Activity-Dependent A-to-I RNA Editing in Rat Cortical Neurons

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ABSTRACT Changes in neural activity influence synaptic plasticity/scaling, gene expression, and epigenetic modifications. We present the first evidence that short-term and persistent changes in neural activity can alter adenosine-to-inosine (A-to-I) RNA editing, a post-transcriptional site-specific modification found in several neuron-specific transcripts. In rat cortical neuron cultures, activity-dependent changes in A-to-I RNA editing in coding exons are present after 6 hr of high potassium depolarization but not after 1 hr and require calcium entry into neurons. When treatments are extended from hours to days, we observe a negative feedback phenomenon: Chronic depolarization increases editing at many sites and chronic silencing decreases editing. We present several different modulations of neural activity that change the expression of different mRNA isoforms through editing.

CHANGES in neural activity can modulate synaptic strength at the level of individual neurons (Burrone *et al.* 2002) and whole networks (Turrigiano *et al.* 1998), influence developmental and differentiation decisions (Borodinsky *et al.* 2004), and bias neurons toward inclusion in novel memory formation (Zhou *et al.* 2009). At the mRNA level, immediate early genes (IEGs) are transcribed rapidly in response to increased neural activity and have been described as a "genomic action potential" (Clayton 2000). The promoter regions of these and other genes involved in neuronal plasticity can undergo activity-dependent alterations in their chromatin structure, such as cytosine demethylation and histone acetylation (Tsankova *et al.* 2004; Ma *et al.* 2009). In addition to the major, genome-wide epigenetic changes known to occur during early development, recent studies have found activity-driven epigenetic modifications in post-mitotic neurons during fear memory recall, exposure to drugs of abuse, and emotional stress (Renthal *et al.* 2007). We sought to understand if neural activity influences a post-transcriptional genetic modification, adenosine-to-inosine (A-to-I) RNA editing.

A-to-I RNA editing is an enzymatically catalyzed, site-specific nucleotide change (deamination) in pre-mRNA that changes adenosine into inosine, which reads as guanosine during translation (Bass 2002; Nishikura 2010). The best-known example is the developmentally regulated editing of the glutamate receptor subunit *Gria2*, where A-to-I editing at a single site controls whether GRIA2-containing AMPA receptors are permeable to Ca^{2+} ions (Sommer *et al.* 1991). In neurons, many transcripts undergo editing: Previous large-scale genomic screens in *Drosophila* and human cells found that synapse-related transcripts are enriched in RNA-editing sites (Paul and Bass 1998; Hoopengardner *et al.* 2003; Li *et al.* 2009). More generally, inosine is greatly enriched in brain tissue compared to other tissues (Paul and Bass 1998) and the mammalian adenosine deaminases acting on RNA (ADARs) tend to be preferentially or exclusively expressed in the brain (Melcher *et al.* 1996). Knockout of ADARs in mice, *Drosophila*, and *Caenorhabditis elegans* leads to aberrant neurological and behavioral phenotypes (Brusa *et al.* 1995; Tonkin *et al.* 2002; Savva *et al.* 2012). This strongly supports that RNA editing influences neural function, but it is not well understood whether changes in neural activity can affect RNA editing to exert control over transcript diversity and protein function. Previously, it has been shown that editing at two sites in serotonin 2C (*Htr2c*) pre-mRNA is regulated in a serotonin-dependent manner

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without any change in transcript expression levels (Gurevich *et al.* 2002); early life stress and the common antidepressant fluoxetine can also alter *Htr2c* editing (Englander *et al.* 2005; Bhansali *et al.* 2007). These studies suggest a possible role for neural activity in inducing changes in RNA editing, but this connection has not been systematically explored on a genome-wide scale with newer sequencing techniques. In this work, we characterize the effects of membrane potential depolarization and synaptic activity on RNA editing across many editing sites from the coding regions of a diverse group of transcripts.

Results

Acute depolarization of rat cortical neurons for 6 hr but not 1 hr alters A-to-I editing in the coding regions of diverse transcripts

We depolarized primary cultures of neonatal rodent cortical neurons by iso-osmotically increasing potassium to 60 mM for 1 or 6 hr (see Supporting Information, File S1 for full methods). In four biological replicates, we extracted RNA and reverse-transcribed cDNA. To validate the efficacy of our activity induction, we measured the expression of two IEGs, *Arc* and *Bdnf*, and observed similar results as reported previously (Figure 1A) (Lin *et al.* 2008). *Arc* is rapidly transcribed after 1 hr depolarization (>20-fold), whereas *Bdnf* transcripts increase significantly only after 6 hr of depolarization (>50-fold). For all experiments, we used the expression of these two IEGs to validate the membrane potential or synaptic activity modulation, as *Arc* and *Bdnf* transcripts have previously been shown to increase after depolarization and decrease after inhibition (Shepherd *et al.* 2006; Aid *et al.* 2007; Lin *et al.* 2008). Depolarization for either 1 or 6 hr does not change transcript expression of any of the adenosine deaminases (*Adar1-3*) required for A-to-I editing (Figure 1B).

After depolarizing neurons, we quantified the level of RNA editing occurring at 25 rat exonic positions that are conserved and known to be edited in humans (Table 1). Each region was first PCR-amplified, and the amplified transcripts were pooled and sequenced using an Illumina GAIIx (Figure 2A). Compared to standard RNA sequencing (RNA-seq), this targeted deep-sequencing approach can reliably quantify small changes and do so even in rare transcripts, since all editing sites are sampled with roughly the same (large) number of reads (Figure S1). In a single Illumina flow cell, we sequenced reads from multiple experimental treatments and multiple editing sites simultaneously. As shown in Figure 2B, reads are properly identified using treatment-specific barcodes and a Burrows–Wheeler short-read aligner (Li and Durbin 2009). The average number of reads per editing site was 20,000 ± 900 reads (mean ± SEM, n = 25 sites), allowing us to quantify the editing level with <1% error (upper bound on the 95% confidence interval).

