

Fig. S1. Down-regulation of dicer using siRNAs in HEK 293T cells causes a loss of dicer-immunoreactivity as detected with anti-dicer C-terminal antibody.

HEK 293T cells were passaged in DMEM/10% fetal calf serum, then transfected in suspension with siRNAs using the Silencer siRNA Transfection II kit (Ambion) and siPORT Amine transfection agent in OptiMeM I medium. Dicer siRNA against human sequence was synthesized, hybridized and purified by HPLC (sense = GCA GCU CUG GAU CAU AAU Att; antisense = UAU UAU GAU CCA GAG CUG Ctt), and transfected at 5, 10 or 30 nM final concentration. As a negative control, GAPDH siRNA was transfected at 30 nM. The next morning, the cells were placed in Neurobasal/B27 serum-free medium. Three days after transfection, cells were lysed in ice-cold homogenization buffer containing 0.1% Triton X-100 and centrifuged at 20,000 x g for 20 minutes. Protein assay was performed, the supernatant was methanol precipitated, and equal amounts of protein (10 rg) were loaded per lane on SDS-PAGE followed by immunoblotting. MW markers indicate 250, 150, 100 and 75 kDa.

The immunoblot shows that full-length dicer (220 kDa) is almost undetectable in cells transfected with dicer siRNA 5-30 nM. A pattern of large dicer-immunoreactive fragments is observed in HEK 293T cells (albeit the sizes are not identical to that observed in mouse brain), and there is a significant loss of these bands as well. The blot was reblotted with anti-GAPDH antibody (Ambion) to confirm that this protein was downregulated in the cells treated with GAPDH siRNA, and also reblotted with antibody against beta-actin to confirm that equal amounts of protein were loaded per lane. Similar results were also observed when cell lysates were immunoblotted with the chicken anti-dicer antibody recognizing an internal epitope (not shown).

