Alternative 3’ UTRs Modify the Localization, Regulatory Potential, Stability, and Plasticity of mRNAs in Neuronal Compartments

Graphical Abstract

Highlights
- Neuronal mRNAs usually possess multiple 3’ UTR isoforms
- 3’ UTR isoforms of a transcript family can be enriched in different compartments
- 3’ UTR isoforms enriched in the neuropil have longer half-lives
- Enhanced neural activity alters the 3’ UTR isoforms present in each compartment

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In Brief
Tushev, Glock, et al. use 3’ end sequencing together with hippocampal slice microdissection to separately examine transcripts arising from somata or the neuropil. They discover a huge diversity in neuronal mRNA 3’ UTRs, which give rise to differences in localization, stability, translation, and plasticity.
Alternative 3' UTRs Modify the Localization, Regulatory Potential, Stability, and Plasticity of mRNAs in Neuronal Compartments

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SUMMARY

Neurons localize mRNAs near synapses where their translation can be regulated by synaptic demand and activity. Differences in the 3' UTRs of mRNAs can change their localization, stability, and translational regulation. Using 3' end RNA sequencing of microdissected rat brain slices, we discovered a huge diversity in mRNA 3' UTRs, with many transcripts showing enrichment for a particular 3' UTR isoform in either somata or the neuropil. The 3' UTR isoforms of localized transcripts are significantly longer than the 3' UTRs of non-localized transcripts and often code for proteins associated with axons, dendrites, and synapses. Surprisingly, long 3' UTRs add not only new, but also duplicate regulatory elements. The neuropil-enriched 3' UTR isoforms have significantly longer half-lives than somata-enriched isoforms. Finally, the 3' UTR isoforms can be significantly altered by enhanced activity. Most of the 3' UTR plasticity is transcription dependent, but intriguing examples of changes that are consistent with altered stability, trafficking between compartments, or local “remodeling” remain.

INTRODUCTION

Neurons distinguish themselves from other cells by their extreme structural and functional compartmentalization, accomplished via morphologically complex dendritic and axonal arbors and synaptic specializations. The number of synapses and the information processing accomplished by synaptic protein networks necessitate an efficient machinery for local information processing (for review, see Hanus and Schuman, 2013). The localization of mRNAs and regulated local translation allows neurons to deal rapidly with the compartmentalized function of synapses, underlying important cellular processes such as memory, dendrite formation, synapse formation, axon guidance, survival, and proteostasis (for review, see Holt and Schuman, 2013). Within neurons, the number of localized mRNA transcripts numbers in the thousands (Cajigas et al., 2012; Gumy et al., 2011; Zivraj et al., 2010).

Within mRNA molecules, information in the 3' untranslated region (3' UTR) can regulate their targeting, translational efficiency, and stability (Tian and Manley, 2017). As such, an understanding of the repertoire of 3' UTR isoforms in localized neuronal transcripts is required to understand the logic and function of local and global protein synthesis in neurons. Studies in worms, flies, and fish have examined 3' UTR heterogeneity and have observed that many 3' UTR isoforms are differentially expressed in a developmentally regulated and/or tissue-specific manner (Hilgers et al., 2011; Ji et al., 2009; Mangone et al., 2010; Ulltsky et al., 2012) and that there is an increase in the average 3' UTR length in neuronal tissue. Similarly, in mouse and human samples, extended 3' UTRs have been documented in neuronal tissue (Miura et al., 2013).

In order to investigate the diversity of 3' UTRs in mature neuronal processes, we microdissected hippocampal slices to separate the somatic and neuropil layers, giving rise to RNA samples enriched in cell somata versus neuronal projections (Cajigas et al., 2012; Mishchenko et al., 2010). Using 3' end sequencing and high-resolution in situ hybridization, we analyzed the 3' UTR heterogeneity of neuropil and somata samples and found that localized transcripts, enriched in either somata or the neuropil, are endowed with longer 3' UTR isoforms that include both novel and repeated regulatory motifs that can drive localization, mRNA stability, and/or the potential regulation of translation. Neuropil-localized transcripts also exhibit longer half-lives, on average, when compared to their somatic counterparts. Following a period of enhanced activity, there is an alteration of 3' UTR isoforms present in each compartment. While most of the 3' UTR plasticity was transcription dependent, some 3' UTR isoforms are altered by a transcription-independent mechanism, suggesting plasticity-induced stability changes, trafficking of 3' UTR isoforms between compartments, or local remodeling.

RESULTS

3' End Sequencing of Hippocampal Transcripts

To investigate the diversity of mRNA polyadenylation and 3' UTRs in the rat hippocampus, we isolated RNA after microdissection of the somata and neuropil layers (s. pyramidale

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and s. radiatum + s. moleculare) in the CA1 region (Figure 1; Figure 2A). We purified total RNA and conducted 3' end sequencing (MACE sequencing, see STAR Methods) of the somata and neuropil samples, obtaining two replicates (tissue for each replicate was obtained from nine animals; data from replicates were highly correlated with one another) (Figure 1; Figure S1A). In order to quantify the 3' UTR diversity of the identified transcripts, we developed a pipeline to identify sequence motifs within the 3' UTRs that support polyadenylation (poly(A)-supported sites [PASS]; Figures S1B–S1H; see STAR Methods). For each transcript identified, we counted the number of distinct 3' UTRs (or PASS) detected. We found that the majority of hippocampal transcripts possessed more than one 3' UTR isoform, with some transcripts exhibiting three or more isoforms (Figure 2B).

As the hippocampal somata and neuropil samples comprise not only neurons, but also glia, we next devised a strategy to identify 3' UTR isoforms that arise from each of these cell types by evaluating them in relative isolation via the preparation of cell-type-enriched cultures (see STAR Methods). We sequenced the 3' ends of polyadenylated RNAs acquired from neuron-enriched (AraC-treated) mature (Figure S2A) hippocampal cultures or from glia-enriched cultures (Figure S2B). Combining the neuron- and glia-enriched datasets, we obtained 21,603 3' UTR isoforms from 11,301 gene loci (with 82.2% 3' UTR and 92.6% gene loci overlap with the hippocampal slice data described above) (Figure S2C; Table S1). Using these data, we developed a classifier to identify three groups of 3' UTR families: (1) neuron-enriched, (2) glia-enriched, or (3) non-enriched (Figure S2B; see STAR Methods). Most (93.4%) 3' UTR isoform members of a transcript family showed a faithful enrichment, e.g., all 3' UTR isoforms were enriched in a single (neuronal- or glial-) cell type. There was, however, a very small subset of transcript families in which 3' UTR isoforms were split between neurons and glia, e.g., one 3' UTR isoform enriched in neurons and one enriched in glia (6.6% of all enriched genes; Figure S2D); these transcripts were not analyzed further but are identified in Table S1. These data suggest that most of the transcript diversity between neurons and glia is due to differences in the genes expressed rather than differences in the 3' UTR isoforms of the same genes. We validated the relative enrichment and de-enrichment of neuronal- and glia-related genes, respectively, using qRT-PCR (Figure S2E) and bioinformatically using published datasets (Figures S2F and S2G). We then evaluated the number of 3' UTR isoforms that each group possesses and found that neuron- and glia-enriched transcripts exhibited a significantly higher number of 3' UTR isoforms than the non-enriched transcripts, with neuron-enriched transcripts exhibiting the highest number of 3' UTR isoforms overall (Figure 2C).

