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Unifying Views of Autism Spectrum Disorders: A Consideration of Autoregulatory Feedback Loops

Caitlin Mullins¹, Gord Fishell¹, and Richard W. Tsien¹

¹Department of Neuroscience and Physiology, Neuroscience Institute, NYU Langone Medical Center, New York, NY 10016, USA

Abstract

Understanding the mechanisms underlying autism spectrum disorders (ASD) is a challenging goal. Here we review recent progress on several fronts, including genetics, proteomics, biochemistry and electrophysiology, that raise motivation for forming a viable pathophysiological hypothesis. In place of a traditionally unidirectional progression, we put forward a framework that extends homeostatic hypotheses by explicitly emphasizing autoregulatory feedback loops and known synaptic biology. The regulated biological feature can be neuronal electrical activity, the collective strength of synapses onto a dendritic branch, the local concentration of a signaling molecule, or the relative strengths of synaptic excitation and inhibition. The sensor of the biological variable (which we have termed the homeostat) engages mechanisms that operate as negative feedback elements to keep the biological variable tightly confined. We categorize known ASD-associated gene products according to their roles in such feedback loops, and provide detailed commentary for exemplar genes within each module.

Introduction and plan of presentation

Understanding the combined dysfunction of social communication and repetitive behavior of individuals with autism spectrum disorders (ASD) presents a formidable challenge. Despite the uncertainties of success for discovering therapeutic treatments for ASD (Mullard, 2015), even limited progress could be leveraged into the broader realm of other neuropsychiatric diseases. Further, we can gain great insights into the normal function of the brain by studying it in cases of dysfunction. Pursuing hopes of ameliorating brain disorders, investigators are presented with an unprecedented chance to “get under the hood” of cognition in a mechanistic way.

Current work on ASD is extremely multidisciplinary. The field offers an abundance of data that is intriguing but often difficult to synthesize. Relating genetic underpinnings to normal or dysfunctional behavior requires connecting synapses, cells, circuits and networks. To

Correspondence to: Richard W. Tsien.

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support this effort, we aim this review at a broad audience that includes readers interested in genetics, neurophysiology, and behavior. As one of us has worked with individuals with ASD, another studies neuronal development, and a third explores synaptic signaling, the diversity of our experience reflects the breadth of areas involved in this disorder.

Current high-water marks of thinking in the field are represented by the following categories of papers, each with both value and limitation:

- A.** Research publications that focus on a mouse model of monogenic ASD, often followed up by attempted amelioration of ASD-like behavior with pharmacological intervention (Aguilar-Valles et al., 2015; Bear et al., 2004; Braat et al., 2015; Chevere-Torres et al., 2012; Choi et al., 2011; Gkogkas et al., 2014; Nageshappa et al., 2015). This approach admirably takes direct aim at issues of necessity and sufficiency, but by definition is only credible for a subset of ASD, even when the scope is broadened by considering the pathways that may be affected (Mullard, 2015).
- B.** Reviews of ASD that categorize genes according to properties of the proteins they encode, such as their anatomical location (Ebert and Greenberg, 2013; Toro et al., 2010; Uzunova et al., 2014), their protein-protein interactions with other gene products (Sakai et al., 2011), or their transcript levels in correlation with other mRNAs (Voineagu et al., 2011). Each provides valuable groupings of proteins, but not an explicit hypothesis about their mechanistic relationship to the disorder as a whole.
- C.** Perhaps most influential are reviews that emphasize a relatively novel concept or particularly vulnerable process: (1) ASD arises from too high a ratio of excitation/inhibition (E:I imbalance) (Rubenstein and Merzenich, 2003), as recently reviewed (Nelson and Valakh, 2015; Rubenstein, 2010); (2) ASD originates from an excess of local protein translation (Darnell and Klann, 2013; Kelleher and Bear, 2008); (3) ASD is caused by a defect in neuronal homeostasis (Bourgeron, 2015; Ramocki and Zoghbi, 2008; Toro et al., 2010; Wondolowski and Dickman, 2013); (4) ASD stems from a dysfunction of activity-dependent gene expression, particularly regulation of nuclear transcription (Ebert and Greenberg, 2013). These ideas have energized research efforts, but it is presently unclear whether they fully differentiate between cause and effect, or how one possible mechanism relates to another.

Researchers can try to make sense of the diversity of hypotheses by subdividing ASD into distinct disorders, or by trying to unify the disparate elements, as attempted here. In line with the advice of George Box, “all models are wrong but some are useful,” attempts to organize genes and signaling pathways, aided by concepts from neurophysiology, neural plasticity, autoregulatory mechanisms, Ca²⁺ signaling, and neurotransmission could help provoke argument and encourage fresh experimentation.

In this effort, we will advance a framework for uniting current themes about ASD under an overriding pathophysiological tent that explicitly emphasizes feedback regulation, building upon an oft-mentioned unidirectional chain of events:

Genetics → altered proteins → pathophysiology → circuit dysfunction → ASD behavior

This one-way street of causality is likely true in part, but does not fully capture the theme of neural adaptability, which must involve feedback in some way. To lay the foundation for our scheme we briefly review genetic findings and set out our reasons for putting genes and gene products into functional rather than anatomical categories. In turn, this organization spurs us to offer some fresh perspective to multiple themes in ASD research. With respect to *signaling from synapse to nucleus* (Ebert and Greenberg, 2013), we reopen the question of how cascades of ASD-related proteins (Ma et al., 2014) may control the expression of other ASD-related genes (Tian et al., 2014). Regarding synaptic plasticity by *local regulation of protein synthesis* (Aguilar-Valles et al., 2015; Fernandez et al., 2013; Kelleher and Bear, 2008; Kelleher et al., 2004; Niere et al., 2012), we meld the concept of coordination amongst neighboring dendritic synapses (Bourne and Harris, 2012; Rabinowitch and Segev, 2008) with ideas about ASD as dysfunctional homeostasis (Huguet et al., 2013; Ramocki and Zoghbi, 2008; Toro et al., 2010). Along the way, we combine morphological (Gilman et al., 2011; Jiang et al., 2013; Penzes et al., 2011; Varea et al., 2015; Zoghbi, 2003) and functional (Bateup et al., 2011) representations of synaptic plasticity, and attempt to broaden thinking about “E:I balance” to a more flexible view of “*E:I coordination*.” We then place each of these themes into a framework of feedback loops for autoregulation of neurons and circuits. Defects in any component of these control systems can lead to related patterns of dysfunction and ASD, which nicely meshes with the disparate mechanisms suggested by others.

Rapid progress in ASD genetics

The challenge and promise of ASD genetics can be telegraphed by a series of numbers. The reported prevalence of ASD is ~1% of the total U.S. population, with estimates abruptly jumping from 1/88 to 1/68 based on surveillance studies conducted in 2008 and 2010. ASD is highly heritable, as judged by heightened risk for individuals with an affected twin or sibling (Bailey et al., 1995; Folstein and Rutter, 1977a, b). Quantitative measures of heritability are derived from rates of concordance for monozygotic twins, dizygotic twins, and siblings, with some evidence coming from a 2014 survey of >2 million Swedish families, the largest population-based study of ASD so far (Sandin et al., 2014). With one notable exception (Hallmayer et al., 2011), heritability estimates range from 52-90% (Chen et al., 2015; De Rubeis and Buxbaum, 2015a, b; Gaugler et al., 2014; Rosenberg et al., 2009; Sandin et al., 2014; Toro et al., 2010). Thus, genetic factors contribute substantially, and likely combine with environmental effects to guide clinical outcome (Bourgeron, 2015).

The genetic landscape of ASD is extremely complex, involving some 400-1200 genes according to current best estimates (De Rubeis and Buxbaum, 2015a; Geschwind and State, 2015; Ronemus et al., 2014; Willsey and State, 2015). In a handful of rare syndromes, autistic features arise from dysfunction of only one gene, as exemplified by Rett (*MECP2*), Angelman (*UBE3A*), Timothy (*CACNA1C*), Fragile X (*FMRI*) and Tuberous sclerosis (*TSC1,2*) syndromes. While highly penetrant and instructive, these syndromic forms are rare; pooling them together accounts for less than 5% of individuals on the ASD spectrum. This leaves geneticists with the daunting challenge of sifting through the 3 billion

nucleotides of the human genome, looking amongst the 1% that differ between two randomly selected people (Geschwind and State, 2015) and determining which differences, or combinations thereof, are causal to ASD amidst the vast sea of those that are not (McCarroll and Hyman, 2013). Thus, it seems remarkable that within just the last few years, genetic studies have identified a sizeable fraction of the sought-after ASD genes, roughly 20-30% according to most estimates (Bourgeron, 2015; Krumm et al., 2014; Schaaf and Zoghbi, 2011).

A wide array of approaches

Multiple genetic efforts have been mounted and have contributed to varying extents. Genome Wide Association Studies (GWAS) have not yet reached desired reproducibility, most likely because they have been underpowered (typically 2,000-5,000 patients, far fewer than the ~50,000 that drove a highly successful GWAS in schizophrenia (Cross-Disorder Group of the Psychiatric Genomics et al., 2013)). Much recent progress has come with the intensive genetic analysis of ASD families, including the finding of recessive genes with powerful effects (Jamain et al., 2003; Morrow et al., 2008). Exome sequencing has uncovered *de novo* single nucleotide variations (SNVs) and insertion/deletion (indel) mutations that segregate with ASD. Four independent studies, all published in the same watershed year (Iossifov et al., 2012; Neale et al., 2012; O'Roak et al., 2012b; Sanders et al., 2012), advanced our knowledge of the genetic complexity of ASD and greatly improved the reliability of gene discovery. Identification of additional genes has accelerated with recent sequencing papers (De Rubeis et al., 2014; Iossifov et al., 2014; O'Roak et al., 2012a). Together, these studies have yielded gene lists with stringent false-discovery rates and suggested possible interrelationships between genes. Further progress in genetics will no doubt continue as the cost of sequencing decreases and the size of cohorts increases.

Genetic Variants and Networks

How newly discovered genetic variations affect gene expression remains a pressing issue. Mouse models are readily available for highly penetrant syndromic forms of ASD (Bader et al., 2011; Crawley, 2012; Guy et al., 2001; Horev et al., 2011; 1994; Yu et al., 2006), but many more studies will be needed to explore how different mutations affect function at the level of protein, neuron, circuit and whole animal. We can expect important advances in the use of model organisms (mouse, zebrafish, fruitfly and possibly even yeast)(Kozol et al., 2015), and in new molecular shortcuts to explore the full range of genetic variants through emerging genome editing methods (Zhang, 2015). Given the effort involved in exploring even one biological hypothesis, distinguishing between high and low confidence hits is essential.

Various approaches have recently been developed to query networks of functionally related genes. For example, systematic study has uncovered hundreds of direct protein-protein interactions amongst products of genes previously implicated in ASD, including a network involving SHANK and TSC1 (Sakai et al., 2011). Direct protein-protein interactions are informative but challenging to interpret because of the diversity, and in some cases, promiscuity of such interactions.

