Emerging Roles of Activity-Dependent Alternative Splicing in Homeostatic Plasticity

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Homeostatic plasticity refers to the ability of neuronal networks to stabilize their activity in the face of external perturbations. Most forms of homeostatic plasticity ultimately depend on changes in the expression or activity of ion channels and synaptic proteins, which may occur at the gene, transcript, or protein level. The most extensively investigated homeostatic mechanisms entail adaptations in protein function or localization following activity-dependent posttranslational modifications. Numerous studies have also highlighted how homeostatic plasticity can be achieved by adjusting local protein translation at synapses or transcription of specific genes in the nucleus. In comparison, little attention has been devoted to whether and how alternative splicing (AS) of pre-mRNAs underlies some forms of homeostatic plasticity. AS not only expands proteome diversity but also contributes to the spatiotemporal dynamics of mRNA transcripts. Prominent in the brain where it can be regulated by neuronal activity, it is a flexible process, tightly controlled by a multitude of factors. Given its extensive use and versatility in optimizing the function of ion channels and synaptic proteins, we argue that AS is ideally suited to achieve homeostatic control of neuronal output. We support this thesis by reviewing emerging evidence linking AS to various forms of homeostatic plasticity: homeostatic intrinsic plasticity, synaptic scaling, and presynaptic homeostatic plasticity. Further, we highlight the relevance of this connection for brain pathologies.

Keywords: alternative splicing, homeostatic plasticity, repressor element 1 silencing transcription factor (REST), homer1, P/Q-type Ca^{2+} channels

INTRODUCTION: FROM GENES TO FUNCTION

Over the last two decades, a vast array of homeostatic plasticity adaptations, which enable neuronal networks to stabilize their activity in the face of external perturbations, have been identified. These involve adjustments in synaptic strength by means of pre- and postsynaptic mechanisms (homeostatic synaptic plasticity) and in intrinsic excitability (homeostatic intrinsic plasticity). Ultimately, both synaptic and intrinsic forms of homeostatic plasticity depend on changes in expression or activity of ion channels and synaptic proteins, which may occur at the gene, transcript, or protein level (**Figure 1**).

By far, the most extensively investigated homeostatic mechanisms involve changes in protein function or localization by means of posttranslational modifications affecting protein–protein interactions and trafficking (reviewed in Turrigiano, 2011; Davis and Müller, 2015; Fernandes and Carvalho, 2016; Cingolani et al., 2019).

Chronic changes in network activity can also be counteracted by regulating protein translation. For example, increased surface expression of the GluA1 subunit of AMPA-type glutamate receptors (AMPARs) compensates blockade of network activity within a few hours. This form of homeostatic synaptic plasticity, known as synaptic upscaling, requires local protein synthesis because it is prevented by dendritic application of the protein synthesis inhibitors anisomycin or emetine (Sutton et al., 2006), and it involves downregulation of miR92a (Letellier et al., 2014; Dubes et al., 2019). Further, the transcription of hundreds of genes was recently shown to be up- or downregulated at early (2 h) and late (24 h) stages of the homeostatic response (Schanzenbächer et al., 2016, 2018).

FIGURE 1 | Genes to function in homeostatic plasticity.

Synaptic upscaling following tetrodotoxin (TTX)-induced suppression of network activity is dependent also on gene transcription because the transcription inhibitor actinomycin D (ActD) blocks effectively upscaling of miniature excitatory postsynaptic currents (mEPSCs) and dendritic accumulation of the AMPAR subunit GluA2 (Ibata et al., 2008). More recently, chronic suppression of network activity was shown to alter the transcription of tens of genes, including that for the AMPAR clustering protein neuronal pentraxin-1 (*Nptx1*); Ca^{2+} entry *via* T-type voltage-gated Ca^{2+} channels (VGCCs) appears essential for this signaling pathway (Schaukowitch et al., 2017). Conversely, chronic augmentation of network activity leads to Ca^{2+}-dependent changes in the expression of hundreds of genes (Flavell and Greenberg, 2008; Schaukowitch et al., 2017), some of which, such as brain-derived neurotrophic factor (BDNF), calcineurin, and MeCP2, are known players in homeostatic synaptic plasticity (Fernandes and Carvalho, 2016). Neuronal activity also increases the expression levels of immediate early genes, such as Arc (aka Arg3.1), which induces a counterbalancing internalization of AMPARs (Shepherd et al., 2006) and, when localized in the nucleus, decreases transcription of the AMPAR subunit GluA1, thereby reducing synaptic strength (Korb et al., 2013).