After 1 hr depolarization, we found no differences in editing levels at any of the sites when compared to neurons treated with a control buffer with sodium substituted for potassium. The editing levels in rat cortical neuron culture were generally in good agreement with the corresponding editing levels reported in humans (Table 1). However, after 6 hr of high potassium depolarization, we found small but significant decreases at 9 of the 25 editing sites ($P < 0.05$, n = 4 cultures, two-sample, unpaired t-test, Benjamini–

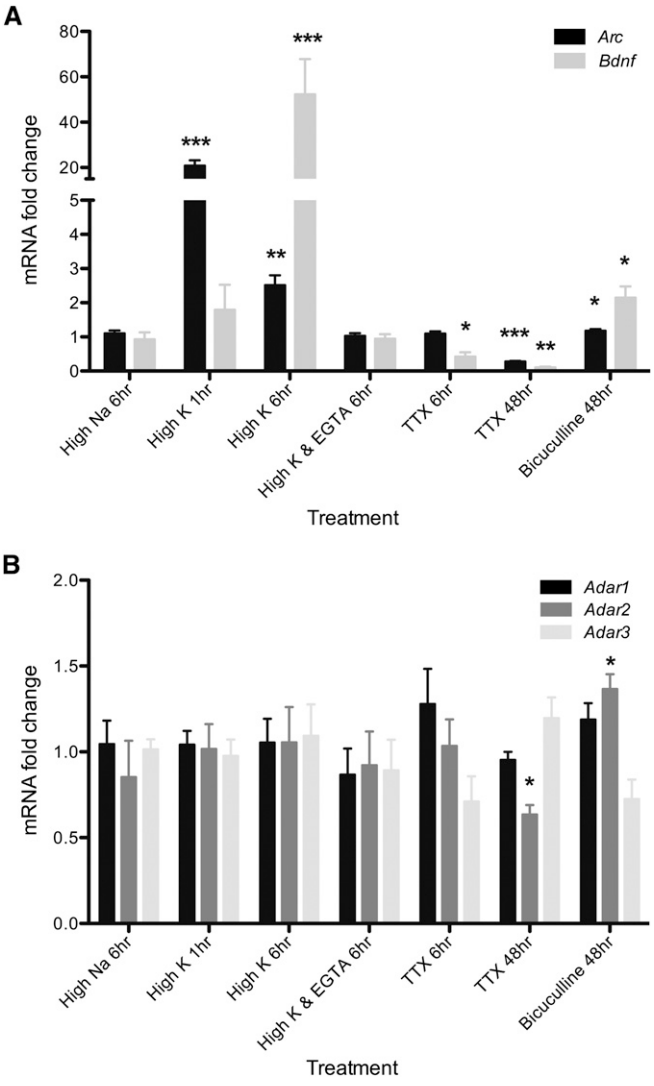

Figure 1 Transcription of immediate early genes after neuron activity modulation. (A) Comparison of the transcript levels of the immediate early genes *Arc* and *Bdnf* between indicated treatments and matched controls using quantitative RT-PCR (qRT-PCR). The High K treatments used 60 mM potassium depolarization buffer. The High Na treatments used a control buffer with sodium substituted for potassium. The High K & EGTA treatment consisted of pretreatment for 30 minutes with 2 mM EGTA and then 6 hours with 60 mM potassium depolarization buffer and 2 mM EGTA. Matched controls were the following: High Na 1 hr (for High Na 6 hr, High K 1 hr); High Na 6 hr (for High K 6 hr, High K and EGTA 6 hr, TTX 6 hr); Neurobasal 48 hr (for Bic 48 hr, TTX 48 hr). Asterisks indicate significant differences in expression from the matched control (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $n = 4$ cultures, one-sample, two-tailed t-test). Error bars indicate SEM. (B) Relative change in the transcript levels of the adenosine deaminases *Adar1*, *Adar2*, and *Adar3* between indicated treatments and matched controls using qRT-PCR. Treatments and matched controls are the same as in A.

Table 1 Comparison of RNA editing percentage with human homologs

Transcript and editing site in rat RefSeq	Mean editing level in Na 1-hr treatment (%, n = 4 cultures)	Standard error of the mean (%)	Previously reported human editing levels at homologous site (%)
Blcap K15R	16.5	1.1	11–18, various brain regions (Li *et al.* 2009)
Blcap Q5R	28.0	1.0	8–16, various brain regions (Li *et al.* 2009)
Blcap Y2C	42.9	0.6	∼30, cerebellum (Kwak *et al.* 2008)
Cadps E1186G	37.8	1.2	20–26, various brain regions (Li *et al.* 2009)
Cyfip2 K320E	50.7	0.6	84, cerebellum (Kwak *et al.* 2008); 41–83, various brain regions (Li *et al.* 2009)
Flna Q2333R	4.5	0.5	14, cerebellum (Kwak *et al.* 2008); 27–40, various brain regions (Li *et al.* 2009)
Flnb Q2272R	20.1	2.8	
Gabra3 I342M	57.6	1.9	57, frontal lobe (Li *et al.* 2009)
Gria2 Q607R	99.5	0.0	95–100, motor neurons (Kawahara *et al.* 2004); 57–98, various brain regions (Li *et al.* 2009)
Gria2 flip R764G	51.8	1.3	58, gray matter (Maas *et al.* 2001); 17–65, various brain regions (Li *et al.* 2009)
Gria2 flop R764G	54.7	3.0	
Gria3 flip R769G	83.4	1.3	48–95, various brain regions (Li *et al.* 2009)
Gria3 flop R769G	77.0	4.6	
Grik1 Q636R	41.4	2.5	47, frontal lobe (Li *et al.* 2009); ∼50, cerebellum and corpus callosum (Barbon *et al.* 2003)
Grik2 I567V	46.7	0.5	
Grik2 Q621R	45.2	2.2	
Grik2 Y571C	50.2	0.2	
Htr2c A	79.0	2.4	74–80, various brain regions (Niswender *et al.* 2001)
Htr2c B	76.5	2.9	40–60, various brain regions (Niswender *et al.* 2001)
Htr2c E	3.8	1.7	16–22, various brain regions (Niswender *et al.* 2001)
Htr2c C	37.6	4.7	60–66, various brain regions (Niswender *et al.* 2001)
Htr2c D	62.8	6.4	46–66, various brain regions (Niswender *et al.* 2001)
Kcna1 I400V	16.5	2.1	20, cerebellum (Kwak *et al.* 2008); 26–59, various brain regions (Li *et al.* 2009)
Kcnma1 S41G	2.5	0.7	
Neil1 K242R	1.0	0.1	

