From Methods Aakalu et al., (2001) Neuron 30(2): 489-502
pcDNA3.1-5′dGFP3′: The CamKII-α 3′UTR sequence obtained from plasmid (Mayford et al., 1996) was PCR amplified (forward primer: 5′-ttatatttgcggccgcggtcgctaccattaccagtt-3′; reverse primer: 5′-ggcgctctctcgagtttaaatttgtagct-3′) and cloned into the NotI and XhoI sites of the pcDNA3.1 vector (Invitrogen). The resulting vector was then cleaved with BamHI and NotI for insertion of the destabilized EGFP ORF (from pd2EGFP, Clontech). The CamKII-α 5′UTR was released from a plasmid (obtained from J. Fallon) and inserted at the HindIII-BamHI sites, yielding pcDNA-5′dGFP3′. pSinRep5-5′dGFP3′: The 5′dGFP3′ fragment was released with PmeI-ApaI (blunted) and ligated into pSinRep5 (Invitrogen). pcDNA3.1-5′$_{myr}$dGFP3′: The d2EGFP ORF (from pd2EGFP, Clontech) was PCR amplified (forward primer: 5′-cgactctagagtgagcaagggcgaggagctg-3′; reverse primer: 5′-tctagagtcgcggccgcatctacaca-3′), digested, and inserted into the XbaI-NotI sites of pBSK. To generate the myristoylation signal, two oligos corresponding to the N-terminal 10 amino acids of p10 were annealed (myr1: 5′-gatccatgggcacggtgctgtccctgtctcccagct-3′; myr2: 5′-ctagagctgggagacagggacagcaccgtgcccatg-3′), digested, and inserted into the BamHI-XbaI sites of pBSK-d2EGFP. The $_{myr}$dGFP was subcloned into the BamHI-NotI sites of pcDNA3.1-5′dGFP3′. pSinRep5-5′$_{myr}$dGFP3′: The 5′$_{myr}$3 dGFP3′ fragment was released with PmeI-ApaI and subcloned into the StuI-ApaI sites of pSinRep5 (Invitrogen). Sindbis viroids were produced according to the Experimental Procedures provided by Invitrogen. Contrary to observations in other cell types, it appears that single-stranded RNA viruses of the α family do not shut down protein synthesis in neurons (K. Lundstrom, personal communication, Hoffmann-LaRoche, Basel, Switzerland).