Supplementary Information

Primary neuron culture, acute neuron isolation and electrophysiological recording

Acute isolation of neurons The hippocampi of Wistar rats were dissected and cut into approximately 1 mm thick slices in ice-cold oxygenated extracellular solution containing (mM): NaCl (140), KCl (5.4), 1.3 $CaCl_2$ (1.3), HEPES (25), glucose (33) and tetrodotoxin (0.001), pH 7.4, osmolarity was adjusted to 310–320mOsm. CA1 neurons were isolated from hippocampal slices by tapping the region with a pair of fine forceps.

Electrophysiological recordings Neurons were bathed in the extracellular solution containing (mM): NaCl (140), KCl (5.4), 1.3 $CaCl_2$ (1.3), HEPES (25), glucose (33), tetrodotoxin (0.001) and glycine (0.003), pH 7.4, osmolarity 310–320 mOsm. Recording pipettes were made from thin-walled borosilicate glass capillaries pulled to a diameter of 1-2μm at the tip, and filled with intracellular solution containing (mM): CsCl or CsF (140), HEPES (10), $MgCl_2$, (2), BAPTA (1) and K_2ATP (4), pH 7.2 and osmolarity was adjusted to 290–300 mOsm.

Whole-cell currents were recorded using an Axopatch 1D or Axopatch 200B amplifier (Molecular Devices) under the voltage-clamp conditions at a holding potential of -60mV except where indicated. Current-voltage (I/V) relationships of NMDA receptors were calculated by subtracting the currents recorded during voltage ramps from -60 to +60 mV in the absence of L-aspartate or NMDA application from that recorded in the presence of L-aspartate or NMDA application. On-line data acquisition and off-line analysis were performed using pClamp8 software (Molecular Devices).

Hippocampal slice preparation and electrophysiological recording

The ACSF contains (mM) NaCl (124), KCl (3), $CaCl_2$ (2.6) , $MgCl_2$ (1.3), $NaHCO_3$ (26), NaH_2PO_4 (1.25) and glucose (10), osmolarity was adjusted to 310–320mOsmol, pH 7.4, at room temperature. Recording electrodes were filled with the intracellular solution containing (mM) K-gluconate (135.5), KCl (17.5), HEPES (10), EGTA (0.2), Mg-ATP (2), GTP (0.3) and QX 314 (5) with osmolarity adjusted to 290–300mOsm and pH 7.2. DC resistance: 3–5mΩ.

Biochemical experiments

Subcellular fractionation Adult male rat forebrains were dissected and homogenized in homogenization buffer containing (mM): sucrose (320), Tris-HCl (10, pH 7.4), $NaHCO_3$ (1, pH 7.4), EDTA (2), sodium orthovanadate (1) and 1% (v/v) protease inhibitor cocktail. Homogenate (H) was subsequently centrifuged at 1,000g for 15min to remove nuclei and other large debris (P1). The supernatant (S1) was centrifuged at 10,000g for 30min to obtain a crude synaptosomal fraction (P2), which was then lysed in a hypo-osmotic buffer containing 35.6mM sucrose on ice for 30min before centrifuged at 25,000g for 30min to pellet a synaptosomal membrane fraction (LP1). The resulting supernatant was centrifuged at 165,000g for 60min to pellet a synaptic vesicle-enriched fraction (LP2). The supernatant (S2) above P2 was centrifuged at 165,000g for 60min to obtain a cytosolic fraction (S3) and a light membrane/microsome-enriched fraction (P3).

For preparing the PSD fraction, rat forebrain was homogenized in 1:10 (w/v) sucrose buffer (0.32M sucrose, 1mM $NaHCO_3$, 1mM $MgCl_2$, 0.5mM $CaCl_2$, 1% protease inhibitor cocktail) and centrifuged at 1,400g for 10min. The resulting supernatant was centrifuged at 13,800g for 20min to obtain the pellet which was then re-suspended in the 0.32M sucrose buffer and overlaid with discontinuous sucrose density gradient (0.85M, 1.0M, 1.2M sucrose). After centrifugation at 82,500g for 120min, the band between

1.0M and 1.2M sucrose was removed and incubated with equal volume of 1% Triton-X-100 for 15min. The PSD-I pellet was then obtained by centrifugation at 32,000g for 20min. The pellet was re-suspended in the 0.32M sucrose buffer and incubated a second time with equal volume of 1%Triton-X-100 for 15min, and then centrifuged at 201,800g for 60min to obtain the PSD-II pellet, which represents the concentrated and final PSD isolate. Extracts from individual fractions were solubilized in 1% sodium dodecyl sulphate (SDS) and centrifuged at 15,000g for 5min for Western blot analysis.