In comparison to the above outlined molecular mechanisms, little attention has thus far been devoted to whether and how homeostatic adaptations are achieved at the level of alternative splicing (AS) of pre-mRNAs (**Figure 1**). As detailed below, this lack of attention may come as a surprise because some AS events are well-known for being controlled by neuronal activity and because AS is ideally suited to optimize protein function to new challenges (Raj and Blencowe, 2015; Vuong et al., 2016; Baralle and Giudice, 2017). Here, we review recent findings linking homeostatic plasticity to AS and discuss the relevance of activity-dependent AS to achieve homeostatic control of neuronal output in health and diseased states.

ALTERNATIVE SPLICING

During RNA maturation, intervening noncoding RNA sequences (introns) are removed while coding sequences (exons) are joined together, thus contributing to transforming a newly transcribed mRNA (pre-mRNA) into a mature mRNA. RNA splicing is performed by a multi-molecular RNA–protein complex, the spliceosome, which binds to specific sequences on the pre-mRNA. These include a donor site (5′ end of the excised intron), an acceptor site (3′ end of the intron), and, upstream of the 3′ site, a polypyrimidine tract and a branch point. For some genes, rather than being univocal, the splicing process creates a range of mature mRNAs, each with a unique exon composition. If translated, these mRNA splice isoforms will produce multiple protein variants with potentially distinct functions. We talk in this case of AS. AS is regulated by *cis*-acting elements (regulatory RNA sequences), which act as splicing enhancers or repressors by recruiting *trans*-acting splicing factors (proteins or ribonucleoproteins) that favor or inhibit different steps of the splicing reaction (Matera and Wang, 2014).
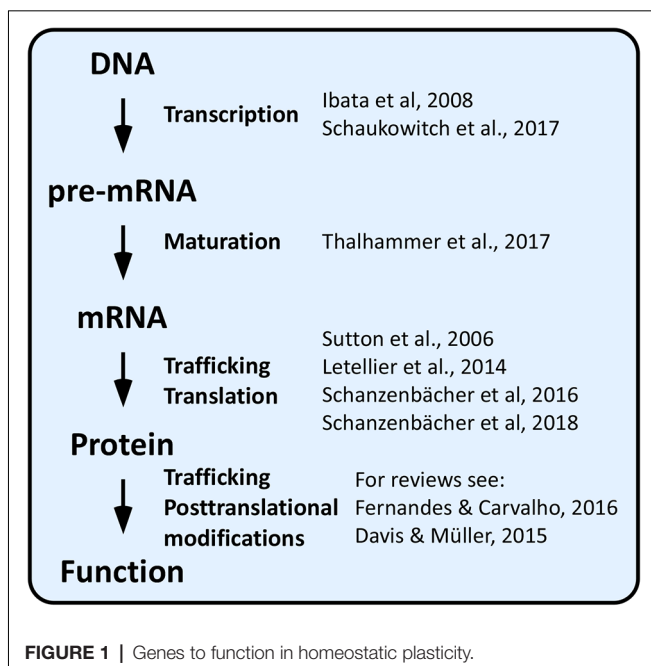
In higher eukaryotes, AS has the potential to convert a limited number of genes into an astounding variety of proteins depending on developmental stage, brain region, and cell types. For example, thousands of mRNA splicing isoforms were found to be different between neurons and glial cells when comparing purified brain cell populations (Zhang et al., 2014). Indeed, some splicing factors display cell-type specific expression (Nguyen et al., 2016; Furlanis and Scheiffele, 2018), while others regulate specific splicing events during brain development (Norris and Calarco, 2012). Transcriptomic and proteomic studies indicate that more than 90% of mammalian genes undergo AS, with the brain exhibiting the most complex repertoire of splice variants (Pan et al., 2008; Wang et al., 2008; Kim et al., 2014; Schreiner et al., 2015). In some cases, as for neurexins and calcium channels, one single gene can give rise to potentially thousands of different mRNA isoforms (Ullrich et al., 1995; Soong et al., 2002; Lipscombe et al., 2013; Schreiner et al., 2014; Treutlein et al., 2014), many of which have been identified at the protein level (Kim et al., 2014; Schreiner et al., 2015). It should also be noted that AS is not limited to diversifying the coding sequence of an mRNA but can also modify the selection of 5' and 3' untranslated regions (UTRs), thus affecting stability, subcellular localization and translation of mRNAs (Hermey et al., 2017; Mauger and Scheiffele, 2017).

