CellPress

Neuron Review 30 THREE DECADES OF EXCITING NEUROSCIENCE SINCE 1988

Activity-Regulated Transcription: Bridging the Gap between Neural Activity and Behavior

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https://doi.org/10.1016/j.neuron.2018.10.013

Gene transcription is the process by which the genetic codes of organisms are read and interpreted as a set of instructions for cells to divide, differentiate, migrate, and mature. As cells function in their respective niches, transcription further allows mature cells to interact dynamically with their external environment while reliably retaining fundamental information about past experiences. In this Review, we provide an overview of the field of activity-dependent transcription in the vertebrate brain and highlight contemporary work that ranges from studies of activity-dependent chromatin modifications to plasticity mechanisms underlying adaptive behaviors. We identify key gaps in knowledge and propose integrated approaches toward a deeper understanding of how activity-dependent transcription promotes the refinement and plasticity of neural circuits for cognitive function.

Introduction

The cells in each tissue of the body adapt to ever-changing external environments as they serve the essential needs of an animal. Neurons of the brain are no different, but they face a unique challenge relative to other cell types in the body. As postmitotic cells, neurons must remain highly adaptable and dynamic throughout the lifetime of an animal while also reliably encoding both short- and long-term memories of the animals' experiences. Whereas most tissues in the body continuously grow and regenerate through cell division, neurons coordinately use their genome and synapses to store the requisite information and perform computations on demand yet also adapt as new experiences and stimuli are encountered throughout their lifetime.

Neurons have thus evolved multiple interlinked strategies to both respond dynamically to their immediate environment and store information stably. One strategy that has been extensively characterized over the last half century involves the neurons' utilization of the richness and diversity of their synaptic properties to maximize the magnitude and types of information that they can retain. Substantial progress has been made in our understanding of how neurons—via their synapses—can be modified to store information on timescales in the order of tens of milliseconds to an hour (Zucker and Regehr, 2002). However, considerably less is understood about how synapses store information for longer periods of time, as is often required for learning of a specific task or memory formation and consolidation. To store information for long periods of time, neurons appear to have evolved an additional strategy, which involves the coupling of their synapses to another key information hub, the nucleus.

The coupling of synaptic activity to the nucleus serves several critical purposes. It promotes new gene transcription and induces modifications of the DNA itself, the latter of which are often referred to as epigenetic phenomena. Gene transcription produces the mRNA transcripts necessary for new protein synthesis. A long-standing view of how experiences are stabilized over time posits that new mRNA and protein synthesis must occur to provide substrates for the structural and functional modifications of synapses that support memory consolidation (Hernandez and Abel, 2008). Importantly, this neuronal activity-dependent transcription is in addition to, and should be distinguished from, the basal gene expression that already occurs in the cell to replenish proteins that are degraded over time. This distinction is essential for our understanding of how experience sculpts the brain. For example, in establishing the role of new mRNA and protein synthesis for memory consolidation, early studies utilized pharmacological inhibitors of transcription and mRNA translation. While these studies have been pivotal in establishing our view of how activity-dependent gene expression supports higher-order cognitive functions, the fact that these inhibitors shut down the basal gene expression machinery that is critical for the standard operation of a cell have clouded interpretations of these findings. In a similar vein, many genes whose expression is induced in response to neuronal activity (described below) are often also expressed to some extent in the basal state. This has complicated attempts to ascertain the role of activity-dependent gene expression in circuit plasticity, as essentially all loss- or gain-of-function manipulations to date have also invariably affected the basal level of expression of these genes. Therefore, novel approaches and technologies that dissect the specific roles of activity-regulated processes will be essential to understand the mechanistic basis of long-term information storage in the nervous system and how these processes go awry in neurological disorders.

It is noteworthy that despite more than three decades of substantial progress in the field of activity-dependent gene transcription, we are only beginning to understand how this process gives rise to behavioral adaptations. While experience-dependent transcription has been shown to regulate numerous cellular processes critical for the development and plasticity of the central nervous system, the evidence that activity-dependent

transcription is indeed necessary for instructing changes to dynamic behavioral states at the organismal level, though promising, is in its infancy. In the next decade, application of single-cell transcriptomic and epigenomic analyses, novel mouse genetic and viral tools, CRISPR/Cas9 gene editing, and two-photon microscopy for chronic imaging of neurons in awake-behaving animals under various behavioral contexts should facilitate a great leap in knowledge in this area. These methods should allow us to concurrently characterize how the activity-dependent gene transcription program contributes to the emergence and regulation of ensembles of behaviorally relevant neurons within specialized networks and how the dynamics of these active ensembles in turn form the basis of various learned behaviors.

The significance of this endeavor cannot be overstated, especially in light of the growing body of evidence indicating that neurodevelopmental and neuropsychiatric disorders, including autism spectrum disorders, schizophrenia, intellectual disability, major depressive disorders, and addiction, are either linked to mutations in components of the activity-dependent transcriptional pathways (Ebert and Greenberg, 2013; Nestler et al., 2016) or associated with genetic variants that map to non-coding regulatory regions in the genome. Indeed, large-scale genome-wide association studies (GWAS) have revealed a significant enrichment of common risk variants in *cis*-regulatory elements (Maurano et al., 2012; Xiao et al., 2017), the accessibility of many of which appears to be dependent on activity-dependent transcription factors (Maurano et al., 2015). Together, these observations point to alterations in activity-dependent transcription as key biological mechanisms underlying diseased states of the nervous system. In addition to insights from disease-risk variants, recent comparative studies have uncovered augmentations to the activity-dependent transcriptional programs in higher-order primate neurons that may contribute, at least in part, to the advanced cognitive abilities of humans. Remarkably, these evolutionary adaptations have been shown to arise from the acquisition of activity-dependent non-coding regulatory sequences in the primate genome (Ataman et al., 2016; Hardingham et al., 2018), once again underscoring the critical role of signal-dependent regulatory elements and activity-dependent transcription in the evolution of expanded cognitive capacities in humans.

In this Review, we begin by providing an overview of the field of activity-dependent gene transcription that led to the emergence of our current understanding of how sensory experience sculpts the developing and adult brain. We then discuss recent work that sheds light on how the delicate interplay between the neuron's transcriptome and epigenome within the context of neural circuits gives rise to cellular processes that manifest in behavioral adaptations. Finally, we look to the future and explore lines of investigation and technological advances that promise to break open a new and paradigm-shifting era that hopefully will allow us to obtain a deeper understanding of how activity shapes the brain. We argue that the study of neuronal activity-dependent gene expression serves as a point of convergence for molecular neuroscience and emerging systems-level framework for understanding information processing and storage in neural circuits.

A Molecular Perspective

Immediate Early Gene Induction as First Signals for Long-Term Adaptations

To facilitate discussion of the underlying principles of activity-dependent transcriptional control of neural circuit form and function, we begin with a focus on the canonical immediate early gene *Fos*. Discovered by Greenberg and Ziff in 1984 (Greenberg and Ziff, 1984), the rapid and transient induction of *Fos* transcription provided the first evidence that mammalian cells could respond to the outside world within minutes by means of rapid gene transcription, in particular through the activation of specific genes (Cochran et al., 1984; Greenberg et al., 1985, 1986; Kruijer et al., 1984; Lau and Nathans, 1987; Müller et al., 1984). At the time, the idea that *trans*-synaptic signals regulate the activity or synthesis of certain neuropeptides and enzymes was recognized (Black et al., 1985; Chen et al., 1983; Zigmond and Ben-Ari, 1977; Zigmond and Mackay, 1974), but this regulation had been found to occur over a period of days, and it was unclear whether the observed effects were mediated by transcriptional or post-transcriptional mechanisms. Moreover, it was known at the time that sensory stimulation is accompanied by an increase in RNA synthesis (Berry, 1969; Kernell and Peterson, 1970) and that long-term changes to synapses occur in response to various forms of learning in an RNA- and protein-synthesis-dependent manner (Glassman, 1969; Goelet et al., 1986; Schwartz et al., 1971). Therefore, the discovery that synapses communicate rapidly with the nucleus by activating the transcription of *Fos* represented a fundamental advance because it provided the first mechanistic framework by which to understand the molecular and cellular events underlying these learning-induced long-term synaptic changes. The fact that the induction of *Fos* transcription is a widespread event that occurs in many different cell types (Sheng and Greenberg, 1990), coupled with the finding that the *Fos* gene encodes a nuclear protein (Curran et al., 1984), immediately prompted the speculation that Fos, by activating subsequent programs of gene transcription, might mediate cell-type-specific functions, including long-term adaptations that underlie learning and memory in neurons.

