aldrich, catalog number: G6501)
19. Aprotinin (Sigma-Aldrich, catalog number: A1153)
20. Leupeptin hydrochloride (Sigma-Aldrich, catalog number: L0649)

 

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21. Pepstatin (Sigma-Aldrich, catalog number: P4265)
22. Phenylmethylsulfonyl fluoride (Sigma-Aldrich, catalog number: 78830)
23. Phospho-S845-GluR1 [Anti-phospho-GluR1 (Ser845) Antibody, clone EPR2148, rabbit monoclonal] (Merck Millipore Corporation, catalog number: 04-1073)
24. Phospho-T185/Y187-ERK2 [Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)] (Cell Signaling Technology, catalog number: 9101)
25. Phospho-S235/236-rpS6 (Cell Signaling Technology, catalog number: 2211)
26. Phospho-S209-eIF4E (Cell Signaling Technology, catalog number: 9741)
27. β-actin [AC-15] (Abcam, catalog number: AB6276)
28. Synaptoneurosome buffer (see Recipes)

## Equipment

1. Swinnex filter holder (Merck Millipore Corporation, catalog number: SX0002500)
2. 4 ºC Eppendorf table centrifuge

## Procedure

*Note: This technique has been used to evaluate biochemical changes after pharmacological treatments in synaptoneurosome preparations of the striatum (Biever et al., 2015). However, the same protocol can be applied to other brain regions or after the performance of behavioral paradigms.*

1. Kill the mouse by cervical dislocation or decapitation and rapidly immerse the head in liquid nitrogen for 4 sec to rapidly cool down the brain without allowing it to freeze (this step helps for the brain dissection and to better preserve protein phosphorylation). We recommend to euthanize the mouse with physical methods such as cervical dislocation rather than using anesthetics or carbon dioxide, but this step should be adapted to local ethical committee guidelines.
2. Remove the brain, remove the meninges, and dissect the striatum of the 2 hemispheres (≈15-20 mg) on an ice-cooled glass dish as it is shown in Video 1.

**Video 1. Striatum extraction**


 

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3. Homogenize the striata in a glass dounce homogenizer (10 strokes of the loose pestle followed by 10 strokes of the tight pestle) with 1 ml of synaptoneurosome buffer kept on ice. The number of strokes might vary depending on the individual homogenizer/pestles and the size of the brain area. All the steps are performed at 4 °C (either on ice or in a 4 °C room). Representative pictures of the homogenates at each step are shown in Figure 1.
4. Transfer the homogenate into an Eppendorf tube and aliquot 100 µl as an input.
5. Prepare all the material required for the synaptoneurosome preparation procedure as indicated in Figure 2.
6. Pre-wet 3 Nylon net filters (100 µm pore) in synaptoneurosome buffer on ice.
7. Attach a 5 ml syringe without the plunger (syringe #1) to a swinnex filter holder (#1) containing the 3 pre-wetted Nylon net filters (100 µm), as illustrated in Figure 3. Then, attach this swinnex to another 5 ml syringe (syringe #2) without the plunger, which has been attached at the dispensing tip end to a swinnex filter holder containing 1 Mitex membrane filter (10 µm pore) (swinnex filter holder #2). Place on the other side of the swinnex #2 a 1.5 ml Eppendorf tube on ice to collect the sample.
8. With a 1-ml pipette, load the sample into the end of the barrel of the syringe #1 attached to a swinnex filter holder #1, insert the plunger and push down on the plunger until all the air is gone from the syringe.
9. Remove the 5 ml syringe #1 and replace it by a 60 ml syringe with the plunger being pulled back. Once attached to the swinnex filter holder #1 containing the 3 Nylon net filters (100 µm), push down on the plunger until all the air is gone from the syringe. This step helps to push down the sample to the barrel of syringe #2.
10. Remove the swinnex filter holder #1 containing the 3 Nylon net filters (100 µm), place the plunger of the 5 ml syringe #2, which is connected to the swinnex filter holder #2 containing 1 Mitex membrane filter (10 µm), and push down until all the air is gone from the syringe.
11. Remove the syringe #2 and replace it with a 60 ml syringe with the plunger being pulled back. Once attached to the swinnex filter holder #2, push down on the plunger until all the air is gone from the syringe. At this step, the homogenate should be in the 1.5 ml Eppendorf tube placed on ice.
12. Centrifuge 1 min at 4,000 *x g* at 4 °C. Pipette the supernatant into a new Eppendorf tube and discard the pellet. A representative image of how the pellet looks is shown in Figure 1b.
13. Centrifuge the supernatant for 4 min at 14,000 *x g* at 4 °C. Discard the supernatant and resuspend the pellet in 100 µl synaptoneurosome buffer. A representative image of how the pellet looks is shown in Figure 1c. (The volume to re-suspend the pellet might change depending on the protein concentration desired. From the striata of one mouse the synaptoneurosome preparation obtained has a protein concentration around 1-2 µg/µl). The pellet can be stored at -80 °C until used.