In order to be instructive for homeostatic plasticity, AS needs to fulfill two criteria: (i) it must be regulated by neuronal activity; and (ii) the outcome of the splicing process must result in a homeostatic compensation. We will explore the requirement of AS in homeostatic plasticity in the next paragraphs following three exemplary cases.

NSR100, MICROEXONS, AND REST IN HOMEOSTATIC PLASTICITY

Some splicing factors, such as Nova-1/2, Rbfox-1/2/3, Ptbp1/2, and nSR100 are highly enriched in neurons. Among these, the Ser/Arg repeat-related protein of 100 kDa (nSR100, aka SRRM4) binds to intronic enhancer UGC elements close to the 3' splice sites to promote microexon inclusion (**Figure 2A**; Raj and Blencowe, 2015). Microexons are a class of cassette exons (exons that can be included or not in the mature transcript) that tend to be located in surface loops and intrinsically disordered regions. They generally have a length of 9–21 nucleotides (nt), often in multiples of three nt, hence leading to alternative versions of a protein with altered functions, protein–protein interaction motifs, or posttranslational modifications. Microexons are especially important in the brain, where they constitute nearly one third of all neural-regulated splicing events. They are frequently misregulated in the brain of individuals with autism spectrum disorder; this is likely due to increased neuronal activity, often associated with autism spectrum disorder, resulting in a rapid decrease in nSR100 expression and increased skipping of microexons (Irimia et al., 2014; Quesnel-Vallières et al., 2016).

Although generally frame preserving, microexon inclusion promoted by nSR100 can also disrupt the reading frame of a gene. For example, one well-known downstream target of nSR100 is the transcriptional repressor REST (repressor element 1 silencing transcription factor; aka NRSF, neural restrictive silencing factor), which silences a multitude of neural genes (Raj et al., 2011). In this case, nSR100 promotes the inclusion of a 16-nt-long microexon located between the third and fourth exons, leading to a frameshift introducing a stop codon at the beginning of the fourth exon. The resulting isoform, REST4, is truncated and lacks the domains required for transcriptional repression of target genes (Raj et al., 2011). When neuronal activity increases, nSR100 expression is rapidly downregulated (Quesnel-Vallières et al., 2016), resulting in skipping of the 16-nt-long microexon and production of the active isoform of REST (**Figure 2A**). Accordingly, REST is upregulated in primary neuronal cultures after 48–96 h of network hyperactivity, and this decreases the expression of its targets, including the sodium channel $Na_V1.2$, the calcium channel $Ca_V3.2$, and various presynaptic proteins (SNAP-25, Synapsin-1, Synaptotagmin-2, and vGlut-1; van Loo et al., 2012; Pozzi et al., 2013). Downregulation of $Na_V1.2$ makes it more difficult for a neuron to elicit action potentials, thus contributing to homeostatic intrinsic plasticity (Pozzi et al., 2013). Decreased expression of presynaptic proteins correlates with a reduction in the number of docked synaptic vesicles and in the frequency of mEPSCs, thus contributing to presynaptic homeostatic plasticity, a prominent form of homeostatic synaptic plasticity (**Figure 2A**; Pecoraro-Bisogni et al., 2018).

ALTERNATIVE SPLICE ISOFORMS OF HOMER1 IN SYNAPTIC SCALING

The Homer1 gene generates long and short splice isoforms. The major isoforms, Homer1b, Homer1c, and Homer1d, are long, constitutively expressed, and act as scaffold proteins at postsynaptic sites (Fagni et al., 2002; Shiraishi-Yamaguchi and Furuichi, 2007). In response to various stimuli, such as electroconvulsive seizures, cocaine, kainate or nicotine exposure, two truncated isoforms of Homer1, Homer1a and Ania3, which have all the characteristics of immediate early gene products, are rapidly (1–4 h) induced (Brakeman et al., 1997; Kato et al., 1997; Berke et al., 1998; Bottai et al., 2002). This is due to myocyte enhancer factor 2 (MEF2) family transcription factors, which boost transcription of the Homer1 gene and to a concomitant termination of transcription within the large central intron between exons 5 and 6, leading to use of alternative poly(A) sites. Because of this coordinated increase in transcription rate and premature transcription termination, only the short isoforms of Homer1 are induced by neuronal activity (**Figure 2B**; Bottai et al., 2002; Flavell et al., 2008).