As the idea that specific transcriptional events are rapidly activated by synaptic transmission became established, subsequent years saw a flurry of studies characterizing the immediate early gene (IEG) program of signal-dependent transcription. IEGs are defined as a class of genes that, like *Fos*, is rapidly and transiently induced by extracellular stimuli, without a requirement for new protein synthesis. Many IEGs encode sequence-specific DNA-binding proteins that function as transcription factors (TFs) and regulate a subsequent wave of late-response gene (LRG) expression, which is now known to be cell-type specific and tailored to the specific function of the cell within a neural circuit (Mardinly et al., 2016; Sheng and Greenberg, 1990). For the purpose of discussion, we refer to LRGs as targets of IEG TFs, although there are delayed-response genes that do not appear to require *de novo* transcription for their expression (Tullai et al., 2007). Early on, several questions arose regarding IEGs in the brain: (1) what are the signaling mechanisms by which neurotransmitters drive the induction of IEGs, (2) what are the IEG TF-regulated LRGs, (3) how does a common set of IEG TFs regulate cell-type-specific

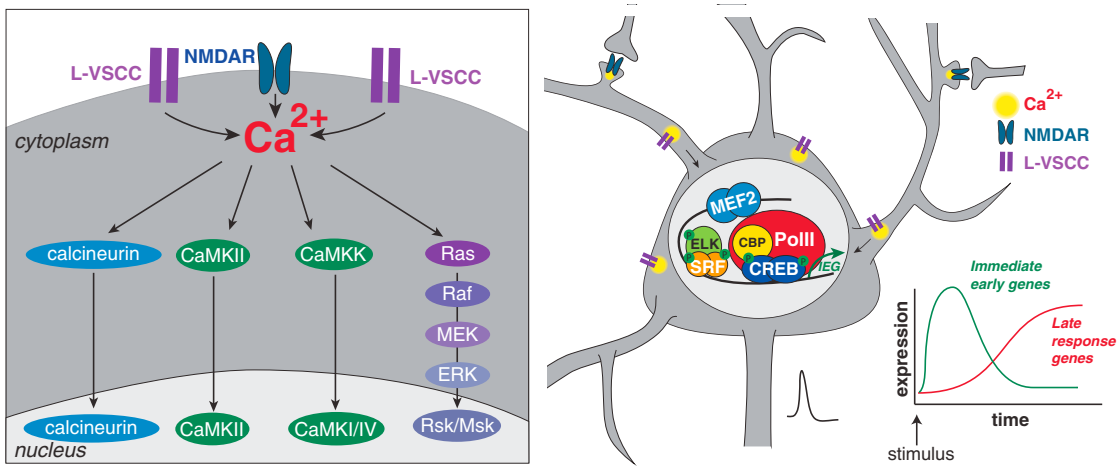

Figure 1. Schematic of Signaling Mechanisms Driving Activity-Dependent Transcription of Immediate Early Genes and Late-Response Genes

Neurotransmitter signaling leads to the generation of action potentials in the neuron. Membrane depolarization induces the opening of L-type voltage-sensitive calcium channels (L-VSCCs). Stimulus-dependent calcium entry via L-VSCCs preferentially leads to the activation of the Ras-MAPK pathway, calcium/calmodulin-dependent protein kinases, and calcineurin-dependent signaling. Note that calcium influx can also occur via activation of NMDA receptors. Decades of work from numerous laboratories have elucidated the molecular mechanisms of these calcium-dependent signaling cascades, which have been simplified in this schematic. Cell-type-specific differences in signaling mechanisms that are not illustrated here have also been described (e.g., see Cohen et al., 2016). These pathways lead to the activation of pre-existing transcription factors CREB, SRF/ELK, and MEF2, which regulate the expression of immediate early genes (IEGs) such as *Fos*. Many IEGs encode transcription factors that regulate a subsequent wave of late-response genes, which have now been shown to be cell-type specific.

LRGs, and (4) what cellular processes do the activity-dependent gene programs regulate? While great strides have been made in addressing the first question, the latter questions have seen relatively slower progress and are the major focus of this Review.

Over the years, work from a number of laboratories has elucidated the signaling mechanisms that drive the activation of IEGs, including *Fos* (Figure 1). Neurotransmitter-dependent induction of IEGs invariably requires an influx of extracellular calcium into the neuron. The resulting increase in cytoplasmic calcium stimulates a cascade of signaling events, including the activation of the Ras-mitogen-associated protein kinase (MAPK), calcium/calmodulin-dependent protein kinases (CaMKs), and calcineurin-mediated signaling pathways (Bito et al., 1996; Hardingham et al., 1997; Sheng et al., 1991; Xing et al., 1996). In addition to mediating local changes at synapses, such as the surface expression or internalization of glutamate receptors, local mRNA translation, and post-translational modifications of proteins (Holt and Schuman, 2013; Martin and Zukin, 2006; Thomas and Huganir, 2004; Wayman et al., 2008), activation of these signaling cascades induces the transcription of activity-regulated genes. Importantly, IEG induction can be triggered by calcium influx through ligand-gated ion channels, such as the N-methyl-D-aspartate-type (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate-type (AMPA) glutamate receptors, and voltage-gated calcium channels, as well as through the release of calcium from intracellular stores (West et al., 2001). However, various studies have shown that, in particular, calcium entry through the L-type voltage-sensitive calcium channels (L-VSCCs) preferentially drives gene transcription. This is thought to be due to the localization of L-VSCCs in cell bodies and proximal regions of dendrites (Westenbroek et al., 1990) and thus their relative proximity to the nucleus, as well as their calcium conductance and gating properties (Simms and Zamponi, 2014; Wheeler et al., 2012) and their physical association with signaling molecules (e.g., calmodulin) important for driving transcription (Deisseroth et al., 1998; Dolmetsch et al., 2001; Ma et al., 2014). With the advent of next-generation genetically encoded calcium indicators, further characterization of the source of calcium fluctuations and the ionic concentrations and time courses that drive activity-dependent transcription in awake-behaving animals is likely to provide deeper insight into the mechanisms of calcium-dependent gene transcription.

The extremely rapid induction of *Fos* and other IEGs suggests that their activation is not dependent on protein synthesis but instead relies on pre-existing transcription factors that are activated rapidly and then drive IEG transcription. Indeed, these constitutively expressed TFs have been identified and include the cyclic adenosine monophosphate (cAMP)-responsive element binding protein (CREB), serum response factor (SRF), and myocyte enhancer factor 2 (MEF2), the former two of which have been shown to control *Fos* transcription (Norman et al., 1988; Sheng et al., 1988). Since CREB, SRF/ELK, and MEF2 are constitutively expressed rather than induced in response to neuronal activity, their activation is dependent instead on their ability to integrate signaling from multiple calcium-dependent pathways and undergo post-translational modifications, such as phosphorylation (Aizawa et al., 2004; Chawla et al., 1998; Deisseroth et al., 1996; Flavell et al., 2006; Janknecht and Nordheim, 1992; McKinsey et al., 2002; Rivera et al., 1993; Shalizi et al., 2006). The literature on the signaling mechanisms that trigger the transcription of activity-regulated genes is impressively rich and has been comprehensively reviewed elsewhere (Benito and Barco, 2015; Deisseroth and Tsien, 2002; Flavell and Greenberg, 2008; Hagenston and Bading, 2011; Lonze

and Ginty, 2002). Importantly, mutations in components of these signaling pathways lead to developmental neurological disorders, including Timothy syndrome (i.e., L-VSCC), Coffin-Lowry syndrome (i.e., ribosomal S6 kinase 2), and Rubenstein-Taybi syndrome (i.e., CREB-binding protein CBP) (Ebert and Greenberg, 2013; Hong et al., 2005; Mullins et al., 2016). These findings underscore the need for continued molecular characterization of the activity-dependent transcriptional machinery to facilitate our understanding of neurological disorders from the perspective of neural circuits (Südhof, 2017).

Once in the nucleus, Fos, together with its partner Jun, form the major heterodimer of the activating protein complex 1 (AP-1). Additional members of the AP-1 family of TFs include Fosb, Fosl1, and Fosl2 and Junb and Jund, any of which can substitute for Fos or Jun, respectively, to form the AP-1 heterodimer (Sheng and Greenberg, 1990). The biological basis of the high level of redundancy within this complex TF family and the specific functions of its individual members remain to be determined. In addition to members of the AP-1 family, IEGs, including the Egr and Nr4a family of TFs, as well as the neuronal-specific TF Npas4, are activated in neurons in response to activity (Christy and Nathans, 1989; Lin et al., 2008; Milbrandt, 1988). The advent of chromatin immunoprecipitation (ChIP) and RNA sequencing has enabled the identification of the genome-wide binding sites of these TFs and revealed specific LRGs regulated by these activity-dependent TFs. Based on genome-wide assessments, there are an estimated 10^4 binding sites for activity-dependent TFs, such as Fos and Npas4, and an estimated 300–500 LRGs regulated by these TFs in neurons (Benito and Barco, 2015; Kim et al., 2010; Malik et al., 2014; Mardinly et al., 2016).

LRGs typically encode effector proteins that regulate cellular processes such as dendritic growth, spine maturation, synapse elimination, and the development of proper excitatory/inhibitory balance (West and Greenberg, 2011). Although considerable progress has been made in describing the molecular and cellular functions of individual LRGs (Leslie and Nedivi, 2011), the sheer number of LRGs that remain to be characterized indicates that additional work will be required to determine the functions of the activity-dependent gene program as a whole. To probe the function of this gene network, one approach has been to disrupt the activity of individual TFs, such as CREB, SRF, MEF2, or IEG TFs, and assess the effects on aspects of neuronal function. However, the functional redundancy of these TFs and the compensatory effects that arise when the function of individual TFs is disrupted, as well as the emerging evidence of cooperativity among many of these TFs (Kim et al., 2010), have presented challenges to these lines of investigation. To begin to tackle these challenges, the concurrent manipulation of a larger number of related TF family members with either classical genetic knockout approaches or novel multiplexed CRISPR-based perturbations (described below) will be critical. Moreover, these challenges underscore the need for new strategies to uncover how each of the activity-dependent TFs may have evolved specific roles in mediating transcription, either through distinct functions at specific regulatory elements across the genome, or by conferring specificity through cooperative action with other TFs.

Diverse Activity-Dependent Gene Programs for Diverse Cell Types

The mammalian brain is populated by numerous cell types with distinct anatomical, electrophysiological, and transcriptomic identities. An emerging hypothesis is that these distinct features of cell types influence the coupling of neuronal activity with transcription, resulting in distinct transcriptional responses to activity. However, early studies of activity-dependent transcription were limited in scope due to the lack of cell-type resolution and the use of pharmacological methods to induce neuronal activity (Lin et al., 2008; Majdan and Shatz, 2006). Several recent studies have revealed that the activity-dependent transcriptome is neuronal subtype-specific both in cell culture and when analyzed *in vivo*. Using RNA sequencing to identify activity-dependent transcripts in cultures of embryonic excitatory or inhibitory neurons, one particular study (Spiegel et al., 2014) demonstrated that immediately following stimulation (within 60 min), both excitatory and inhibitory neurons express largely overlapping sets of genes, which are enriched for the classically known activity-dependent transcription factors (e.g., *Egr1*, *Fos*, *Fosb*, and *Npas4*). However, at later time points (>120 min after stimulation), excitatory and inhibitory neurons begin to express divergent patterns of gene expression. These findings were reinforced by subsequent work that used ribosome tagging (Sanz et al., 2009) to isolate mRNAs from specific neuronal subtypes *in vivo* (Mardinly et al., 2016), which further revealed unique activity-dependent gene programs in three different inhibitory neuronal subtypes, the parvalbumin (PV)-, somatostatin (SST)-, and vasoactive intestinal peptide (VIP)-expressing interneurons of the forebrain.

