File S1

Supporting Materials and Methods

Primary cortical neuron culture

Dissociated P0 rat cortical neurons were obtained from animals of either sex using a protocol similar to one described elsewhere (BANKER and GOSLIN 1998; SANJANA and FULLER 2004). Briefly, cortical tissues were proteolyzed with papain for 40 min at 37ºC, followed by rinsing and trituration in the plating medium. The plating medium consisted of serum-free Neurobasal-A without phenol red, with 2% B27 and 2 mM Glutamax (Gibco/Invitrogen) (BREWER *et al.* 1993). Cells were plated at a density of 200,000/mL on tissue-culture-treated plastic and placed in a 37ºC 5% CO_2 incubator. Feeding of the culture was done with the same serum-free medium (50% replacement every 4 days in vitro).

Neuron activity modulation

For short-term activity modulation, neurons were depolarized for 1 or 6 hours with high potassium (60 mM) buffer (LIN *et al.* 2008). The depolarization buffer consisted of 120 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 10 mM HEPES (pH 7.35, 285 mOsm). The control buffer was identical except 120 mM NaCl was substituted for KCl. Approximately 1 volume of the appropriate medium was added to the culture medium to achieve the desired final concentration. For short-term activity reduction, we bathed neurons in 2 µM TTX (Sigma) for 6 hours. In experiments where was Ca^{2+} blocked, cultures were pre-treated with 2 mM EGTA for 30 minutes before adding a modified depolarization buffer with 2 mM EGTA.

For long-term activity modulation, neurons were placed in culture media supplemented with 2 µM TTX, 20 µM bicuculline methiodide (Tocris), or the same amount of new Neurobasal-A (control) for 48 hours (TURRIGIANO *et al.* 1998; WIERENGA *et al.* 2006). After 24 hours, half of the culture media was replaced with fresh media and/or drug treatments.

RNA extraction and cDNA library creation

Total RNA and gDNA were purified from neuron cultures (Qiagen AllPrep). For each sample, 0.5-1 µg of isolated RNA was reverse-transcribed in a 20-40 µl reaction volume using a mixture of random hexamer and oligo-dT primers (qScript cDNA SuperMix). To ensure no gDNA contamination was introduced during RNA isolation, PCR was conducted on cDNA templates (50 cycles, Invitrogen Platinum PCR SuperMix High Fidelity 12532-016). We used two exon-spanning primers sets (*Actb*, *Flna*) that produce smaller amplicons for cDNA templates and one primer set (*Htr2c*) that only hybridizes to an intronic sequence (Supplementary Figure 2). (See Supplementary Table 1 for primer sequences.)

Quantitative PCR of immediate early genes and adenosine deaminases

Relative transcript abundance was calculated by the ΔΔCt method with primer efficiencies derived from standard curves (Microsoft Excel 2008) (PFAFFL 2001). For primer sequences, see Supplementary Table 2. Two-tailed *t*-tests (α=0.05) were used to test for significant differences in expression from a matched control after first testing for equality of variances using Bartlett's test (Matlab R2009a). Data with unequal variance was first log-transformed and then re-tested for equal variance before applying *t*-tests.

Identification of A-to-I editing sites in rat exons

Recent studies have found that A-to-I RNA editing is extremely abundant in human transcripts (ATHANASIADIS *et al.* 2004; BLOW *et al.* 2004; KIM *et al.* 2004; LEVANON *et al.* 2004). However, most of these sites are located in regions that are not evolutionary conserved and have no corresponding position in the rat genome. In order to study RNA editing in the rat, we collected almost all of the known evolutionary conserved, exonic editing sites in human (ATHANASIADIS *et al.* 2004; BLOW *et al.* 2004; HOOPENGARDNER *et al.* 2003; LI *et al.* 2009) and found their position in the rat genome using the Batch Coordinate Conversion (liftOver) utility at the UCSC Genome Browser (FUJITA *et al.* 2011). The location of each site was verified manually by comparison of the human and rat protein sequence at the position.

Editing transcript PCR and Illumina sequencing library preparation

For each cDNA sample, a PCR reaction was carried out with primer pairs flanking the editing site(s) in each transcript (see Supplementary Table 3 for primer sequences). We set up a 20 µl PCR reaction containing 10 µl of KAPA SYBR FAST Universal 2X qPCR Master Mix, 0.5 µl of cDNA template, and 500 nM each of forward and reverse primers. At the 5' end of the forward primer, we added a common sequence (Supplementary Table 4) followed by a 4-base unique barcode sequence (Supplementary Table 5) and then the editing site-specific sequence (Supplementary Table 3). At the 5' end of the reverse primer, we added similar site-specific and common sequences but no barcode. The PCR program is: 95°C for 3 minutes, 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds, and 72°C for 3 minutes. Twelve PCR products with different barcodes were pooled. For each pool, we carried out a 100 µl of PCR reaction to attach sequencing adaptors containing 50 µl of KAPA SYBR FAST Universal 2X qPCR Master Mix, 5 µl of mixed PCR products, and 500 nM each of PAGE-purified primers. The PCR program used was: 95°C for 3 minutes, 8 cycles of 95°C for 3 seconds and 60°C for 30 seconds, and 72°C for 3 minutes. After purification (Qiagen QiaQuick), each pool of 12 samples was sequenced in one lane of Illumina GAIIx.

Sequencing read alignment and RNA editing quantification

Reads were converted into Sanger FASTQ format using the Mapping and Assembly with Qualities (MAQ) package (LI *et al.* 2008) and 4-base barcodes were used to separate reads into experimental conditions (Figure 2b). Barcodes differed by at least 2 bases from each other (Supplementary Table 5), preventing any sample misidentification due to single nucleotide sequencing error. Almost all reads (>98%) had a perfect match to one of the 12 possible 4-base barcodes in their first 4 nucleotides. Reads without a perfect barcode match in this location were discarded and not analyzed further. Reads were aligned to reference sequences using BWA (Burrows-Wheeler Aligner) (LI and DURBIN 2009) with the maximum number of allowable mismatches equal to maximum number of editing sites expected in a single read. To detect significant changes in editing levels, we conducted two-sample *t*-tests for each editing site with Benjamini-Hochberg false-discovery rate multiple hypothesis correction (BENJAMINI and HOCHBERG 1995) with type I error rate α=0.05 (Matlab R2009a). The margin of error in the editing proportion at 95% confidence was estimated using Bernoulli parameter p=0.5, making it an upper bound: margin of error $\approx 0.98/(\text{reads})^{0.5}$. The mean and standard error of the change in editing across 4 biological replicates for each experiment is shown in Supplementary Table 6.

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