Immunoprecipitation and Western blot analysis Proteins from the P2 fraction of adult rat forebrain or cell pellets were dissolved in a lysis buffer containing (mM): Tris-HCl (50, pH 9.0), NaCl (150), 0.5% (v/v) Triton X-100, 1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, EDTA (2), sodium orthovanadate (1), and 1% (v/v) protease inhibitor cocktail.

Solubilized proteins (800μg) were incubated with antibodies as indicated overnight at 4°C. The immune complexes were collected with protein G-sepharose beads (Amersham Biosciences) for 2hs at 4°C. The blotting analysis was performed by repeated stripping and successive probing with antibodies as indicated.

For protein quantification, signal detected with ECLplus (Amersham Biosciences) in Western blot were scanned with Amersham Storm 860 fluorescent scanning system (Molecular Dynamics). Protein band intensity was calculated with the software, ImageQuant 5.0 (Molecular Dynamics).

cDNA transfection cDNAs transfected were full length NR1-1a (8μg), full length or C-terminal truncated NR2A (32μg), v-Src (as where indicated, a gift of Dr. T. Pawson, University of Toronto), Csk (1μg, a gift of Dr. K. Harder, Royal Melbourne Hospital), wild-type (2μg) or mutant neuronal Src (n-Src, 2μg, Y535F or K303R/Y535F, provided by Dr. S. Hanks, Vanderbilt University)[1]. cDNA sequence encoding the C-tail truncated forms of NR2A (NR2AΔCT) was amplified by PCR, and was constructed into *EcoRI* site of vector pcDNA3 (Invitrogen). Primers for constructing NR2AΔCT were 5'-CGGAATTCACCATGATGGGCAGATTGG-3' and 5'-CGGAATTCCTACCAGATGAAGGTGATG-3'.

In-vitro GST pull-down assay DNA sequences encoding the C-tails of NR1 (aa 834-938) and NR2A (aa 838-1464) were amplified by PCR. The PCR products were constructed into BamHI-EcoRI sites of vector pcDNA3 (Invitrogen) for in-vitro transcription and translation. Primers for constructing C-tails of NR1 and NR2A are 5'-TTTGGATCCATGGAGATCGCCTACAAGCG-3' and 5'-TTTGAATTCTCAGCTCTCCCTATGACGGG-3', 5'-CGGGATCCACCATGATGGAGCACCTCTTC-3' and 5'-CGGAATTCTTAAACATCAGATTCGATAC-3'. pGEX2th-Csk-ST plasmid was kindly provided by Dr. G. Sun at the University of Rhode Island [2]. Csk S109C mutation was made using QuikChange XL Site-Directed Mutagenesis Kit (Stratagene) as described by the manufacturer with pGEX2th-Csk-ST as template. Point mutation was confirmed by DNA sequencing. The GST and GST fusion proteins conjugated with Csk or Csk S109C were produced in *E. Coli* (BL-21 strain, Invitrogen) with isopropyl-β-D-thiogalactopyranoside (IPTG, 0.1mM) induction.

Characterization of Csk and Chk antibodies Corresponding antibodies of high specificity, and showing no cross reactivity, are required in order to distinguish between

expression of Csk and Chk. We therefore examined the selectivity and cross reactivity of these antibodies initially by using GST-fusion proteins coupled to Csk and Chk. These antibodies demonstrated a high selectivity between Csk and Chk with no cross reactivity (Supplementary Fig. 2a). Furthermore, this selectivity was confirmed using cell lysates from the adult brain and the liver. The liver contains no Chk [3 4, 5] and served as a negative control (Supplementary Fig. 2d). We also examined the ability of anti-Csk to distinguish levels of expression of Csk in additional ways. Firstly, we examined its signal with differing levels of expression of recombinant Csk expressed in fibroblasts grown from Csk-/- transgenic mouse embryos (Supplementary Fig. 2b). Secondly, we tested the ability of the antibody to detect differential levels of expression Csk in adult brain lysates taken from wildtype and Csk+/- mice which are deficient in Csk (Supplementary Fig. 2c). These results validate our subsequent use of these antibodies.