Despite these advances, significant challenges to our understanding of the diversity of activity-dependent transcription remain. One challenge is that currently available genetically defined mouse lines, although numerous and varied with respect to neuronal subtype, do not comprehensively span the universe of cell types in the brain. However, the advent of single-cell RNA-sequencing (scRNA-seq) technologies (Klein et al., 2015; Macosko et al., 2015) has led to the identification of the full spectrum of known neuronal subtypes and has illuminated the transcriptomes of previously unappreciated or inaccessible cell types in multiple regions of the nervous system (Zeisel et al., 2015). Building upon this technological advance, several recent studies in the cortex, amygdala, and hippocampus have begun the task of comprehensively characterizing the experience-dependent transcriptomes in all cell types (Hrvatin et al., 2018; Hu et al., 2017; Lacar et al., 2016; Wu et al., 2017) (Figure 2A). In addition to identifying divergent transcriptional responses in inhibitory neuron subtypes and excitatory neurons in different laminar zones of the cortex, these studies identified a largely unexplored response to extracellular stimuli in non-neuronal cells (e.g., oligodendrocytes, endothelium, microglia, astrocytes, pericytes, and macrophages) of the nervous system (Figure 2B). The emergence of a rich landscape of stimulus-dependent transcriptomes in diverse neuronal and non-neuronal cell types reveals new avenues for understanding the role of experience-dependent transcription within each region of the brain. For example, analyses of stimulus-dependent transcriptional responses in the endothelium, oligodendrocytes, and astrocytes

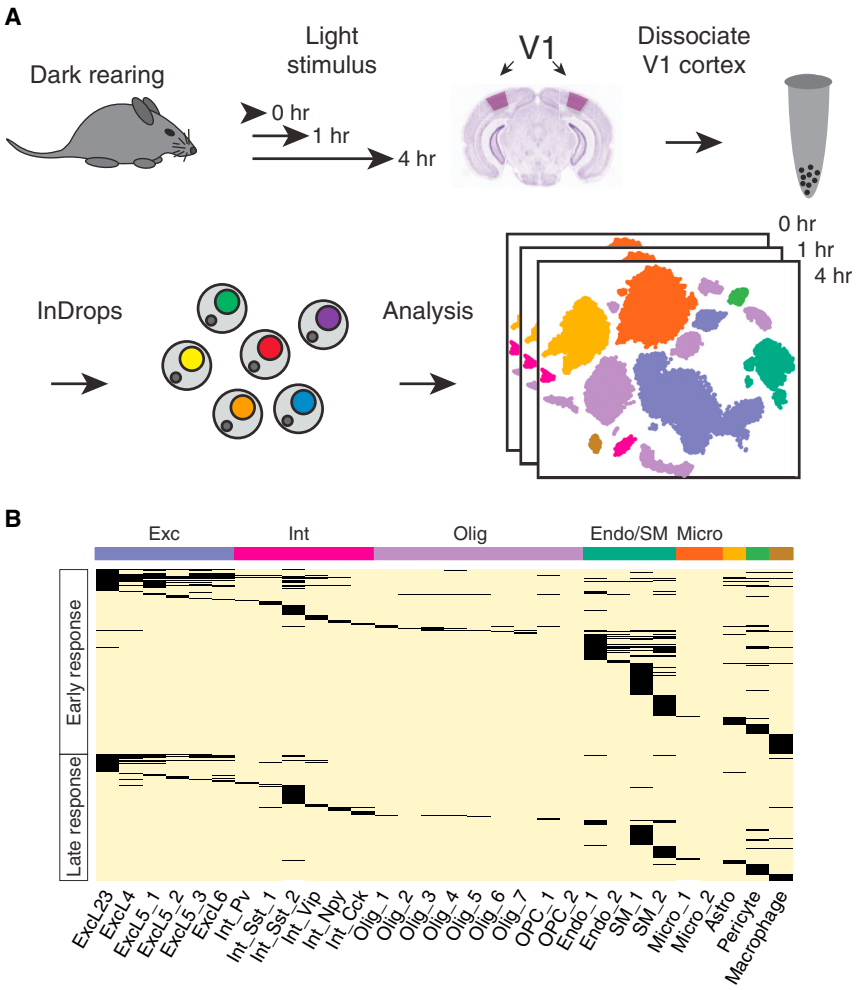

Figure 2. Cell-type Specificity of the Activity-Dependent Gene Programs
(A) Single-cell RNA-sequencing technologies have enabled unprecedented characterization of the diversity and cell-type specificity of experience-dependent transcriptomes in the brain.
(B) Divergent activity-dependent transcriptional responses in neuronal and non-neuronal cell types depicted by heatmap of 611 stimulus-regulated genes (horizontal black lines) grouped into early-response and late-response genes by cell type. Exc, excitatory neurons; Int, interneurons; Olig, oligodendrocytes; endo/SM, endothelium, smooth muscle; Micro, microglia. This figure is adapted with permission from Hrvatin et al. (2018).

have uncovered many new experience-regulated genes of as yet unknown function. Activation of these non-neuronal cells may be a secondary consequence of neurotransmitter-mediated signaling, such as the release of neurotrophic factors and neuromodulators from neurons or changes in blood flow or oxygen levels (Attwell et al., 2010; Mount and Monje, 2017). Additional studies of these non-neuronal activity-dependent genes will likely reveal new mechanisms of structural and functional communication between neurons and non-neuronal cells that underlie neurovascular coupling, myelination, and neurotransmitter reuptake (Andreone et al., 2015; Barres, 2008; Purger et al., 2016).

As a complementary approach to scRNA-seq, several high-throughput methodologies that enable the spatial resolution of a large number of RNA transcripts within the same cells (e.g., FISSEQ, MERFISH, and STARmap) (Chen et al., 2015a; Lee et al., 2015; Wang et al., 2018) may soon transform the study of neuronal activity-dependent transcription, especially as these technologies become increasingly optimized for the localization of RNA molecules to specific subcellular compartments, including distal dendritic regions. The ability to spatially localize thousands of nascent activity-regulated transcripts in specific cell types with subcellular resolution, within the context of a larger anatomical region, has the potential to clarify the relationship between transcriptional regulation and local translation at specific synapses in response to neuronal activity (Poo et al., 2016).

Developmental Specification of Cell-type-Specific Activity-Dependent Gene Programs

The discovery that activity-dependent gene programs are cell-type specific not only has profound implications for the functional diversity of neural circuits but also raises the question of how this diversity of activity-dependent transcriptional responses becomes specified during cellular differentiation. In particular, the question of how a stereotyped set of IEG TFs induced in virtually all mammalian cell types activates a unique set of LRGs in each cell type in the brain has garnered significant interest.

Recent progress toward understanding how cell-type specificity of activity-dependent gene transcription is achieved has come from studies of Fos/Jun heterodimers. The investigation of Fos function gained momentum in the 1990s when it was revealed that this family of nuclear proteins interacts with members of the Jun family via a hydrophobic dimerization motif, termed the leucine zipper, to form a positively charged DNA-binding domain that selectively interacts with the AP-1 consensus sequence, 5′-TGA(C/G)TCA-3′ (reviewed in Sheng and Greenberg, 1990). Studies with reporter genes in transient transfection assays suggested that Fos/Jun heterodimers primarily bound to AP-1 sites within promoters of their target genes to activate transcription (Eferl and Wagner, 2003). Based on these findings, the prevailing view in the literature was that Fos/Jun complexes might bind to different promoters in each cell type to regulate cell-type-specific programs of gene expression. However, over the last several years, genome-wide approaches for mapping TF binding sites revealed that Fos/Jun complexes bind instead to gene distal enhancer elements (Malik et al., 2014). It is now appreciated that enhancers enable the fine-tuning and spatio-temporal control of gene expression levels (Long et al., 2016). Indeed, recent studies have indicated that enhancers, rather

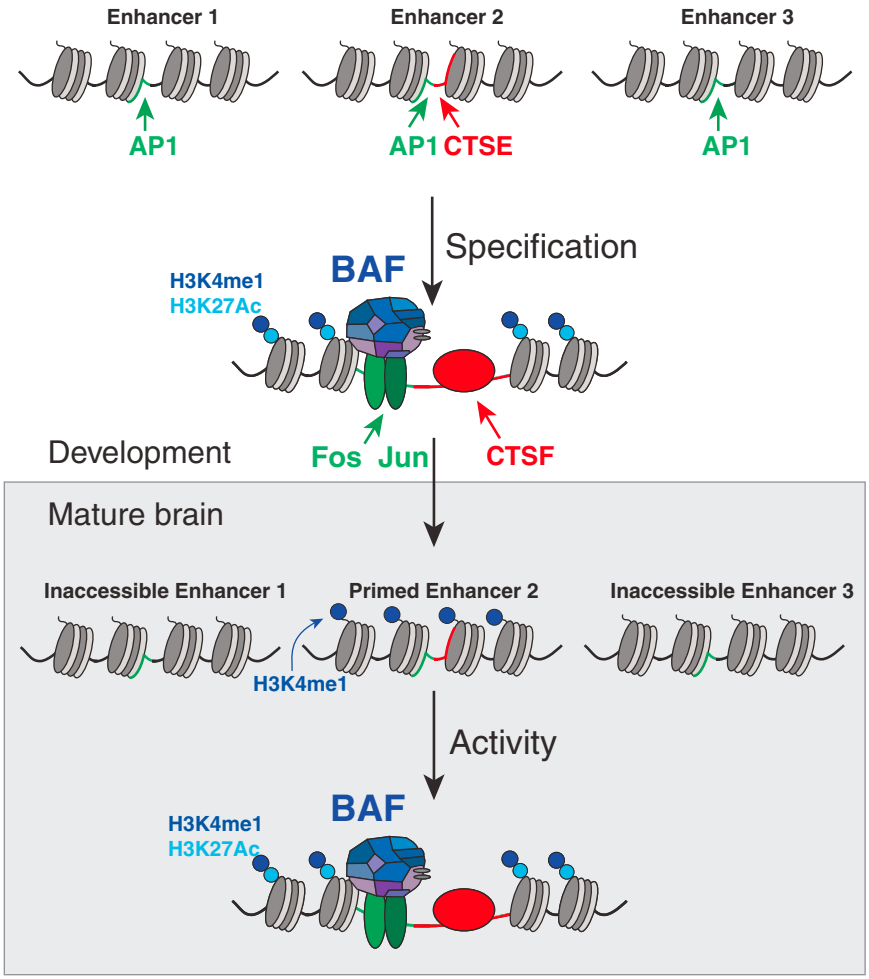

Figure 3. Model of Developmental Specification of Cell-type-Specific Activity-Dependent Gene Programs by AP-1 and Cell-type-Specific Pioneer Factors