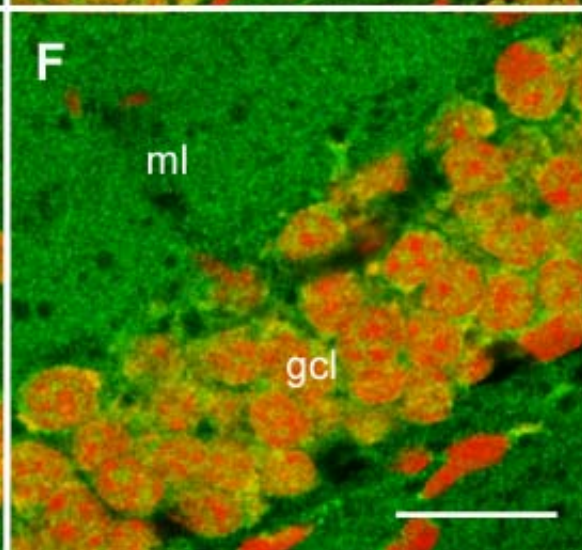
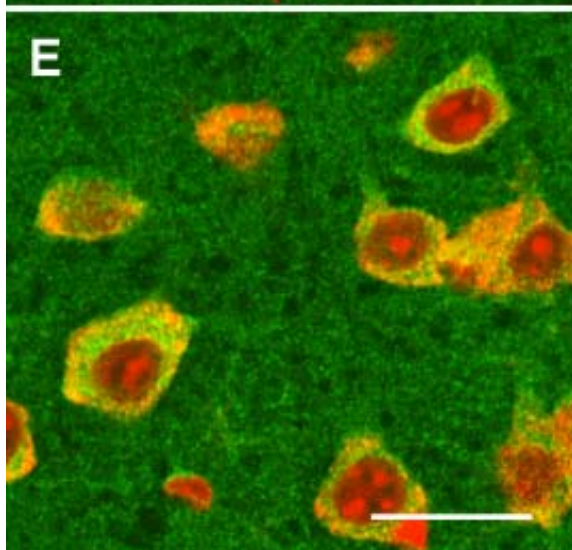
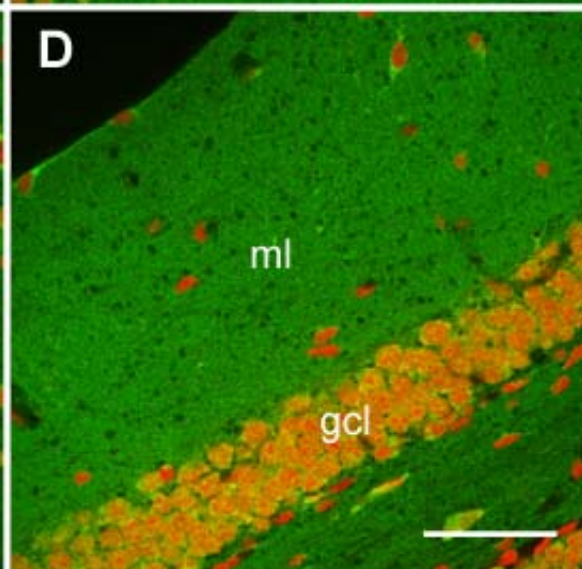
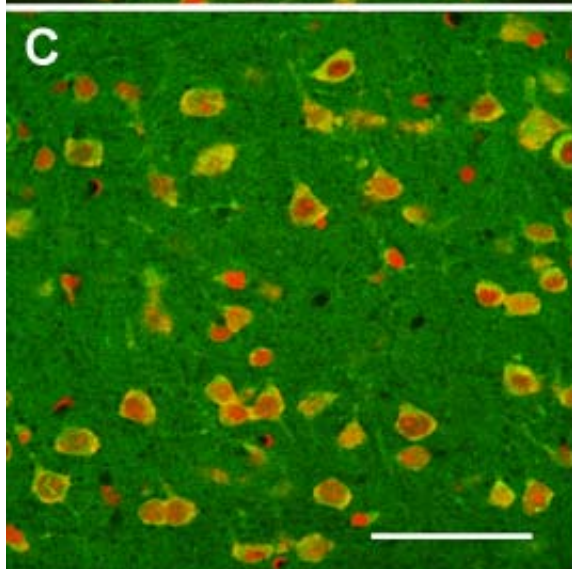
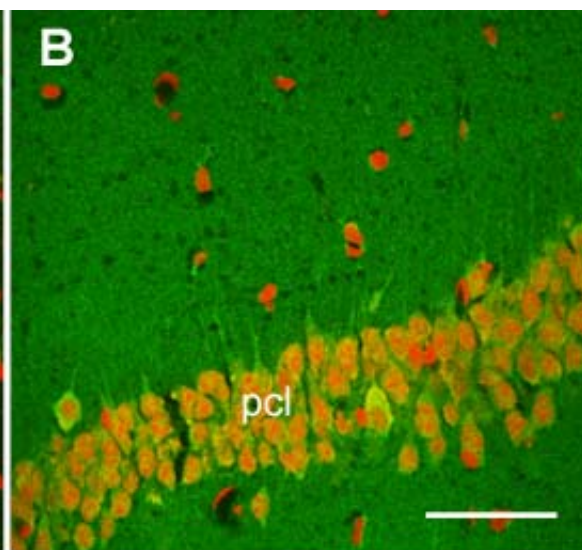
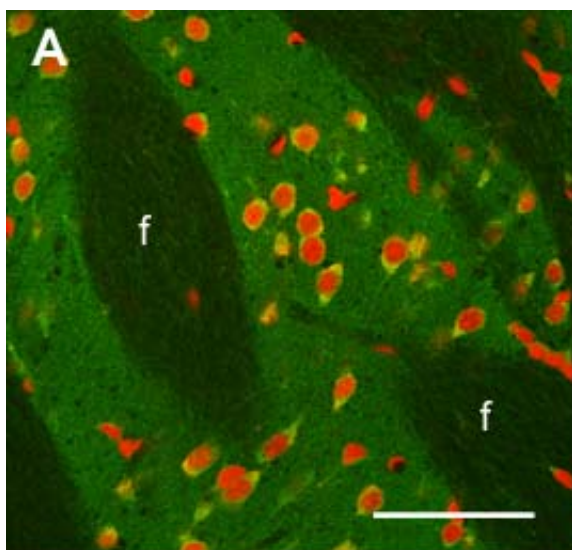


Fig. S2. Immunolocalization of Dicer (green) in multiple brain regions.