Reference List

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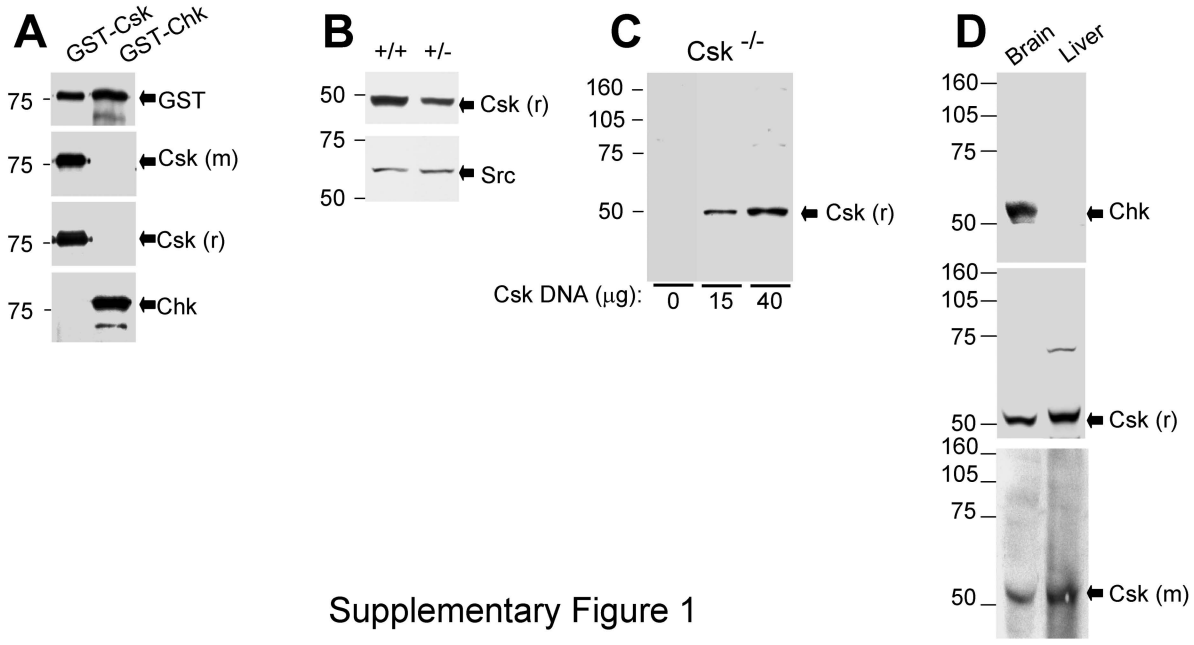
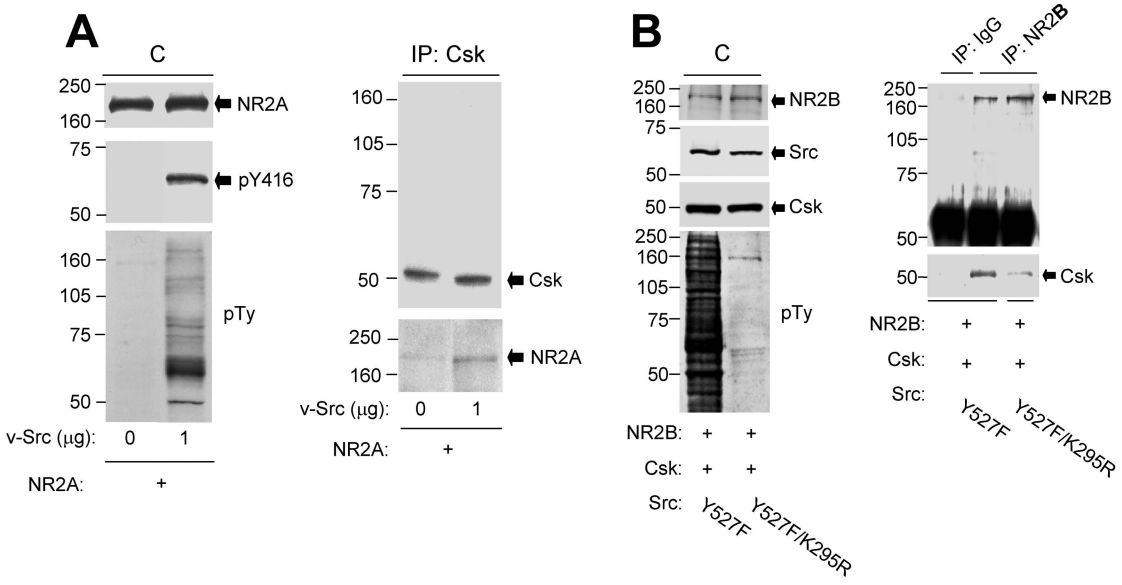


Supplementary Figure 1



Supplementary Figure 2