(Top) Current model of stimulus-dependent enhancer selection posits that during differentiation of a given cell type, AP-1 sites across the genome are occluded by nucleosomes and thus inaccessible. Adjacent to each AP-1 site, it is often possible to identify additional sequence motifs that are more cell-type specific (CTSE, cell-type-specific element) and predicted to bind cell-type-specific so-called pioneer factors (CTSFs). Once Fos/Jun complexes are expressed in response to extracellular stimuli, they most likely cooperate with CTSFs to recruit the chromatin remodeling BAF complex to cell-type-specific enhancer elements. This leads to chromatin remodeling, enhancer selection, and thus activation of late-response gene transcription in a cell-type-specific manner.

(Bottom) Once these cell-type-specific enhancers have been specified during development, even after Fos and Jun decay away, the enhancers are thought to remain primed via specific post-translational histone modifications. These enhancers are then ready for activation in the mature brain the next time Fos/Jun complexes are induced in response to neuronal activity.

than promoters, are most often the gene regulatory elements that confer the cell-type specificity of gene expression during development and in mature organisms (Heintzman et al., 2009; Long et al., 2016).

In addition to the realization that Fos/Jun complexes predominantly bind to enhancers, recent work has demonstrated that even though across the mammalian genome there are approximately 10^6 AP-1 binding sites in each cell type, Fos/Jun complexes bind to only approximately 10^4 of these sites (Kundaje et al., 2015; Vierbuchen et al., 2017). When the binding of Fos/Jun complexes was analyzed in different cell types, it became clear that upon activation by extracellular stimuli, these complexes bind to almost completely distinct repertoires of enhancers in each cell type (Malik et al., 2014; Vierbuchen et al., 2017). Taken together, these data suggest a model in which AP-1 factors may be involved in the specific selection of an enhancer repertoire in each cell type during mammalian development to determine the cell-type specificity of activity-dependent gene expression.

The mechanisms by which AP-1 factors select enhancers in each cell type are beginning to be uncovered (Heinz et al., 2013; Vierbuchen et al., 2017). Although the consensus AP-1 binding sites are present in virtually all cell types, adjacent to each site, it is often possible to identify additional DNA sequence motifs that are more cell-type specific and predicted to bind so-called pioneer TFs that, in contrast to Fos/Jun complexes, are expressed in a more cell-type-specific manner. As the genetic ablation of Fos/Jun TFs has been challenging due to redundancy within the AP-1 family of TFs, further insight into the role of AP-1 in enhancer selection has come from comparisons of enhancers in fibroblasts and macrophages from distinct genetically divergent inbred mouse strains, which contain tens of millions of single nucleotide polymorphisms (SNPs) across their genomes (Heinz et al., 2013; Link et al., 2018; Vierbuchen et al., 2017). These experiments identified hundreds of SNPs within enhancers that led to loss of enhancer activity in one mouse strain, but not the other. Unexpectedly, SNPs that disrupted the AP-1 binding site were the most frequently observed mutations in these enhancers (Vierbuchen et al., 2017), suggesting that AP-1 TF binding is required for the selection of these enhancers.

These and additional findings have led to a model of stimulus-dependent enhancer selection that posits that early during the differentiation of a given cell type, most late-response gene enhancer sequences are wrapped around histones to form nucleosomes and are therefore not accessible to Fos/Jun heterodimers (Figure 3, top panel). However, once expressed in response to extracellular stimuli, Fos/Jun complexes most likely cooperate with cell-type-specific pioneer TFs to evict nucleosomes at specific enhancers in each cell type, thus rendering them primed for subsequent activation. Interestingly, AP-1 factors were found to interact directly with the SWI/SNF

ATP-dependent chromatin remodeling complex (also referred to as the Brg1-associated factor [BAF] complex) (Vierbuchen et al., 2017), a 12–15 subunit complex that can evict or move histone octamers from enhancer sequences, thus promoting the binding of additional TFs.

In the brain, the pioneer TFs that work together with AP-1 factors to select enhancers in a neuronal subtype-specific manner to bring about unique activity-dependent transcriptional responses in each neuronal subtype should be the subject of future investigation. Candidate TFs based on motif analyses of active enhancers in hippocampal neurons include the bHLH factors and members of the Egr family (Su et al., 2017; Vierbuchen et al., 2017). Another future avenue of investigation pertains to the role of the BAF complex in the brain. The composition of BAF subunits has been shown to be unique in neurons (Wu et al., 2007), thus providing a possible means for specialization of transcriptional responses to neuronal activity. Notably, there is now mounting evidence that mutations of at least eight of the subunits of the BAF complex can lead to intellectual disability or autism spectrum disorders in humans (Ronan et al., 2013). However, future experiments will be critical to determine whether activity-dependent gene programs are deregulated in the absence of BAF and to identify the specific consequences of this deregulation in the pathogenesis of these disorders.

As genes that are critical for brain function tend to have multiple enhancer elements with redundant functions, ascribing function to individual enhancers in the brain is a significant challenge. Therefore, while there is presently no direct evidence that individual activity-dependent enhancers are required for neural circuit development or function, evidence is accumulating that this is likely to be the case. Perhaps the most compelling insight has come from genetic studies indicating that disease-associated non-coding variants tend to be found within *cis*-regulatory elements and not protein coding sequences (Maurano et al., 2012). Intriguingly, a recent large-scale study of accessible *cis*-regulatory elements across multiple human tissue types further revealed that SNPs within AP-1 motifs are a common cause of changes in chromatin accessibility (Maurano et al., 2015). Taken together, these findings suggest that activity-regulated AP-1 TFs are critical for enhancer selection and activation of LRG transcription in a cell-type-specific manner. The next decade of research will undoubtedly continue to inform this model and lend further mechanistic insight into the process of experience-dependent brain development and the pathogenesis of various neuropsychiatric disorders.

Neuronal Activity Shapes the Brain's Epigenome

In the previous section, we have discussed how extracellular stimuli, through inducible transcription factors, regulate the selection of enhancers to give rise to diverse activity-dependent gene programs. Once cell-type-specific enhancers have been commissioned during development, the short-lived Fos/Jun heterodimers decay away and the enhancers again become occluded by nucleosomes. Nevertheless, the selected enhancers appear to remain primed for activation, such that re-induction of Fos/Jun leads to recruitment of BAF to remodel the nucleosomes and enhance chromatin accessibility for activation of gene transcription in mature neurons (Vierbuchen et al., 2017; Wu et al., 2007) (Figure 3, bottom panel).

In addition to recruiting chromatin remodeling complexes, neuronal activity has also been shown to regulate chromatin accessibility by controlling the turnover and exchange of histones (Maze et al., 2015; Yang et al., 2016; Zovkic et al., 2014). For example, once activity-regulated genes are induced, they must be turned off again. A recent study suggests that this process is mediated by recruitment of the nucleosome remodeling and deacetylase complex (NuRD) to promoters, where it promotes a change in histone H2A.z composition, resulting in the shutoff of activity-dependent transcription, which appears to be crucial for dendritic pruning and sensorimotor neural coding (Yang et al., 2016).

While activity-regulated changes in gene transcription are relatively short-lived, occurring over a period of hours, learned behaviors and memories can persist for weeks to months in mice and for years to decades in humans. This leaves open the question of how information is stored in the neuron for extended periods of time after neuronal activity has subsided and activity-dependent gene expression has returned to its basal level. One possibility is that these transient changes in gene expression lead to changes in synaptic connectivity that are somehow stabilized so that they last well beyond the period of inducible gene expression. Another relatively nascent but emerging hypothesis is that neuronal activity, in addition to inducing gene expression via the mechanisms described above, can trigger long-lasting modifications of the genome that in turn affect the gene expression profile of the neuron, permitting the encoding of a memory of prior experiences within the neuronal genome itself. Specifically, the deposition or removal of cytosine methylation across the neuronal genome have been postulated to fulfill this function (Bird, 2002).

Until recently, most studies of the function of cytosine methylation in the brain have focused on methylation at CG (mCG) sequences. Early studies examining whether changes in DNA methylation occur during learning revealed changes in mCG patterns at a number of activity-regulated genes, including *Brain-derived neurotrophic factor* (*Bdnf*) and *Activity-regulated cytoskeleton-associated protein* (*Arc*) (Lubin et al., 2008; Miller and Sweatt, 2007). In addition, these studies demonstrated that pharmacological inhibition of DNA methyltransferases (DNMT), the enzymes responsible for depositing methylation across the genome, leads to alterations in the mCG states of specific genes and is correlated with impaired memory formation (Levenson et al., 2006). Despite these intriguing initial findings, it remains to be determined whether these correlations between the methylation state of individual activity-regulated genes and behavioral outputs are causally related.