Comparison of adenosine-to-inosine editing levels found in this study (rat cortical neurons) and the previously reported levels for the homologous editing sites in humans. All editing levels are given as percentage inosine (or a range of the percentage inosine).

Hochberg false-discovery rate correction) (Figure 3A). Editing levels changed in a diverse group of transcripts, including AMPA- and kainate-type glutamate receptor subunits (*Gria2*, *Grik2*), a fragile X mental retardation 1 interacting protein (*Cyfip2*), actin-interacting cytoskeletal proteins (*Flna*, *Flnb*), and a tumor suppressor protein (*Blcap*).

We observed that, within a single transcript, not all editing sites show activity-based modulation: For example, the fraction of inosine-containing transcripts at the *Gria2* Q/R site is unchanged whereas its R/G site shows a decrease in editing (Figure 3A). We speculate that such decreases in editing within a single transcript cannot be attributed to a simple increase in transcript levels due to the different effects of activity at the two editing sites. However, without additional quantification of transcript levels, a transient uncompensated change in transcript levels cannot be ruled out. For *Gria2*, editing at the Q/R site has been shown to be a prerequisite for splicing and transcript maturation (Higuchi *et al.* 2000), which could explain the differential editing. Alternatively, since the Q/R site is edited solely by ADAR2 whereas the R/G site is edited by both ADAR1 and ADAR2, it is possible that activity-induced changes in *Gria2* editing are mediated solely by ADAR1. Even when the same ADAR deaminates multiple sites, it is likely that the local double-stranded RNA (dsRNA) structure at each site or other sequence-specific differences can result in different ADAR-binding affinity and degrees of editing. Also, different ADAR isoforms, such as an alternatively spliced ADAR2 truncation with no deaminase activity and a SUMOylated ADAR1 with reduced deaminase activity, and other dsRNA-binding proteins may also influence editing in a site-selective manner through competitive inhibition (Wahlstedt and Ohman 2011).

Neuron activity-dependent A-to-I editing requires calcium entry

Many activity-regulated changes in neurons, such as IEG expression and LTP, depend on calcium entry through *N*-methyl-D-aspartate (NMDA) and voltage-gated calcium channels (Turrigiano *et al.* 1998; Guzowski *et al.* 2000; Greer and Greenberg 2008; Lin *et al.* 2008). To determine if activity-dependent RNA editing also requires calcium entry, we blocked extracellular calcium by pretreating cultures for 30 min with the calcium chelator ethylene glycol-bis-(2-aminoethyl)-N,N,N′, N′-tetraacetic acid (EGTA) before placing them in high-potassium depolarizing buffer that also