The long isoforms of Homer1 consist of two major domains: (i) an N-terminal Enabled/Vasp homology 1 (EVH1) domain, which binds to proline-rich sequences in Group 1 metabotropic glutamate receptors (mGluR1 and 5), inositol-1,4,5-trisphosphate (IP_3) receptors, ryanodine receptors, TRPC1 ion channels, and the scaffold protein Shank; and (ii) a C-terminal coiled-coil (CC) structure followed by leucine zipper motifs, which favor oligomerization of homer proteins (Szumlinski et al., 2006). The long isoforms of Homer1 are
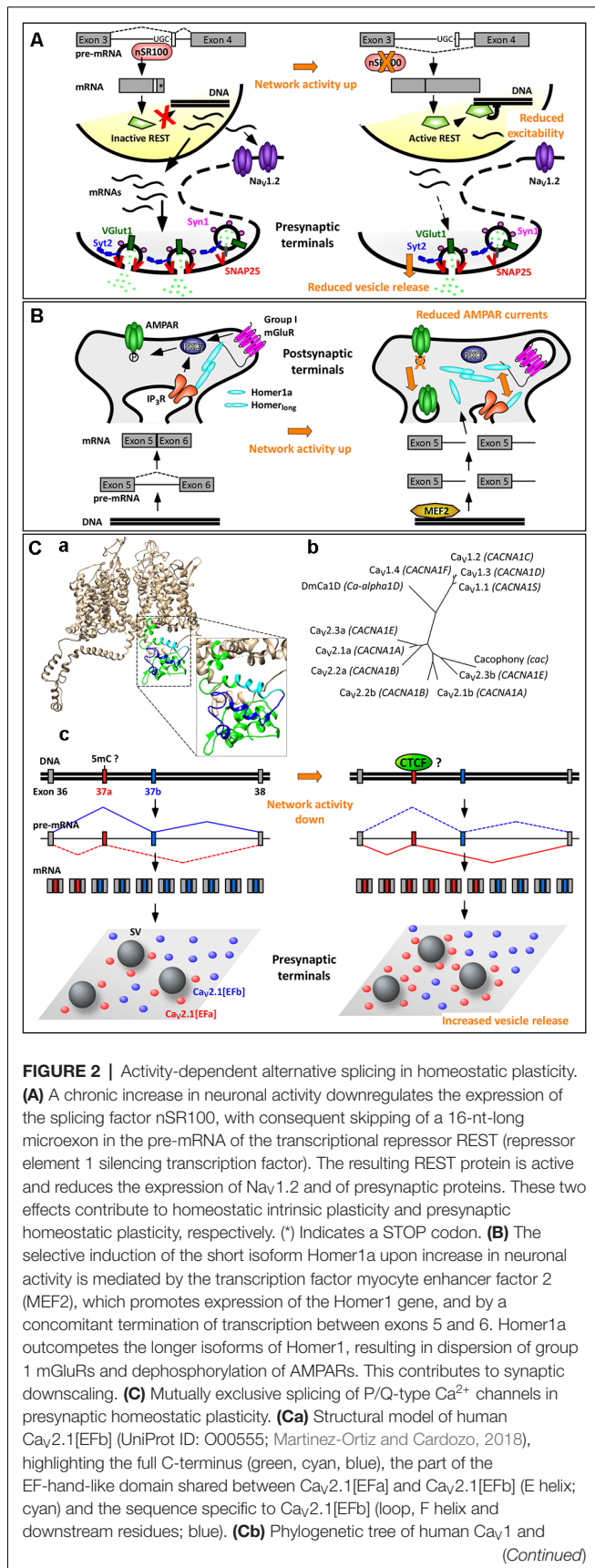
FIGURE 2 | Continued