To examine whether 3' UTR diversity is related to protein function, we used gene ontology (GO) to examine the functional categories comprising the neuron- or glia-enriched 3' UTR isoforms and compared these functional categories to the groups of transcripts represented by multiple 3' UTRs or single 3' UTRs as determined in our analysis of 3' UTRs in hippocampal slices (Figure 1). Hierarchical clustering of significant functional GO categories revealed that the family of neuron-enriched transcripts (closely related to multiple 3' UTR hippocampal slice transcripts; Figure 2D) was significantly enriched for genes that code for neuronal and synaptic function (Figure 2E; Table S2). Glia-enriched transcripts (with overlap with single 3' UTR transcripts and, to a lesser extent, multiple 3' UTR transcripts; Figure 2D) showed enrichment for terms associated with the extracellular space and adhesion (Figure 2E). Single 3' UTR variants, by contrast, were enriched for general cellular functions, including components of the extracellular matrix and basic cellular functions (Figure 2E).

**Localization of Neuronal Transcripts in Somata and Neuropil**

We used the neuron-enriched transcript dataset identified above to search for 3' UTR isoforms enriched in the microdissected somata or neuropil of area CA1 from hippocampal slices. We found that both the neuropil and the somata contain hundreds of 3' UTR variants that are significantly enriched (Figure 3A); these transcripts are in good agreement with previously published data (Figure S3A). All 3' UTR isoforms detected and their relative expression in the somata and neuropil are shown in Table S1. We used qRT-PCR to validate the enrichment and de-enrichment of 22 identified transcripts in each compartment (neuropil or somata) and found very good agreement (R² ≈ 0.966) with the sequencing data (Figure 3B). We also used GO to analyze the somatic- and neuropil-enriched transcripts and found a significant enrichment of terms associated with axon-, dendrite-, or synapse-related functions in both compartments (Figure 3C; Figures S3B and S3C; Table S2). Some somatically localized transcripts could code for membrane proteins as the bulk of their synthesis and post-translational modifications might occur in the abundant somatic endoplasmic reticulum (ER) and Golgi (Hanus et al., 2016). Indeed, we found that membrane proteins were often, but not always, represented by somatically enriched transcripts, whereas cytoplasmic proteins were often...
represented by neuropil-enriched isoforms (Figure S3D). We also examined the relevance of localized 3' UTR isoforms for protein families associated with neurodevelopmental, neuropsychiatric, or neurodegenerative diseases and found a significant enrichment for many diseases (Figure S3E; Table S2).

We next analyzed the length of the 3' UTR isoforms and found that localized 3' UTR isoforms, enriched in either the neuropil or the somata, possessed significantly longer 3' UTRs than the non-enriched transcripts, with neuropil 3' UTRs exhibiting the longest 3' UTRs reported to date (Figure 3D; Figure S3F). Furthermore, neuron-enriched transcripts tend to have longer 3' UTRs than glia-enriched transcripts, while both of these families have significantly longer 3' UTRs than non-enriched isoforms (Figure 3D). Using published data (Derti et al., 2012; Grillo et al., 2010), we analyzed 3' UTRs across tissues and species and discovered that 3' UTRs are significantly longer for transcripts expressed in brain relative to other tissues, as observed by others (Hilgers et al., 2011; Miura et al., 2013; Smibert et al., 2012), and also noted an increase in 3' UTR length from plants to primates (Figure 3D). In contrast to a previous study of developing neurites or cell lines (Taliaferro et al., 2016), we found that neuropil-enriched 3' UTRs were not enriched for mRNAs containing alternative last exons (ALEs) (less than 13% of all predicted sites; see STAR Methods). Taken together, these data suggest that longer 3' UTRs are necessary platforms that might favor both the asymmetric distribution of transcripts within cells and enhanced regulatory potential.

Significantly enriched among the neuropil-localized long 3' UTRs are transcripts that code for synaptic proteins (Figure S3C; Table S2). In order to validate the localization of long 3' UTRs in the neuropil, we chose 19 representative transcripts, which express multiple 3' UTRs as indicated by our sequencing data. Eight of these transcripts exhibited enriched expression of the long 3' UTR in the neuropil and 11 exhibited enriched expression of the long 3' UTR in the somata. We developed in situ hybridization probes against the unique region of these long 3' UTR isoforms and examined their localization in cultured hippocampal neurons and hippocampal slices (Figures 4A–4F; Figures S4A–S4D). As shown in Figures 4A–4G and S4, the in situ hybridization probes probes against the unique region of these long 3' UTR isoforms and examined their localization in cultured hippocampal neurons and hippocampal slices (Figures 4A–4F; Figures S4A–S4D). As shown in Figures 4A–4G and S4, the in situ
hybridization signal is strongest for the long 3' UTR isoforms in the expected compartment (neuropil for CamKIIα and Calm1; somata for Grin2a; Figure 4) in both dissociated neurons and hippocampal slices. Taken together, the neuropil-enriched 3' UTR isoforms exhibited a significantly higher dendritic in situ hybridization signal than the somata-enriched 3' UTR isoforms (Figure 4H; Figure S4C).

We next asked if a 3' UTR isoform of a transcript exhibits localization in either (neuropil or somata) compartment, how do the other isoforms of the same transcript family behave? To address this, we plotted the relative expression of short and long isoforms in the somata and neuropil compartments and evaluated the prevalence of two different scenarios: the differential expression of short and long 3' UTR isoform variants in somata versus neuropil (e.g., the short enriched in one compartment while the long is enriched in the other, or vice versa; Figure 5A, top left and bottom right quadrants) or the coordinate enrichment of both short and long 3' UTR isoform variants in a compartment (e.g., both the short and the long enriched in either the soma or the neuropil; Figure 5A, top right and bottom left quadrants). In the scatterplot, we observed a positive and significant correlation between the localization biases of different 3' UTR isoforms of the same gene (R = 0.3040; p = 3.10E–128), indicating a coordinate enrichment of both long and short isoforms within compartments (Figure 5A). There exists, however, evidence for the differential enrichment of isoform variants in different compartments, with...
a greater number of transcript families sorting their longest isoform to the neuropil rather than to the somata (Figures 5A and 5B). This is illustrated in Figure 5A by a comparison of the number of paired isoforms in the top left quadrant (274, long enriched in neuropil and short enriched in somata) and the lower right quadrant (122, short enriched in neuropil and long enriched in somata) as well as the cumulative frequency distribution (Figure 5B). We evaluated whether the neuropil-localized 3' UTR isoform distribution patterns were enriched for any particular functions using GO and found a significant enrichment for postsynaptic density and membrane, dendritic spine, memory, and other groups (Figure S5A; Table S2). We determined the sufficiency of a long 3' UTR to drive localization of a transcript to the neuropil by cloning a series of reporter constructs in which the coding sequence for GFP was flanked by the three different CamKIIa 3' UTRs, the long 3' UTR for Kcnab2 (enriched in the neuropil; Figure 3B; Table S1), or no 3' UTR as a control. Following transfection of the reporters into neurons, we used fluorescence in situ hybridization (FISH) with probes anti-sense to the GFP coding sequence to detect the mRNA in neuronal somata and dendrites (Figure 5C). We found that the long CamKIIa 3' UTR resulted in the highest number of transcripts detected throughout the dendritic arbor relative to somata; the short and the middle CamKIIa 3' UTRs resulted in mRNA signal only in the proximal (approximately <40 μm) segment of the dendrite (Figure 5D). The long Kcnab2 3' UTR isoform was also sufficient to drive the localization of the reporter to dendrites (Figures S5B–S5D).