In a complementary approach, transcriptomic studies in postmortem tissue have uncovered clusters of genes (so-called modules) whose transcripts co-vary. The co-variation is revealed agnostically by a clustering procedure known as weighted gene co-expression network analysis (WGCNA) (Zhang and Horvath, 2005), and is used to infer a functional relationship. The co-regulation may occur among individual ASD probands and controls (Voineagu et al., 2011), among multiple brain regions (Ben-David and Shifman, 2012; Voineagu et al., 2011), in various cortical layers (Parikshak et al., 2013; Willsey et al., 2013), and over the course of development (Parikshak et al., 2013; Willsey et al., 2013). Once the transcripts are grouped into modules, their behavior is compactly summarized by the module eigengenes (first principal component). Voineagu *et al.* performed comparative transcriptomic analysis of post-mortem ASD brain samples and found two modules highly correlated with ASD status (Voineagu et al., 2011). One of these modules (M12) was downregulated in ASD cases, contained genes known for involvement in synaptic function, vesicular transport, and neuronal projection, and was inferred to be causally involved in ASD pathophysiology.

Progressing from gene discovery to functional gene groupings to pathophysiology

With the identification of a substantial fraction (20-30%) of the full panoply of ASD-related genes, researchers are now striving to use putative gene groups to understand the pathophysiology of ASD. Traditionally, genes are grouped according to Gene Ontology (GO) terms (e.g., ion channels), by cellular location (e.g., in the postsynaptic spine), by developmental expression (e.g. during early circuit formation), or by compatibility with a prevalent hypothesis (e.g. E:I balance). Strategies based on grouping genes and gene products offer promise for inferring mechanism and would be further boosted by the addition of a more physiological view of neuronal autoregulation, as described below.

Dysfunctional feedback regulation as a unifying theme in ASD

Here we expand upon previous work by providing a general framework for ASD that combines genetic information with ideas about the pathophysiology of homeostatic feedback loops. The starting point is a hypothetical scheme for the autoregulation of neuronal firing, proposed by Abbott, Golowasch, Marder, and Turrigiano at Brandeis in a prescient series of papers two decades ago (LeMasson et al., 1993; Marder et al., 1996; Siegel et al., 1994) (Fig. 1). We have adapted this computational model to diagram neuronal firing as the *controlled variable* whose variations are delimited by a feedback system. Changes in a neuron's firing rate result in alterations in Ca^{2+} channel activity, which serves as a *sensor* that drives an *effector mechanism*. The effector mechanism works through a *feedback connection* to counteract variation of the sensed variable from its designated set point. The feedback loop can be likened to a temperature-control system in which the controlled variable is the temperature in a room, the sensor a thermostat, the effector mechanism a remote heating/cooling unit, and the feedback connection the ductwork between the heating/cooling unit and the room itself. In neuronal homeostasis, as in HVAC systems, the feedback connection operates to oppose change, hence its designation as negative feedback. Proper control depends upon successful operation of each of the components; failure of a

component disables the entire loop, while a weakening of function can be compensated for by other elements in the loop, but only within certain limits.

This is but one example of a servo loop mediating autoregulation in the healthy brain, whose operation can go awry in response to genetic lesions or environmental stressors. It exemplifies a variety of feedback mechanisms — not just those involved in regulating electrical activity as illustrated here, but also extending to autoregulation of neuronal biochemistry, cell biology, and microcircuit performance.

The components of this autoregulatory system provide a logical framework for grouping ASD-implicated genes (Fig. 2). Here we form a separate category in addition to those found in previous papers: a homeostatic sensor (sometimes referred to as a “homeostat”). Like the metaphorical room thermostat, it detects perturbations or dysfunction in other parts of the system, and can itself be prone to failure. In turn, the sensor triggers compensatory changes in feedback elements such as nuclear gene expression, local protein translation, and changes in synaptic function and structure. A prototypical homeostat protein would be a Ca^{2+} channel that couples membrane potential changes to downstream biochemical events (see *Diverse systems for sensor-mediated control of gene expression*). Indeed, pore-forming and auxiliary subunits of several Ca^{2+} channels are encoded by known autism-associated genes. Although genetic studies also implicate sodium and potassium channels, their roles in ASD are activity-related in a somewhat different way. While Ca^{2+} channels can send an intracellular signal, Na^{+} channels merely drive electrical firing, and neither Na^{+} nor K^{+} ions are established biochemical messengers; their ultimate impact is likely mediated by Ca^{2+} channels.

With this distinction in mind, we generalize the concept of homeostat proteins to sensors that respond to chemical signals and support downstream biochemical signaling. The chemical ligand can be a neurotransmitter like glutamate, a neurotrophin like BDNF, or a glycoprotein such as Wnt. Once again, the homeostatic sensor can detect variations in the controlled variable and trigger one or more effector mechanisms, including altered nuclear or dendritic events. The feedback can result in altered levels or properties of signaling proteins, modifying excitability or synaptic properties as part of the servo loop to oppose perturbations of the controlled variable (Fig. 2).

This scheme has several advantages for thinking about homeostatic responses in the context of both typical and atypical neuronal activity. It allows the putative assignment of genes into functional rather than anatomical groupings, as we have attempted in Table 1. Mindful of the importance of work identifying genes with high reliability, we have used a color-code to indicate the degree of confidence currently placed on individual genes (see Table 1 legend). Many of the entries do not reach genome-wide significance based on current data but are included because of their fit with a pathophysiological scenario, and are annotated with a footnote. The inclusion and classification of individual genes will likely need revision, but the overall organization conveyed by Figure 2 and Table 1 may help hypothesis-building about possible effects of mutations on downstream events. Preliminary distinctions between sensors, effectors, and feedback elements provide a bridge between gene discovery and thinking about biochemical signaling systems. In the homeostatic framework in Figure 2, a

mutation in any individual element can have effects throughout the loop, and can also impact different routes through the overall loop. The scheme permits a transition between overtly successful compensation to perturbations, to outright failure when the system is challenged beyond its limits.

In the next two sections, we take a tour of the homeostatic loop, beginning with genetic modifications in homeostat proteins and their consequences for nuclear production of mRNA, and then progressing to local protein translation of messages into synaptic proteins.

Diverse systems for sensor-mediated control of gene expression

We illustrate the importance of sensor regulation of transcription by reviewing two ASD-related signaling pathways, one responding to chemical signals, the other to electrical activity. Although rarely summarized side-by-side, these systems address similar biological challenges and are replete with components and biomarkers associated with neuropsychiatric disorders, including autism.

Wnt signaling and ASD

The Wnt signaling system has long been implicated in neuronal overgrowth, as is often observed in the autistic brain (Chenn and Walsh, 2002; Courchesne et al., 2001). Signaling downstream of the proteolipid ligands known as Wnts can proceed via the canonical Wnt/ β -catenin pathway (Nelson and Nusse, 2004), or through multiple non-canonical pathways, not mediated by β -catenin (Gordon and Nusse, 2006; Rosso et al., 2005; Salinas and Zou, 2008), with some involving Ca^{2+} signaling (Kohn and Moon, 2005). Canonical Wnt signaling acts, indirectly, to enhance the stability of β -catenin, allowing its translocation from the cell surface to the nucleus, thereby connecting extracellular signals to the regulation of nuclear gene expression by downstream transcriptional machinery (Fig. 3). Mutation of the gene encoding β -catenin, *CTNNB1*, has been found among suggestive *de novo* ASD risk contributing mutations (de Ligt et al., 2012; O'Roak et al., 2012b); furthermore, ~40% of those 126 mutations map to a highly interconnected β -catenin/chromatin remodeling network (O'Roak et al., 2012b). However, it is worth noting that β -catenin also participates in cell-cell interactions via cadherin (*CDH8* being a candidate ASD gene, see Table 1), independent of Wnt signaling. Otherwise, the evidence favors the involvement of genes co-regulated with the canonical Wnt/ β -catenin pathway. One supporting argument derives from the clear role of *CHD8* (not to be confused with *CDH8*), an ASD gene highlighted by multiple genetic studies (Krumm et al., 2014). There is mounting evidence from work in both fly and humans that CHD8 negatively regulates β -catenin (*CTNNB1*) function, either through direct binding to β -catenin, or by recruitment to promoter regions of β -catenin-responsive genes (Thompson et al., 2008). This may explain why putative loss of function mutations of *CHD8* and *CTNNB1* exert opposite effects on brain growth in ASD probands (Chenn and Walsh, 2002; Krumm et al., 2014). *CHD8* provides a leading exemplar of an autism-associated gene product that regulates other autism risk genes (Cotney et al., 2015).

Surface-to-nucleus signaling by β -catenin is subject to positive and double negative (disinhibitory) regulation, both dependent on elements linked to ASD (Fig. 3, ASD-related genes shown in bold colors, matching the scale in Table 1). On one hand, MET tyrosine

kinase, which has been implicated in ASD (Judson et al., 2011), responds to hepatic growth factor by liberating β -catenin from binding to surface cadherins (e.g., *CDH8*). On the other hand, free cytosolic β -catenin is phosphorylated by GSK3 β (part of a destruction complex), and thus marked for proteosomal degradation. Finally, various species of Wnt molecules, including Wnt2, signal across the surface membrane through interaction with a frizzled receptor (mostly subtype FZD9) and an auxiliary receptor LRP5/6, which have tantalizing but at present unsubstantiated linkages to ASD. Dishevelled (*DVL*), which inhibits the destruction complex, was knocked out in the first mouse model of social dysfunction (Lijam et al., 1997), an early predecessor to more recent findings connecting Wnt related signaling molecules to ASD (De Ferrari and Moon, 2006; O'Roak et al., 2012a; Parikshak et al., 2013). The canonical-Wnt signaling system overlaps with multiple branches of biochemical signaling, each amply dotted with ASD-implicated proteins (Fig. 3). The availability of druggable-components in these signaling events presents intriguing opportunities for pharmacological manipulation, building upon experience with Wnt signaling and cancer therapeutics (Moon et al., 2004). However, as clearly summarized by Kalkman (Kalkman, 2012), one must recognize the multiple roles of Wnt signaling, not only in cell adhesion and dendritic remodeling but also in early brain development (Rosso et al., 2005; Salinas and Zou, 2008; Ueda et al., 2008; Wayman et al., 2006; Yu and Malenka, 2003). This is but one example of signaling proteins that participate in both neuronal development and ongoing function (Box 1; next section).

Activity-dependent gene expression in ASD involves calcium channels operating as homeostats

The idea that defects in activity-induced gene expression contribute to ASD was initially based on a provocative realization: while only 3% of the transcriptome is expressed in an activity-dependent manner, a far greater percentage of ASD implicated genes display activity-dependent expression (Kelleher and Bear, 2008; Morrow et al., 2008). Likewise, ASD-associated mutations that alter neurotransmission ultimately disrupt activity-dependent signaling to the nucleus (Ebert and Greenberg, 2013). Thus, it is widely believed that defects in activity-dependent gene expression may be a common feature of diverse forms of ASD. Just as Wnt signaling via frizzled receptors provides a mechanism for neurons to monitor their local extracellular environment, activity-dependent gene expression provides an ongoing readout of neuronal firing. Communication of neuronal depolarization proceeds by a variety of signaling cascades and regulates intranuclear events such as enhancer recruitment (Kim et al., 2010), transcription factor activation (Ebert and Greenberg, 2013) and alternative pre-mRNA splicing (Li et al., 2007). Further, multiple forms of activity-dependent regulation can operate in coordination, for example, Ca²⁺ channel control of nuclear gene expression by excitation-transcription (E-T) coupling or excitation-alternative splice (E-AS) coupling could act in concert with transmitter-driven alterations of local translation in dendrites (see next section).