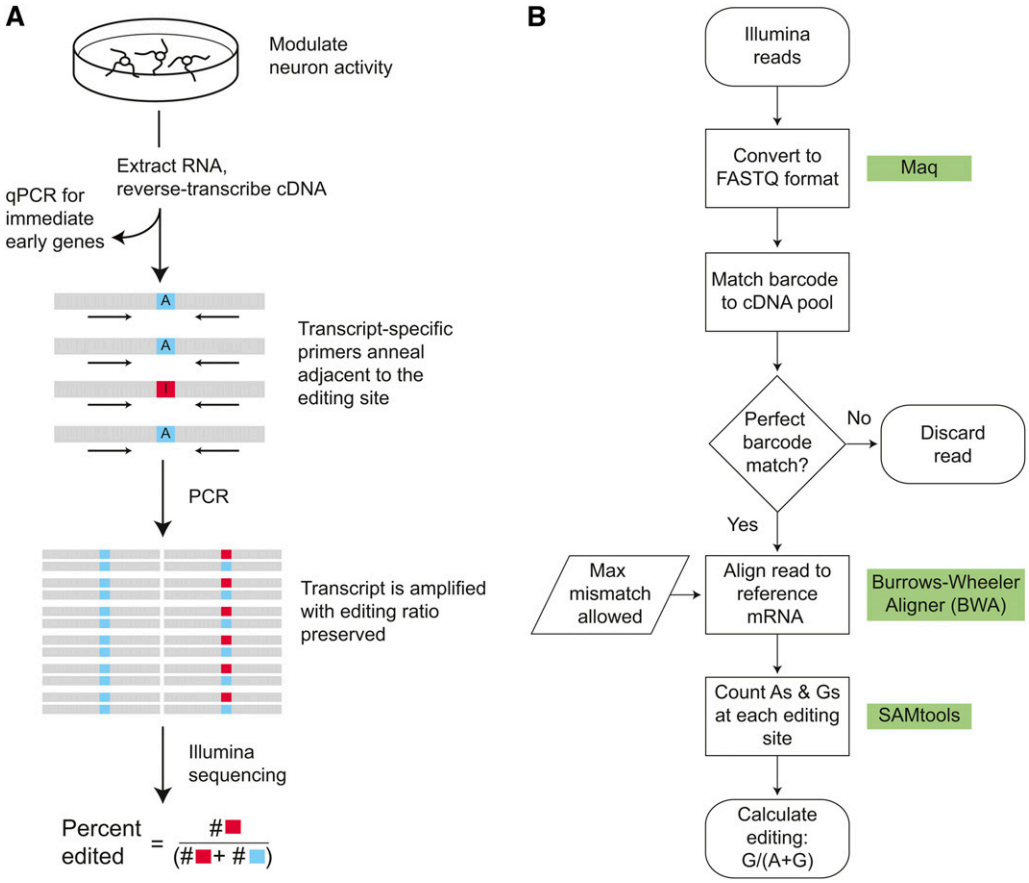

Figure 2 Targeted deep-sequencing assay for RNA editing. (A) Schematic of RNA editing percentage quantification. Primary cortical neuron cultures are exposed to various activity-increasing and -decreasing drug treatments, followed by RNA extraction and reverse transcription to cDNA. PCR was used to check for gDNA contamination of the cDNA (Figure S2 and Table S1). To validate activity-modulating treatments, immediate early gene expression is measured using qPCR (Table S2). For each transcript in each condition/replicate, a separate PCR is done to amplify the transcript using primers that anneal a few bases before the editing site(s) of interest (Table S3). Only one transcript and one editing site are shown in the schematic; our experiments included 25 editing sites in 14 transcripts. Blue squares indicate a transcript that is unedited (adenosine at editing site) and red squares indicate an edited transcript (inosine at editing site, which is read as guanosine). A separate PCR is used to attach barcodes and sequencing primers (Table S4 and Table S5), and then all transcripts are sequenced with Illumina. Reads are aligned, and the ratio of reads with guanosine at the editing site over the total reads (adenosine and guanosine) gives the editing percentage. This can be assayed even when the transcript has very few copies in the original cDNA library. (B) Flowchart of data-processing steps to compute the editing percentage at each editing site from Illumina reads. Green squares indicate steps where existing open-source tools were used. Briefly, all Illumina reads were first converted to the standard FASTQ format. Then barcodes were examined to assign each of the (multiplexed) reads back to their respective experimental conditions; barcodes were removed from the read. Any read without an exact-match barcode in the first four bases of the read was discarded entirely. Then reads were aligned to a database of reference sequences surrounding each editing site with the maximum allowable mismatch between the read sequence and the reference sequence determined by the maximum number of editing sites in a single read. Pileup statistics were computed at each editing site for all aligned reads, allowing direct calculation of the editing percentage.

contained EGTA for 6 hr. Previous work has shown that blocking calcium entry prevents immediate early gene transcription (Greer and Greenberg 2008; Lin *et al.* 2008), and, similarly, we found that neither *Arc* ($P = 0.77$) nor *Bdnf* ($P = 0.61$) expression was significantly different from that of the control. When blocking calcium and depolarizing, none of the 25 editing sites were significantly changed, indicating that entry of extracellular calcium is necessary for activity-dependent RNA editing (Figure 3B).

Blockade of neuron activity induces changes in A-to-I editing in the opposite direction from those induced by depolarization

Since increasing activity alters editing, we wondered if reducing activity could also influence editing. We placed cultured neurons in 2 μM tetrodotoxin (TTX) to block all action potentials for 6 hr and again assayed IEG expression and RNA-editing levels. We found a decrease in *Bdnf* expression (0.43 ± 0.12, $P = 0.04$) compared to control cultures (Figure 1A), reflecting the decrease in activity. TTX treatment for 6 hr increased editing at nine different editing sites in six transcripts (Figure 3B). Compared to high-potassium depolarization, we observed RNA-editing changes in the opposite direction—increased editing—at five of the same sites. Editing at these five sites (in *Blcap*, *Cyfip2*, and *Gria2* transcripts) is differentially modulated depending on whether neural activity increases or decreases, indicating that some editing sites have bidirectional plasticity in their activity-dependent editing changes. As with high-potassium depolarization, we did not observe any changes in *Adar1-3* transcript levels (Figure 1B) after TTX blockade of action potentials.

Chronic activity modulation produces distinct changes in A-to-I editing that are largely opposite to those induced by acute changes in neuron activity

Although changes in RNA editing are present after just 6 hr of activity modulation, we also chronically decreased or increased activity for 48 hr using 2 μM TTX or 40 μM GABA-A antagonist bicuculline, respectively, to see if different