Ca$_V$2 channels and of Cacophony and DmCa1D from *Drosophila melanogaster* for the amino acidic region corresponding to exons 37 of Ca$_V$2.1 (Clustal Omega www.ebi.ac.uk/Tools/msa/clustalo/, rendering using TreeDyb, http://www.phylogeny.fr/one_task.cgi?tasktype=treedyn, Chevenet et al., 2006); UniProt IDs: Ca$_V$1.1: Q13698, aa: 1414–1446; Ca$_V$1.2: Q13936, aa: 1587–1589; Ca$_V$1.3: Q01668, aa: 1497–1529; Ca$_V$1.4: O60840, aa: 1474–1506; Ca$_V$2.1b: O00555, aa: 1843–1875; Ca$_V$2.2b: Q00975, aa: 1741–1773; Ca$_V$2.3b: Q15878, aa: 1756–1788; Ca$_V$2.1a: O00555-4, aa: 1844–1876; Cacophony: P1645, aa: 1370–1402; DmCa1D: Q24270, aa: 1959–1991; sequences for Ca$_V$2.2a and Ca$_V$2.3a are as in Thalhammer et al. (2017). The three exons 37a cluster together as do the three exons 37b, suggesting conservation of these mutually exclusive exons across Ca$_V$2 channels; the corresponding region of Cacophony from *D. melanogaster* is more tightly related to exon 37b. **(Cc)** The increased expression of the isoform Ca$_V$2.1[EFa] upon chronic activity deprivation might occur following demethylation of the exon 37a locus with consequent binding of the chromatin organizer CCCTC-binding factor (CTCF) to it. Ca$_V$2.1[EFa] localizes in close proximity to fuse-competent synaptic vesicles, thereby supporting effectively vesicle release and presynaptic homeostatic plasticity. Drawing of relative exon/intron length is to scale only in **(Cc)**; numbers of mRNAs and proteins are not intended to be quantitative.

FIGURE 2 | Activity-dependent alternative splicing in homeostatic plasticity. **(A)** A chronic increase in neuronal activity downregulates the expression of the splicing factor nSR100, with consequent skipping of a 16-nt-long microexon in the pre-mRNA of the transcriptional repressor REST (repressor element 1 silencing transcription factor). The resulting REST protein is active and reduces the expression of Na$_V$1.2 and of presynaptic proteins. These two effects contribute to homeostatic intrinsic plasticity and presynaptic homeostatic plasticity, respectively. (*) Indicates a STOP codon. **(B)** The selective induction of the short isoform Homer1a upon increase in neuronal activity is mediated by the transcription factor myocyte enhancer factor 2 (MEF2), which promotes expression of the Homer1 gene, and by a concomitant termination of transcription between exons 5 and 6. Homer1a outcompetes the longer isoforms of Homer1, resulting in dispersion of group 1 mGluRs and dephosphorylation of AMPARs. This contributes to synaptic downscaling. **(C)** Mutually exclusive splicing of P/Q-type Ca^{2+} channels in presynaptic homeostatic plasticity. **(Ca)** Structural model of human Ca$_V$2.1[EFb] (UniProt ID: O00555; Martinez-Ortiz and Cardozo, 2018), highlighting the full C-terminus (green, cyan, blue), the part of the EF-hand-like domain shared between Ca$_V$2.1[EFa] and Ca$_V$2.1[EFb] (E helix; cyan) and the sequence specific to Ca$_V$2.1[EFb] (loop, F helix and downstream residues; blue). **(Cb)** Phylogenetic tree of human Ca$_V$1 and

(*Continued*)

therefore essential in cross-linking multiple postsynaptic proteins. Conversely, the short isoforms of Homer1 lack the C-terminal domain involved in oligomerization; once induced, they act as dominant-negative regulators disrupting the binding between Homer1 long isoforms and their effectors (Xiao et al., 1998; Kammermeier and Worley, 2007).

Increasing network activity, therefore, upregulates transiently the expression of Homer1a, which, among other things, disrupts the protein–protein interactions clustering group 1 mGluRs at perisynaptic sites. In addition, Homer1a acts as an endogenous allosteric modulator of mGluR1/5; that is, it supports a glutamate-independent activity of these mGluRs (Ango et al., 2001). This is essential in promoting homeostatic downscaling of synaptic AMPARs both *in vitro* and *in vivo* (**Figure 2B**; Hu et al., 2010; Diering et al., 2017), as reviewed elsewhere in this topic (Cingolani et al., 2019).