Ca²⁺ channels are perfectly suited to act as sensors of electrical activity. By transducing membrane potential changes into protein conformational changes and Ca²⁺ influx these channels serve as homeostats, transmitting information about neuronal activity to downstream effector systems. The pore-forming α_1 subunit (e.g. *CACNA1C*) and the

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auxiliary subunits β (e.g. *CACNB2*) and $\alpha_2\delta$ (*CACNA2D3*) were all prominent hits in a large-scale genetic screen across multiple neuropsychiatric diseases (Cross-Disorder Group of the Psychiatric Genomics et al., 2013). Further, there is clear evidence from Timothy syndrome (TS), a rare genetic disorder arising from mutation of either *CACNA1C* or *CACNB2*, that improper function of a Ca^{2+} channel can engender ASD with a penetrance as high as 60-80% (Breitenkamp et al., 2014; Splawski et al., 2005; Splawski et al., 2004). The pathogenic effects of genetically altered Ca^{2+} channel subunits likely revolve around their ability to generate a conformational signal in response to membrane depolarization (Li, Tadross & Tsien, accepted for publication in *Science*). The point mutations that give rise to ASD generally sensitize voltage-dependent gating of Ca^{2+} channels, shifting its activation to more negative potentials by ~ 10 mV (e.g. G406R in *CACNA1C*, but not G402S). Thus, the neurologically penetrant mutation gives an abnormal voltage setpoint, in keeping with the premise of a disturbed regulatory feedback loop. Finally, exome sequencing of simplex libraries has repeatedly pointed to *CACNA1D*, the pore-forming subunit of another L-type channel, as another ASD-associated gene (De Rubeis et al., 2014). In this case, neurologically active mutations also cause a negative shift of voltage-activation (Pinggera et al., 2015), as expected from studies of *CACNA1C*.

What signaling pathways lie downstream of Ca^{2+} channel opening? The $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ proteins encoded by *CACNA1C* and *CACNA1D* are localized in postsynaptic compartments (spines and somatodendritic areas, respectively). The Ca^{2+} rise that matters for signaling to the nucleus must occur within 1-2 μm of the channel mouth (Deisseroth et al., 1996), but is linked to the nucleus by a signaling pathway (Ma et al., 2014), depicted in Fig. 4.

Communication is initiated by a signaling complex near the dendritic Ca_v1 channel, which sends a shuttle protein to the nucleus upon activation. In excitatory neurons γCaMKII operates as the shuttle, gathering Ca^{2+} /calmodulin ($\text{Ca}^{2+}/\text{CaM}$) from the cytoplasm and transporting it into the nucleus. There, $\text{Ca}^{2+}/\text{CaM}$ is released and activates CaMKK and its substrate CaMKIV, the protein kinase responsible for CREB phosphorylation and other nuclear events. Once activated by the CaMK cascade, CaMKIV is able to phosphorylate another important nuclear factor, CREB binding protein/p300 (CBP, gene name *CREBBP*) (Impey et al., 2002; Kwok et al., 1994). Mutations in *CREBBP* give rise to Rubenstein-Taybi syndrome, a disorder with prominent autistic features (Petrij et al., 1995). Phospho-CBP acts as a requisite co-factor of multiple transcription factors, including CREB and the Wnt-dependent TCF-LEF1 complex.

Strikingly, genes encoding the key molecular players have been repeatedly implicated in neuropsychiatric disorders including autism, depression, and schizophrenia (Fig. 4, ASD-associated genes in bold, colors according to Table 1). How can alterations in individual signaling or structural proteins be involved in such a diverse array of neuropsychiatric diseases? Genetic studies have revealed many disease-related alleles, but much work remains to evaluate their effects on the magnitude and even the sign of the functional differences. This effort has begun for an intronic SNP of *CACNA1C* (rs1006737) that crops up in multiple neuropsychiatric disorders (Ferreira et al., 2008; Green et al., 2010; Moskvina et al., 2009; Nyegaard et al., 2010), including ASD (Li et al., 2015). $\text{Ca}_v1.2$ mRNA levels and current densities were both consistently increased in induced human neurons homozygous for the risk genotype compared with non-risk genotypes (Yoshimizu et al.,

2015). The consistency bodes well for therapeutic approaches, but offers no specific clues as to the differences between ASD and these other disorders. It remains possible that other alleles may cause disorder-specific effects on protein abundance or function; alternatively, those changes could be similar (Yoshimizu et al., 2015), but the final functional outcome in the brain might vary according to the genetic or environmental context. Knowledge of environmental factors is growing, as exemplified by converging evidence that fetal testosterone may contribute to the preponderance of ASD in males vs. females (~4:1) (Baron-Cohen et al., 2011).

With overall outcome in mind, we now turn from the dysfunctional calcium channel homeostat to the altered programs of gene expression that it triggers. Abnormalities were observed in neuronal precursors derived from individuals with Timothy syndrome; the expression of >200 genes were altered, many of them CREB-dependent (Pasca et al., 2011). When subjected to an unbiased systems biology analysis (Tian et al., 2014), the pattern of differences in gene expression was compatible with involvement of known Ca²⁺-dependent transcriptional regulators, including CREB, NFAT, MEF2, and FOXO. The analysis uncovered Ca²⁺-dependent co-expression modules that reflect distinct aspects of TS, including intellectual disability and ASD-related phenotypes. The approach also uncovered several players that provide unexpected connections to other signaling modules. Thus, multiple feedback loops may interconnect (see Summary and Future Outlook). Looking at gene expression beyond transcription, activation of the nuclear CaMKIV also controls alternative splicing of transcriptionally derived pre-mRNA (E-AS coupling). For example, CaMKIV phosphorylation regulates the activity of RBFOX1, a factor that controls the alternative-splicing and stability of many mRNAs involved in synaptic and neuronal signaling, including *CAMK2G*, *GRIN1*, *CNTNAP2*, *NRCAM* and *RBFOX1* itself (Gehman et al., 2011). Voineagu, Geschwind and colleagues compared normal and RBFOX1-deficient samples (therein referred to as A2BP1) and found hundreds of variations in splicing events, many of them in transcripts of ASD-linked genes (Voineagu et al., 2011). Overall, RBFOX1 target genes were enriched for synaptic proteins, actin-binding proteins and other molecules involved in cytoskeleton reorganization, processes crucial to proper neuronal structure and connectivity. Taken together, studies of E-AS coupling and E-T coupling underscore the power of activity-dependent, Ca_v1-regulated signaling and its impact on expression of ASD-linked genes and neuronal pathobiology.

Dysfunction of Local mRNA Translation and Synaptic Plasticity

Decades after local dendritic protein translation was first proposed (Steward and Levy, 1982), there is now wide agreement that it plays an integral part in synaptic plasticity (Schuman et al., 2006). Furthermore, consensus exists that disturbance of dendritic mRNA translation contributes to ASD (Darnell and Klann, 2013; Kelleher and Bear, 2008). Here we compare two modes of local translational regulation, each involving well-known ASD-related signaling proteins, FMRP and TSC1/2. We point out that these regulatory modes have sharply different effects on synaptic plasticity, and put forward a pathophysiological hypothesis that yokes together their dichotomous effects on both synaptic structure and activity.

Genetic alterations in the Fragile X mental retardation 1 gene (*FMR1*) give rise to Fragile X Syndrome (FXS) (Verkerk et al., 1991). This relatively common form of inherited intellectual disability is often associated with symptoms of ASD, including perseverative behaviors, speech and sensory difficulties, and disrupted sleep (Kidd et al., 2014). Encoded by the *FMR1* gene, Fragile X mental retardation protein (FMRP) mostly operates as an mRNA-binding protein (Fig. 5B2) that represses translation of an emerging subset of mRNAs (Brown et al., 2001; Darnell et al., 2001; Darnell et al., 2011; Santoro et al., 2012), among other functions (Contractor et al., 2015). FXS is often compared to another single-gene syndromic disorder, Tuberous sclerosis (TSC). Frequently associated with deficits in long-term and working memory, along with intellectual disability, ASD, and epilepsy, TSC arises from heterozygous mutations in the *TSC1* or *TSC2* genes (Ehninger et al., 2009; Ehninger et al., 2008; Kong et al., 2014; Tsai et al., 2012). The TSC1/2 protein works through negative regulation of the mTOR cascade involving Rheb, TORC1, and the EIF4E/EIF4G complex to dampen translation. The net result is that TSC1/2, like FMRP, operates as a negative regulator of local translation. Strikingly, genetic manipulations that relieve inhibition by either FMRP or TSC1/2 exert sharply different effects on classic forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD) (Fig. 5). Closer examination of these disparities is illuminating.

In hippocampal slices from *Tsc2*^{+/-} mice, LTP is enhanced (Fig. 5A1); as a result, protocols that normally induce a decaying form of potentiation (E-LTP) result in the generation of long-lasting LTP (L-LTP) (Ehninger et al., 2008). Furthermore, LTD is attenuated, both in *Tsc2*^{+/-} (Auerbach et al., 2011; Bateup et al., 2011), and in a loss of function mutant, *Tsc2* *RG*, that weakens translational braking (Chevere-Torres et al., 2012). In accord with the consensus-signaling pathway (Fig. 5A1,A2), Ehninger *et al.* further demonstrated that abnormalities of LTP and deficiencies in hippocampal-dependent learning tasks in *Tsc2*^{+/-} animals, in which mTOR-signaling is abnormally heightened, can be rescued by the mTOR inhibitor rapamycin.

In contrast to the loss of TSC function, deletion of *FMR1* gives rise to dramatically different alterations of both potentiation and depression. The *fmr1* KO spares L-LTP (Zhang et al., 2009), while LTD is famously enhanced (Huber et al., 2002) (Fig. 5B1). With either low frequency stimulation (LFS, Fig. 5B1) or application of a metabotropic glutamate receptor agonist (Huber et al., 2002), removal of FMRP braking causes an increase in the amplitude and persistence of LTD.

Taken together, these findings suggest that the physiological outcome of increased translation differs, depending on the mode of regulation. The distinction between FMRP- and TSC-mediated control and their physiological outcomes has not been emphasized in the literature. Focusing on these differences begs the question: how might various modes of elevated protein synthesis be linked to synaptic plasticity and behavior?

Relating local translation to synaptic plasticity and disease

How might malfunctioning protein synthesis impair regulation of synaptic plasticity? As a starting point, we focus on the duality between synaptic strengthening and weakening observed in long-term potentiation (LTP) and long-term depression (LTD). Textbooks show

LTP and LTD as functionally opposite outcomes, either of which can occur at the level of individual synapses (all true). Sometimes LTP is presented as the inscription of a tiny piece of memory, and LTD as its erasure. This implicitly portrays memory storage as rooted in the strengths of single synapses, with each individual spine operating as a discrete unit independent of its neighbors. It is unlikely that changes in the nervous system during such events are simply a net increase or decrease in total synaptic activity as this simplified model would indicate. A more integrated view is a partnership in which LTP and LTD work in parallel, rather than in opposition, to redistribute synaptic strength in support of behavioral plasticity.