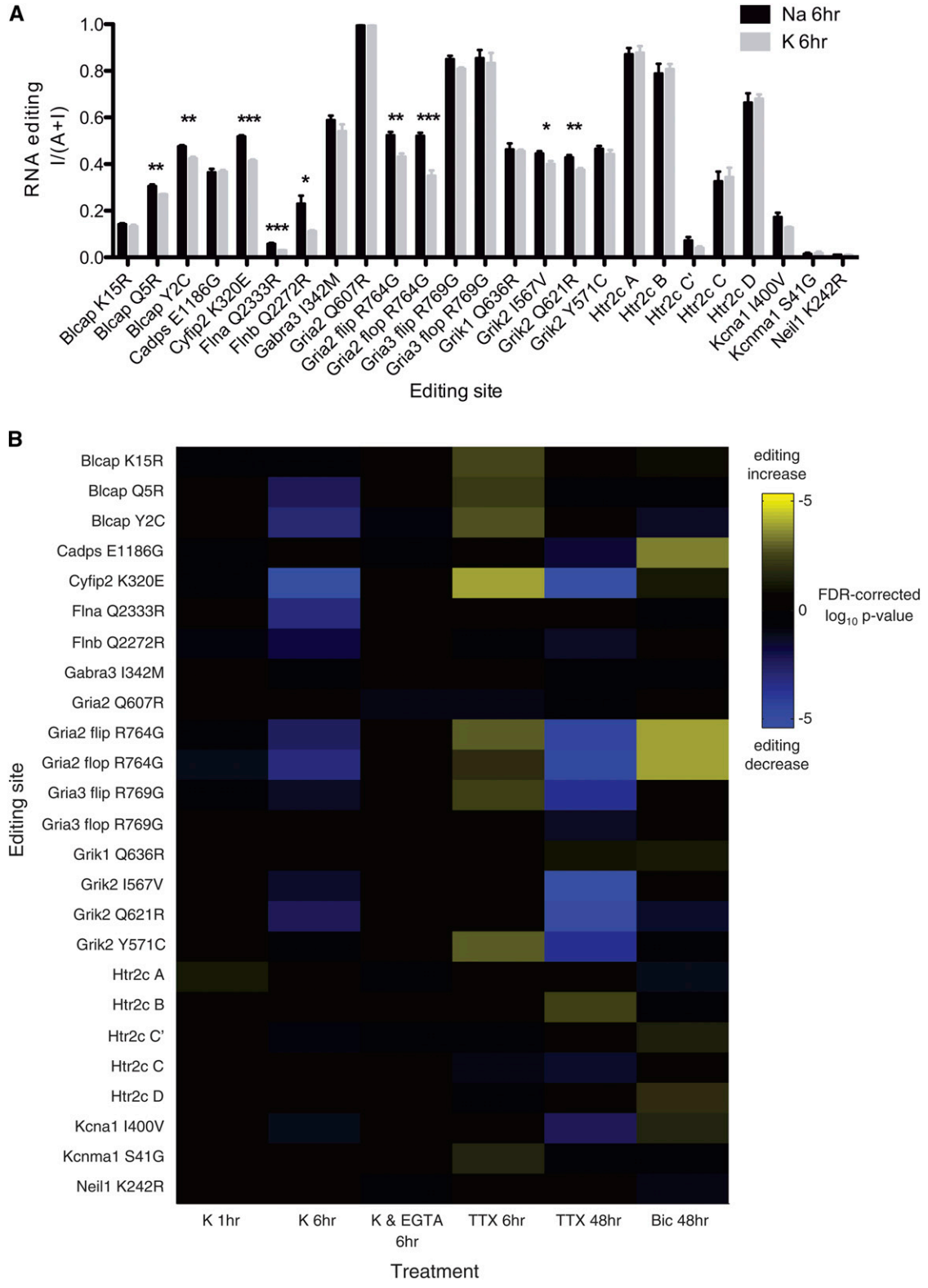

Figure 3 Activity-dependent changes in RNA editing at 6 and 48 hr. (A) Editing levels (fraction inosine) for High Na 6 hr (control) and High K 6 hr (depolarizing) treatments at each editing site. Asterisks indicate significant differences between the two treatments at that editing site (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $n = 4$ cultures, two-sample unpaired *t*-test, Benjamini–Hochberg false-discovery rate correction). Error bars indicate SEM. (B) Heat map showing significance (*P*) value for differential editing (change in percentage of inosine) at each editing site. Yellow indicates significant increases in editing percentage and blue indicates significant decreases. Intensity denotes log significance. Editing sites are labeled *Gene XNY*, where *Gene* denotes the transcript name, *X* is the amino acid specified if the site is not edited, *N* is the amino acid position in the rat RefSeq protein record, and *Y* is the amino acid specified if the site is edited. Differential editing is calculated between each treatment and matched controls (see Figure 1). The difference in editing levels for each site in each condition is presented in Table S6.

changes in RNA editing occurred after longer periods of activity modulation. When using TTX to block action potentials for 48 hr, *Arc* and *Bdnf* expression was reduced to 0.28 ± 0.02 ($P = 4 \times 10^{-4}$) and 0.11 ± 0.02 ($P = 2 \times 10^{-3}$) of control, respectively (Figure 1A). With chronic TTX, significant changes in editing occurred at 12 sites, more than in any other treatment, and, unlike the acute TTX treatment, all except 1 of the 12 sites reflected decreases in editing from control (Figure 3B).

When using bicuculline to block inhibitory synaptic currents and thus increase membrane depolarization for 48 hr, there were small elevations in *Arc* (1.18 ± 0.05, $P = 0.03$) and *Bdnf* (2.15 ± 0.33, $P = 0.02$) expression (Figure 1A). As in the case of chronic activity blockade, the changes after 48 hr of increased excitability were in the opposite direction of those seen with acute (6 hr) activity increase (Figure 3B). Of the sites with decreased editing after 6 hr of high-potassium depolarization, only one of them, *Grik2* Q621R, had a similar decrease after the 48-hr bicuculline treatment. Both splice isoforms of *Gria2* had increases instead of decreases in editing at the R/G site. In *Htr2c*, chronic bicuculline induced editing at two different editing sites (D and E) from chronic TTX (B and C), suggesting possible opposing signaling roles for these editing sites.

Unlike the acute activity modulations, chronic depolarization and inhibition also induce modest changes in *Adar2* transcription (but not in *Adar1* or *Adar3*) that are complementary to the majority of changes in editing seen in each condition (Figure 1B). After chronic TTX treatment, *Adar2* transcript is reduced to 0.63 ± 0.06 ($P = 7 \times 10^{-3}$) and, after chronic bicuculline, *Adar2* increases to 1.37 ± 0.09 ($P = 0.02$). ADAR2 deaminates several of the sites with corresponding decreases in editing after chronic TTX and increases in editing after chronic bicuculline, such as *Cyfip2* K320E, *Kcna1* I400V, and *Gria2* R764G. The only site that shows increased editing after chronic TTX, *Htr2c* B, is deaminated exclusively by ADAR1 and thus would not be expected to display reduced editing in response to decreased ADAR2.