Properties and Elements within Neuronal 3' UTRs
We analyzed the properties and elements of the diverse 3' UTRs described above, focusing on the localized (neuropil or somata) versus non-localized transcripts. We first examined sequence conservation along the length of 3' UTRs of genes with multiple or single 3' UTRs and compared it to other genomic regions. We found that brain 3' UTRs are more conserved than intergenic sequences and introns but are less conserved than exons (Figure S6A). We next analyzed the guanine-cytosine (GC) content...
and secondary structure element predictions and observed that, in comparison to non-localized transcripts, somata-enriched transcripts possess a lower GC content and less predicted secondary structure, whereas neuropil-enriched transcripts exhibit the opposite trend with significantly higher GC content and predicted secondary structure (Figure 6A). As expected, brain 3' UTRs, in general, also exhibit higher GC content and predicted secondary structure than intergenic regions (Figures S6B and S6C). The structure stability evident in the neuropil 3' UTRs likely provides binding platforms for RNA-binding proteins to regulate the localization, translation, and stability (see below) of these transcripts.

Do the nucleotide sequences added during 3' UTR extension provide new or redundant regulatory potential for the upstream transcript? To answer this question, we analyzed all 3' UTR isoforms in transcript families with multiple 3' UTRs for the presence of 7-mers (see STAR Methods). The presence of these potential regulatory motifs were then categorized as follows: (1) “novel,” a motif that is present once and exclusively in the unique sequence of the long 3' UTR; (2) “repeat between,” a regulatory motif that is already present in the shorter 3' UTR isoform and is repeated in the unique sequence of the longer 3' UTR isoform; or (3) “repeat within,” a regulatory motif that is introduced and repeated exclusively within the unique sequence of the long 3' UTR isoform.
As such, novel and repeat-within elements add new regulatory potential to a transcript. Both repeat-between and repeat-within elements increase the likelihood of regulation by competing for a limited pool of regulatory binding elements (e.g., microRNAs [miRNAs], RNA-binding proteins, etc.) with repeat within adding this potential exclusively to the long 3'UTR isoform. We found that the dominant type of regulatory element in a longer isoform was in the novel category, although the frequency of novel motifs was less than predicted by chance alone, suggesting an active deselection of these motifs (Figure 6B). By contrast, the repeat elements, although low in total number, were present at significantly higher levels than expected by chance or by comparison with intergenic sequences (Figure 6B). This general pattern was also observed when we allowed for a mismatch within the group of significant 7-mers (Figure S6D). Taken together, these analyses indicate that additional 3' UTR elements add new possibilities for regulation but also significantly increase regulatory motifs already present, potentially increasing the “regulatory competitiveness” of an mRNA isoform. We also considered whether differences in secondary and tertiary structural elements exist between 3' UTR isoforms. We did not, however, detect any significant differences in GC

Figure 6. Regulatory Potential of Localized 3' UTR Isoforms
(A) GC content (left) and secondary structure element prediction (normalized minimum free energy, MFE) of non-, somata-, and neuropil-localized 3' UTRs. The GC content of somata- and neuropil-localized isoforms was significantly de-enriched or enriched, respectively, relative to non-localized 3' UTR isoforms. The MFE of neuropil-localized transcripts was significantly lower than either somata- or non-localized 3' UTRs. ****p < 0.0001, Mann-Whitney U test.
(B) Scheme below the plot shows detected regulatory elements that were categorized into novel (square) representing a single introduction of 7-mer in the unique part of the long 3' UTR, repeat between (circle) representing a duplicate of a motif present in the shared 3' UTR isoform of a family member, and repeat within (triangle) representing an element introduced and repeated within the unique part of the long 3' UTR. Graph shows the relative fraction of 7-mers (including miRNA binding sites) that fall into the categories: novel, repeat within, or repeat between. To assess expected distribution of these fractions, we repeated the analysis for intergenic regions (1 kB downstream of the poly(A) site) and dinucleotide shuffled unique 3' UTR isoforms.
(C) Relative expression and significant enrichment of 7-mers in somata and neuropil. 18.4% of all 7-mers show a compartment-specific enrichment in either the neuropil (red dots) or the somata (blue dots). Also highlighted are the established motifs interacting with the RNA-binding proteins CPE, FMRP, Mbnl1, and Zbp1.
(D) Relative expression and significant enrichment of miRNA binding sites in somata and neuropil. 22% of all binding sites for miRNAs expressed in area CA1 show a compartment-specific enrichment (neuropil, red dots; somata, blue dots) in a 3' UTR isoform.
See also Figure S6 and Tables S3 and S4.

[Legend on next page]
content or minimum free energy for the short, middle, or long 3' UTR isoforms (Figures S6B and S6C).

We next addressed whether there are specific 7-mers that are overrepresented in 3' UTR isoforms localized to the somata or neuropil. We found 3,017 7-mers, representing 215 families (allowing an edit distance of 2; see STAR Methods), enriched in localized transcripts relative to non-localized transcripts, with 1,637 (138 families) and 1,380 (126 families) 7-mers enriched in the neuropil- and somata-localized 3' UTR isoforms, respectively (Figure 6C; Table S3). We searched for previously identified consensus binding motifs for several well-known neuronal RNA-binding proteins and found (Table S4), for example, that the two motifs identified for the zip-code-binding protein (Zbp1; Kim et al., 2015) are enriched in neuropil-localized 3' UTRs (Figure 6C). The motifs for two other neuronal RNA-binding proteins, fragile X mental retardation protein (FMRP; Anderson et al., 2016; Ascano et al., 2012) and muscle-blind (Mbnl1; Koniecny et al., 2014; Wang et al., 2012), were enriched in distinct somatic- and neuropil-localized 3' UTRs. The cytoplasmic polyadenylation element (CPE) is recognized by the CPE-binding protein (CPEB) and usually promotes the cytoplasmic polyadenylation of mRNAs (de Moor and Richter, 1999) important, for example, for the chemotropic responses in axons (Lin et al., 2009). Surprisingly, we found that the CPE motifs were significantly enriched in somata-localized 3' UTRs rather than neuropil 3' UTRs, although we note that several neuropil 3' UTRs do contain a CPE, for example, CamKIIα (Figure S5E), as noted by Wu et al. (1998).

We conducted a similar analysis seeking to identify miRNA seeds present in 3' UTR isoforms (see STAR Methods). In total, we identified 298 miRNA seed sequences present in the somata and neuropil (Figure 6D; Table S3). To illustrate the abundance and diversity of miRNA elements in a single 3' UTR, we plotted the miRNA seeds and other regulatory motifs within the CamKIIα complete 3' UTR (Figure S5E). Using Nanostring, we next addressed whether the specific miRNAs that bind to the identified seeds are expressed in the somata and neuropil. We observed that 191 out of the total 423 miRNAs that are measured with Nanostring are expressed in the hippocampal CA1 region. Furthermore, 42 of the detected miRNAs have binding sites that are significantly enriched in the somata (20) or neuropil (22) (Figure 6D).

Neuropil Localized Transcripts Are Long Lived

Differences in mRNA half-life might also play an important role in both the localization and function of neuropil miRNAs. To determine the half-lives of neuronal transcript 3' UTR isoforms, we blocked transcription (e.g., Akbalik et al., 2017) and then collected cultured hippocampal neuron samples at successive time points (0 hr, 2 hr, 4 hr, 9 hr, and 16 hr) (Figure 7A). We performed 3' end sequencing on these samples and, after normalization (see STAR Methods and Figures S7A and S7B), fit an exponential decay function to the time course data of each 3' UTR isoform measured. This allowed us to determine the half-life of each 3' UTR isoform, yielding a median transcript half-life of 7.38 hr (Figure 7A; Figure S7C). To determine whether some groups of biologically related transcript isoforms are particularly long or short lived, we performed a gene set enrichment analysis (GSEA) on the half-life ranked list of 3' UTR isoforms (Figure 7B). Interestingly, we found that the long-lived 3' UTR isoforms are enriched for GSEA terms representing dendritic, axonal, or synaptic protein functions, while the GSEA terms for the less stable transcript groups were enriched for genes whose products primarily function in the nucleus or nucleoplasm, such as transcription factors (Figure 7B; Table S2). We then examined the relative half-life of synaptic genes (Zhang et al., 2007) compared to all other neuron-enriched transcripts and found that synaptic 3' UTR isoforms exhibited significantly longer half-lives (Figure 7C). We also analyzed the extent to which the properties of the 3' UTR isoforms described above (single 3' UTR versus a short, middle, or long family member, localized or not) affect transcript half-life. Although the different categories of 3' UTR isoforms have similar median half-lives, transcripts localized to the neuropil have significantly longer...
Figure 8. Elevated Neuronal Activity Results in Compartment-Specific Plasticity of 3′ UTR Isoforms

(A) Change in 3′ UTR isoform expression in neuropil (y axis) versus somata (x axis) following 4 hr of elevated activity (elicited by bicuculline 40 μM). In total, 783 3′ UTR isoforms exhibited significant alterations in expression following enhanced activity. In some cases (top left and bottom right quadrants), there was differential plasticity of 3′ UTR isoforms between the somata and the neuropil compartments, whereas in other cases (top right and bottom left quadrants), there was coordinate regulation in the two compartments. Upregulated 3′ UTR isoforms are shown in purple, and downregulated 3′ UTR isoforms are shown in gray.