Bidirectional changes at nearby spines

In work not previously highlighted in the context of local translation, synaptic biologists postulated and observed local coordination between strengthening and weakening of synapses, advantageous for both network stability and for optimizing information storage. For example, Rabinowitch & Segev theorized about dendritic mechanisms of local regulation, where the strengthening of single spines by Hebbian plasticity is chained to compensatory mechanisms for weakening other spines nearby (Fig. 6A) (Rabinowitch and Segev, 2008). These ideas are supported by the anatomical studies of Bourne & Harris, who compared the morphology of serially reconstructed dendrites from hippocampal tissue, which had or had not undergone saturating LTP (Bourne and Harris, 2012). After LTP, single spines enlarged and the postsynaptic area increased, as expected. But remarkably, the density of spines along the dendritic length fell by almost half; as a result, the aggregate postsynaptic area over the examined stretch of dendrite was no different in LTP compared to control conditions (Fig. 6B). The implication is that spines in the neighborhood of the strengthened synapses were subject to weakening or disappearance, very much as Rabinowitch & Segev suggested. The kind of coordination Bourne & Harris observed would help keep the overall synaptic input of a dendritic segment within reasonable bounds, while allowing selective inputs to strengthen. Both theory and experiment point out the usefulness of considering spines populating a section of dendrite collectively, and reckoning with the possibility of disparate changes in synaptic strength (Fig. 6C1,C2).

Neither Rabinowitch & Segev nor Bourne & Harris explicitly addressed how coordination between strengthening and weakening of neighboring spines might come about. Knowing that LTP is induced by a strong input and a strong release of glutamate onto a soon-to-be-strengthened spine, it becomes clear that this must be accompanied by glutamate spillover to nearby spines. By laws of diffusion, the local concentration of glutamate ([Glu]) must fall as a function of distance from the source. Thus, nearby spines must experience a decline in local [Glu] such that the more sensitive mGluRs, but not NMDARs, are strongly activated, leaving mGluR signaling to predominate in a penumbra of spines near the one destined to be strengthened via LTP. According to the mGluR scenario (Bear et al., 2004), the neighboring spines would undergo rapid PP2A-, FMRP-mediated de-repression of local translation. This in turn will increase the abundance of regulatory proteins such as Arc, CaMKII α , PSD95, and CaMKII β (Darnell et al., 2011; Muddashetty et al., 2011; Niere et al., 2012; Park et al., 2008; Stefanovic et al., 2015), setting up the spine for a binary decision, to undergo LTD or LTP.

Sharp dichotomy in regulating synaptic strength

The morphological picture from Bourne and Harris suggests that individual spines experience a threshold phenomenon that determines whether they are to be strengthened or weakened, presumably guided by the inputs they receive during LTP induction. How is the decision point between one or the other determined? FMRP-regulated gene products such as Arc, β CaMKII and SAPAP (Brown et al., 2001; Darnell et al., 2011; Niere et al., 2012; Park et al., 2008) could contribute to a biochemical mode of discrimination. Okuno *et al.* have shown that β CaMKII serves as an activity sensor; when Ca^{2+} is low, CaM-free, kinase-deactivated β CaMKII interacts directly with Arc and helps facilitate Arc-mediated endocytosis of AMPARs (Okuno et al., 2012). In contrast, when Ca^{2+} is high, activated β CaMKII can participate in SAPAP trafficking and increase incorporation of AMPARs, enabling spine remodeling and synaptic strengthening (Shin et al., 2012). Furthermore, NMDAR-dependent mechanisms could work through α CaMKII accumulation to drive AMPAR incorporation and LTP (Huganir and Nicoll, 2013; Lisman et al., 1997). Thus, the decision between strengthening and weakening likely employs local protein synthesis as a platform, but is ultimately governed by β CaMKII as a local sensor of activity-dependent rises in Ca^{2+} .

Sharp decision between LTP- and LTD in single spines, but coexistence along a dendrite

Published data supports that negative feedback connections between translational modes are regulated by FMRP and TSC1/2. Two possible players are a protein kinase (S6K1) and a protein phosphatase (PP2A), each known to play multiple roles (Fig. 5A2, B2). S6K1 has been shown to act downstream of TORC1 to drive EIF4E-regulated initiation of translation (Richter et al., 2015), and dampens translation of FMRP-regulated mRNAs by phosphorylating FMRP (Santoro et al., 2012). Furthermore, PP2A not only removes the FMRP brake (Niere et al., 2012) but also interferes with NMDA signaling by dephosphorylating and deactivating ERK1/2 (Alessi et al., 1995). With this negative cross-talk, individual spines are expected to select sharply between one translational program or the other (depicted schematically as mutual inhibition between LTP and LTD modules, Fig. 6C1, C2). In synapses with exaggerated LTD, as in FXS, LTD is further linked to synapse elimination via the involvement of the nuclear transcription factor MEF2 (Pfeiffer et al., 2010). Huber and colleagues showed that MEF2-mediated transcription and local FMRP-regulated translation work together to eliminate excitatory synapses. This provides a satisfying explanation for the thinning out of non-potentiated postsynaptic spines in Fig. 6B (Bourne and Harris, 2012).

To recapitulate this composite scenario for *synapse weakening* (Darnell et al., 2011; Gross et al., 2015; Huber et al., 2002; Niere et al., 2012):

Low, steady $\uparrow[\text{Glu}]$ (or mGluR type I agonist) \rightarrow mGluR activation \rightarrow PP2A activation \rightarrow FMRP derepression \rightarrow \uparrow Arc;

\uparrow Arc, working with non-active CaMKII β \rightarrow GluA1/2 removal \rightarrow synapse weakening \rightarrow LTD, and possibly synapse elimination

This stands in contrast with, and in opposition to another hypothetical scenario, a composite view of *synapse strengthening* (Banko et al., 2005; Bateup et al., 2011; Kelleher et al., 2004):

High, brief \uparrow [Glu] \rightarrow NMDAR act \rightarrow \uparrow MAPK signaling \rightarrow inhibition of TSC complex function \rightarrow inhibition of Rheb inhibition \rightarrow mTOR activation \rightarrow EIF4E activation \rightarrow GluA1,2 synthesis \rightarrow GluA1 incorporation upon \uparrow CaMKII α,β activation \rightarrow LTP \rightarrow secondary morphological changes

Brief recap

Our working hypothesis depicts individual spines as veering between two kinds of activity-dependent regulation of mRNA translation. Taking advantage of the greater glutamate sensitivity of mGluR versus NMDAR, they support dichotomous changes in postsynaptic properties that favor LTD in one case and LTP in the other. We hypothesize that these processes are mutually antagonistic within single spines, but can work side by side at nearby spines. This model provides a means for putting Hebbian plasticity at one spine in close proximity to LTD at a near neighbor (Rabinowitch and Segev, 2008) and thus redistributing synaptic weights (Bourne and Harris, 2012). Proteins locally synthesized under control of FMRP or TSC1/2 are critical for LTD and LTP. Accordingly, dysfunction of either mode of regulation would be disruptive for overall LTP:LTD coordination (and potentially circuit-wide E:I coordination), and thus deleterious for behavioral plasticity.

E:I coordination and altered synapse structure

A dozen years ago, Rubenstein & Merzenich (R&M) put forth a provocative hypothesis: that an excess of excitation relative to inhibition was a major cause of autism (Rubenstein and Merzenich, 2003). As they argued, and as recent studies have reminded us, 30% of patients with an ASD also have epilepsy, and 30% of patients with epilepsy have been diagnosed with an ASD (Bolton et al., 2011; Brooks-Kayal, 2010; Tuchman et al., 2009). R&M went on to note that 50-70% of EEG or MEG sleep recordings from children with autism show evidence of sharp spike activity, indicative of unstable cortical networks. The concept of “E:I balance” has greatly stimulated studies of brain physiology and ASD pathogenesis, even though on closer examination the “balance” is neither complete nor desirable. During normal synaptic communication, the inhibitory current is typically several-fold larger than the excitatory current at the neuron's resting potential. As a result, the E:I ratio cannot be 1.0 as the word “balance” implies. In feedforward networks excitation typically occurs out-of-sync with inhibition, preceding it by milliseconds. This pattern of activity creates a compound synaptic event that drives spiking with much greater temporal precision than could be achieved by excitation alone (Pouille et al., 2009; Pouille and Scanziani, 2001). Indeed, a fixed moment-to-moment relationship between E and I would not be effective, either for throughput of single spikes, or for more complicated and powerful forms of network activity such as sharp wave ripples, which require E and I to be out of step with each other (Stark et al., 2014). In several ways then, “E:I balance” does not accurately depict the temporally asynchronous, proportional-but-not-equal, temporarily adjustable relationship between the magnitudes of E and I required for proper network activity. We utilize “E:I

coordination” as a more faithful characterization of the neuronal state, amply supported by experimental evidence that neuronal circuits can enforce coordination of E and I strength (Xue et al., 2014)(Fig. 7B), even down at the level of single dendritic branches (Liu, 2004) (Fig. 7A). Terminology aside, understanding E:I coordination is a pressing issue for neuroscience in general, and for the pathogenesis of ASD in particular.

So how good is the evidence that ASD results from dysfunctional E:I coordination? Does ASD arise from too much E relative to I, as R&M proposed, or is the opposite just as dangerous? These issues are addressed in a thoughtful review by Nelson and Valakh (2015). Two of their important messages are that: 1) perturbations of excitation or inhibition must be considered within the broader context of complex neuronal circuits, and 2) it is hard to disentangle the impact of a primary insult from the compensation that ensues. The first point is vividly illustrated by Rothwell, Malenka, Sudhof *et al.*, who have uncovered a circuit basis for repetitive motor behaviors arising from ASD-based manipulations of NLGN3: altered synaptic inhibition of medium spiny inhibitory neurons in ventral striatum (Rothwell et al., 2014). The second point is extended here by putting proteins encoded by ASD genes into explicit feedback loops for neuronal autoregulation.

Regardless of the specific circuit, adjustments of inhibitory neuron firing or inhibitory transmission can operate much more rapidly than feedback by transcriptional or translational loops. As a consequence, E:I coordination can be viewed as made of the same cloth as transcription and translation autoregulation — with the control of inhibition filling in a critical kinetic gap. Thus, adjustment of inhibition provides a particularly powerful and temporally flexible form of autoregulation. Conversely, it stands to reason that dysfunction of inhibition would be a major component of ASD pathogenesis.

There is little doubt that E:I coordination would be affected by genetic perturbations of a variety of synaptic components, including postsynaptic components (*PSD95*, *SHANK1*, *-2*, *-3*; *SYNGAP1*), and proteins involved in trans-synaptic interaction (*NRXN2*, *NGLN1*, *-3*, *-4*). For example, the ASD-associated SHANK family are scaffolding proteins present in the postsynaptic density that serve a crucial role in synapse formation and maintenance by connecting proteins at the membrane with the actin cytoskeleton of the dendritic spine (Guilmatre et al., 2014). *SHANK3* is one of the genes with the strongest statistical links to ASD, but as the protein functions postsynaptically in both excitatory and inhibitory populations, its effects on E:I coordination, while very important, are not simple to pin down. In the previous section, “Dysfunction of Local mRNA Translation and Synaptic Plasticity,” we discussed aspects of excitatory synaptic strength that can go awry in ASD. Here we briefly focus on a narrower set of examples where ASD may arise specifically from a defect in inhibition, to highlight the idea that dysfunction of E:I coordination can play a causal role in ASD.