 

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*Note: In this type of synaptoneurosome preparation we obtained samples containing enrichment of both presynaptic and postsynaptic markers, a substantial reduction of glial markers and absence of nuclear markers (Biever et al., 2015). Moreover, phospho-proteins can be detected as shown in Figure 4.*

## Representative data

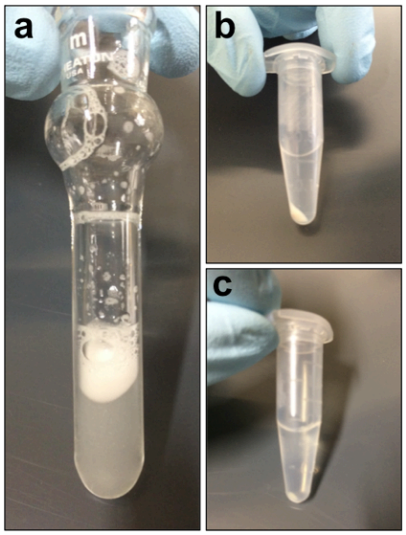


**Figure 1**. **Representative images of the homogenates at different steps.** a. Sample obtained after the homogenization of the striata of one mouse as indicated in step 3. b. Sample obtained after the first centrifugation as indicated in step 12. c. Sample obtained after the second centrifugation as indicated in step 13.

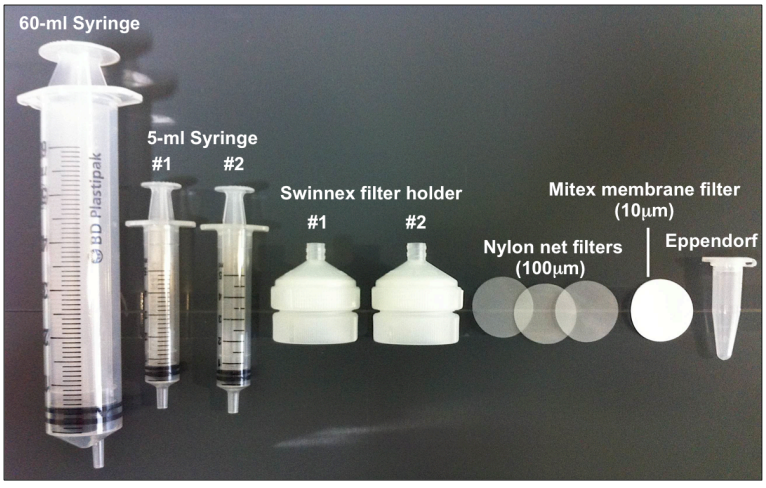


**Figure 2**. **Materials required for synaptoneurosome preparation procedure.** Place three pre-wetted Nylon net filters (100 µm) in swinnex filter holder #1 and one Mitex membrane filter (10 µm) in swinnex filter holder #2. Mount the apparatus in the following order from the bottom to the top: Eppendorf on ice < swinnex filter holder #2 < 5 ml syringe #2 without plunger < swinnex filter holder #1 < 5 ml syringe #1 without plunger. After

 

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loading the sample with a pipette into the barrel of syringe #1, add the plunger and push down until all the air is gone from the syringe. Remove the 5 ml syringe #1 and replace it by a 60 ml syringe with the plunger being pulled back. Once attached to the swinnex filter holder #1, push down on the plunger until all the air is gone from the syringe. This step helps to push down the sample to the barrel of syringe #2. Continue the protocol as indicated from step 10.