(B) GO terms representing significantly enriched protein function groups for upregulated and downregulated 3′ UTR isoforms following elevated activity.

(legend continued on next page)
half-lives when compared to somata- and non-localized mRNAs (Figure 7D; Table S1). Following from the above observation, within a transcript family, the longer 3′ UTR isoform possesses longer half-lives (Figure 7E). We also observed that the half-lives of short and long isoforms of the same transcript family are not correlated (Figure S7D). Furthermore, we observed no significant relationship between the stability of a given 3′ UTR isoform and its structural stability, GC content, level of expression, or length (Spies et al., 2013).

To determine whether specific sequences influence mRNA half-life differences in our data, we searched for k-mers significantly associated with either shorter- or longer-lived 3′ UTR isoforms. We identified 464 motifs (comprising 121 families, allowing an edit distance of 2) significantly associated with shorter 3′ UTR half-lives and 344 motifs (105 families) associated with longer half-lives (Figure 7F; Table S3). Previously identified (Ray et al., 2013; Spies et al., 2013) destabilizing and stabilizing motifs were also detected and in good correspondence with our data (Figure 7F).

Similarly, we identified miRNA seed sites within 3′ UTRs that were also significantly associated with either shorter- or longer-lived transcript isoforms (Figure 7G; Table S3). To validate the efficacy of some miRNA seed sites in shortening transcript half-lives, we constructed firefly luciferase 3′ UTR reporter constructs possessing or lacking the seed sites for four different destabilizing miRNAs (miR-425, miR-146a, miR23a, and miR-154) (Figure 7H). These constructs, together with their cognate miRNAs, were transfected into cells, and transcript levels were assessed using qRT-PCR. In three of the four cases examined, the presence of the miRNA seed together with the miRNA was sufficient to destabilize the reporter construct when compared to a 3′ UTR lacking the miRNA seed (Figure 7I). Taken together, our data indicate that neuropil-localized transcripts are endowed with longer half-lives and that both somatic-localized and neuropil-localized 3′ UTRs are enriched with specific regulatory elements, including miRNA seed sites, that are important for localization and stability.

3′ UTR Plasticity Following Neural Activity

We next determined whether 3′ UTR diversity is altered by enhanced activity in neural networks. To stimulate neural activity, we treated hippocampal slices with the GABA_A receptor antagonist bicuculline (40 μM, 4 hr). Following enhanced activity, hippocampal slices were microdissected to obtain the somata and neuropil fractions that were then subjected to 3′ end sequencing. We found that increased neural activity led to significant alterations in 3′ UTR isoforms evident in both compartments (Figures S8A and S8B). Enhanced neural activity led to the upregulation of 367 and 408 3′ UTR isoforms and the down-regulation of 416 and 375 isoforms in the somata and neuropil, respectively (Figure 8A). Within this dataset, there were examples of coordinate regulation of the same 3′ UTR isoform in both compartments (Figure 8A, bottom left and top right quadrants) as well as oppositional regulation in the compartments (Figure 8A, top left and bottom right quadrants). We used a GO analysis to evaluate the potential enrichment of protein function groups represented by altered (up or downregulated) 3′ UTRs following bicuculline (Figure 8B; Table S2). Protein groups that were significantly enriched for downregulated 3′ UTR isoforms included membrane proteins and protein-kinase-associated proteins, for example. Significantly enriched protein groups represented by both up- and downregulated 3′ UTRs included long-term memory and ionotropic glutamate receptor binding (Figure 8B). When analyzed at the population level, we found that elevated activity resulted in a global and significant shortening of the 3′ UTR isoforms in both compartments, especially for the neuropil-localized isoforms (Figure 8C). Approximately 14% of the 3′ UTRs that were regulated and 18% of the 3′ UTRs that were shortened by bicuculline were also regulated by a chemical long-term potentiation (LTP) protocol in a recent study (Fontes et al., 2017); this degree of overlap is not significantly higher than one would predict by chance (Figure 8E). We validated the activity-dependent changes in 3′ UTRs for several transcripts in each compartment using qRT-PCR (Figure S8F).

Elevated neural activity is known to elicit changes in gene transcription and polyadenylation site choice (Flavell et al., 2008). As such, we reasoned that the activity-induced changes in 3′ UTR isoforms are most likely the result of regulated alternative poly(A) site choice following activity-induced changes in transcription. In order to examine the dependence of the above changes on
transcription, we blocked transcription during the enhanced activity and again conducted 3’ end sequencing (Figures S8C and S8D). As shown in Figures 8D and 8E, the majority (76.6%) of the activity-induced changes in 3’ UTR isoform usage were prevented by transcription inhibition, suggesting a role for transcription dependent alternative polyadenylation. There was, however, a substantial population of compartment-specific 3’ UTR isoform plasticity that persisted despite transcriptional inhibition (Figures 8D, 8F, and 8G). These transcription-independency (activity-dependent) changes might be the result of altered stability, trafficking of 3’ UTR isoforms between the two compartments (soma and neuropil), or remodeling (e.g., shortening or lengthening) of 3’ UTR isoforms within a compartment. The median half-life of our transcripts (~7.5 hr) is well beyond the duration of our activity manipulation, making it unlikely that extending the half-life can account for increases in most 3’ UTR isoforms, although reductions in 3’ UTR isoforms could be accounted for by enhanced turnover. We further considered the other two possiblities, regulated trafficking or local remodeling of 3’ UTR isoforms, with additional criteria to examine their feasibility as “simple” mechanisms for transcription-independent changes in local 3’ UTR composition (Figure 8F; Figure S8G). For the case of regulated trafficking of 3’ UTR isoforms between compartments, we reasoned that an increase (or decrease) in an isoform in one compartment (e.g., the neuropil) should be associated with a decrease (or increase) in that isoform in the other compartment (e.g., the soma). Applying these criteria, we found 32 (49.2%; 21 in the neuropil and 13 in soma) cases of 3’ UTR isoform regulation consistent with inter-compartmental trafficking (Figures 8F and 8H). For the case of local remodeling of a 3’ UTR isoform, we consider the example case in which there is a transcription-independent increase in a short isoform. We reasoned that, for this phenotype to be consistent with local remodeling (e.g., shortening), there should be a commensurate depletion in any longer isoform family member in the same compartment. Applying these criteria, we found 22 cases (38.8%; 17 in the neuropil and 5 in soma) of 3’ UTR isoform regulation consistent with local remodeling (Figures 8F and 8I). Taken together, these data indicate that neuronal activity can drive 3’ UTR isoform changes in specific compartments; while most (76.6%) of these changes require transcription, some 3’ UTR changes may occur in the cytoplasm and involve the differential trafficking or the local remodeling (e.g., shortening) of 3’ UTR isoforms.