The advent of newly developed whole-genome sequencing methods for assessing cytosine methylation at single-base pair and single-cell resolution has allowed for several additional key discoveries to be made (He and Ecker, 2015; Luo et al., 2017). Perhaps, most importantly, these analyses revealed that neurons accumulate high levels of mCA during the first few weeks of postnatal mouse development when sensory experience is actively promoting the refinement of neural circuits. In humans, these non-CG methylation marks gradually accumulate in neurons during the first two decades of life (Lister et al., 2013). In particular, mCA is deposited across the transcribed regions of

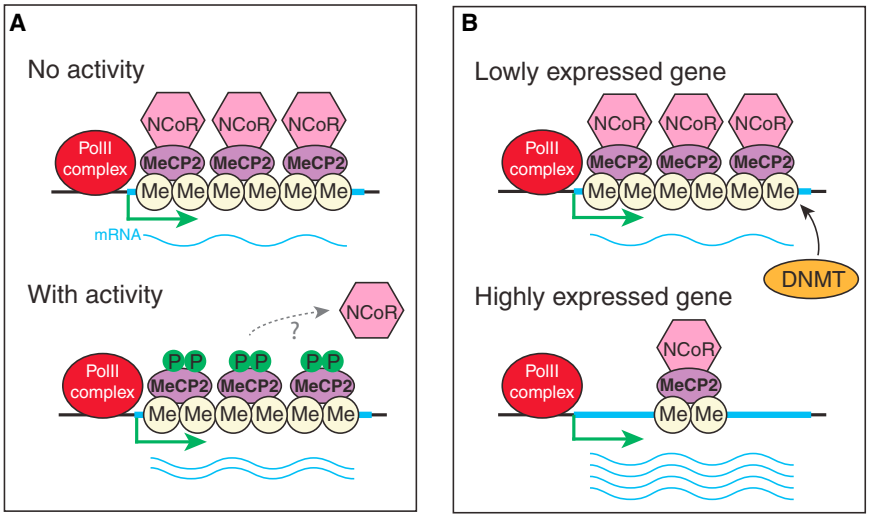

Figure 4. Model of MeCP2-Dependent Regulation of Gene Repression

(A) Simplified schematic showing that in the absence of neuronal activity, MeCP2, a reader of mCA, acts as a repressor of gene transcription in part through the recruitment of repressive co-factors (e.g., NCoR complex). In response to neuronal activity, MeCP2 is rapidly phosphorylated at multiple sites. This modification of MeCP2 potentially leads to the regulated release of NCoR, thus relieving gene repression.

(B) MeCP2 loss-of-function models suggest that MeCP2 represses highly methylated genes that contain a large number of mCA sites within these genes. DNA methyltransferases (DNMTs) deposit methylation across the genome.

lowly expressed genes in a neuronal subtype-specific manner (Mo et al., 2015; Stroud et al., 2017). At these genes, readers of mCA, such as the methyl-CpG binding protein 2 (MeCP2), are found to act as repressors of gene transcription in part through the recruitment of histone deacetylases (Chen et al., 2015b; Ebert et al., 2013; Gabel et al., 2015; Lagger et al., 2017; Lyst et al., 2013; Sugino et al., 2014), though much work remains to be done to clarify the mechanisms by which MeCP2 represses gene expression. Interestingly, mutations in MeCP2 give rise to Rett syndrome in humans and also lead to significant neuronal dysfunction and behavioral abnormalities in mouse models (Chahrour and Zoghbi, 2007). Furthermore, deletion of the DNA methyltransferase Dnmt3a, which deposits mCA across the neuronal genome in the postnatal period, results in a disruption of the active maintenance of mCA in mature neurons (Guo et al., 2014; Stroud et al., 2017), leading to behavioral deficits reminiscent of those observed in Rett syndrome (Elliott et al., 2016; LaPlant et al., 2010).

Perhaps most provocative is recent evidence indicating that neuronal activity affects the function of Dnmt3a and MeCP2 by regulating the deposition and reading of mCA across the neuronal genome, respectively. For example, heightened levels of neuronal activity in early life decrease the binding of Dnmt3a at activity-regulated genes, thus reducing the levels of mCA across their transcribed regions in a manner that persists throughout the life of the organism (Stroud et al., 2017). Additionally, the MeCP2 protein is rapidly phosphorylated at multiple sites in response to neuronal activity, potentially leading to the regulated release of repressive co-factors from the neuronal genome (Ebert et al., 2013; Lyst et al., 2013) (Figure 4A). It is noteworthy that the gene expression deficits associated with MeCP2 loss-of-function models have been suggested to be restricted to highly methylated long genes due to the large number of mCA sites in these genes (Gabel et al., 2015; Sugino et al., 2014) (Figure 4B). Furthermore, these deficits are partially rescued by the application of topoisomerase inhibitors, which were found to suppress gene transcription in a length-dependent manner (Gabel et al., 2015; King et al., 2013). Intriguingly, topoisomerases have also been implicated in controlling the rapid induction of activity-dependent genes by resolving topological barriers imposed at promoters (Madabhushi et al., 2015). Taken together, these findings raise the possibility that neuronal activity leads to modification of the activity or binding of the protein complexes responsible for depositing and reading mCA, potentially greatly lengthening the time period during which neuronal activity might affect the neuronal transcriptome.

A Cellular and Circuit Perspective

Experience-Dependent Transcriptional Instruction for the Developing Brain

Insight into how activity-dependent transcriptional mechanisms control neural circuit function has been informed by efforts to characterize the role of neuronal activity in regulating the many stages of brain development and maturation as well as learning and behavioral adaptations. The development of the mammalian brain is controlled by both genetics and the environment. At birth, while the brain, like most organs, is largely formed, it continues to mature over a prolonged period in response to sensory experience. Beginning with the landmark work of Hubel and Wiesel that established the role of visual experience in shaping ocular dominance columns in the visual cortex (Hubel and Wiesel, 1970), the significance of sensory experience in fine-tuning neural connectivity in the postnatal brain has become increasingly evident (Hensch, 2005; LeBlanc and Fagiolini, 2011).

There are several distinct stages of postnatal nervous system development, each of which is controlled at least in part by activity-dependent transcription. Neurons first undergo axonal growth and dendritic arborization, followed by a period of exuberant synapse formation. Subsequently, synapse elimination occurs, and the number and strength of synapses are calibrated to ensure proper connectivity and excitatory-inhibitory balance within neural networks (West and Greenberg, 2011). Each of these steps is highly regulated by calcium-dependent processes that induce activity-regulated genes, many of which encode synaptic effector molecules. To date, a number of activity-regulated genes that coordinate various aspects of synapse maturation and function during critical periods, defined as periods of increased sensitivity to environmental influences in early

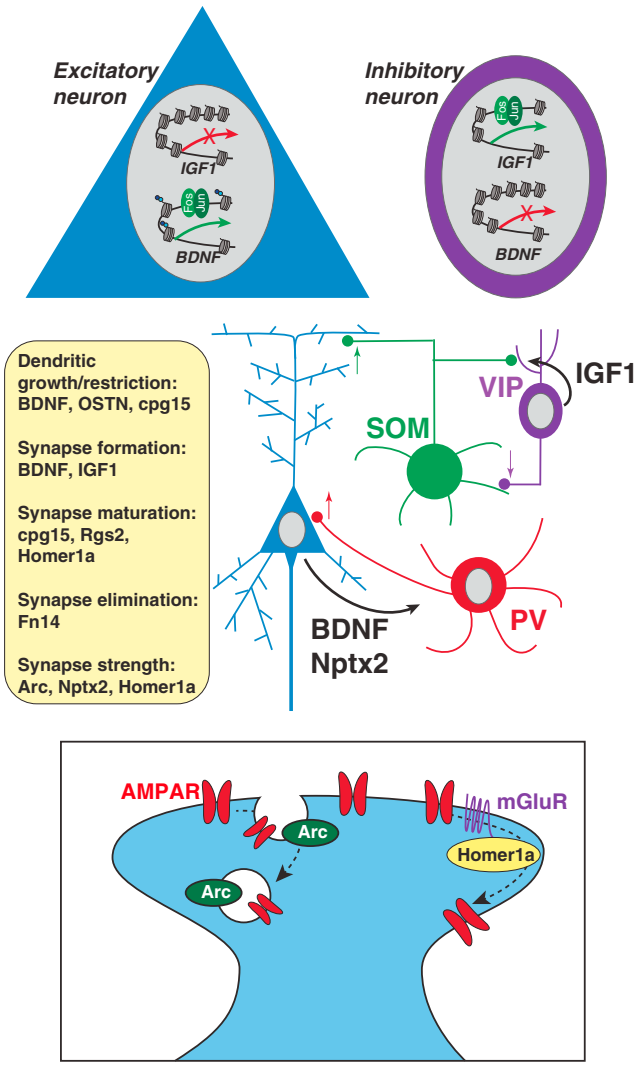

Figure 5. Cell-type-Specific Activity-Dependent Gene Programs Are Tailored to Specific Functions of Cells within a Neural Circuit

Each cell type in the brain possesses a distinct set of activity-regulated genes (top panel) that allow each cell type to interact with and modify specific synaptic inputs within their resident neural circuit (middle panel, right). For example, IGF1 is secreted from VIP-expressing interneurons and recruits inhibitory inputs onto VIP-expressing interneurons themselves, while BDNF is secreted from excitatory neurons and recruits inhibitory inputs onto the cell bodies of excitatory neurons. Activity-regulated genes have been shown to regulate cellular processes, such as dendritic growth and restriction, synapse formation, maturation, elimination, and strength (middle panel, left). For example, Arc and Homer1a have been shown to function as negative regulators of AMPA receptor expression at synapses (bottom panel).

postnatal life (LeBlanc and Fagiolini, 2011), have been identified and characterized. These genes include *Bdnf*, *Arc*, *Homer homolog 1a* (*Homer1a*), *Neuronal pentraxin 2* (*Nptx2*), and *Neuritin 1* (*Nrn1* or *cpg15*) (Flavell and Greenberg, 2008; Korb and Finkbeiner, 2011; Leslie and Nedivi, 2011; Shepherd and Bear, 2011) (Figure 5).

