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A high-throughput semi-automated preparation for filtered synaptoneurosomes

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HIGHLIGHTS

- New high-throughput, semi-automated homogenization and filtrations steps for synaptoneurosomes preparation.
- Dramatically reduces time to prepare synaptoneurosomes.
- Significantly increases sample recovery and enrichment for synaptic proteins.
- The steps are compatible with biosafety regulations for pathogen infected brain tissue.
- Important technical advance for studying synaptic proteins in valuable brain samples.

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ABSTRACT

Background: Synaptoneurosomes have become an important tool for studying synaptic proteins. The filtered synaptoneurosomes preparation originally developed by Hollingsworth et al. (1985) is widely used and is an easy method to prepare synaptoneurosomes. The hand processing steps in that preparation, however, are labor intensive and have become a bottleneck for current proteomic studies using synaptoneurosomes. For this reason, we developed new steps for tissue homogenization and filtration that transform the preparation of synaptoneurosomes to a high-throughput, semi-automated process.
New method: We implemented a standardized protocol with easy to follow steps for homogenizing multiple samples simultaneously using a FastPrep tissue homogenizer (MP Biomedicals, LLC) and then filtering all of the samples in centrifugal filter units (EMD Millipore, Corp).
Results and comparison with existing methods: The new steps dramatically reduce the time to prepare synaptoneurosomes from hours to minutes, increase sample recovery, and nearly double enrichment for synaptic proteins. These steps are also compatible with biosafety requirements for working with pathogen infected brain tissue.
Conclusions: The new high-throughput semi-automated steps to prepare synaptoneurosomes are timely technical advances for studies of low abundance synaptic proteins in valuable tissue samples.

1. Introduction

The synaptoneurosomes preparation has become an important tool for studying synaptic proteins, especially when measuring low abundance synaptic proteins in valuable tissue samples (Suzdak et al., 1986a, 1986b; Morrow et al., 1990; Weiler et al., 1997; Wu et al., 1998; Quinlan et al., 1999a, 1999b). The popularity of synaptoneurosomes comes from the simple protocol developed by Hollingsworth et al. (1985) that used a filtration step to process homogenized cortical tissue into synaptoneurosomes. A number of research groups have contributed further developments to the preparation by using different homogenization techniques (Chandler and Crews, 1990; Villasana et al., 2006; Chang et al., 2012), low speed centrifugation times (Titulaer et al., 1997), and filter pore sizes (Weiler and Greenough, 1991; Titulaer et al., 1997). Current protocols for synaptoneurosome preparation (Lugli and Smalheiser, 2013), however, continue to be done by hand and one sample at a time. In contrast to the time-consuming hand preparation protocols for synaptoneurosomes, Western blotting systems have been moving rapidly to high-throughput platforms. Even standard Western blotting protocols use stripping and reprobing methods to increase throughput for

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quantifying protein expression. The bottleneck for synaptoneurosomes, however, remains the labor intensive procedures used to prepare each sample. We have addressed this obstacle by developing a new high-throughput, semi-automated protocol to prepare synaptoneurosomes.

New tissue homogenization and filtration technologies are available to alleviate the time consuming process of synaptoneurosome preparation. These new devices process many samples at once, increasing throughput, and helping to address the variability that happens when preparing individual samples by hand. For example, current homogenization protocols list different numbers of strokes or pulses and in practice the number used depends on the species and storage of the tissue sample. Filtration protocols also vary in the number of filtrations, size of filter pores, and description of the pressure needed to push the homogenate through the filters. Regardless of how well filtration is performed, there is dead space in the syringe filter holders where valuable tissue is lost in the filter holder.

We developed a new high-throughput semi-automated synaptoneurosome preparation by replacing the homogenization and filtration steps with a high-speed bench top homogenizer and centrifugal filtration process. We compared our new protocol with the hand preparation technique and found three main advantages: preparation time was significantly reduced; more sample was recovered after filtration; and there was greater enrichment of synaptic proteins.

2. Materials and methods

2.1. Tissue sample collection

A total of 24 samples were collected from somatosensory cortex (A/P −4 to −2 mm, M/L 2–5 mm) of Long Evans rats ($n = 8$). An additional set of 16 tissue samples were collected from adult cat frontal cortex ($n = 4$). Animals were euthanized with Euthanyl (150 mg/kg rats; 165 mg/kg cats), and perfused transcardially with cold 0.1 M phosphate buffered saline (PBS) until circulating fluid was clear. The brain was quickly removed from the skull and immersed in cold PBS, one or two samples were cut from each hemisphere (approx. 2 mm × 2 mm) then quickly frozen on dry ice and stored at −80 °C.

All experimental procedures were approved by the McMaster University Animal Research Ethics Board.