The expression of Homer1a is increased in the CA1 region of the hippocampus in schizophrenic patients (Matosin et al., 2016) and up- or downregulated in different brain regions of patients with either bipolar disorder or major depression (Leber et al., 2017). Furthermore, in *Fmr1* knockout mice, a model for fragile X syndrome, mGluR5 is preferentially associated to Homer1a, leading to an enhanced glutamate-independent activation of this receptor and consequent neocortical circuit dysfunctions and behavioral abnormalities. Some of these defects are rescued by genetic deletion of Homer1a (Giuffrida et al., 2005; Ronesi et al., 2012), which is consistent with brain function being dependent on an appropriate ratio between short and long isoforms of Homer1.

ALTERNATIVE SPLICING OF P/Q-TYPE Ca^{2+} CHANNELS IN PRESYNAPTIC HOMEOSTATIC PLASTICITY

Mutually exclusive splicing is a form of AS, whereby the splicing of two or more exons is coordinated in such a way that

only one is retained while the others are spliced out from the mature mRNA (**Figure 2Cc**). Mutually exclusive exons are generally highly similar possibly because they originated from exon duplication. However, far from being redundant, they usually allow the formation of protein isoforms that differ in the function of specific domains while preserving the overall structure and size. Indeed, mutually exclusive splicing in many genes is spatially and temporally regulated (Pohl et al., 2013). Recent data indicate that mutually exclusive exons may be much more frequent in mammals than previously thought and that they are overrepresented in genes encoding for ion channels (Hatje et al., 2017). Interestingly, the occurrence of pathogenic single-nucleotide polymorphisms (SNPs) in mutually exclusive and cassette exons is significantly higher than in other types of exons, suggesting that these two forms of AS are especially susceptible to pathogenic mutations. For mutually exclusive splicing, the pathogenic SNPs tend to be present in only one of the two possible exons. Thus, the second mutually exclusive exon cannot normally replace the defective one either because of functional diversification or because of differential spatiotemporal expression patterns (Hatje et al., 2017).

A well-characterized case of mutually exclusive exons occurs in the proximal C-terminus of the pore-forming α_1 subunit of the Ca_V2 VGCCs ($Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$; Bourinet et al., 1999; Bell et al., 2004; Gray et al., 2007; Hatje et al., 2017), which serve as primary Ca^{2+} entry for the release of synaptic vesicles at most presynaptic terminals. The 97-nt-long mutually exclusive exons 37a and 37b encode part of an EF-hand-like domain, thus creating two variants of it (EFa and EFb; **Figure 2Ca**; Bourinet et al., 1999; Chaudhuri et al., 2004; Thalhammer et al., 2017). This motif is not specific to Ca_V2 channels but conserved across Ca^{2+} and Na^+ channels (Babitch, 1990; Ben-Johny et al., 2014); in particular, exons 37a and 37b in Ca_V2 channels exhibit a high level of similarity with the corresponding exons in Ca_V1 channels (**Figure 2Cb**).

Which are the functions of the EF-hand-like domain and why do Ca_V2 channels need two variants of it? Three major, not mutually exclusive, functional differences have been proposed. In N-type Ca^{2+} channels ($Ca_V2.2$), mutually exclusive splicing at exons 37 has been shown to regulate sensitivity of the channel to voltage-independent inhibition by G protein-coupled receptors (GPCRs). That is, several GPCRs, including opioid receptors, inhibit $Ca_V2.2$[EFa] but not $Ca_V2.2$[EFb] through kinase phosphorylation of a tyrosine residue (Y1743) present exclusively in the former isoform (Raingo et al., 2007; Andrade et al., 2010). Because $Ca_V2.2$[EFa] is enriched in capsaicin-responsive nociceptors of dorsal root ganglia (Bell et al., 2004), this isoform-specific regulation mediates analgesia, for example, by morphine (Andrade et al., 2010).