Bdnf encodes a secreted protein that regulates the excitatory-inhibitory balance that is required for critical period plasticity (Greenberg et al., 2009; Hensch, 2005; Timmusk, 2015). In one study, a mouse line was generated that contains a subtle knockin mutation in *Bdnf* promoter IV, which, among eight known promoters in mice, is the predominant promoter that drives neuronal activity-dependent *Bdnf* transcription in the cortex. The knockin mice displayed a significant decrease in the number and strength of GABAergic synapses that form on cortical pyramidal neurons, suggesting that activity-dependent *Bdnf* transcription regulates synaptic inhibition (Hong et al., 2008). This knockin line contains mutations in the cAMP response element (CRE), which were sufficient to disrupt CREB binding and thus CREB-dependent *Bdnf* transcription. Importantly, this specific manipulation affected neuronal activity-dependent transcription of *Bdnf*, but not basal transcription from the other *Bdnf* promoters. This dissociation was key in allowing for the precise determination of the physiological function of neuronal activity-dependent *Bdnf* transcription. It is noteworthy that a subtle change within one of multiple regulatory elements that control the expression of *Bdnf* can profoundly affect neural wiring, providing support for the idea that additional variants in activity-dependent regulatory elements will be found to affect circuit connectivity and function.

Neuronal pentraxins are also dynamically regulated by activity and have been shown to promote neurite outgrowth (Tsui et al., 1996). In a recent study, *Nptx2* was shown to be crucial for the recruitment of excitatory synapses onto a specific subtype of GABAergic interneurons, the PV-expressing interneurons (Pelkey et al., 2015) (Figure 5). Secreted Nptx2 does this by regulating the clustering of GluA4, a subunit of the AMPA receptor. A consequence of loss of Nptx2 is the disruption of excitatory synaptic transmission in PV interneurons, which leads to impaired PV-mediated feedforward inhibition. This defect in PV-mediated inhibition in turn prolongs the critical period, leading to a deficiency in circuit rhythmogenesis, hyperactivity, increased anxiety, and deficits in spatial working memory.

Homer1a is an activity-regulated gene that encodes a postsynaptic scaffold protein that functions as a negative regulator of AMPA receptor expression at synapses (Diering et al., 2017; Shan et al., 2018). This finding is consistent with the observation that *Homer1a* transcription is mediated by the activity-regulated transcription factor MEF2, which has also been found to restrict the number of excitatory synapses (Flavell et al., 2006). *Arc* is another target of MEF2 that promotes the endocytosis of AMPA receptors. Thus, MEF2 serves as a versatile negative regulator of excitatory synapse development (Barbosa et al., 2008; Chowdhury et al., 2006; Rial Verde et al., 2006; Wilkerson et al., 2014) (Figure 5). By contrast, cpg15, a small extracellular protein anchored to the membrane, promotes synapse stabilization (Fujino et al., 2011; Harwell et al., 2005). Adding to this growing list, a recent study describes a novel function for *Fibroblast growth factor-inducible 14* (*Fn14*), an activity-regulated gene that is expressed in the dorsolateral geniculate nucleus and regulates synaptic refinement in the vision-dependent phase of retinogeniculate synapse maturation (Cheadle et al., 2018).

These activity-regulated genes are only a small subset of the hundreds that remain to be characterized, and thus, the next decade will undoubtedly witness specific functions being ascribed to many more activity-regulated genes. These studies highlight the diversity and exquisite tailoring of activity-regulated

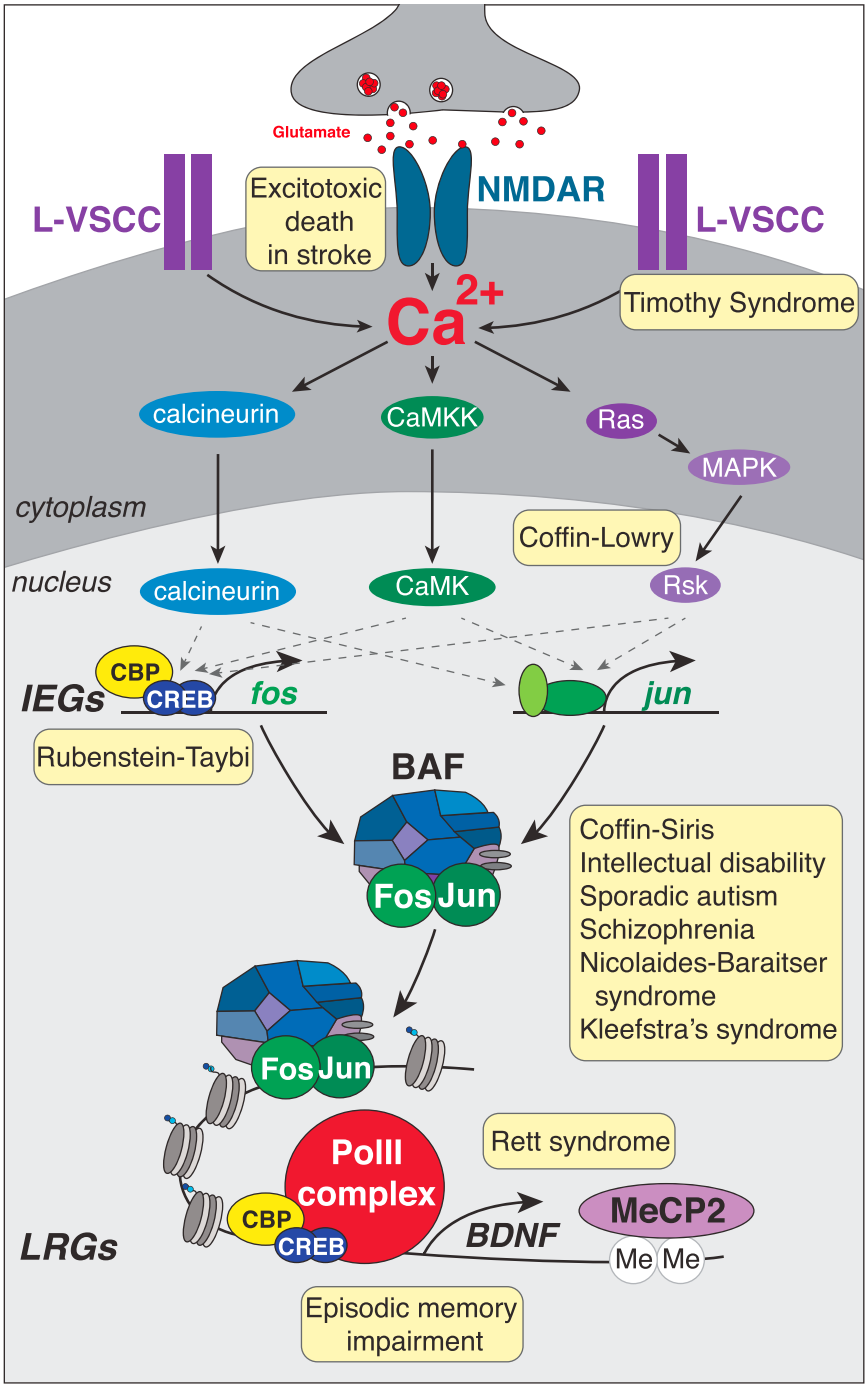

Figure 6. Mutations in Specific Components of the Activity-Dependent Transcriptional Pathway Have Been Implicated in Various Neurodevelopmental and Neurological Disorders in Humans

Simplified schematic depicting that mutations in L-VSCCs have been implicated in Timothy syndrome, Rsk2 in Coffin-Lowry syndrome, and CREB-binding protein CBP in Rubenstein-Taybi syndrome. Mutations in multiple subunits of the BAF complex have been implicated in various neurological disorders, including sporadic autism and intellectual disability (see Ronan et al., 2013). Mutations in MeCP2 lead to Rett syndrome. *Bdnf* is an example of a late-response gene whose defects in expression or function have been associated with impaired episodic memory and depression, among others.

effector molecules to distinct developmental processes in specific cell types and brain regions. Importantly, progress in characterizing the function of additional activity-regulated genes will have to be made concurrently with efforts to understand the overall effect of the network of activity-regulated genes in the developing brain. This is especially crucial given that various neurodevelopmental disorders, including autism spectrum disorders, schizophrenia, and intellectual disability, manifest in a common disruption of excitatory-inhibitory balance during critical periods (LeBlanc and Fagiolini, 2011; Mullins et al., 2016; Nelson and Valakh, 2015). Although defects in specific components of the activity-dependent gene network have been implicated in these polygenic disorders (Figure 6), a future challenge will be to identify unifying pathophysiological mechanisms that underlie them. This may be achieved by interrogating the activity-dependent transcriptional regulatory mechanisms at play in a systematic manner, using high-throughput methodologies and mouse models of these disorders.

Activity-Dependent Transcription Is Activated and Serves as a Reporter of Neuronal Activity in the Mature Brain

Once neural circuits have matured, experience-dependent plasticity manifests broadly as a mechanism for adaptations to diverse physiological drives, including hunger, thirst, sleep, fear, pain, cold, and warmth, as well as social interactions. These distinct internal states give rise to various forms of goal-oriented learning as an organism interacts with the external world and seeks to meet its physiological needs. Some forms of learning can occur gradually over a period of days or weeks and may require the development of long-term memories, which sometimes last the lifetime of the animal.

To understand how neuronal activity-driven transcription regulates circuit dynamics and behavior in the mature brain, it would first be necessary to understand the cellular and behavioral features *in vivo* that lead to the induction of activity-dependent gene programs. This was initially accomplished using immunohistology and *in situ* hybridization approaches to monitor IEG

expression in specific brain regions in response to distinct behavioral states (see Tischmeyer and Grimm, 1999). These studies demonstrated the high fidelity and utility of IEGs as markers of neuronal activation in various behavioral paradigms and also raised the possibility that IEGs might directly regulate synaptic plasticity and thus behavioral adaptations.