Discussion

Although changes in A-to-I RNA editing were previously known to influence neural function (Hoopengardner *et al.* 2003; Hideyama *et al.* 2010), this study is the first demonstration that changes in membrane potential and synaptic receptor activity can modulate RNA editing at many different sites in protein-coding regions. In this study, we focused on characterizing the scope of the activity-induced editing using a targeted deep sequencing approach, in which the absolute abundance (or lack thereof) of a particular transcript is not a bottleneck for accurate quantification of editing. Compared to standard RNA-seq, this helpful dissociation allows examination of editing at many sites simultaneously and requires virtually no optimization to examine new loci. Recently, others have also used a targeted sequencing approach for even quantification of editing at transcripts of variable abundance, such as in mice during development (Wahlstedt *et al.* 2009) and after antidepressant treatment (Abbas *et al.* 2010; Morabito *et al.* 2010) and in human patients with schizophrenia and bipolar disorder (Silberberg *et al.* 2011). For many of the sites, this is the first report of their editing in rats, and we find that the level of editing at these sites is largely conserved with the corresponding human orthologs (Table 1).

For some of the transcripts that undergo activity-dependent editing, such as glutamate and serotonin receptor subunits, defects in editing have been implicated in common nervous system disorders, including epilepsy, amyotrophic lateral sclerosis, and depression (Maas *et al.* 2006; Hideyama *et al.* 2010), yet the individual functional changes resulting from editing, *e.g.*, biochemical, electrophysiological, etc., are known for only a few sites. Since the changes in editing that we observe are often small in magnitude, it is vital to understand the functional impact—if any—of small changes in transcript editing on the nervous system. With the recent expansion of newly identified coding and noncoding editing sites (Li *et al.* 2009) and the development of new sequencing and analysis techniques (Ramaswami *et al.* 2012), it is now possible to understand how the ensemble of editing changes allows neurons to adapt to changes in network activity. This initial characterization of how activity can alter RNA editing increases the rich diversity of mechanisms that neurons can use to control the expression of different protein isoforms.

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Activity-Dependent A-to-I RNA Editing in Rat Cortical Neurons

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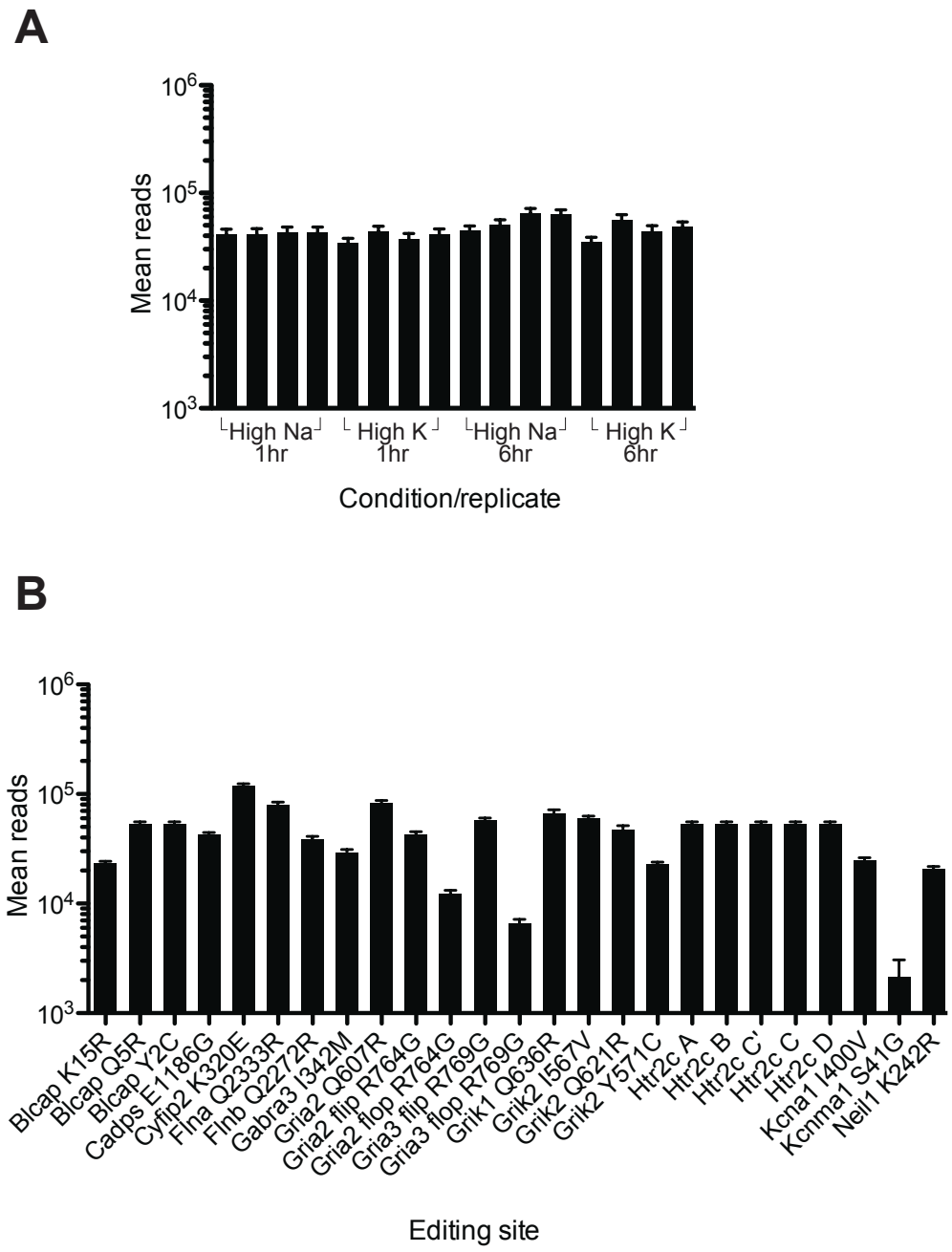

Figure S1 Read uniformity across conditions and editing sites. Counts of the average number of sequencing reads per editing site and per condition in one multiplexed Illumina sequencing run. **a,** Mean number of sequencing reads per condition/replicate averaged across editing sites (*n*=25). In each condition in the sequencing run (High Na 1hr, High K 1hr, High Na 6hr, High K 6hr), reads counts are plotted for 4 separate biological replicates. **b,** Mean number of sequencing reads per editing site averaged across different conditions/replicates (*n*=12). In both panels, error bars indicate s.e.m.

