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Supplemental Figure 1. Validation of anti-Nav1.1, anti-Nav1.2 and anti-Nav1.6 specificities. **A-B.** Nav1.1 **(A)** and Nav1.2 **(B)** were immunoprecipitated (IP) from rat brain hippocampus lysates and immunoblotted (IB) with anti-Nav1.1, anti-Nav1.2, and anti-Nav1.6 antibodies (lanes 1-4). **C.** HEK293FT cells were transfected (TF) with human Nav1.1 (lane 1), rat Nav1.2 (lane 2) or mouse Nav1.6 (lane 3) and immunoblotted with anti-Nav1.6 antibody. Hippocampal lysate (lane 4) and a pre-adsorption peptide (lane 5) were used as a positive and negative control, respectively. The blocking peptide, made up of the same sequences as the Nav1.6 antibody, specifically blocks expression of Nav1.6 from the hippocampal lysate. **D.** HEK293FT cells were transfected (TF) with eGFP (green fluorescence) and human Nav1.1 (top left 2 panels), rat Nav1.2 (top, right 2 panels) or mouse Nav1.6 (bottom 3 panels), and immunolabeled with anti-Nav1.6 with detection using AlexaFluor-568-conjugated secondary antibody (red fluorescence). All cells expressing Nav1.6 co-expressed eGFP (arrows). Untransfected cells (bottom right) served as a negative control. Mr, relative molecular mass (in kDa). Bar 75 μ m. See figure 1 for magenta-green images.

Figure 2. Nav subtypes are expressed in soma, dendrites and axons of hippocampal CA1 and DG. **A.** Schematic diagram of CA1, CA3, and dentate gyrus (DG) in rat hippocampus. **B-C.** High magnification light microscopic photomicrographs (top) of Nav1.1 (left), Nav1.2 (middle), and Nav1.6 (right) in rat CA1 (**B**) and DG (**C**). Nav labeling is evident in stratum oriens (SO), pyramidal cell layer (PCL), stratum radiatum (SR), and stratum lacunosummoleculare (SLM) in CA1 (**B**, top) and in molecular layer (ML), granular cell layer (GCL), and central hilus (CH) in DG (**C**, top). Bar 200 μ m. Examples of immunofluorescence localization of Nav1.1 (left), Nav1.2 (middle), and Nav1.6 (right) in rat hippocampal cultures from CA1 (**B**, bottom) and DG (**C**, bottom). Bar 15 μ m. **D.** Immunofluorescence images showing double labeling of Nav1.1 (red) with excitatory axon terminal marker vesicular glutamate transporter (vGlut; green, left) and inhibitory axon terminal marker glutamic acid decarboxylase 65 (GAD 65; green, right). Bar 5 μ m. See figure 2 for magenta-green images.