2.2. Synaptoneurosome preparation

In this study, we developed a high-throughput, semi-automated process for preparing filtered synaptoneurosomes and compared it with the hand preparation technique (Fig. 1). First, we describe

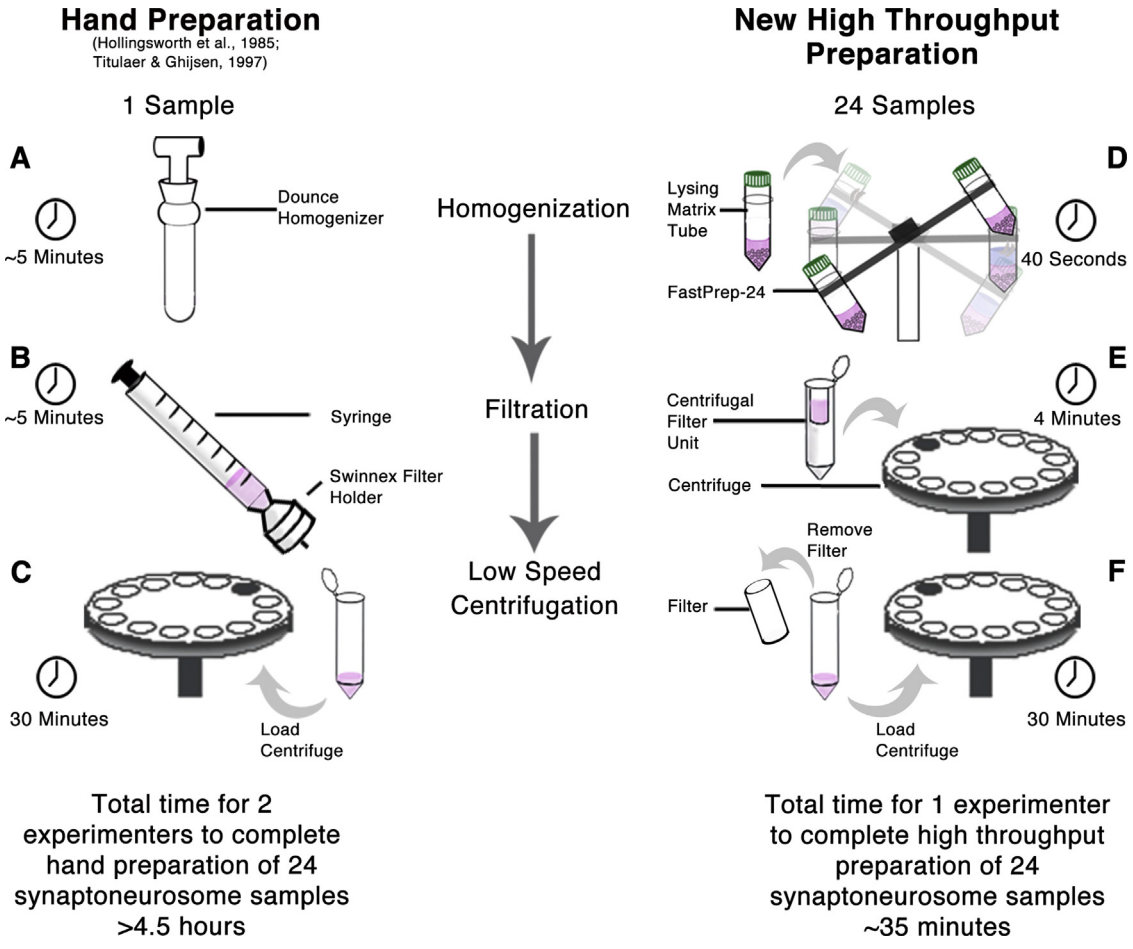

Fig. 1. Schematic overview of steps for the hand and new high-throughput, semi-automated synaptoneurosome preparations providing an outline of the processes from homogenization to filtration, and low speed centrifugation. (A) Two experimenters worked together to complete the steps for the hand preparation, which began by homogenizing samples one at a time by hand using a Dounce homogenizer. (B) The homogenate was then filtered through hydrophobic membranes, contained in the Swinnex filter holder, by applying hand-pressure to a syringe. (D) One experimenter completed the high-throughput preparation, starting with placing samples in lysing matrix tubes, and homogenization in the Fast-Prep®-24 Tissue and Cell Homogenizer for 40 s. (E) Homogenate was transferred to centrifugal filter units, and centrifuged for 4 min. The filter was removed from the centrifugal filter unit before low speed centrifugation. (C and F) In both preparations, samples were centrifuged at low speed for 30 min. The total time for two experimenters to complete hand preparation of 24 synaptoneurosome samples was over 4.5 h. In contrast, one experimenter can complete the preparation of 24 synaptoneurosome samples following the high-throughput preparation in about 35 min.