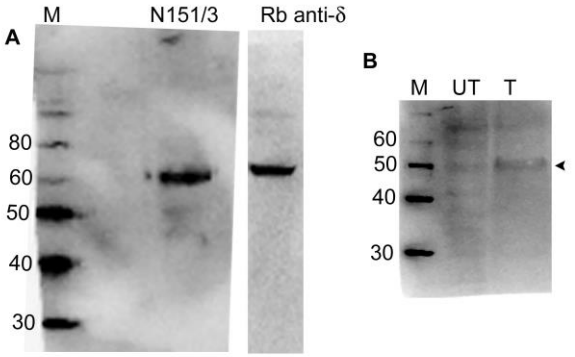
NMDA receptor activation down-regulates expression of δ subunit-containing $GABA_A$ receptors in cultured hippocampal neurons

Suchitra Joshi and Jaideep Kapur

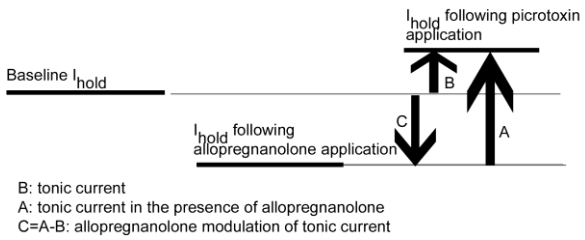
Department of Neurology, University of Virginia Health Sciences Center,

Box 800394, Charlottesville, VA 22908-0394

Journal title: Molecular Pharmacology



Supplementary figure 1: Characterization of mouse monoclonal anti-δ subunit antibody. **A**: Comparison of reactivity of mouse monoclonal anti-δ subunit antibody (clone N151/3.3) and rabbit anti-δ subunit antibody (Millipore) in a Western blotting assay. Expression of δ subunit was determined in 50 μg of rat cerebellar proteins using 3 μg/ml of anti-δ subunit antibody (N151/3.3) or 1:500 diluted rabbit anti-δ subunit antibody (Millipore). Both antibodies reacted with a single protein of approximate size 55 kDa corresponding with predicted size of the δ subunit of GABARs. **B:** A representative Western blot demonstrating reactivity of anti-δ subunit antibody (N151/3.3) with 50 μg proteins from HEK293 cells expressing rat δ subunit (T). Proteins from untransfected (UT) cells were used as a control. Lane M shows standard molecular size marker.



Supplementary figure 2: A schematic representation of measurement of tonic current and its neurosteroid modulation. Average pre-drug and post-drug holding currents were measured as described in the materials and methods. Tonic current was measured as the difference between pre-drug holding current and holding current following picrotoxin application (B). Allopregnanolone modulation of tonic current was the difference between pre-drug holding current and holding current following allopregnanolone application once a stable response was obtained (C).