ARX

(Aristaless related homeobox gene), an X-linked gene (Xp22.1), encodes the first transcription factor (TF) implicated in causing autism (Rubenstein and Merzenich, 2003; Turner et al., 2002). Boys that inherit mutations in *ARX* primarily manifest epilepsy, mental retardation or movement disorders, and some also have ASD (Turner et al., 2002). *ARX*

mutations have pleiotropic effects (Rubenstein and Merzenich, 2003; Sherr, 2003; Turner et al., 2002), not atypical of ASD-causing genes. In the mouse, *Arx* is expressed in several regions and cell types within the forebrain. For ASD, its most pertinent role is in a hierarchy of TFs critical for GABAergic interneuron development. Loss of *ARX* severely impairs interneuron development (Vogt et al., 2014), as *Arx* is a repressor that functions to orchestrate interneuron differentiation. Of the 84 genes found to be abnormally regulated in *Arx*^{-/-} mice (Fulp et al., 2008), many are involved in neuronal development (cell migration, axonal guidance, neurogenesis) and some have been implicated in ASD, epilepsy, and mental retardation, befitting clinical features seen in patients with *ARX* mutations. While much work is needed to clarify the molecular and behavioral effects of *Arx* regulation (Marsh and Golden, 2012), *Arx* already provides a clear example of the severe impact of interneuron loss on ASD as well as epilepsy (Rubenstein and Merzenich, 2003).

SCN1A

Mutations of this sodium channel gene are most prominent in Dravet syndrome (DS), also known as severe myoclonic epilepsy in infancy (SMEI). This disorder is characterized by infantile-onset epilepsy, both febrile and afebrile seizures resistant to medication, and cognitive dysfunction. DS occurs from loss of function of *SCN1A*, which encodes the α -subunit of the voltage-gated sodium channel Na_v1.1, prominently expressed in the soma and axon initial segment of neurons (Cheah et al., 2012). Identification of >300 mutations in *SCN1A* exons accounts for ~70% of DS cases; and most mutations are truncations or deletions that disrupt voltage sensing or ion conduction, with the remaining 30% likely acting through their impact on *SCN1A* expression (Oakley et al., 2011). *SCN1A* loss-of-function impairs sodium currents and spiking in hippocampal GABAergic interneurons, without a detectable effect on excitatory pyramidal neurons. In turn, decreased inhibitory firing leads to dysregulation of inhibition, the likely cause of seizures in DS patients (Oakley et al., 2011). Closer examination of autistic features in 37 DS patients showed that ~25% met the criteria for autism, and ~95% presented with intellectual disability, with more severe deficits in those with autism. Interestingly, there was no apparent difference in the epileptic features of DS patients with or without autism (Li et al., 2011). Do autistic-like behaviors arise as sequelae of frequent seizures, or directly from reduced GABA transmission? To address this, Catterall's group treated *Scn1a*^{+/-} mice with the benzodiazepine clonazepam, a positive allosteric modulator of GABA_A receptors that enhances opening of GABA receptor chloride channels in response to presynaptically released GABA. Low doses of clonazepam completely rescued the abnormal social behaviors and deficits in fear memory typically presented by *Scn1a*^{+/-} mice (Han et al., 2012), demonstrating the impact of restoring the proper coordination of inhibitory transmissions.

Overview

By reducing the number or excitability of inhibitory neurons, *ARX* and *SCN1A* weaken GABAergic transmission in distinct ways. Taken together with other genes (e.g., *GABRB3*, *CNTNAP2* (Sanders et al., 2015), and possibly *CNTNAP4* (Karayannis et al. 2014)), these ASD-associated genes illustrate the importance of E:I coordination. We note that many of the genetic actions do not encourage sharp distinctions between inhibitory neuron function or structure, or even between effects on excitatory or inhibitory neurons. Further, E:I

dyscoordination is capable of influencing a variety of other neuronal functions, including plasticity and activity-dependent gene expression. How these functions may connect and interact is challenging to predict, particularly as the downstream effects will vary greatly. Nevertheless, the concept of E:I coordination will remain a pillar of ASD pathogenesis.

Summary and Future Outlook

This review extends traditional schemes that link genes \rightarrow behavior in terms of a chain or tree of causality. We have emphasized a rethinking of those logical relationships: gene candidates, signaling proteins, and physiological variables, organized in a set of dynamic feedback loops (exemplified by the scheme in Fig. 2). Our emphasis on feedback was motivated in part by prevailing groupings of ASD-related genes. As categorized by putative function (activity-dependent transcription, glutamate regulation of local protein synthesis and synaptic plasticity, and alterations of E:I coordination and synaptic structure), all of these groups display a prominent autoregulatory aspect. We build upon excellent reviews on ASD that highlight the role of homeostasis (Ramocki and Zoghbi, 2008; Toro et al., 2010; Wondolowski and Dickman, 2013), the bidirectional communication between postsynaptic receptors and dendritic protein synthesis (Santini and Klann, 2014), and the influence of homeostasis on aberrant E:I coordination (Nelson and Valakh, 2015). We also take note of valuable categorizations of ASD-associated genes based on anatomical location or protein-protein interactions. The physiological framework reviewed here builds upon the recent identification of interconnected signaling hubs (Krumm et al., 2014; Parikshak et al., 2013; Voineagu et al., 2011; Willsey et al., 2013), and a venerable literature on feedback in neurons, ranging from action potential generation (Hodgkin and Huxley, 1952) to transcription-factor based neuronal differentiation (Hobert, 2011; Weinberg et al., 2013). If constellations of ASD-associated genes support the servo control of neuronal excitability, gene expression, and local synaptic strength in the healthy brain, it is reasonable to think that faulty dynamic feedback regulation will also be important for dysfunction in the autistic brain.

To introduce the power of feedback, we began with a unitary track and a specific mechanism for autoregulation of neuronal excitability (Fig. 1). However, the expanded feedback arrangement in Fig. 2 offers multiple choices for sensor, effector mechanisms, feedback connections and controlled variables, among further possibilities not enumerated. In combination, such options greatly expand the possible ways that a neuron or circuit could use feedback to keep physiological variables close to their setpoints. Servo loops for different variables may also interact (e.g., dysfunction of Wnt signaling and dendritic morphology would alter E:I coordination). Thus, the scheme provides ample opportunity for rich regulatory interactions, but adds complexity to the task of deciphering ASD.

Implications of dysfunctional autoregulation

If malfunctioning servo loops help manifest ASD, researchers will encounter a mix of good and bad news. On the positive side, stressed-out feedback systems will likely alter levels of proteins, protein phosphorylation, and signaling molecules, thus providing useful biomarkers as to the identity of the dysfunctional pathway. Furthermore, a therapeutic strategy could

aim to repair the loop as a whole, rather than the specific molecular lesion itself. Confusingly, however, a specific change in cellular biochemistry could be part of the pathogenic mechanism or a beneficial homeostatic response to it. Disambiguating these possibilities would be needed to understand cause and effect, but will present the challenge of devising better experimental methods for directly and specifically varying the affected players. Analysis of genetic modifications will remain the gold standard for causative inference. Nonetheless, transcriptomic, proteomic or metabolic changes will also be useful for the identification of dysfunctional pathways well before their pathogenic or homeostatic impact is readily determined.

The hypothetical framework is put forward at the level of neurons and not any particular brain circuit. However, the expression of perturbations and autoregulatory sequelae will depend on the particular circumstances of individual circuits and their cellular components. Thus, abnormal physiological feedback would affect different circuits in varying ways, depending on their cellular and synaptic makeup (Rothwell et al., 2014). Pathology at the cellular level may even reverberate around multiple interacting circuits. The diverse impact in individual circuits likely contributes to clinical complexity, but may eventually help diagnostic efforts once underlying principles can be deciphered.

Predictions for the future

We close this review with largely optimistic predictions.

1. Relating the pathophysiology of ASD to the physiology of the normal brain is underexplored, but will be of mutual benefit.
2. Despite its current bewildering complexity, the genetics of ASD (~500 genes) will prove useful in seeking a unifying pathophysiological scheme. Here we side with those who prefer to keep focused on the entire spectrum (like Hans Asperger and the committee that made DSM-V) rather than split it into sub-disorders (like Leo Kanner and the creators of DSM-IV). Consideration of autoregulatory loops suggests a wide diversity of specific pathophysiological sub-scenarios, possibly easing the ongoing tension between lumping and splitting (Silberman, 2015). Finding yet more causative genetic modifications might prove helpful in delineating those pathophysiological sub-scenarios.
3. Extrapolating from excitation-transcription coupling (Fig. 4) and local glutamate-translation coupling (Fig. 5), we expect that coupling between excitation and chromatin remodeling will be a compelling process for further investigation (Kim et al., 2010), with likely relevance to ASD. Furthermore, we anticipate further pursuit of functional synergies between the effects of LTP and LTD, aided by local translation of mRNA in dendrites.
4. Medium- to high-throughput methods will be developed to determine the physiological impact of genetic perturbations. This will be needed to sort the individual mutations according to their impact on signaling, which may not always be equivalent to their gene ontology class. A rare but exemplary study examined a non-coding variant in *CACNA1C* of wide distribution across

neuropsychiatric diseases and found gain of function in transcript abundance and L-type current density in a great majority of instances (Vogt et al., 2015; Yoshimizu et al., 2015). In general, if altered function of an ASD-related protein can be traced further to changes in transcription, splicing, translation or trafficking, varied polygenic interactions are to be expected with gene products controlling those processes.

5. ASD-related signaling pathways that appear in genetic screens could also be targets of environmental agents. Surface signaling proteins such as calcium channels and NMDA receptors are very susceptible to toxins, drugs and antibodies (Cline et al., 1996). Research on environmental influences such as maternal inflammation (Hsiao et al., 2012; Patterson, 2011), the gut microbiome (Hsiao et al., 2013), and ASD-provoking drugs such as valproic acid may offer new understanding. Not all environmental factors will prove to be deleterious. Sleep, fever, and behavior modification each have beneficial influences that are not well understood but might influence compensatory feedback signaling.
6. Complementary experimental and computational approaches will be productively harnessed in studying feedback systems that interlock at cellular, circuit, and network levels. Homeostatic compensation for dysfunctional E:I coordination (Nelson and Valakh, 2015) will be an interesting proving ground for computational analysis (Rosenberg et al., 2015). The agent bumetanide, now in clinical trials for treatment of ASD (Lemonnier and Ben-Ari, 2010), enhances GABAergic inhibition and may thereby beneficially readjust activity-dependent feedback.
7. Network analysis will aid the development of biomarkers for dysfunctional signaling, which to be most useful, cannot be viewed statically or in isolation. As a diagnostic strategy, the time-synchronized read-out of multiple biomarkers may be a first step in designing and tuning therapeutic interventions.

