SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Animals – Male wild-type C57BL/6 (3 – 4 months old) were obtained from the National University of Singapore, Laboratory Animal Centre. All procedures involving animals were approved by the National University of Singapore, Institutional Animal Care and Use Committee.

Preparation of rat and mouse brain lysates – After decapitation, the brains were removed immediately and washed three times in ice-cold phosphate buffered saline (PBS) (in mM: 137 NaCl, 2.7 KCl, 10 Na_2HPO_4, 2 KH_2PO_4, pH 7.4). Hippocampi from the rat and mice brains were dissected. The remaining rat brain was homogenized in either ice-cold non-denaturing radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris, 150 mM NaCl, 0.5% sodium deoxycholate, 1% NP-40, pH 7.4) or Tris-Triton-X buffer (TTB, 0.1M Tris, pH 7.2/0.1% Triton X-100) (31) without protease inhibitors. Rat and mice hippocampi were homogenized in TTB. The homogenized lysates were subsequently centrifuged at 15,000 x *g* for 30 min and the supernatants were retained. Protein concentration of the lysates were measured using Pierce BCA protein assay kit (Thermo Scientific, Rockford, USA).

Supplementary Figure 1. Rat brain lysate (RBL) proteins (40μg) were treated with increasing concentrations of Actilyse® for 20 h at 37°C in the presence or absence of tPA-STOP™. A reduction in NR2B immunoreactivity upon exposure to Actilyse®, suggesting the degradation of NR2B proteins, was observed (n = 4). Single blot was probed for NR2B and GAPDH.

Supplementary Figure 2. tPA degrades NR2B but not NR1 or GluR2. *A*. RBL (10μg, prepared using non-denaturing RIPA buffer), RBL (25μg, prepared using Tris-Triton-X buffer (TTB)), rat hippocampal lysate (Rat Hipp, 25μg, prepared using TTB) and mouse hippocampal lysate (Ms Hipp, 25μg, prepared using TTB) were treated with 10μg/ml of tPA (Actilyse®) for 30min at 37°C in the presence or absence of tPA-STOP™ (500 - 600μM). In all conditions, reductions in NR2B immunoreactivities were observed upon exposure to tPA. Presence of tPA-STOP™ prevented the degradation of NR2B proteins. Despite the decrease in NR2B immunoreactivites, NR1 and GluR2 were not found to be degraded, suggesting that degradation of NR2B by tPA was substrate-specific. Single blot was probed for NR2B and GAPDH, stripped and reprobed for NR1 and GluR2. *B*. Quantification showing the effects of tPA treatment on NR2B, NR1 and GluR2. The decrease in NR2B immunoreactivities upon tPA treatment in the various lysate preparations were found to be statistically significant when compared to the untreated control of the corresponding lysate preparation (two-tailed paired t-test, n = 3 for each lysate preparation). However, there were no statistically significant changes in NR1 and GluR2 immunoreactivities in all lysate preparations (non-denaturing RIPA buffer *vs* TTB, rat *vs* mouse) upon tPA treatment. * and ** denote $p < 0.05$ and $p < 0.01$, respectively (against corresponding control). Dotted line denotes quantified protein level (100%) of untreated control.

Supplementary Figure 3. Rat brain lysate (RBL) proteins (10μg) were treated with 20 μg/ml of tPA for up to 3 h at 37°C and probed with anti-NR2B (Santa Cruz Biotechnology, Inc). Addition of D-Phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) (20 μM), a serine protease inhibitor, prevented degradation of NR2B proteins.

Supplementary Figure 4. Mean maximal current responses for NR1/NR2BWT and NR1/NR2B-ΔATD-R67 expressed in *Xenopus* oocytes. Current responses (μA) were measured using two-electrode voltage clamp in saturating concentrations of glutamate (100 μM) and glycine (100 μM) at pH 7.3. Each current response obtained from individual oocytes is denoted by an open circle (NR1/NR2BWT) or an open triangle (NR1/NR2B-ΔATD-R67). The mean maximal current for the truncated NR1/NR2B-ΔATD-R67 (0.073 ± 0.017 μA, n = 16) was statistically significantly smaller than the NR1/NR2BWT receptors (0.674 ± 0.188 μA, n = 17, $p < 0.0001$; two-tailed Mann Whitney test).