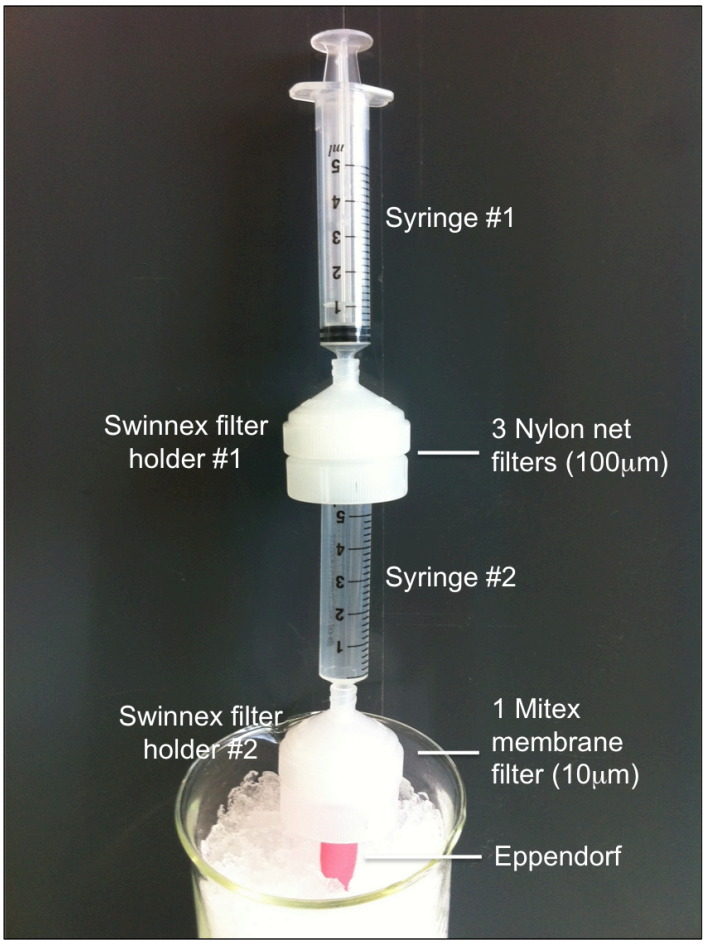


**Figure 3**. **Illustration of the mounted apparatus for synaptoneurosome preparation procedure.** A 5 ml syringe (syringe #1) is attached to a swinnex filter holder containing 3 Nylon net filters (100 µm) (swinnex filter holder #1) and connected to another 5 ml syringe (syringe #2), which is attached to a second swinnex filter holder containing a Mitex membrane filter (10 µm) (swinnex filter holder #2). A 1.5 ml Eppendorf tube is placed on ice on the other side of the swinnex to collect the sample that was initially loaded into the syringe #1.

*Note: Syringes #1 and #2 could be replaced with syringes of different sizes depending on sample volume.*

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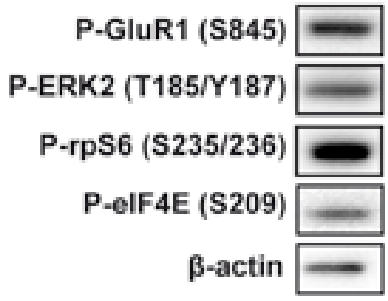


**Figure 4. Representative western blots of phospho-proteins in striatal synaptoneurosome preparations.** Ten µg per lane of synaptoneurosomal samples were separated in 13% SDS-polyacrylamide gel before electrophoretic transfer onto Immobilon-P membranes. A regular western blot protocol was used including the following antibodies: phospho-S845-GluR1, phospho-T185/Y187-ERK2, phospho-S235/236-rpS6 phospho-S209-eIF4E and β-actin.

## Acknowledgments

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## Recipes

1. Synaptoneurosome buffer (use sterile water of high purity, such as Milli-Q water)
   2.5 mM $CaCl_2$
   124 mM NaCl
   3.2 mM KCl
   1.06 mM $KH_2PO_4$
   26 mM $NaHCO_3$
   1.3 mM $MgCl_2$
   10 mM D-(+)-Glucose
   20 mM HEPES (pH 7.4)
   0.15 µM aprotinin
   11 µM leupeptin
   1.5 µM pepstatin
   0.6 mM phenylmethylsulfonyl fluoride
   1 mM sodium orthovanadate*

 

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100 mM sodium fluoride*

5 mM sodium pyrophosphate decahydrate*

40 mM Glycerol phosphate disodium salt hydrate*

*Note: The buffer should be freshly prepared the day of the experiment. Buffer components marked with an asterisk are only required if phospho-proteins are analyzed in the synaptoneurosome preparations.*

## References

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