**DISCUSSION**

We report, for the first time, the 3’ UTR complexity in a mammalian brain tissue, the hippocampal CA1 region, containing mature neurons with fully developed synapses. We examined the diversity of 3’ UTRs in (hippocampal) brain mRNAs to discover differences that influence the localization, stability, and potential translatational regulation of different mRNA isoforms. We microdissected hippocampal slices and used RNA samples enriched in cell somata or axons and dendrites (a.k.a. the neuropil; Cajigas et al., 2012; Mishchenko et al., 2010) for 3’ end sequencing. As previously reported (Derti et al., 2012; Elkon et al., 2013; Shi, 2012; Tian et al., 2005), we found that more than 50% of all genes and more than 70% of the neuron-enriched genes express multiple 3’ UTR isoforms. Hundreds of 3’ UTR mRNA isoforms show enrichment in either the neuropil or the somatic compartment. We found that localized transcripts, whether enriched in the somata or the neuropil, possessed longer 3’ UTRs than non-localized mRNA isoforms. This is in contrast to a study of developing neurons or a neural cell line in which no enrichment for longer isoforms was found in very young, nascent projections (Taliaferro et al., 2016).

The neuropil-localized 3’ UTR isoforms are significantly enriched for proteins associated with dendritic spines and synapses. Prominent among this category is the much-studied CamKIIα mRNA, one of the most abundant mRNAs in dendrites (Cajigas et al., 2012). Mayford et al. (1996) originally showed that the CamKIIα 3’ UTR in its entirety is sufficient for dendritic targeting and then went on to show (Miller et al., 2002) that the 3’ UTR is required for the localization of the mRNA and, importantly, a majority of the synthetically localized CamKIIα protein. Others have identified distal regions (Blichenberg et al., 2001; but see Mori et al., 2000) or elements (Huang et al., 2003; Subramanian et al., 2011) within the CamKIIα 3’ UTR that influence localization. Notably, the above distal regions and elements reside in the long, neuropil-localized 3’ UTR that we have identified and characterized here (Figure S5E).

Interestingly, we found that many of the somatically localized 3’ UTR isoforms code for dendritic or synaptic membrane proteins, which likely make use of the relatively abundant somatic and Golgi apparatus (Cui-Wang et al., 2012). Perhaps the most-studied synaptic membrane proteins are the excitatory neurotransmitter-gated ion channels, the AMPA and NMDA receptor families. We found that all of the AMPA- and NMDA-type glutamate receptors express multiple 3’ UTs (except Grin2d), which under basal conditions are mostly enriched in the somat or not enriched in either compartment (except Grin2c, which has an isoform enriched in the neuropil; Table S1). It is important though to distinguish between enrichment (a significantly higher quantity present in one compartment over the other) and presence or “localization.” The mRNAs and 3’ UTR isoforms of these receptors are clearly present in the neuropil, detected in our 3’ end sequencing analyses here (Figure S1) and also by in situ hybridization data shown here (see Gria1 and -2 and Grin2a and -b puncta in the dendrites and neuropil; Figure 4F; Figure S4) and in other publications (e.g., Cajigas et al., 2012; Grooms et al., 2006; Swanger et al., 2013). In addition, the local translation of some AMPA and NMDA receptors has also been observed (Swanger et al., 2013; tom Dieck et al., 2015). Our data show that regulation of particular 3’ UTR isoforms following plasticity can, in principle, change the local abundance, turnover, and translatability of a transcript. We speculate that some members of multi-subunit synaptic membrane complexes may be produced in the soma and distributed globally but that the final receptor complex composition can be fine-tuned by local translation of the complex member mRNAs that are enriched in dendrites.

The sequence motifs present in localized 3’ UTRs, particularly the extensions, add new regulatory potential but also exhibit a significant overrepresentation of redundant motifs, including mRNA seed sequences. Our previous work indicated that, in the CA1 region of the hippocampus expressed, mRNAs...
possess, on average, 500 potential mRNA targets (Sambandan et al., 2017). The addition of redundant motifs may thus enhance the competitive potential for regulation by a limited pool of individual miRNAs. In addition, we searched for 7-mers or miRNA seeds significantly enriched in either somatodendritic or neuropil-localized 3’ UTR isoforms and detected hundreds of motifs that provide the substrate for regulated localization, stability, and translational regulation. Kim et al. (2004) previously demonstrated that, in dissociated cortical neurons, miR-326 localizes with polyribosomes, the active sites of translation. We found that the binding site for miR-326 was significantly enriched in the neuropil-localized 3’ UTRs, suggesting a role for miR-326 in the regulation of local translation.

We measured the stability of the different 3’ UTR isoforms and found that the 3’ UTR isoforms associated with synaptic proteins are longer lived than the isoforms that code for proteins associated with transcriptional regulation, for example. In general, the 3’ UTRs associated with synaptic proteins and present in the neuropil were longer lived than other isoforms. Within transcript families, the longer 3’ UTRs were significantly longer lived than their shorter counterparts, supporting the notion that the longer half-life might help to establish localization to distal regions of the dendrite or axon. This is in contrast to a study in fibroblasts, which found no difference in stability between short and long 3’ UTR isoforms (Spies et al., 2013). Interestingly, a potentially destabilizing 3’ UTR Grik4 variant has been associated with neuropsychiatric disorders. We also identified hundreds of stabilizing and destabilizing motifs and miRNA seed sequences, which are significantly associated with both prolonged and reduced 3’ UTR isoform half-life. The sufficiency of several destabilizing miRNA seed sites was validated using qRT-PCR. These destabilizing motifs have the potential advantage of resetting the local mRNA pool via the degradation of a population of mRNAs with a particular regulatory potential and the subsequent replacement with a different mRNA population. Alternatively, the use of 3’ UTR isoforms that are inherently unstable can lead to a temporally discrete epoch of translation, for example, associated with the early phase of synaptic plasticity.

We found that following an epoch of enhanced neuronal activity, the 3’ UTR isoforms in both compartments, the somata and the neuropil, were altered, with an overall shortening of 3’ UTRs observed. A shortening of 3’ UTRs was recently reported in hippocampal slices following a chemical-LTP protocol (Fontes et al., 2017). Most of the activity-dependent changes in 3’ UTR isoforms were transcription dependent, consistent with alternative polyadenylation identified as an important mechanism for diversifying 3’ UTRs in both dendrites (Fontes et al., 2017) and developing axons (Shigeoka et al., 2016; Zhang et al., 2016). Surprisingly, though, a substantial fraction (~25%) of activity-induced changes in 3’ UTR isoforms were independent of transcription. The isoform- and compartment-specific changes could be due to regulated degradation; the enhanced turnover of 3’ UTR isoforms is certainly a viable mechanism that warrants further exploration. Alternatively, we considered two additional possibilities for the transcription-independent changes: regulated trafficking of 3’ UTR isoforms between somata and neuropil and local “remodeling” within a compartment. We found many expression profiles consistent with trafficking (decrease of an isoform in one compartment and the coordinate increase in the corresponding compartment). We also found many expression patterns consistent with local remodeling. For example, there were many cases of 3’ UTR isoform plasticity in which enhanced expression of the shorter 3’ UTR isoform was accompanied by a decrease in the long isoform in the same compartment. The mechanism for such shortening is unknown but would require either the excision (e.g., splicing) of 3’ UTR elements or the degradation of nucleotides (e.g., by an exo- or endonuclease) and the re-adenylation of the newly shortened transcript. Cytoplasmic splicing factors (Cajigas et al., 2012; Glanzer et al., 2005), poly(A) polymerases, and deadenylases have been identified and, in some cases, visualized in the dendrites of neurons (Udagawa et al., 2012). The functional consequences of enhanced expression of short 3’ UTRs will also be interesting. Others have reported relatively enhanced translation of short 3’ UTRs (Mayr and Bartel, 2009; Sandberg et al., 2008), presumably owing to the loss of miRNA-mediated repression. In our data, the local conversion (shortening) of a long 3’ UTR isoform would allow for a given isoform to be first localized (using information present in the long isoform) but then exhibit the relatively faster turnover and translational regulation inherent to the shorter isoform.

