SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS & MATERIALS

Preparation of synaptosome-enriched fractions

Synaptosomal fractions were prepared as described previously (Jeannotte and Sidhu 2007). Neocortical samples were washed once with PBS and resuspended in 10% w/v synaptosome homogenizing buffer (0.32 M sucrose, 10 mM Tris-Cl, 1 mM EDTA; pH 7.4). Tissue was homogenized in glass-Teflon homogenizers on ice. Total homogenates were centrifuged at 500 g for 10 min at 4 °C. The supernatant was then spun at 25,000 g for 15 min at 4 °C. The pellet was resuspended in a small volume of homogenizing buffer and the protein concentration was determined by the Lowry protein assay.

The distributions of SY as an additional synaptic vesicle marker and GFAP as a marker for astrocyte enrichment were analyzed in the same blots as LDH isoenzymes. SYP immunostaining revealed a single band migrating at 37 kDa and GFAP staining revealed a single band at 49 kD (Fig. S1C).

Enzyme-Linked Immunosorbent Assay (ELISA)

All ELISAs were performed using Maxisorp plates (Nunc, Roskilde, Denmark) coated for overnight incubation at 4°C, with 20 μg/ml of fractions (100 μl/well), followed by blocking with PBS/T/BSA (PBS with 0.05% Tween 20, 1% BSA) for 2 h at 37°C. ELISA assays were conducted with anti-LDH-A and anti-LDH-B antibodies as previously described for Western blot analysis (see Materials and Methods). After that, the wells were washed with PBS/T and incubated with 100 μl/well of HRP-labeled anti-rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:2,000 in the blocking buffer and incubated for 1 h at 37°C. An *O*-

phenylenediamine dihydrochloride/peroxidase solution (Thermo Scientific, Rockford, IL) was used as the chromogen to visualize the reaction product. After sufficient color development the reaction was stopped with 40% H_2SO_4 and optical densities of wells were then read at 490 nm, subtracting 650 nm (background). The optical density of the sample was compared to a standard curve, which is a serial dilution of a known concentration for muscle tissue and heart tissue homogenate, LDH standards in PBS.

RESULTS

Characterization of synaptosomal fraction

Synaptosomal isolation provides an enrichment of cellular components found at the synapse. It should be noted that these fractions tend to contain the complete presynaptic terminal, including mitochondria and synaptic vesicles, along with the postsynaptic membrane and the postsynaptic density (PSD) (Bai and Witzmann 2007) (Fig. S1A). Synaptosomal fractions were obtained by using homogenization buffer containing 0.32 M sucrose, which is widely accepted (Yura et al. 1996; Jeannotte and Sidhu 2007). The neuronal enrichment and glial contamination of synaptosomal fractions was tracked using Western blot analysis of a presynaptic marker, SYP, and the glial marker GFAP as previously described (Hoogland et al. 1999). Figure S1B-C shows a graphical representation and a typical example of a Western blot loaded with homogenate (right panel) and synaptosomal fraction (left panel) from brain tissue of primates.

SUPPLEMENTAL FIGURES

Figure S1 Characterization of synaptosomal fraction **A.** Schematic depiction of the derivation of synaptosomes (adapted from Phelan and Gordon-Weeks, 1997). **B.** Bar graph represents the

relative enrichment of SYP and GFAP in the synaptosomal fraction and total homogenate. Data were calculated as the signal ratios of the protein of interest to β-actin first, and then, for the purpose of normalization, the ratio observed in the total cortical homogenates was set as 100%. **C.** Representative Western blots using anti-GFAP and anti-synaptophysin antibodies are shown. β-actin was used as a loading control.