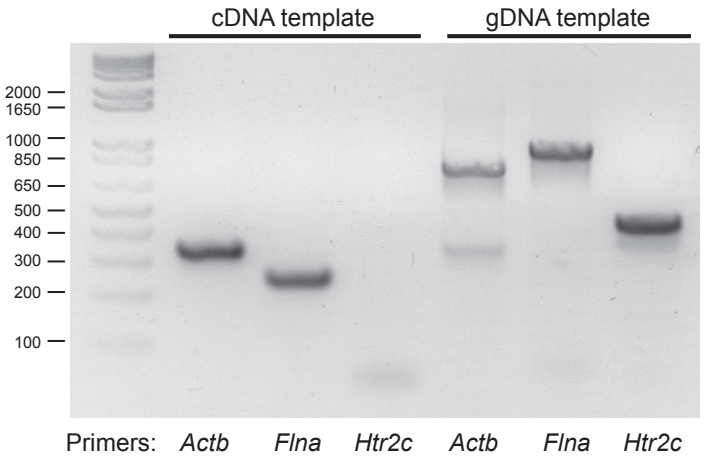

Figure S2 Saturation PCR for gDNA contamination in cDNA. Sample gel result from saturation PCR using 3 primers that produce different size bands depending on whether gDNA is present or not. (See Supplementary Table 1 for template-dependent band sizes.) The template used (cDNA from isolated RNA or gDNA) is labeled above the gel image and the primer is labeled below. None of the gDNA bands are present when cDNA is used as a template. This procedure was repeated for all extracted RNA to verify absence of gDNA contamination.

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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Table S1 Primers to test cDNA for gDNA contamination

Intron-spanning or intron-annealing primers to verify absence of gDNA in prepared cDNA libraries. *Actb* and *Flna* are exon-spanning and *Htr2c* only amplifies an intronic sequence.

Gene	Forward primer	Reverse primer	RNA band (bp)	DNA band (bp)
Actb	CTAAGGCCAACCGTGAAAAG	GCTCGAAGTCTAGGGCAACA	339	805
Flna	AAAGGATGGCTCTTGTGGTG	CTATGCACCTTGGCATCAAT	246	988
Htr2c	AACCGATCAAACGCAATGTT	CAATGCTATCATCACTGGGAAA	None	454

Table S2 qRT-PCR primers for immediate early genes

Forward and reverse primers used for quantitative reverse-transcription polymerase chain reaction (qRT-PCR) to measure expression levels of activity-induced immediate early genes *Arc* and *BDNF.*

Gene	Forward primer	Reverse primer
Arc	CCTCTCAGGGTGAGCTGAAG	AGTCATGGAGCCGAAGTCTG
Bdnf	GCGGCAGATAAAAAGACTGC	GCAGCCTTCCTTCGTGTAAC
Gapdh	GCATCCTGCACCACCAACTG	ACGCCACAGCTTTCCAGAGG

Table S3 Editing site sequences for primers

Primers for amplification of short amplicons that include editing sites within a few bases of one of the primers. For some transcripts, editing sites were spaced further than could reliably be captured in a single short read: For those transcripts, multiple primer pairs were used to capture all editing sites. *Italics* indicates sequence/primer closest to editing site(s).

Gene	Forward primer	Reverse primer
Blcap (1)	*CTGCCCGTCCTCCTCATC*	AATCGGAGCAGTGGTACAGG
Blcap (2)	*GCCCGGCAGAGATCATGT*	CAGGAAGACCAGGGCACATA
Cadps	*GGATGTCCTTCGTGATAAGGTC*	CCGATTTCGAATGGTCTCAT
Cyfip2	AGCTGGATGCCAAGAAGAGA	*CTCTTCATAGTGAGCACTGGTCT*
Flna	*CGCCGCCTTACTGTTTCTAGT*	GGATGAAACGCACAGCATAC
Flnb	*GCTGCCTCACTGTTCTGAGC*	CATTCTCATGGGGGATGAAG
Gabra3	GGCTACTTTGTCATCCAGACC	*GAAAATACAAAGGCATAACAGACG*
Gria2 (1)	*TTTCCTTGGGTGCCTTTATG*	CTTTCGATGGGAGACACCAT
Gria2 (2)	*CATCGCCACACCTAAAGGAT*	CAATCAAAGCCACCAGCATT
Gria3	*GCAACCCCTAAAGGCTCAG*	TATAGAACACGCCTGCCACA
Grik1	*TGGAGTTGGAGCTCTCATGC*	ATGGGGGATTCCATTCTTTC
Grik2 (1)	*TCTCCCCTGATATCTGGATGTA*	AGAGCTCCAACTCCAAACCA
Grik2 (2)	ATGGAATGGAATGGTTCGTG	*GCACACAACTGACACCCAAG*
Grik2 (3)	*TGGAGTTGGAGCTCTCATGC*	ATGCGTTCCACAGTCAGAAA
Htr2c (1)	*ATCGCTGGACCGGTATGTAG*	TCACGAACACTTTGCTTTCG
Htr2c (2)	AGATATTTGTGCCCCGTCTG	*GAATTGAACCGGCTATGCTC*
Kcna1	*CATCGCTGGTGTGCTGAC*	CCTGCCTGTAGTGGGCTATG
Kcnma1	CAGCAGTAGCAGCAACATGG	*AAGAAGAGGACGCGTCTAGG*
Neil1	TCTAGAGGCCCTGCAACAGT	*TTCCTCTCCACGCTCTGG*

Table S4 Sequences for universal primers

Common primers used for attaching barcodes and sequencing adaptors. The first two primers are used in the first round of pre-sequencing PCR. The last two primers are used in the second round of pre-sequencing PCR.