In recent years, these IEGs, and in particular *Fos* and *Arc*, have become increasingly useful as reporters of neuronal activity in the brain as novel genetically encoded mouse and viral tools have been generated (DeNardo and Luo, 2017). These tools not only enable the labeling of, but also provide access to, activated neurons either transiently or permanently. The advantages and disadvantages of various activity-based tools, as well as technologies that allow intact brain-wide fluorescent imaging at cellular resolution (Renier et al., 2016; Ye et al., 2016), have been extensively reviewed elsewhere (see DeNardo and Luo, 2017; Kawashima et al., 2014). Briefly, the most basic designs consist of IEG promoters used to drive various effector gene modules, including fluorescent proteins for visualization, ligand-dependent transcription factors or recombinases for downstream expression of additional effector genes, and light- or ligand-gated channels for subsequent control of the labeled populations. Moreover, as is the case with transgenic and viral strategies, enhancer modules, the short regulatory sequences containing the DNA-binding sites of sequence-specific TFs, can be added to these designs to augment the transcriptional responses of the IEG-dependent effectors (e.g., E-SARE and RAM) (Kawashima et al., 2013; Sørensen et al., 2016).

As these genetically encoded tools are largely based on *Fos* and *Arc*, which are expressed in many cell types, they do not provide selective access to specific neuronal subtypes, and thus, these reporters cannot yet be used to perturb the function of subtype-specific neurons that have been activated. The design of the next generation of activity-based tools must necessarily focus on the creation of cell-type-specific activity-dependent reporters. Regulatory elements controlling the induction of *Npas4*, for example, would likely be promising in restricting effector expression to neurons, as *Npas4* expression is selectively induced in response to calcium influx-dependent neuronal activity (Lin et al., 2008). In addition, with the advent of next-generation sequencing technologies, which have enabled the profiling of thousands of activity-dependent regulatory elements (Kim et al., 2010; Malik et al., 2014; Su et al., 2017), the identification and characterization of new enhancer elements that confer both activity- and neuronal-subtype resolution should be within reach.

Experience-Dependent Transcription in the Plasticity of Circuits that Underlie Learning and Memory

The currently available genetically encoded activity-dependent reporters have already proven to be indispensable for establishing the importance of distinct subsets of activated neurons for specific behaviors. For example, a series of studies have used the permanent labeling of Fos-activated neurons during contextual fear conditioning to implicate these activated neurons in contextual fear memory formation, consolidation, and attenuation (Garner et al., 2012; Khalaf et al., 2018; Kitamura et al., 2017; Liu et al., 2012). However, currently little is known about the learning-related structural and functional alterations that occur during the encoding of fear memories in these activated neuronal ensembles. In addition, although within activated neurons protein synthesis-dependent increases in dendritic spine density and synaptic strength have been shown to underlie the ability of an animal to perform natural recall (Ryan et al., 2015), establishing a specific requirement for activity-dependent transcription in consolidation and reconsolidation (Alberini and Ledoux, 2013) will require more precise genetic loss- and gain-of-function manipulations to the activity-dependent transcriptional program.

To date, both loss- and gain-of-function manipulations to numerous activity-regulated genes, including *Arc*, *Homer1a*, *Bdnf*, *Creb*, *Srf*, *Mef2*, *Fos*, *Fosb*, *Egr1*, and *Npas4*, have identified deficits in classical behavioral paradigms such as contextual fear conditioning, spatial memory, and novel object recognition (reviewed in Nonaka et al., 2014; Okuno, 2011). However, these studies have so far employed relatively coarse manipulations that target entire brain regions, thus making it difficult to disentangle cell-autonomous effects from those that are due to network-level perturbations. It is noteworthy that these prior genetic manipulations likely affected a multitude of cell types, including neuronal and non-neuronal cell types, thus complicating identification of the primary and secondary effects of disrupting the function of a specific component of the activity-dependent transcriptional network. While these studies implicate a role for activity-dependent transcription in neural circuit function and behavior, further characterization of the structural and functional changes that activated neurons undergo during learning should shed light on how activity-dependent transcription modifies synapses in order to balance the flexibility and stability required for the proper encoding of memories.

The advent of modern imaging technologies has proven particularly advantageous for understanding the dynamic nature of synapses, from their protein compositions to their structure (Svoboda and Yasuda, 2006). The ability to perform longitudinal imaging of the mammalian brain in awake-behaving animals, with input and cell-type specificity, has been especially informative given the timescales over which transcriptional mechanisms typically manifest. For example, there have been significant strides in understanding experience-dependent structural plasticity through *in vivo* imaging. This has led to the identification of differences in spine dynamics depending on cell type and brain region (Berry and Nedivi, 2016). Several studies have revealed that while spines are impermanent in the hippocampus, they are significantly more persistent in the neocortex, possibly reflecting the different durations of information retention in each region (Attardo et al., 2015; Holtmaat and Svoboda, 2009). Moreover, in contrast to the stability of excitatory synapses in the visual cortex, nearby inhibitory synapses were found to remodel continuously, with the rate of remodeling dependent on changes in sensory input (Rose et al., 2016; Villa et al., 2016). These and other findings provide a synaptic correlate for examining how activity-dependent transcription allows cortical and subcortical circuits to encode information stably while remaining dynamic.

How some synapses persist while others are modified in an experience-dependent manner remains to be determined. By virtue of the hundreds of genes that are activated in response

to sensory stimuli, activity-dependent transcription could potentially play an instructive role in promoting the turnover of particular synapses while simultaneously stabilizing other synapses. Importantly, various mechanisms by which activity-dependent transcription controls the turnover of synapses, including regulated mRNA transport and local translation of distinct mRNA transcripts, have been identified (Fontes et al., 2017; Martin et al., 1997; Van Driesche and Martin, 2018). In the future, determining how the activity-dependent transcriptional program influences the dynamics of individual synapses will require a comprehensive understanding of the net effect of the multitude of activity-regulated effector genes that are induced and the ability to visualize their mRNAs or protein products and their spatial distribution across many synapses in a neuron *in vivo*. In addition, as animals employ multiple concurrently active forms of plasticity during learned behaviors, defining coherent mechanisms of the activity-dependent transcriptional program will be greatly facilitated by chronic imaging of neurons over prolonged periods to detect the different forms of plasticity that underlie learning and memory.

A recent study represents a start to achieving these goals (El-Boustani et al., 2018). Using two-photon microscopy to measure multiple forms of spike-timing-induced plasticity within single dendritic branches while tracking the molecular dynamics of Arc and visualizing AMPA receptor endocytosis, El-Boustani et al. (2018) address how local coordination of different forms of plasticity shapes neuronal responses to visual inputs in awake animals. By this analysis, Arc was found to be critical for both the Hebbian strengthening of activated synapses and the heterosynaptic weakening of adjacent synapses. In the future, longitudinal imaging of the same sets of spines over days will provide additional insight into how various plasticity mechanisms, via activity-regulated transcription and LRG expression, regulate the strengths of these synapses. In addition, recent work has identified a viral capsid-like property of Arc protein that packages *Arc* mRNA in extracellular vesicles in synaptic boutons (Ashley et al., 2018; Pastuzyn et al., 2018). These vesicles are released upon neuronal stimulation and trafficked across the synaptic cleft and into postsynaptic neurons, providing an input-specific mechanism by which Arc can regulate plasticity at distinct activated synapses. The prevalence of this form of synaptic plasticity in the mammalian brain and its interplay with spike-timing-dependent plasticity, as described above, would be of great interest for future studies.

As our understanding of the activity-dependent transcriptome has deepened, the possibilities for uncovering the activity-dependent transcriptional regulation of various plasticity mechanisms underlying learned behaviors have increased. For example, a notable observation from recent cell-type-specific activity-dependent gene expression studies (Hrvatin et al., 2018; Mardinly et al., 2016; Spiegel et al., 2014) is that each cell type possesses a distinct set of activity-dependent transcripts, a subset of which encodes secreted molecules. These secreted factors allow each neuronal subtype to interact with and modify specific synaptic inputs within their resident neural circuit (Figure 5). For example, there is mounting evidence indicating that in the CA1 region of the hippocampus, *Bdnf* is selectively induced in excitatory neurons and critical for the recruitment of inhibitory inputs onto the somatic region of excitatory neurons (Bloodgood et al., 2013; Spiegel et al., 2014). Interestingly, this regulation of perisomatic inhibition is mediated by transcription of the neuronal-specific IEG *Npas4*, which also restricts dendritic inhibition in a manner that permits the dendritic neighborhood of activated pyramidal neurons to become more receptive to excitatory inputs and thus more permissive of plasticity. However, the activity-regulated genes that directly mediate this change in dendritic inhibition are not yet known. In contrast to these observations in the hippocampus, in the visual cortex in response to light, *insulin-like growth factor 1* (*Igf1*) was found to be selectively induced in VIP-expressing interneurons, where it recruits inhibitory inputs onto the VIP-expressing interneurons themselves, thereby imposing a sensory experience-dependent brake on cortical plasticity (Mardinly et al., 2016). These results underscore the intricacy of the neuronal subtype-specific activity-dependent gene programs and thus their far-reaching implications for the plasticity of subtype-specific inhibitory synapses in response to learning.

Learning-induced changes in GABAergic interneuron subtypes that are consistent with the timescale of activity-dependent transcription have been well demonstrated in many systems. As an example, motor learning can induce dendritic compartment-specific reorganization of spines, coincident with changes to local inhibitory circuitry (Chen et al., 2015c). Specifically, SST-positive interneurons, which largely mediate dendritic inhibition, show a learning-dependent reduction in bouton density over days. In contrast, the number of PV-positive axonal boutons increase with motor learning, implying an enhancement of control over action potential output of pyramidal neurons in the motor cortex. As another example, learning-induced increases in the selectivity of pyramidal neurons in the visual cortex for a rewarded stimulus are accompanied by increases in selectivity of PV-positive interneurons for the same stimulus and concurrent decorrelation of SST-driven activity from the network (Khan et al., 2018). Understanding the mechanisms by which activity-dependent gene transcription gives rise to learning-induced reorganization of inhibition and the subsequent enhancement of cortical representations of task-relevant stimuli would represent a significant step in linking neuronal activity to long-term changes in neural circuit function.

The regulation of several additional forms of plasticity by activity-dependent transcription has also been described. For example, in a recent study, the activity-dependent transcription factor *Mef2c* was found to be crucial for the promotion of local excitatory inputs onto layer 2/3 pyramidal neurons in the somatosensory cortex and a simultaneous downregulation of the strength of long-range excitatory inputs originating from contralateral regions (Rajkovich et al., 2017). These findings underscore the capacity for activity-dependent transcription to determine not only local network function, but also inter-hemisphere communication in the brain.