Development Box

Autism spectrum disorders are often framed as the outcome of aberrant development and interconnectivity of brain structures (Berg and Geschwind, 2012; Courchesne et al., 2001; Geschwind and Levitt, 2007). This prompts the question of whether ideas about dysfunctional homeostasis in ASD might be applicable to neurodevelopment. If homeostasis provides negative feedback to minimize deviations from a fixed set point, it might appear at odds with neurodevelopment, a fundamentally transitional process. In fact, negative feedback mechanisms could also support developmental trajectories if driven by a gradually varying set point, like a graded setting on a thermostat or a changing command potential in a voltage clamp system. Alternatively, certain stages of neurodevelopment may not be driven by feedback regulation, but may harness a unidirectional chain of events like a transcription factor cascade, tuned by feedback on an evolutionary scale but not by immediate servo control.

Early neurodevelopment relies on cell-to-cell chemical signaling rather cell-autonomous, activity-dependent homeostasis like that in Fig. 1. Nonetheless, autoregulation, involving positive as well as negative feedback, is critical. Positive feedback loops generate a threshold between two discrete developmental states, enforcing all-or-none distinctions between neuronal precursors and their resultant progeny. Classic experiments have defined lateral inhibition between a neuroblast and its neuronal precursor neighbors (Doe and Goodman, 1985), the molecular players in intercellular communication (Louvi and Artavanis-Tsakonas, 2012), and downstream nuclear signaling mechanisms (Imayoshi and Kageyama, 2014). The positive feedback within a neuroblast and negative feedback to adjacent precursor cells safeguards the reservoir of precursors even as neuroblasts emerge from its midst. Analogous principles apply to the asymmetric cell division between neuroblasts and ganglion mother cells that give rise to glia and neurons (Anderson and Jan, 1997; Knoblich, 1997). In the mature state, similar positive feedback loops can operate cell autonomously to enforce sharp separation between diverse neuronal phenotypes (Hobert et al., 2010).

In later stages of circuit development, when neurons have gained excitability and synapse formation has begun, autoregulation by negative feedback is prominent (Ben-Ari, 2015; Feller, 2004; Hensch, 2005; Rosenberg and Spitzer, 2011; Turrigiano and Nelson, 2004). In some cases, the autoregulation is cell-autonomous, in others it is a distributed property of an entire circuit and its state of chemical signaling. Possibly pertinent to autism, electrical events can arise from ion fluxes through chloride, sodium, calcium, or NMDA receptor channels, and the intracellular signaling can involve Ca^{2+} spikes, oscillations or waves, often with the participation of internal Ca^{2+} stores. The various Ca^{2+} signals often exert effects through regulation of gene expression via E-T coupling, or regulation of local translation. The details vary among diverse brain circuits but the overall organization seems generally compatible with the schema in Fig. 2.

Transcriptomics can help define transitions between developmental stages and mechanisms

The transitions between developmental phases have been charted by tracking expression of ASD- and ID-related genes through human cortical development (Parikshak et al., 2013). An early transition (~11 weeks post-conception) is indicated by attenuation of a gene module that opposes neuronal differentiation, giving way to one that favors it. A later transition (~20 weeks post-conception in humans, equivalent to perinatal mice) is marked by enhancement of a gene module that supports activity-dependent synaptic development. Bioinformatics analyses suggest that these processes are connected via translational regulation by FMRP and transcriptional co-regulation by common transcription factors, mechanisms touched upon elsewhere in this review. Developmental transcriptomic data seems compatible with the hypothesis that that cascades of transcription factors and positive feedback autoregulatory loops predominate early in development, but pass the baton to activity-dependent feedback mechanisms that fine-tune neuronal morphology and synaptic connectivity (see (Fishell and Heintz, 2013; Kepecs and Fishell, 2014) for review). Genetic insults that disrupt early developmental transcriptional cascades likely result in lethality and are therefore underrepresented as proximal causes of neuropsychiatric diseases.

Involvement of ASD-associated proteins in development and mature states

There are no sharp boundaries between development and autoregulation of mature function, so proteins encoded by the same ASD-associated genes likely participate in both development and ongoing homeostasis. For example, point mutations in *CACNA1C* that underlie Timothy Syndrome perturb the development of neuronal dendrites (Krey and Dolmetsch, 2007), bones (Ramachandran et al., 2013), and skin (Yucel et al., 2013), as well as ongoing electrophysiological function of heart and brain (Krey and Dolmetsch, 2007; Splawski et al., 2005; Splawski et al., 2004). It remains unclear whether the same pathophysiological mechanism is responsible at all stages. Likewise, Wnt signaling is known to shape early cell-cell interactions (Nelson and Nusse, 2004), and to guide cell migration and differentiation of axons and dendrites (Salinas and Zou, 2008; Yu and Malenka, 2003); nonetheless, Wnt2 and other proteins traditionally associated with neural development may have important roles in post-critical period regulation of neuronal function (Farias et al., 2007; Moon et al., 2004; Wayman et al., 2006). The lack of temporally restricted involvement has interesting implications for discovery of ASD therapies. While some aspects of autoregulatory signaling may be complete when neural development is over, other (dys)function may carry on right through to mature brain and, with suitable caution (Kalkman, 2012; Mullard, 2015), could offer therapeutic opportunities.

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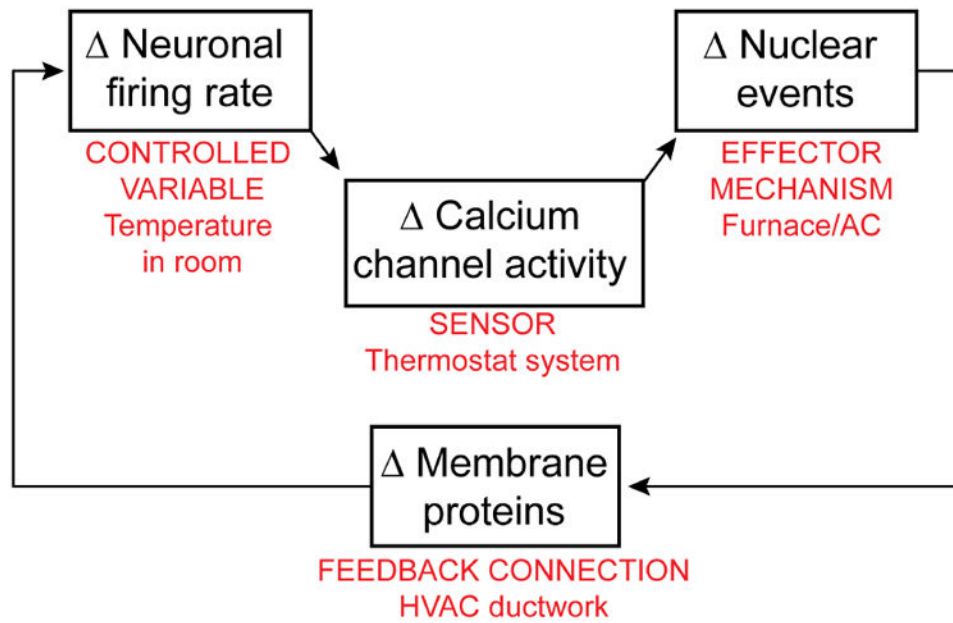


Fig 1. Ca^{2+} regulation of gene expression in neuronal development & homeostasis

A canonical homeostatic feedback loop, proposed by Abbott, Marder *et al.* (LeMasson et al., 1993; Marder et al., 1996; Siegel et al., 1994), hypothesizes that firing \rightarrow Ca^{2+} \rightarrow gene expression \rightarrow membrane channel proteins regulating neuronal excitability. This feedback loop is taken as key to how neuronal firing is kept away from extremes. The components of the feedback loop can be likened to elements of a temperature control system (labeled in red).

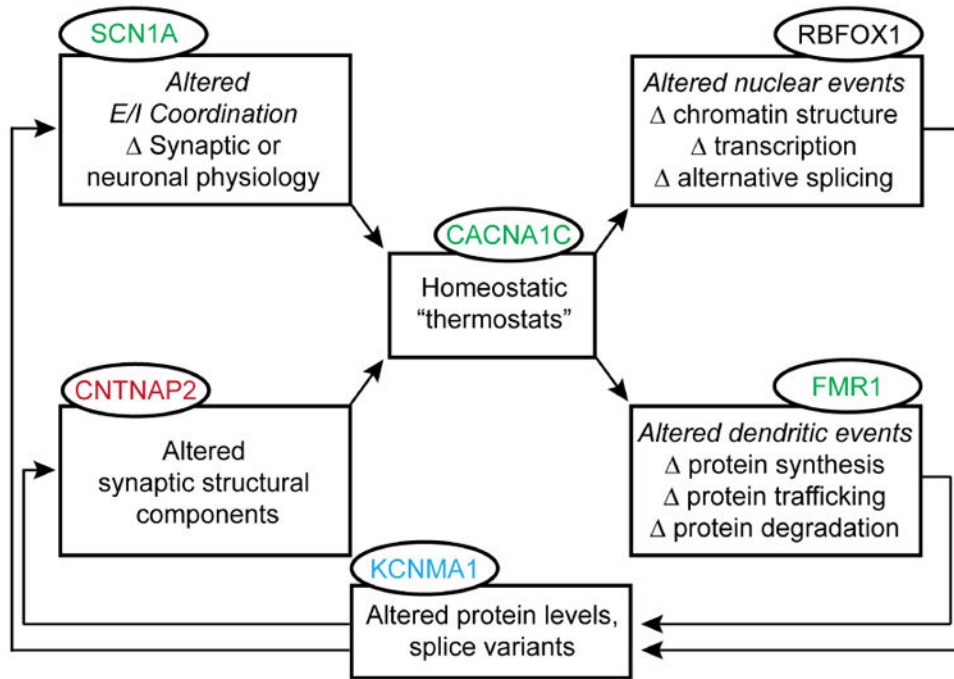


Fig 2. Homeostatic feedback through a multi-pathway loop

Expanding upon Figure 1 to incorporate alterations in both nuclear and dendritic events that arise during a homeostatic response. These effector mechanisms operate via feedback to alter synaptic or neuronal physiology and synaptic structure. ASD-associated mutations have been identified in genes associated with each component of this feedback loop (ovals); dysfunction in any one section can affect the operation of the entire loop to impact function at a variety of levels. This schematic representation of autoregulation appears in simplified versions in later figures (3, 4, and 6), highlighting the specific modules being discussed.