Primer/purpose	Sequence
Forward common (followed by barcode and site-specific forward sequence)	CGACGCTCTTCCGATCT
Reverse common (follows site-specific reverse sequence)	CATACGAGCTCTTCCGATCT
Forward primer to attach sequencing adaptor	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACG ACGCTCTTCCGATCT
Reverse primer to attach sequencing adaptor	CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT

Table S5 Multiplex sequencing barcodes

Barcodes used to pool multiple experimental conditions and/or replicates into a single Illumina flowcell lane. All barcodes differ by at least 2 nucleotides.

	Barcodes		Barcodes
1	CCTC	7	GGCA
2	ACCA	8	ATCC
3	TATA	9	GTCG
4	GACC	10	AGCT
5	CTTA	11	CAGT
6	TGTC	12	TCAG

Table S6 Differences in A-to-I RNA editing for all treatments

Mean difference in editing levels (fraction inosine) between indicated treatment and matched control. Significant differences (p<0.05, n=4 cultures, two-sample unpaired t-test, Benjamini-Hochberg false-discovery rate correction) are indicated by **bold text**.

Site	K 1hr vs. Na 1hr		K 6hr vs. Na 6hr		K+EGTA 6hr vs. Na 6hr		TTX 6hr vs. Na 6hr	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Blcap K15R	-0.02	0.01	-0.01	0.01	0.01	0.01	**0.04**	0.01
Blcap Q5R	0.00	0.01	**-0.03**	0.01	0.01	0.01	**0.05**	0.01
Blcap Y2C	-0.01	0.02	**-0.05**	0.01	-0.01	0.01	**0.06**	0.01
Cadps E1186G	-0.01	0.01	0.00	0.02	-0.01	0.01	0.00	0.01
Cyfip2 K320E	-0.01	0.01	**-0.11**	0.01	0.00	0.01	**0.09**	0.01
Flna Q2333R	0.01	0.01	**-0.03**	0.00	0.00	0.00	0.00	0.00
Flnb Q2272R	-0.05	0.03	**-0.12**	0.03	0.00	0.01	-0.02	0.01
Gabra3 I342M	0.01	0.05	-0.05	0.03	0.02	0.02	0.21	0.11
Gria2 Q607R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gria2 flip R764G	-0.01	0.01	**-0.09**	0.02	0.03	0.02	**0.11**	0.02
Gria2 flop R764G	-0.07	0.03	**-0.17**	0.03	0.02	0.03	**0.09**	0.02
Gria3 flip R769G	-0.02	0.02	-0.04	0.02	0.01	0.01	**0.05**	0.01
Gria3 flop R769G	0.09	0.05	-0.02	0.06	0.00	0.02	0.02	0.03
Grik1 Q636R	0.03	0.04	-0.01	0.03	0.02	0.02	0.02	0.01
Grik2 I567V	0.00	0.01	**-0.05**	0.02	0.01	0.01	0.01	0.01
Grik2 Q621R	0.00	0.03	**-0.05**	0.01	0.00	0.01	0.00	0.01
Grik2 Y571C	0.00	0.01	-0.02	0.02	0.01	0.01	**0.06**	0.01
Htr2c A	0.07	0.03	0.01	0.04	-0.03	0.02	-0.01	0.02
Htr2c B	0.02	0.04	0.02	0.05	-0.01	0.02	-0.01	0.03
Htr2c C'	-0.01	0.02	-0.03	0.02	-0.01	0.01	-0.01	0.01
Htr2c C	-0.03	0.06	0.02	0.06	-0.01	0.02	-0.05	0.02
Htr2c D	-0.03	0.08	0.02	0.04	0.02	0.02	-0.02	0.02
Kcna1 I400V	-0.01	0.03	-0.05	0.02	0.00	0.01	0.01	0.01
Kcnma1 S41G	0.00	0.01	0.00	0.01	0.00	0.01	**0.02**	0.01
Neil1 K242R	0.00	0.00	0.00	0.00	-0.01	0.01	0.00	0.01

Site	TTX 48hr vs. NBA 48 hr		Bic 48hr vs. NBA 48hr	
	Mean	*SE*	*Mean*	*SE*
Blcap K15R	0.00	0.01	0.01	0.01
Blcap Q5R	-0.01	0.01	-0.02	0.01
Blcap Y2C	0.00	0.01	-0.02	0.01
Cadps E1186G	**-0.03**	0.01	**0.02**	0.00
Cyfip2 K320E	**-0.10**	0.01	0.02	0.01
Flna Q2333R	0.00	0.00	0.00	0.00
Flnb Q2272R	**-0.02**	0.01	-0.01	0.01
Gabra3 I342M	-0.06	0.04	-0.03	0.02
Gria2 Q607R	0.00	0.00	0.00	0.00
Gria2 flip R764G	**-0.11**	0.01	**0.06**	0.01
Gria2 flop R764G	**-0.10**	0.01	**0.06**	0.01
Gria3 flip R769G	**-0.14**	0.02	-0.02	0.02
Gria3 flop R769G	-0.16	0.06	0.01	0.03
Grik1 Q636R	0.07	0.02	0.03	0.01
Grik2 I567V	**-0.14**	0.01	0.00	0.01
Grik2 Q621R	**-0.12**	0.01	**-0.03**	0.01
Grik2 Y571C	**-0.13**	0.02	-0.03	0.02
Htr2c A	0.04	0.02	-0.05	0.02
Htr2c B	**0.08**	0.02	-0.04	0.03
Htr2c C'	0.00	0.01	**0.01**	0.00
Htr2c C	**-0.07**	0.02	0.01	0.03
Htr2c D	-0.01	0.06	**0.09**	0.02
Kcna1 I400V	**-0.04**	0.01	**0.03**	0.01
Kcnma1 S41G	-0.01	0.00	-0.01	0.01
Neil1 K242R	0.00	0.00	0.00	0.00