In addition, there is emerging evidence that activity-dependent transcription regulates intrinsic neuronal excitability. Learning-induced phosphorylation and activation of CREB in a subset of neurons has been shown to enhance neuronal excitability (Lisman et al., 2018). This increase in intrinsic excitability facilitates the allocation of CREB-activated neurons to a

subsequent memory trace closely linked in time (Rashid et al., 2016). In contrast to these observations, some studies report higher spontaneous firing rates but no changes in intrinsic excitability in Fos-activated neurons, which may be due to stronger synaptic connectivity among these neurons (Yassin et al., 2010). The causal relationship between activity-dependent transcription and these neuronal properties remains to be determined. Understanding how activity-dependent transcription contributes to the emergence of distinct ensembles of behaviorally relevant neurons through the interplay of global neuron-wide and local synapse-specific mechanisms will be important.

Experience-dependent transcription also plays a prominent role in the regulation of homeostatic plasticity (Turrigiano, 2012). A new study identifies a physiological role for homeostatic scaling-down during sleep, which occurs by an activity-regulated, Homer1a-dependent mechanism involving the removal of AMPA receptors and the weakening of excitatory synapses (Diering et al., 2017). Importantly, the targeting of Homer1a to postsynaptic densities is modulated by the neuromodulator adenosine, which is present at higher levels during the sleep cycle, highlighting the intricate control of brain state-dependent homeostatic synaptic scaling crucial for the consolidation of memories.

Finally, recent evidence indicates that neuronal activity promotes adaptive myelination. This then likely leads to increases in the velocity at which action potentials propagate along axons. Activity-dependent myelination may be advantageous for reinforcing the learning of certain tasks, such as skilled motor actions. Notably, within several hours of activation of excitatory neurons, the proliferation and subsequent differentiation of nearby oligodendrocyte precursor cells (OPCs) ensues (Gibson et al., 2014). Both OPC proliferation and differentiation take several hours to occur and may require activity-dependent gene transcription. One hypothesis is that an activity-regulated gene in excitatory neurons encodes a secreted protein, such as BDNF, that then binds to its receptor (e.g., TrkB) on OPCs and stimulates OPC proliferation and differentiation, thus promoting myelination (see Mount and Monje, 2017). The significance of this form of structural remodeling for neural circuit plasticity has the potential to be far-reaching in both healthy and diseased states of the nervous system.

Future Perspectives and Concluding Remarks

In describing the progress that has been made over the past decades, we have highlighted gaps to be addressed as we seek a better understanding of the activity-dependent transcriptional control of neural circuit function. We conclude by identifying and proposing several additional areas of focus for the future. Importantly, these prospective avenues of research will undoubtedly be defined by innovative combinations of modern sequencing, genome editing, and functional imaging methodologies, which have the potential to yield unprecedented insights into higher-order cognitive processes.

In the brain, the intricate interplay of various plasticity mechanisms during learning gives rise to neuronal ensembles that display population-level representations of behaviorally relevant stimuli (Picardo et al., 2016). Notably, these subnetworks of neurons generate dynamic patterns of activity that underlie various adaptive behaviors. Accordingly, understanding how activity-dependent transcription mediates learned behaviors will require dissecting its role in the emergence and regulation of these neuronal ensembles. To that end, it will first be necessary to characterize the specific cellular and behavioral features that induce IEGs during natural behaviors. This should be possible to achieve by two-photon calcium imaging of populations of neurons while simultaneously monitoring the dynamics of IEG expression *in vivo*. In this regard, special attention must be given to the choice of IEG-based mouse genetic tools to ensure the faithful recapitulation of the endogenous levels and temporal patterns of activity-dependent gene expression, as well as preservation of the function of the IEG protein products.

These *in vivo* calcium imaging experiments will also facilitate the linking of calcium fluctuations and calcium-dependent signaling mechanisms to activity-dependent transcriptional responses. Several studies have suggested that varying patterns of neural activity can give rise to unique activity-dependent gene programs (Belgrad and Fields, 2018; Dolmetsch et al., 1998; Tyssowski et al., 2018; Worley et al., 1993; Wu et al., 2001). However, our understanding of how these patterns of action potentials influence the dynamic flow of information from the synapse to the nucleus and lead to different patterns of gene activation is still lacking. Recent tools that enable the optogenetic control of specific signaling pathways (e.g., Ras/Erk) (Wilson et al., 2017) will further our understanding of the convergence of diverse neurotransmitter, neuromodulator, and neurotrophic factor signaling pathways in the activation of gene transcription.

Once the cellular and behavioral features that lead to the induction of activity-dependent gene programs *in vivo* are known, establishing a causal role for activity-dependent transcription in the representations of learned stimuli will be critical. Progress in this endeavor will require a better understanding of the cell-type specificity of the activity-dependent gene programs, followed by the utilization of methods for genetically accessing and reversibly inhibiting specific components of the activity-dependent transcriptional network in a cell-type-specific manner and in *in vivo* contexts.

The characterization and manipulation of the activity-regulated transcriptional network should be facilitated by employing next-generation single-cell sequencing and CRISPR/Cas9 gene-editing technologies to identify and then reversibly disrupt the function of activity-regulated enhancers. These activity-dependent enhancers represent the predominant genomic modules for binding and regulation by activity-dependent TFs and are critical for the fine-tuning of gene expression in diverse cell types and contexts. However, the neuronal subtype-specific, activity-regulated enhancers that are differentially modulated by various physiological behaviors have not been comprehensively characterized. Moreover, the interactions of active enhancer elements and their gene targets, including their spatial organization within a cell, remain to be clarified. Efforts to address these gaps in knowledge will be worthwhile as this information may allow us to target these enhancer elements to manipulate the activity-dependent expression of a specific gene without affecting the basal expression of the gene. Several studies have underscored the promise of this approach (Hong

et al., 2008; Joo et al., 2016; Smith-Hicks et al., 2010), which should be greatly facilitated by CRISPR/Cas9 methodologies in the next decade.

CRISPR/Cas9-mediated manipulations need not be restricted to single enhancers or genes but have been shown to be amenable to high-throughput, multiplexed, genome-scale interrogations. For example, Perturb-seq, which combines pooled CRISPR-based perturbations with scRNA-seq, has been demonstrated to be a powerful tool for profiling and probing the combinatorial, nonlinear effects of multiple transcription factors on gene expression signatures and cell states in heterogeneous cell types (Adamson et al., 2016; Datlinger et al., 2017; Dixit et al., 2016; Jaitin et al., 2016). This high-throughput approach for probing gene regulatory networks may be advantageous compared to classical loss- or gain-of-function approaches that are sometimes limited due to TF redundancy. These newer approaches have the potential to fundamentally advance our understanding of how the dynamic states of the brain are generated by the molecular regulatory circuits of heterogeneous cell types in the central nervous system.

As the coupling of synaptic activity to gene transcription is conserved across species, it will also be important to use non-human primate models and human neurons to uncover evolutionary mechanisms that lead to the emergence of species-specific activity-dependent regulatory elements, protein-coding genes, and non-coding RNAs that shape the human brain in response to experience (Hardingham et al., 2018). Indeed, several recent studies have begun to identify primate-specific activity-regulated genes, such as *OSTN*, *ZNF331*, and *CAMTA1*, and non-coding RNAs, such as *LINC00473* (Ataman et al., 2016; Pruunsild et al., 2017; Qiu et al., 2016). Importantly, a unifying evolutionary mechanism emerged from these studies, namely that the species evolution of activity-dependent transcription arises from the acquisition of signal-dependent TF binding sequences in regulatory elements. Future progress in understanding the signal-dependent transcriptional mechanisms underlying cognitive abilities in humans will require characterization of the human cell-type-specific activity-dependent transcriptomic and epigenomic landscapes at single-cell resolution. While *in vitro* studies have identified the function of several activity-regulated genes, such as *OSTN* in the restriction of activity-dependent dendritic growth, a major challenge for the future would be in employing non-human primate models for *in vivo* characterization of the function of the human activity-dependent transcriptome.

In conclusion, future studies of activity-dependent transcription will undoubtedly reveal deeper molecular insights, including knowledge of the dynamic regulation of protein compositions at single synapses, and the discovery of novel activity-regulated chromatin complexes and their multidimensional organization in the nucleus. At the cellular level, understanding the activity-dependent transcriptional regulation of various developmental and adaptive processes with greater cell-type and brain-region specificity will also be essential. Unifying these molecular and cellular studies with systems-level approaches for probing the interdependent relationship between gene regulatory networks and neural activity patterns that form the basis of integrated motor, sensory, and cognitive functions will be a major frontier. Finally, understanding the evolutionary forces that shape activity-dependent gene transcription in humans will complement the decades of discoveries in simpler systems and hopefully lead to effective treatments for human neurological and neuropsychiatric disorders.

ACKNOWLEDGMENTS

We apologize to researchers whose work we were not able to cite because of space limitations. We thank Dr. J. Zieg for help with the figures and Dr. N. Sharma, Dr. D.D. Ginty, Dr. S.R. Datta, Dr. C.D. Harvey, Dr. J.M. Gray, Dr. A.E. West, Dr. E.C. Griffith, Dr. E.A. Pollina, Dr. T. Vierbuchen, Dr. L.D. Boxer, and Dr. S. Ashrafi for critical feedback on this Review. E.-L.Y. is supported through the Stuart H.Q. and Victoria Quan and the Department of Neurobiology Graduate Fellowships. Work on this subject in the Greenberg laboratory is supported by the NIH (R37 NS028829), Rett Syndrome Research Trust, and the Paul G. Allen Frontiers Group. M.E.G. would like to dedicate this Review to his postdoctoral mentor, Dr. Edward B. Ziff, at the time of his retirement.

DECLARATION OF INTERESTS

M.E.G. is on the Board of Directors of and holds equity in Allergan, plc.

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