Lastly, we note that the diversity of 3’ UTRs discovered here represents an almost entirely unexplored landscape for dysregulation during neurodevelopmental, psychiatric, and degenerative disorders. We found that disease-related proteins are significantly more likely to make use of mRNAs with multiple 3’ UTRs. Indeed, a recent study reported that the Huntingtin transcript, Htt, contains multiple 3’ UTRs, and expression of the shorter 3’ UTR is associated with more protein aggregates and cell death in cultured cells (Xu et al., 2017). As most disease-related sequencing analyses have focused on protein-coding exons and often failed to find mutations, more attention should be focused on the 3’ UTRs of these genes.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

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DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION
Supplemental Information includes eight figures and five tables and can be found with this article online at https://doi.org/10.1016/j.neuron.2018.03.030.

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AUTHOR CONTRIBUTIONS
G.T., C.G., and E.M.S. designed experiments; C.G., M.H., and A.B. conducted experiments; G.T., C.G., and M.J. analyzed experiments; E.M.S. wrote the manuscript; and all authors edited and revised the manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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SUPPORTING CITATIONS
The following reference appears in the Supplemental Information: Zeisel et al. (2015).

REFERENCES


## STAR METHODS

### KEY RESOURCES TABLE

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| Plasmid: AcGFP Kcnab2 long 3' UTR | This paper | N/A |
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| Plasmid: pmirGLO_Ctxn1-3' UTR-miR23 | This paper | N/A |
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| Plasmid: pmirGLO_Arl2bp-3' UTR-miR154 | This paper | N/A |
| Plasmid: pmirGLO_Arl2bp-3' UTR-miR154del | This paper | N/A |

### Software and Algorithms

| Fiji | Schindelin et al., 2012 | https://imagej.net/Welcome |
| MATLAB | MathWorks | https://de.mathworks.com/ |
| NeuroBits | This paper | https://github.molgen.mpg.de/MPIBR/NeuroBits |
| STAR | Dobin et al., 2013 | https://github.com/alexdobin/STAR |
| Bowtie | Langmead et al., 2009 | http://bowtie-bio.sourceforge.net/index.shtml |
| Samtools | Li et al., 2009 | http://samtools.sourceforge.net/ |
| Tabix | Li et al., 2009 | https://github.com/samtools/tabix |
| FastSeqStats | This paper | https://software.scic.brain.mpg.de/projects/MPIBR-Bioinformatics/fastqSeqStats |
| PASSFinder | This paper | https://software.scic.brain.mpg.de/projects/MPIBR-Bioinformatics/PASSFinder |
| ClusterPASS | This paper | https://github.com/MPIBR-Bioinformatics/ClusterPASS |
| UCSC Table Browser | Karolchik et al., 2004 | http://genome.ucsc.edu/cgi-bin/hgTables |
| PASSAnnotator | This paper | https://github.molgen.mpg.de/MPIBR-Bioinformatics/PASSAnnotator |
| PASSCountExpression | This paper | https://github.molgen.mpg.de/MPIBR-Bioinformatics/PASSCountExpression |
| edgeR library (v.3.18) | McCarthy et al., 2012 | https://bioconductor.org/packages/release/bioc/html/edgeR.html |
| RNAfold, ViennaRNA v.2.0 | Lorenz et al., 2011 | https://www.tbi.univie.ac.at/RNA/#download |
| MEME v.4.11 | Bailey et al., 2009 | http://meme-suite.org/ |
| mkseed | https://github.com/takayasaito/mkseed | |
| TargetScan | Agarwal et al., 2015 | http://www.targetscan.org |
| miRanda | Enright et al., 2003 | http://www.microrna.org/ |
| GOAnalysis | This paper | https://software.scic.brain.mpg.de/projects/MPIBR-Bioinformatics/GOAnalysis |
| uShuffle | Jiang et al., 2008 | http://digital.cs.usu.edu/~miyang/uShuffle/ |
| Other | | |
| LSM880 | Zeiss | https://www.zeiss.de/mikroskopie/produkte/confocal-microscopes/lsm-880.html |
| StepOnePlus Real-Time PCR System | Thermo Fisher Scientific | Catt#4376600 |
| MACE sequencing | GenXPro | https://genxpro.net/ |
CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Erin M. Schuman (erin.schuman@brain.mpg.de).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The procedures involving animal treatment and care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (DIRECTIVE 2010/63/EU; German animal welfare law; FELASA guidelines). The animals were euthanized according to annex 2 of § 2 Abs. 2 Tierschutz-Versuchstier-Verordnung.

Acute Hippocampal Slices

Sprague Dawley rats were housed in standard cages and fed standard lab chow and water ad libitum. Rats Hippocampal slices (500 μm) were prepared from four-week-old male animals as previously described (Kang and Schuman, 1996).

Primary Hippocampal Cultures

Dissociated rat hippocampal neurons were prepared from P0-1 day-old rat pups as previously described (Aakalu et al., 2001). For neuron-enriched cultures and cultures used for half-life measurements, neurons were plated at a density of 36,000 cells/cm² onto poly-d-lysine-coated 60 mm cell culture dishes. At one day in vitro (DIV), AraC was added to a final concentration of 3 μM. After 2 days, medium was exchanged to pre-conditioned growth medium (Neurobasal-A supplemented with B27 and GlutaMAX, 30% glia-culture supernatant, 15% cortex-culture supernatant) and neurons were cultured until DIV21. For fluorescence in situ hybridization, 30,000 cells were plated onto poly-d-lysine coated glass-bottom Petri dishes (MatTek) and cultured for 21 days in growth medium (Neurobasal-A supplemented with B27 and GlutaMAX). The cultures were maintained in a humidified incubator at 37°C and 5% CO₂. The sex of cells was not determined.

Primary Glia-Enriched Cultures

The hippocampi of P0-1 day old rat pups were isolated and triturated after digestion with papain. Cells were plated on uncoated 60 mm cell culture dishes and grown at 37°C and 5% CO₂ in minimal essential medium supplemented with GlutaMAX and 10% horse serum. At DIV7, medium was changed to growth medium (Neurobasal-A supplemented with B27 and GlutaMAX) and cells were cultured until DIV21. Glial enrichment was confirmed by the over-representation of glial markers (Figures S2F and S2G). The sex of cells was not determined.

Cell Lines

HEK293 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FCS at 37°C in a 5% CO₂ atmosphere.

METHOD DETAILS

Tissue Microdissection, RNA Isolation, and 3' End Sequencing

The somatic and the neuropil layer of the CA1 region were microdissected by hand from each slice. Tissue pieces were first collected in RNA later to stabilize and protect RNA from degradation. Total RNA was extracted using TRIzol reagent and purified using the RNeasy Mini Kit following the manufacturer’s instructions including the DNaseI digestion. Successful isolation of the neuropil was confirmed by de-enrichment of NeuN, a nuclear marker, using an anti-NeuN antibody (1:5000) as previously described in Cajigas et al. (2012). Total RNA was subjected to 3' end sequencing using the MACE sequencing kit (Müller et al., 2014).