In P/Q-type Ca^{2+} channels ($Ca_V2.1$), the two isoforms have been shown to differ in how elevations in intracellular Ca^{2+} regulate the activity of the channel. Specifically, activation of $Ca_V2.1$[EFa], but not $Ca_V2.1$[EFb], is facilitated by preceding Ca^{2+} entry (Ca^{2+}-dependent facilitation, CDF; Chaudhuri et al., 2004). This is in accordance with a large body of evidence indicating that the EF-hand-like domain in the proximal

C-terminus of Ca^{2+} and Na^+ channels, rather than binding directly to Ca^{2+}, represents a general transduction element for the regulation of the channel by Ca^{2+}-calmodulin (Peterson et al., 2000; Ben-Johny et al., 2014; Gardill et al., 2018). Calmodulin itself binds, in a Ca^{2+}-independent manner, to downstream domains in the C-terminus of Ca^{2+} and Na^+ channels (Peterson et al., 1999; Zuhlke et al., 1999; Mori et al., 2000; Erickson et al., 2001).

More recently, experiments in native systems have revealed that the two isoforms of $Ca_V2.1$ regulate neurotransmitter release and short-term synaptic plasticity at hippocampal synapses in opposite directions. While $Ca_V2.1$[EFa] promotes synaptic efficacy and short-term synaptic depression, $Ca_V2.1$[EFb] characterizes synapses with low release probability and prominent short-term synaptic facilitation (Thalhammer et al., 2017). This is contrary to what the isoform-specific CDF, as characterized in non-neuronal cells, would have predicted (Chaudhuri et al., 2004; Weyrer et al., 2019); it likely reflects instead a differential spatial relationship of the two isoforms to fuse-competent synaptic vesicles, with a tight and loose coupling configuration for $Ca_V2.1$[EFa] and $Ca_V2.1$[EFb], respectively (**Figure 2Cc**; Thalhammer et al., 2017). More in general, AS of $Ca_V2.1$ might underlie most of the intra- and inter-synaptic differences in nanoscale topographical arrangements of this channel, as recently revealed (Holderith et al., 2012; Nakamura et al., 2015; Rebola et al., 2019).

Whereas the expression of $Ca_V2.1$[EFb] remains relatively constant throughout postnatal development, that of $Ca_V2.1$[EFa] increases postnatally, in parallel with a tightening of the coupling between VGCCs and the neurotransmitter release machinery. As a result, both isoforms are expressed at similar levels in most regions of the adult brain (Bourinet et al., 1999; Soong et al., 2002; Vigues et al., 2002; Chaudhuri et al., 2004; Thalhammer et al., 2017). The developmental upregulation of $Ca_V2.1$[EFa] occurs in rodents between the second and third postnatal week, the same period when ataxic symptoms become apparent in $Ca_V2.1^{-/-}$ knockout mice (Mark et al., 2011). Further, four point mutations associated with episodic ataxia type II have been identified in the exon 37a of *CACNA1A* (the gene for the α_1 subunit of $Ca_V2.1$) in four unrelated families (Graves et al., 2008; Mantuano et al., 2010), while none has been found, to date, in exon 37b, suggesting that $Ca_V2.1$[EFa] might be more relevant for the etiology of episodic ataxia type II than $Ca_V2.1$[EFb].

At the cellular level, while most neurons express both isoforms to various degrees, parvalbumin interneurons, which rely on P/Q-type Ca^{2+} channels to form synapses characterized by nanodomain coupling, high release probability, and short-term synaptic depression (Eggermann et al., 2012), stand out for expressing exclusively $Ca_V2.1$[EFa] (Huntley et al., 2020), again pointing to functional synaptic specialization of the two $Ca_V2.1$ splice isoforms.

Besides these differences in spatiotemporal expression patterns, the relative synaptic abundance of the two isoforms is regulated by network activity in a homeostatic fashion. Specifically, hippocampal neurons increase exclusively the synaptic expression of $Ca_V2.1$[EFa] in response to activity

deprivation. Because this isoform is the more efficient of the two in driving vesicle release, its higher expression levels appear perfectly suited to counteract the decrease in network activity (**Figure 2Cc**; Thalhammer et al., 2017). These findings provide therefore a precise molecular basis for the involvement of P/Q-type Ca^{2+} channels in presynaptic homeostatic plasticity (Frank et al., 2006; Jakawich et al., 2010; Lazarevic et al., 2011; Zhao et al., 2011; Jeans et al., 2017) and highlight the importance of activity-dependent AS in homeostatic synaptic plasticity.