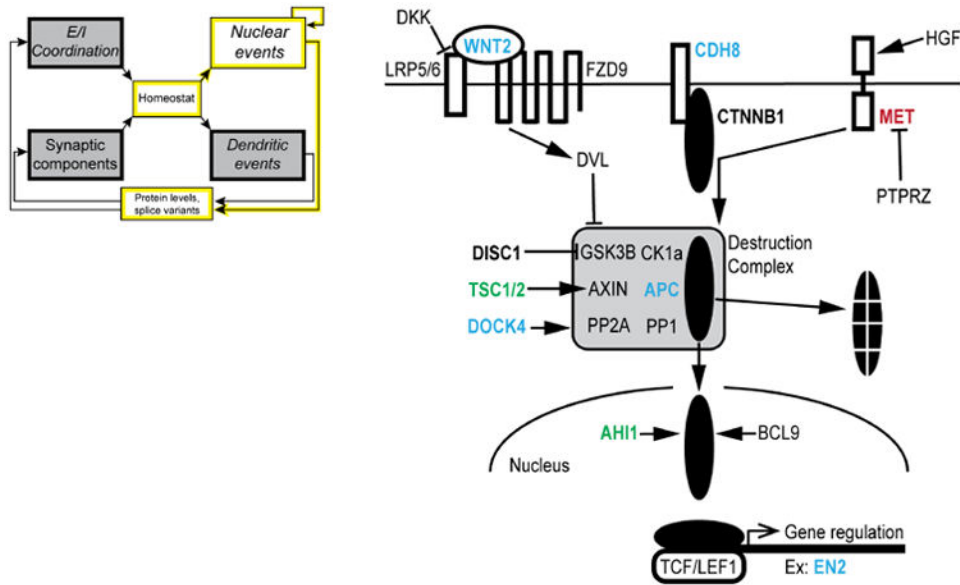


Fig. 3. Canonical Wnt/ β -catenin signaling pathway and ASD-related protein players

Hepatocyte growth factor (HGF) activation of the MET receptor tyrosine kinase releases β -catenin (*CTNNB1*) from surface cadherin molecules. β -catenin in the cytoplasm is bound by proteins that make up a destruction complex (DC). The DC marks β -catenin for proteasomal degradation. Wnt2 signaling via binding with a Frizzled receptor (*FZD9*) and its co-receptor LRP5/6 activates dishevelled (*DVL*), which saves β -catenin from degradation by inhibiting the DC. When *DVL* is active, β -catenin enters the nucleus, where it activates gene transcription by binding with the TCF/LEF1 family of transcription factors. Gene names are shown in bold if associated with ASD and present in Table 1 (utilizing the same color-coding scheme). The icon, simplified from Fig. 2, highlights the specific modules directly involved in the pathway illustrated by this figure (and utilized in the same manner for Figs. 4 and 6).

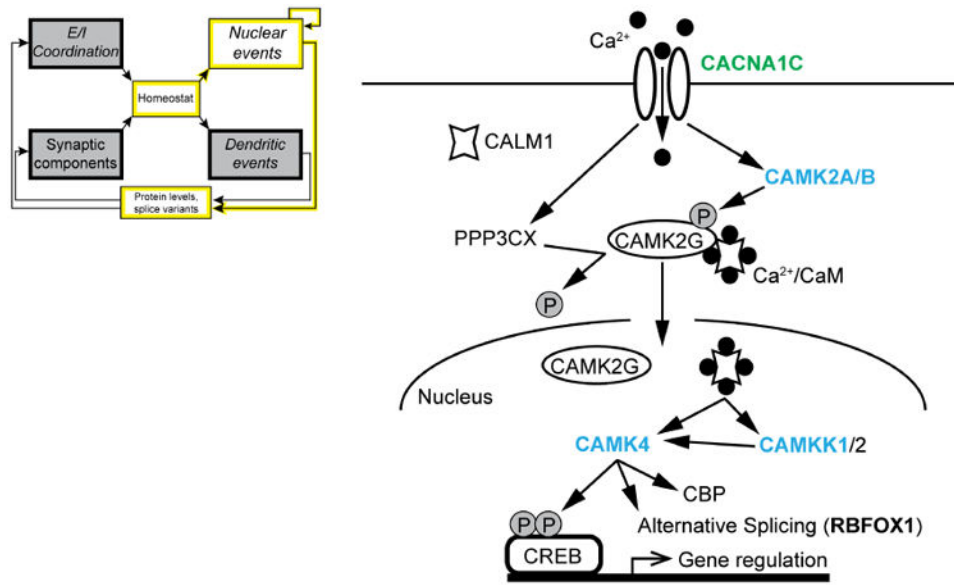


Fig. 4. Linking L-type channels to nuclear events

Calcium entering through $\text{Ca}_V1.2$ channels (*CACNA1C*) binds to calmodulin (*CALM1*, CaM), and triggers activation of α_2/β -CaMKII (*CAMK2A/B*), and calcineurin (*PPP3CC*, CaN). The aggregation of α_2/β -CaMKII at postsynaptic sites may contribute to synaptic tagging, a key step in long-lasting LTP (Frey and Morris, 1997; Hudmon et al., 2005; Redondo et al., 2010). γ CaMKII (*CAMK2G*) is phosphorylated by α_2/β -CaMKII to trap the $\text{Ca}^{2+}/\text{CaM}$ signal in place, and dephosphorylated at a different residue by CaN. This dephosphorylation event exposes a nuclear localization sequence on γ CaMKII and triggers translocation, shuttling $\text{Ca}^{2+}/\text{CaM}$ into the nucleus. Nuclear $\text{Ca}^{2+}/\text{CaM}$ activates CaMKK (*CAMKK1/2*) and CaMKIV (*CAMK4*), which phosphorylates CREB, activating CRE-mediated gene transcription. CaMKIV also phosphorylates CREB binding protein (CBP, *CREBBP*), further favoring transcription, and activates alternative splicing, via splicing factors such as Rbfox1 (*RBFOX1*). In this scheme, both β CaMKII and CaN act in close proximity to Ca_V1 channels, while γ CaMKII appears to operate as a carrier, not a kinase. Gene names are shown in bold if associated with ASD and present in Table 1 (utilizing the same color-coding scheme).

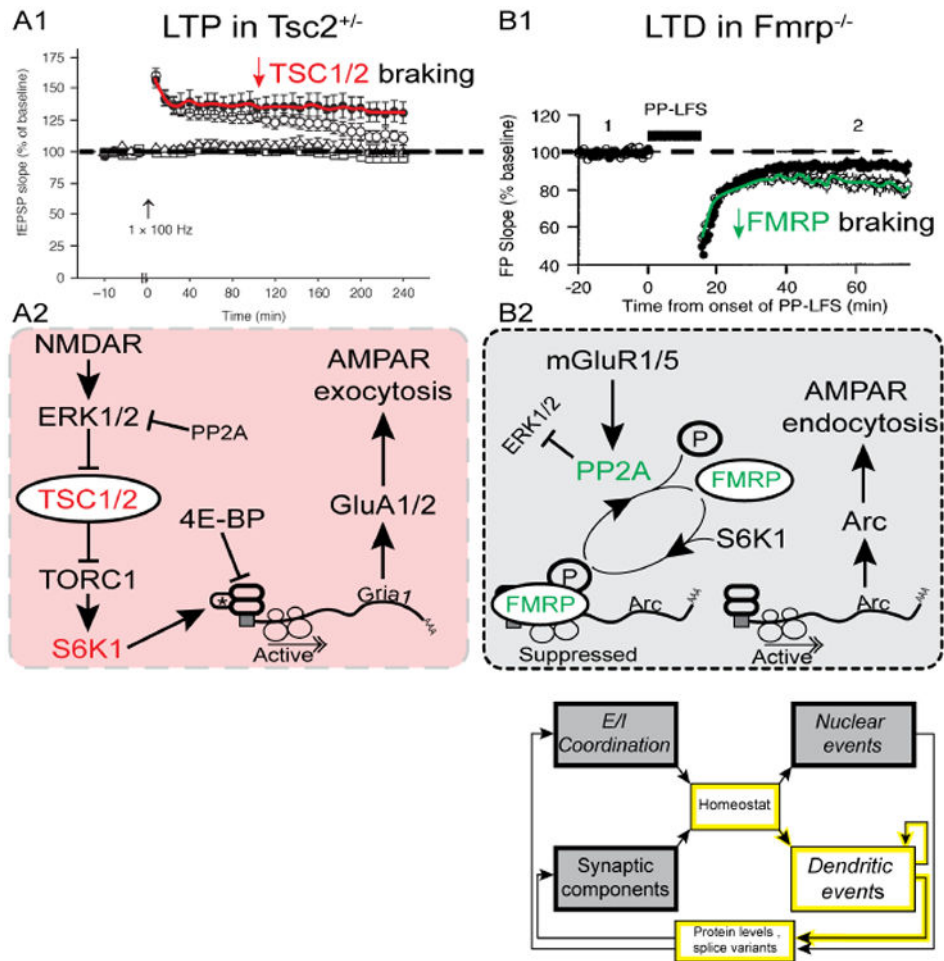


Fig. 5. Contrasting roles of TSC1/2 and FMRP

A1, LTP is exaggerated in *Tsc2*^{+/-} mice. From (Ehninger et al., 2008). A2, Activation of NMDARs leads to the inhibition of TSC1/2, which disinhibits TORC1. Activation of TORC1 leads to the translation of proteins important for LTP, including GluA1/2 subunits. AMPAR exocytosis supports LTP. Loss of TSC1/2 (as seen in tuberous sclerosis) would lead to excessive TORC1 activation, and exaggerated LTP (experimentally supported by A1). B1, LTD is exaggerated in FMRP KO mice. From (Huber et al., 2002)(Copyright 2002 National Academy of Sciences, USA). B2, Putative signaling pathway linking metabotropic GluR action to local translation of Arc and LTD. Activation of mGluR1/5 activates PP2A, which dephosphorylates FMRP. This releases mRNA transcripts from FMRP repression, and allows the local translation of LTD-relevant proteins. Translation of Arc supports readout of low spine activity by β -CaMKII, Arc-dependent AMPAR endocytosis, and LTD (Okuno et al., 2012). Loss of FMRP (as seen in FXS), leads to decreased inhibition of translation, and increased LTD (see panel B1).