Pharmacological Treatments

Rat hippocampal slices (500 μm) were treated with bicuculline (40 μM) or water control for 4 hr on filter paper in a recovery chamber. For transcription inhibition, Actinomycin D (10 μg/ml), DRB (D-ribofuranosylbenzimidazole) (100 μM) and Triptolide (1 μM) were added to the ACSF 30 min before the addition of bicuculline. For RNA half-life measurements, Actinomycin D (10 μg/ml), DRB (100 μM) and Triptolide (1 μM) were added to the culturing medium.

High-Resolution In Situ Hybridization in Primary Hippocampal Neurons and Slices

In situ hybridization was performed using the Quantigene ViewRNA ISH Cell Assay for Fluorescence with probes targeting the unique sequence of the long 3’ UTR. The manufacturer’s protocol was applied with the following modifications: Primary hippocampal neurons (DIV 21) were fixed for 20 min at room temperature using a 4% paraformaldehyde solution (4% paraformaldehyde, 5.4% glucose, 0.01 M sodium metaperiodate, in lysine-phosphate buffer). The Proteinase K treatment was substituted with a Pepsin digestion. For this, neurons were kept for no more than 45 s in 0.01 mg/ml Pepsin, 10 mM HCl in water, pH 2.5. Enzyme activity was halted with PBS pH 7.2 and washed thoroughly. After completion of in situ hybridization, cells were washed with PBS and incubated in
blocking buffer (4% goat serum in PBS) for 1 hr. Dendrites were stained for 1 hr at room temperature using an anti-MAP2 antibody (SySy, 188004; 1:1000), washed three times with 1x PBS and incubated with the secondary antibody (Jackson IR, 106-475-003, DyLight 485; 1:1,000 dilution) for 1 hr at room temperature.

For in situ hybridization in hippocampal slices, 4-week-old male rats were perfused with 1x PBS and 4% (v/v) paraformaldehyde solution in PBS. The hippocampi were dissected, sliced to 2 mm and fixed for 3 hr at room temperature. Slices were cryoprotected in 20% (v/v) sucrose in PBS (DEPC-treated) overnight at 4 °C and cryosectioned at 12 μm thickness. In situ hybridization was performed as described above with the following modifications. Probes were diluted to 1:50. After completion of the in situ hybridization, the slices were blocked for 1 hr in blocking buffer. Dendrites were stained overnight at 4 °C using an anti-MAP2 antibody (SySy, 188004; 1:1000), washed three times with 1x PBS and incubated with the secondary antibody (Invitrogen, A11073, Alexa 488; 1:1,000) for 2 hr at room temperature. Slices were mounted in AquaPolymount. Fluorescence imaging was performed with a LSM880 laser scanning confocal microscope with x40 (NA 1.4) objectives with appropriate excitation laser lines and spectral detection windows. In Figures 4D–4F and Figures S4A and S4B, the mRNA signal in hippocampal cultures was dilated for better visualization. The raw, undilated images were used for analysis.

**GFP Reporter Experiments**

Cultured hippocampal neurons were transfected (using Magnetofectamine) at DIV11-16 with 0.2-1 μg per dish of the reporter construct (CaMK2a promoter-GFP-CaMK2a short 3′ UTR, middle 3′ UTR, long 3′ UTR, Kcnab2 long 3′ UTR or no UTR, respectively). In situ hybridization against the coding sequence of GFP was performed 24 hr post-transfection, as described above.

**cDNA Synthesis and Quantitative Real-Time PCR**

Total RNA was reverse-transcribed with the QuantiTect Reverse Transcription Kit. qRT-PCR was performed using the 2X SYBR Green Master Mix. Reaction setup and cycling parameters were performed according to the QuantiTect primer assay recommendations. qRT-PCR was run on a StepOnePlus Real-Time PCR System. The primer sequences used in this study are indicated in Table S5. Neuron- and glia enriched genes (Figure S2E) were normalized to the geometric mean of GAPDH and 18 s rRNA. Somata- and neuron-pil-enriched genes (Figure 3B) were normalized to the geometric mean of 18 s rRNA and Cdc73. Long 3′ UTR isoforms that were altered with neural activity (Figure S6E) were normalized to GAPDH.

**Transfection of MicroRNA Mimics**

mirVana miRNA Mimics and the respective 3′ UTR-reporter (pmirGLO Dual-Luciferase vector (Promega) expressing firefly flanked by the 3′ UTR containing or lacking the miRNA binding site and renilla) were co-transfected with Lipofectamine 2000 according to the manufacturer’s instructions. For each co-transfection 1 μg of the 3′ UTR-reporter and a final concentration of 30 nM of miRNA mimic was used. 24 hr post-transfection, RNA was isolated using Direct-zol and treated with DNase using the TURBO DNA-free kit to remove potential DNA contaminations. qRT-PCR was performed as described above. Firefly Ct-values were normalized to renilla Ct-values. Then the deltaCt method was used to give the fold change between a reporter with the miRNA binding site and a reporter lacking the miRNA binding site.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Image Analysis**

Neuronal cell soma and processes were segmented using NeuroBits. FISH puncta were detected by locating the center of mass of the soma along the segmented regions. For each puncta the path traveled, and hence the distance to the soma, was calculated with the help of the segmented neuronal tree.

Puncta traveled distances were grouped by in situ probe and a non-parametric test was applied. Analysis of FISH probes against GFP followed a different approach due to the less discrete character of the signal in the GFP-mRNA channel. The signal was smoothed by local averaging along the segmented dendrites and normalized to the average GFP signal in the cell soma. A median of the distance along the dendrite was weighted by the GFP-mRNA signal to produce a final measure of signal localization. Again, each distance measure was grouped by in situ probe and a non-parametric test was applied to assess group differences.

**Detection and Annotation of poly(A) Supported Sites**

The pipeline for the detection and annotation of poly(A) supported sites (PASS) consisted of the following steps:

i) Quality assessment. The tool fastqSeqStats was used to assess read quality. The sequenced reads were fragmented in 300-500 nt regions and sequenced from the 5′ side. The expected elevation of poly(A/T) content toward the 3′ end was observed (Figure S1).

ii) Genome Alignment. Reads alignment was conducted with STAR aligner (Dobin et al., 2013), version (2.5.2) with the following parameters:

STAR-runMode alignReads--runThreadN 6--genomeDir $2--readFilesIn $file--readFilesCommand zcat--outSAMattributes All--outStd Log-outSAMtype BAM SortedByCoordinate--outSAMstrandField intronMotif--outFilterIntronMotifs RemoveNoncanonical-alignSoftClipAtReferenceEnds No--outFilterScoreMinOverLread 0.25--outFilterMatchNminOverLread 0.25

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iii) Poly(A) supported site prediction.

To detect the peaks of poly(A) supported sites we used the in-house developed tool PASSFinder. It relies on the HTSlib API (Li et al., 2009) version (1.4.1).

A genome map of potential internal priming regions was created by aligning a string of 10 consecutives As using Bowtie. The result was converted to a BED record and the mask file was indexed and randomly accessed with Tabix. The 3’ base coverage indicated potential 3’ UTR sites. In order to identify the positions of polyadenylation we implemented a base clustering technique that merges positions in a user-defined window. During the grouping process, information about the best expressed base and best expressed seed (3’ base of reads containing poly(A) tail) is maintained by ClusterPASS. Alternative last exons (ALE) were defined as poly(A) sites detected in intronic regions. Only ~13% of the identified PASS fell into this category. ALEs showed relatively low expression compared to PASS detected in annotated 3’ UTRs, accumulating less than 3% of all reads (Figure S1G).

iv) Annotation assignment.

Annotation was based on a BED map that was downloaded with the UCSC Table Browser tool for Rattus Norvegicus, build rn5. The PASSAnnotator tool used the map and assigned gene features based on upstream proximity.

v) Expression counting.

Expression was determined by read coverage in proximity to each of the predicted poly(A) supported sites. The provided PASSCountExpression.pl script automated that routine.