Tissue was counterstained with propidium iodide (red) to reveal cell nuclei and nucleic-acid rich regions of cytoplasm. A) caudoputamen; B) hippocampal area CA1; C) neocortex; D) dentate gyrus; E) higher magnification view of neocortex; F) higher magnification view of dentate gyrus granule cells showing nuclear staining. Dicer was distributed throughout the brain in neuronal cell bodies, dendrites and as punctate staining in the neuropil. Glial cells were also labeled although white matter as a whole exhibited only weak labeling, as seen in the fiber fascicles (f) running through the caudoputamen (A). f = fiber fascicle; pcl = pyramidal cell layer; gcl = granule cell layer; ml = molecular layer. Scale bars in A-D = 100 μm ; scale bars in E-F = 20 μm .

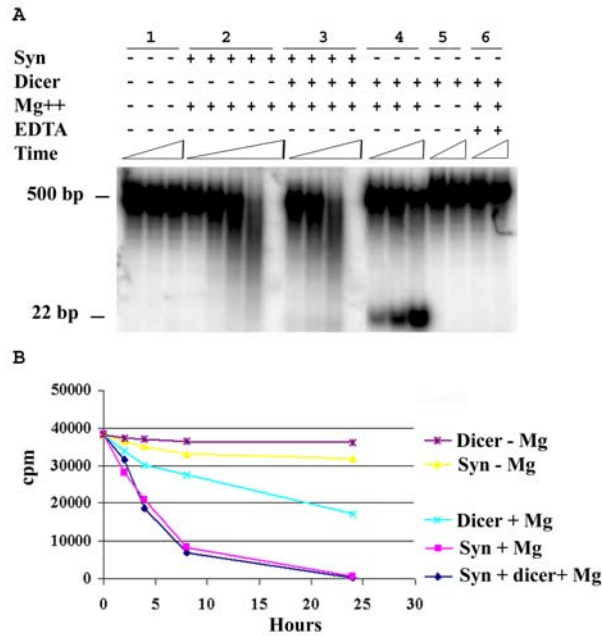


Fig. S3. RNAse III activity assay in lysed synaptoneurosomes.

A: Synaptoneurosomes and recombinant dicer were assayed for RNAse III activity as described in Methods, but synaptoneurosomes were first lysed with 0.1% Triton X-100 in the presence of leupeptin. Reactions were stopped at the times indicated; RNA was extracted with Trizol, resuspended in equal amount of loading buffer, separated on a 4-20% Criterion-TBE gel, and autoradiographed. Expt 1: (incubated 0, 4, 24 hrs). Negative control: In the absence of synaptoneurosomes and dicer, no loss of dsRNA substrate or formation of small RNA products were detected. Expt 2: (0, 2, 4, 8, 24 hrs).

Synaptoneurosome lysates exhibited RNAse III activity as assessed by the loss of dsRNA substrate, but no small products accumulated. Expt 3: (2, 4, 8, 24 hrs). When synaptoneurosomes and recombinant dicer were mixed together, the dsRNA substrate was cleaved but no small products accumulated. Expt. 4: (4, 8, 24 hrs). Positive control: recombinant dicer shows the expected loss of dsRNA substrate, and the formation of small 22 nt. RNA fragments. Expt. 5: (4, 24 hrs). Negative control. Recombinant dicer shows no RNAse III activity when Mg⁺⁺ was omitted. Expt. 6: (4, 24 hrs). Negative control. Recombinant dicer shows no RNAse III activity when excess EDTA was added.

B: To quantify the loss of the 500 bp dsRNA substrate in the experiments just described, an equal amount of all the samples was loaded in a 3.5% Agarose gel, and the bands were excised and counted in a scintillation counter. Only selected experiments are plotted, as indicated.