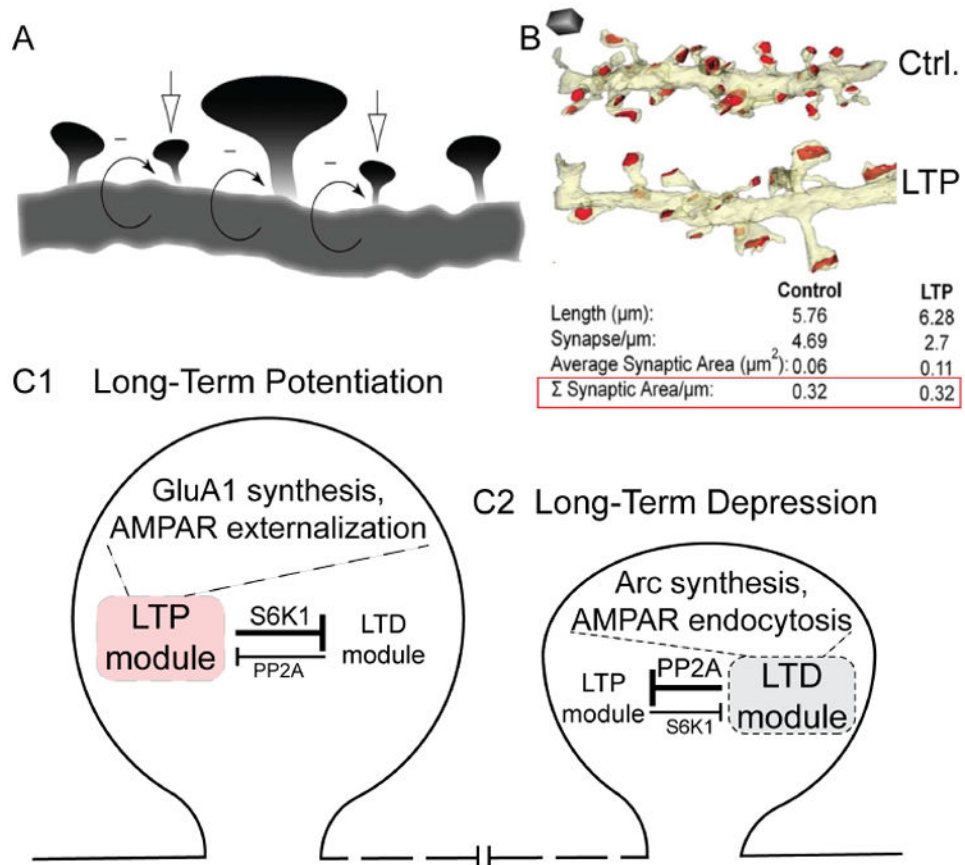


Fig. 6. Competing LTD and LTP processes

A, LTP and LTD are illustrated here as occurring within the same dendritic compartment, at neighboring spines. From (Rabinowitch and Segev, 2008). B, Following LTP-induction protocol, some spines increase in size, without the overall synaptic area changing. From (Bourne and Harris, 2012). C1,C2, LTP and LTD are seen as competing processes within single spines. PP2A activation is critical for the LTD module but inhibits the LTP module, whereas S6K1 is critical for the LTP module but inhibits the LTD module. Mutual inhibition supports a winner-take-all scenario. When the action of S6K1 predominates, the LTP module overrides LTD, and GluA1 synthesis and AMPAR externalization takes place (C1; Fig. 5A2). When the action of PP2A predominates, the LTD module prevails over LTP, and Arc synthesis and AMPAR endocytosis takes place (C2; Fig. 5B2). For clarity, this model focuses on simple aspects of multiple mutually inhibitory interactions between the FMRP and TSC1/2 pathways (Ashley and Warren, 1995; Bian et al., 2015; Darnell and Klann, 2013).

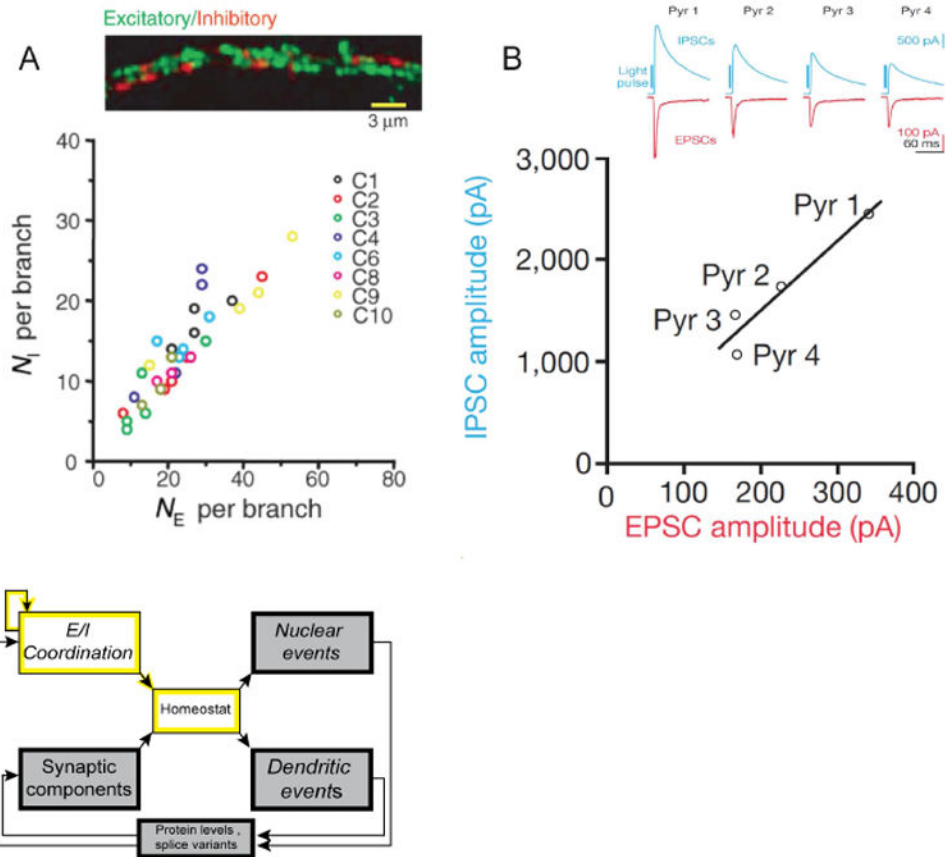


Fig. 7. E:I coordination in neuronal, circuit, and pathophysiological contexts

A, Coordination (but not equality) of the numbers of excitatory and inhibitory synapses per dendritic branch (N_E , N_I respectively). N_I is $\sim 0.5 \times N_E$. From (Liu, 2004). B, Coordination (but not equality) of the collective strengths of E and I transmission, illustrated for 4 pyramidal neurons driven by the same optogenetic stimulation of layer IV feedforward inputs. Inset shows recordings of EPSCs (red) and IPSCs (blue) with |driving force| of ~ 70 mV. |IPSC| is $\sim 7 \times$ |EPSC|, reflecting a ~ 7 -fold ratio of synaptic conductances. From (Xue et al., 2014).

Autoregulatory framework of ASD genes

Table 1

We group ASD-gene candidates into functional modules presented in Figure 2, utilizing a graded scale of confidence based on the Simons Foundation Gene Scoring Module (from red to burgundy, black, and green)(Basu et al., 2009). Gene names listed in blue are included, despite weaker or mixed evidence, based on findings of de novo mutations (see footnotes) and what we feel are interesting areas for future research. [Associated Disorder abbreviations: ADHD- Attention Deficit/Hyperactivity Disorder; BPD- Bipolar Disorder; DD- Developmental Disorder; Ep- Epilepsy; ID- Intellectual Disability; OCD- Obsessive Compulsive Disorder; MDD- Major Depressive Disorder; Scz- Schizophrenia; ALS- Amyotrophic Lateral Sclerosis; TSC- Tuberous Sclerosis].

Gene	Full Gene Name	Function	Chromosome Band	Associated Disorders	Primary Citation
Signaling to the Nucleus					
	Activity-dependent neuroprotector homeobox	Transcription factor (TF)	20q13.13	DD, Ep, ID	(O’Roak et al., 2012)
	Ankyrin repeat domain 11	Inhibits ligand dependent transcription	16q24.3	ADHD, DD, Ep, ID	(Marshall et al., 2008)
	Adenomatosis polyposis coli	Tumor suppressor	5q21-q22	ID	(Barber et al., 1994)
	Ash1 (absent, small, or homeotic)-like (Drosophila)	Histone methyltransferase	1q22		(Willsey et al., 2013)
	Additional sex combs like 3 (Drosophila)	Putative polycomb group (PcG) protein	18q11	DD, ID	(Dinwiddie et al., 2013)
	B-cell CLL/lymphoma 11A	Zinc finger protein	2p16.1	DD, ID, Scz	(Iossifov et al., 2012)
	Calcium/calmodulin-dependent protein kinase alpha	Protein kinase	5q32.1		(Iossifov et al., 2014); (Stephenson et al., 2015 unpublished data)
	Calcium/calmodulin-dependent protein kinase beta	Protein kinase	7p13		(Iossifov et al., 2014)
	Calcium/calmodulin-dependent protein kinase 4	Protein kinase	5q21.3		(Iossifov et al., 2014; Waltes et al., 2014)
	Calcium/calmodulin-dependent protein kinase 1, alpha	Protein kinase	17p13.2		(Iossifov et al., 2014)
	Cadherin 8, type 2	Membrane protein	16q22.1		(Pagnamenta et al., 2011)
	Cyclin-dependent kinase-like 5	Protein kinase	Xp22	ADHD, DD, Ep, ID, OCD,	(Weaving et al., 2004)

⁴Barber et al., 1994 reported on a patient with ASD with an interstitial deletion of chromosome 5, q15q22.3, with confirmed deletion of the APC gene.

- ^bIossifov et al., 2014 identified a *de novo* variant of this gene in an intron in a male proband, and a missense mutation in a male proband and his unaffected sister (Supplementary Table 2); Stephenson et al., found changes in α CaMKII activity as a result of the Iossifov 2014 missense mutation (see- Stephenson J.R., Wang, X., Shonesy, B.C., Sutcliffe, J.S., and Colbran, R.J. (2015). Physiological effects of an ASD-associated mutation in CaMKIIalpha. Program No. 584.14/E17. 2015 Neuroscience Meeting Planner, Chicago, IL: Society for Neuroscience, Online.)
- ^cIossifov et al., 2014 identified a *de novo* missense mutation in this gene in a male proband and his unaffected sister (Supplementary Table 2).
- ^dIossifov et al., 2014 identified a *de novo* missense mutation in this gene in a male proband and his unaffected brother (Supplementary Table 2); Waltes et al., 2014 reported an association between rs25925 of *CAMK4* and ASD after genotyping ten SNPs in two sets of German populations.
- ^eIossifov et al., 2014 identified a *de novo* mutation in an intron of this gene in a male proband and his unaffected sister (Supplementary Table 2).
- ^fPagnamenta et al., 2011 reported rare familial 16q21 microdeletions and expression analyses implicating CDH8 in ASD and ID.
- ^gMartin et al., 2013 found a missense variant in *WNT1* (S88R) that was overrepresented in ASD patients relative to controls.
- ^hMarui et al., 2010 identified three SNPs within *WNT2* that showed significant associations with autism.
- ⁱNeves-Pereira et al., 2009 identified and mapped a *de novo* chromosome breakpoint between 4q and 5q to an alternative transcript of the gene, as well as two ASD patients with single nucleotide insertions in the EIF4E promoter shown to alter activity.
- ^jBamby et al., 2005 reported a significant association with ASD for SNPs within *GRIN2A* for rs1014531 and for intron 10.
- ^kIossifov et al., 2014 identified a *de novo* mutation in an intron of this gene in a male proband and his unaffected sister, and a missense mutation in a female proband and her unaffected sister (Supplementary Table 2).
- ^lLaumonnier et al., 2006 identified a breakpoint in this gene in a patient with a *de novo* translocation, and a second patient with a sequence variation in a highly conserved region of the ion channel encoded by the gene; Neale et al., 2012 identified mutations in this gene in two individual probands, one missense and one silent.
- ^mIossifov et al., 2014 identified a *de novo* missense mutation of this gene in two male probands and their unaffected sisters, and a synonymous mutation in a third male proband and his unaffected sister (Supplementary Table 2).
- ⁿFeyder et al., 2010 reported a significant association between variations in two human *DLC4* SNPs with neural endophenotypes related to ASD.
- ^oIossifov et al., 2014 identified a *de novo* mutation in an intron of this gene in a male proband and his unaffected sister, a missense mutation in a male proband and his unaffected sister, and a frame-shift mutation in a male proband and his unaffected brother (Supplementary Table 2).
- ^pBenayed et al., 2005 replicated an association between two intronic SNPs in this gene with ASD.