Consolidated Set of Experimental Data

Experimental data were sequenced from tissue and cell-culture samples. To facilitate analysis and maintain data integrity, a consolidated set of predicted poly(A) supported site was created. The required conditions were minimum site expression of 2 reads per million in at least one sample and a site position hitting within the 3’ UTR or extended 3’ UTR region (< 20 kb) of detected genes. We intentionally excluded sites falling in introns or coding sequence exons as those propose a model of truncated protein products and are out of the scope of this study.

Classification of Cell-Enriched Genes

Differential 3’ UTR isoforms expression between neuron-enriched and glia-enriched samples was determined using the edgeR library (version 3.18) in R (McCarthy et al., 2012). The trimmed mean of M-values (TMM) normalization was combined with frame design to avoid batch effects. A gene-wise negative binomial generalized linear model with quasi-likelihood test was used to conduct differential expression (DE) analysis. The steps followed closely examples given in the edgeR manual.

The results of neuron- or glia-enriched 3’ UTR isoforms were projected back on the gene level, where a gene was labeled as “neuron-enriched” if at least one isofrom showed DE in a neuron-enriched sample. The same logic was applied for glia-enriched isoforms. Genes with predicted multiple 3’ UTR isoforms, for which one isofrom was DE in the neuron-enriched sample and another was DE in the glia-enriched sample were labeled as “shared” and removed from downstream analysis.

Structural and Sequence-Specific Properties of Neuronal 3’ UTRs

DNA sequence was extracted from the Rattus Norvegicus m5 version genome, starting from the closest stop codon to a predicted poly(A) supported site. Only sequences with a size ranging from 100 to 5000 nucleotides were included in the analysis. The GC content was assessed by counting the number of G or C bases in the sequence and then divided by the number of bases in the predicted 3’ UTR. The ViennaRNA package version 2.0 with RNAfold was used to calculate the minimum free energy per 3’ UTR sequence (Lorenz et al., 2011). A method described by Trotta (2014) was adapted to normalize minimum free energy units for varying 3’ UTR length. Sequences were shuffled preserving the di-nucleotide content by a routine implemented in the Motif based sequence analysis tool MEME, version 4.11 (Bailey et al., 2009).

miRNA Site Prediction

miRNA sites were predicted using three target prediction programs:

- mkseed
- targetscan (Agarwal et al., 2015)
- miRanda (Enright et al., 2003) (pairing score > 155 and energy score < −20)

Reference miRNAs were downloaded from miRBase release 21 (Kozomara and Griffiths-Jones, 2014). The union of the predictions was further compared against Ago-Clip binding sites (Chii et al., 2009) and a score based on the number of Ago-Clip reads spanning each prediction site was calculated. A conservation score was calculated by averaging the base-wise phyloPhast score from 13 animals available for Rattus norvegicus genome version 5 (Tyner et al., 2017). We measured the expression of miRNAs with Nanostring in rat hippocampus (Sambandan et al., 2017) and cross-referenced that data with the predicted seed. Our final predicted miRNA sites were combination of the following criteria: more than one prediction program recognized the site, there are more than 10 Ago-Clip reads spanning the site, average phyloPhast score for the site seed is more than 0.2, and the miRNA is in hippocampus above background levels as reported from Nanostring counter (Sambandan et al., 2017).
K-mer Site Prediction
To define a set of predicted k-mers per 3' UTR region, a sliding window of 7-mers was applied. With each step of the window an average conservation score was calculated based on the phyloPhast Rattus norvegicus version 5. A threshold of 0.2 was applied and each k-mer was recorded in hash data structure. Every time a k-mer was found the localization fold change and stability of the corresponding 3' UTR was recorded to the hashed data. The k-mer localization and stability measure was determined by averaging those results and the difference between one k-mer and the rest of the candidates defined the enrichment score and statistics. The Levenshtein distance of one was used to allow for fuzzy k-mer matches. The k-mers were clustered with the Hobohm clustering algorithm (Hobohm et al., 1992).

Localization of Neuronal 3' UTRs in Subcellular Compartments
To address localization, only genes classified as neuron-enriched (see Classification of Cell-Enriched Genes) were used. Differential expression was determined using the edgeR library as described above (see Classification of Cell-Enriched Genes). Gene ontology analysis was conducted on two unranked lists. The target list comprised all localized genes or CA1 layer-specific genes and the background list comprised all neuron-enriched classified genes. We used the GOAnalysis tool developed in our Bioinformatics source library.

K-mer Content
The k-mer content was investigated in neuron-enriched genes with multiple 3' UTRs. The top two expressed isoforms were labeled as “shorter” and “longer” representing their proximity to the stop codon. We extracted each 7-mer from the “longer” isoform using a sliding window. Three classes were defined describing the content options. Class “repeat between” was counted when the 7-mer is also present in the “shorter” 3' UTR isoform. Class “novel” and “repeat within” were counted in the case of lacking a match in the “shorter” isoform but being present one or multiple times in the “longer” isoform, respectively. To address the expected k-mer content we conducted two controls. First we used di-nucleotide shuffling of the “longer” isoform and repeated the procedure 3 times per gene. Di-nucleotide shuffling was done using the uShuffle procedure (Jiang et al., 2008). Second, we extracted the intergenic sequence 1kb downstream of the last predicted PASS and repeated the same analysis as for the observed 3' UTRs. The Levenshtein distance of one was used to allow for fuzzy k-mer matches. The k-mers were clustered with the Hobohm clustering algorithm (Hobohm et al., 1992).

Classifying Protein Products
Protein products were classified as described in Hanus et al. (2016).

Stability of Neuronal 3' UTRs
Raw counts were first represented as reads per million with minimum threshold of 2 reads per million. In a sequencing experiment with fixed depth in the same RNA pool, stable messages will exhibit an apparent increase in expression over time when transcription is blocked, owing to their enhanced representation in the pool. We used that property to define a normalization scheme. Each time point was individually ranked and ranks per gene were summed to determine an overall rank score. 100 genes with the highest overall rank score were chosen and used for calculating a normalization ratio. To predict 3' UTR stability a non-linear regression with an exponential decay function was applied to each biological replicate. The average of the two replicas was used for half-life value. Only 3' UTR isoforms with stability between 0 and 24 hr were included in our final analysis as higher predictions were unreliable with the chosen time points. GSEA was implemented and conducted as previously described in Subramanian et al. (2005). Gene ontology terms were used for grouping 3' UTR isoforms. To investigate k-mers or miRNAs seeds associated with 3' UTR stability, we conducted an analysis adapted from the above GSEA analysis. Each k-mer or miRNA represented a group in a ranked list of 3' UTR targets. The median half-life in the groups was compared against the median half-life outside the group using the non-parametric Mann-Whitney U-test. The skewness of the distribution was taken to indicate either a stabilizing or de-stabilizing influence. The analysis was corrected for multiple comparisons (Benjamini-Hochberg) and the results were visualized using volcano plots.

Neuronal 3' UTR Plasticity upon Altered Neuronal Activity
Raw expression counts were normalized using the TMM procedure and replica batch effects were removed by edgeR library. To determine differential expression a simpler procedure was applied. 3' UTR isoforms were sorted according to their expression and data points were binned in blocks of size 150. For each bin, data points were Z-transformed and outliers were determined with p value $\leq 0.05$. Multiple hypothesis correction was applied at the end to account for false discoveries.

DATA AND SOFTWARE AVAILABILITY
The accession number for the raw sequencing data reported in this paper is NCBI BioProject: PRJNA390472. Processed data used for analyses in this manuscript are included in Table S1. All scripts used in this study are deposited in https://github.molgen.mpg.de/MPIBR-Bioinformatics/NeuroUTRs.