Although it is not known how network activity regulates this splicing event, it has recently been proposed that inclusion of exon 37a or 37b in $Ca_V2.2$ is consequent to differences in chromatin structure and transcription rates, rather than being directly regulated at the mRNA level (Javier et al., 2019; Lopez Soto and Lipscombe, 2020). Because splicing occurs mostly co-transcriptionally (Luco et al., 2011), rapid transcription of *Cacna1b* (the gene for the α_1 subunit of $Ca_V2.2$) would lead to simultaneous availability to the splicing machinery of the two mutually exclusive exons. Direct competition between them would results in inclusion of the downstream stronger exon 37b. Conversely, a slow transcription rate would favor recruitment of the splicing machinery to the first upstream exon 37a, thus leading to inclusion of this weaker exon into the final transcript. Indeed, the zinc finger DNA-binding protein CCCTC-binding factor (CTCF), a well-known organizer of chromatin architecture, binds to the exon 37a locus of *Cacna1b* to promote inclusion of this exon (Javier et al., 2019; Lopez Soto and Lipscombe, 2020). This is likely because CTCF favors the formation of intragenic chromatin loops and slows down the elongation rate of the RNA polymerase II (Pol II; Shukla et al., 2011; Ruiz-Velasco et al., 2017). Importantly, CTCF binding is not constitutive but prevented by methylation of the *Cacna1b* exon 37a locus, which consequently leads to exon 37a exclusion (Javier et al., 2019; Lopez Soto and Lipscombe, 2020).

Because the methylation level of chromosomal DNA is key to both memory formation and homeostatic synaptic plasticity (Day and Sweatt, 2010; Meadows et al., 2015), it is conceivable that activity-dependent methylation and demethylation might regulate also the inclusion of exon 37a in *Cacna1a* during presynaptic homeostatic plasticity. According to databases of chromatin immunoprecipitation followed by sequencing (ChIP-seq[1], ENCODE Project Consortium, 2012), CTCF binds indeed also to the *Cacna1a* exon 37a locus (**Figure 2Cc**).

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[1]https://screen.wenglab.org/search/?q=CACNa1A&uuid=0&assembly=GRCh38

DISCUSSION: IMPLICATIONS OF ACTIVITY-DEPENDENT ALTERNATIVE SPLICING FOR BRAIN DISORDERS

Genome-wide transcriptomic studies indicate that AS is more prominent in the brain than in other tissues (Yeo et al., 2004; Pan et al., 2008). Accordingly, defects in AS have been implicated in neurological and neurodegenerative disorders (Raj and Blencowe, 2015; Furlanis and Scheiffele, 2018; Montes et al., 2019). AS defects can originate from mutations that alter either *cis*-acting elements on specific genes or *trans*-acting splicing factors affecting the splicing of multiple transcripts. As discussed briefly in this minireview article, the former mutations are prominent in mutually exclusive and cassette exons involved mostly in monogenic brain pathologies such as episodic ataxia type II, the latter are especially critical for multifactorial brain disorders, for example, for autism spectrum disorder (Gehman et al., 2011; Voineagu et al., 2011; Irimia et al., 2014; Quesnel-Vallières et al., 2016; Gonatopoulos-Pournatzis et al., 2018).

In both cases, to fully understand how defective AS alters circuit and brain function, it is important to consider that some AS events in the brain are regulated by network activity and that the outcome of the splicing process can in turn compensate for changes in activity levels, thus establishing negative feedback loops that make brain function especially resilient to damage. Rather than being direct, the effects of defective AS on brain function are therefore likely to be indirectly mediated by deficient or aberrant homeostatic plasticity mechanisms.

Elucidating the interplay between activity-dependent AS and homeostatic plasticity, as well as implementing new technologies, such as genome editing approaches aimed at correcting pathogenic mutations interfering with AS or at rebalancing splice isoform levels (Gapinske et al., 2018; Konermann et al., 2018; Thalhammer et al., 2018; Yuan et al., 2018), will help us to develop new and improved splicing therapies for brain disorders.

AUTHOR CONTRIBUTIONS

AT, FJ, and LC contributed jointly to the manuscript.

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