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Interneuron Types as Attractors and Controllers

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Abstract

Cortical interneurons display striking differences in shape, physiology, and other attributes, challenging us to appropriately classify them. We previously suggested that interneuron types should be defined by their role in cortical processing. Here, we revisit the question of how to codify their diversity based upon their division of labor and function as controllers of cortical information flow. We suggest that developmental trajectories provide a guide for appreciating interneuron diversity and argue that subtype identity is generated using a configurational code of transcription factors that produce attractor states in the underlying gene regulatory network. We present our updated three-stage model for interneuron specification: an initial cardinal step, allocating interneurons into a few major classes, followed by definitive refinement, creating subclasses upon settling within the cortex, and lastly, state determination, reflecting the incorporation of interneurons into functional circuit ensembles. We close by discussing findings indicating that major interneuron classes are both evolutionarily ancient and conserved. We propose that the complexity of cortical circuits is generated by phylogenetically old interneuron types, complemented by an evolutionary increase in principal neuron diversity. This suggests that a natural

neurobiological definition of interneuron types might be derived from a match between their developmental origin and computational function.

Contents

1. INTRODUCTION

Classic studies by Ramón y Cajal first explored cortical interneuron diversity based on their wide range of characteristic morphologies (DeFelipe et al. 2013, Fairen 2007, Petilla Interneuron Nomenclature Group 2008). Beginning in the 1980s, it was recognized that particular interneuron morphologies are associated with the expression of specific molecular markers [e.g., parvalbumin (PV)] and predictable intrinsic physiological properties (e.g., fast spiking); however, the number of subtypes and the basis for their generation remained obscure (Freund & Buzsaki 1996, Krnjevic 1997, McBain & Fisahn 2001). The advent of developmental studies about 15 years ago revealed that the origins of specific interneuronal subtypes could be clearly mapped back to their time and place of origin within the subpallium (Anderson et al. 1997, Butt et al. 2005, Nery et al. 2002,

Wichterle et al. 2001, Xu et al. 2004). Moreover, from a series of genetic fate–mapping efforts, it became clear that all cortical interneurons, as well as those populating other forebrain structures, including the hippocampus, striatum, and amygdala, originate from the subpallium, largely from the medial ganglionic eminence (MGE) and caudal ganglionic eminence (CGE) (Fogarty et al. 2007; Miyoshi et al. 2007, 2010) as well as the preoptic area (Gelman et al. 2009). Tremendous progress has also been made over the past decade in the characterization of cortical interneuron subtypes (reviewed in Fishell & Rudy 2011), the developmental and molecular cascades that generate them (Bandler et al. 2017, Batista-Brito & Fishell 2009, Wonders & Anderson 2006), and the circuit motifs to which they contribute (Hangya et al. 2014, Moore et al. 2010, Turkheimer et al. 2015).

In a previous review (Kepecs & Fishell 2014) we examined the question of interneuron diversity and argued that focusing on their roles in neural computation will be the ultimate arbiter for interneuron classification. We proposed that, based on their developmental origin, interneurons can be classified into a small number of cardinal classes, each with distinct functional roles based on their input and output connectivity and intrinsic properties. Here, we revisit these ideas about interneuron function in light of recent data and discuss their function as controllers of cortical information flow. We then extend our previous ideas about cardinal interneuron types in light of recent transcriptomic data that lend credence to the existence of a low number of cardinal interneuron subtypes, at least at the level of transcription (Hodge et al. 2018; Saunders et al. 2018; Tasic et al. 2016; Zeisel et al. 2015, 2018). We revisit the question of interneuron diversity from a functional vantage point and consider how interneuron diversity arises within and across species. After discussing the classic view that interneuron diversity is specified by a combinatorial transcriptional code (Flames et al. 2007, Gelman et al. 2012), we consider the findings from loss-of-function analysis that are not accounted for by this model (Bandler et al. 2017, Wamsley & Fishell 2017). Instead, we propose an attractor model in which interneuron identity is determined by a configurational code, with individual genes contributing to attractor dynamics of the transcriptional program.

In the years since our previous review, the great success story has been the advent of single-cell transcriptomic methods for understanding neuron diversity (Hodge et al. 2018; Saunders et al. 2018; Tasic et al. 2016, 2018; Zeisel et al. 2015, 2018). Single-cell RNA-sequencing (RNA-seq) methods have been used to delineate both the transcriptional diversity of mature interneuron populations within the cortex (Hodge et al. 2018; Tasic et al. 2016, 2018) and the developmental trajectories through which they emerge (Mayer et al. 2018, Mi et al. 2018). In addition, recent work has started to examine the related questions of how interneuron identities vary across brain regions (Saunders et al. 2018), as well as across species ranging from reptiles to humans (Boldog et al. 2018, Tosches et al. 2018), to understand how they emerged through evolution.

New single-cell transcriptomic data also allow us to consider the role of transcription factors (TFs) in the emergence of interneuron subtypes. We first consider combinatorial codes that imply static assemblies of TFs produce different interneuron subtypes (Flames et al. 2007) and advance an alternative instead in which TFs participate in dynamic gene regulatory networks (GRNs) that generate stable identities through setting up attractor states. In this configurational model, different TFs contribute to specification dynamics to varying degrees, and their network configuration determines the developmental trajectories and defines locally stable identities. This model better accounts for loss-of-function results and explains the robustness of the transcriptional networks both during development and across evolution.

We end this review by speculating on the path forward. Our understanding of interneuron identity is beginning to be further expanded using epigenetic approaches (La Manno et al. 2018; Luo et al. 2017, 2018; Mezger et al. 2018; Nord et al. 2015; Silberberg et al. 2016). With the explosion of

deeper knowledge about the genetic and epigenetic states of individual interneurons, an improved molecular understanding is emerging of how interneurons adapt their genetic program as they integrate into cortical and subcortical circuits, how they maintain their identities in adulthood, and how they arise through evolution. Such studies provide insight as to how interneuron subtypes acquire the particular properties that allow them to function canonically in many cortical circuits.

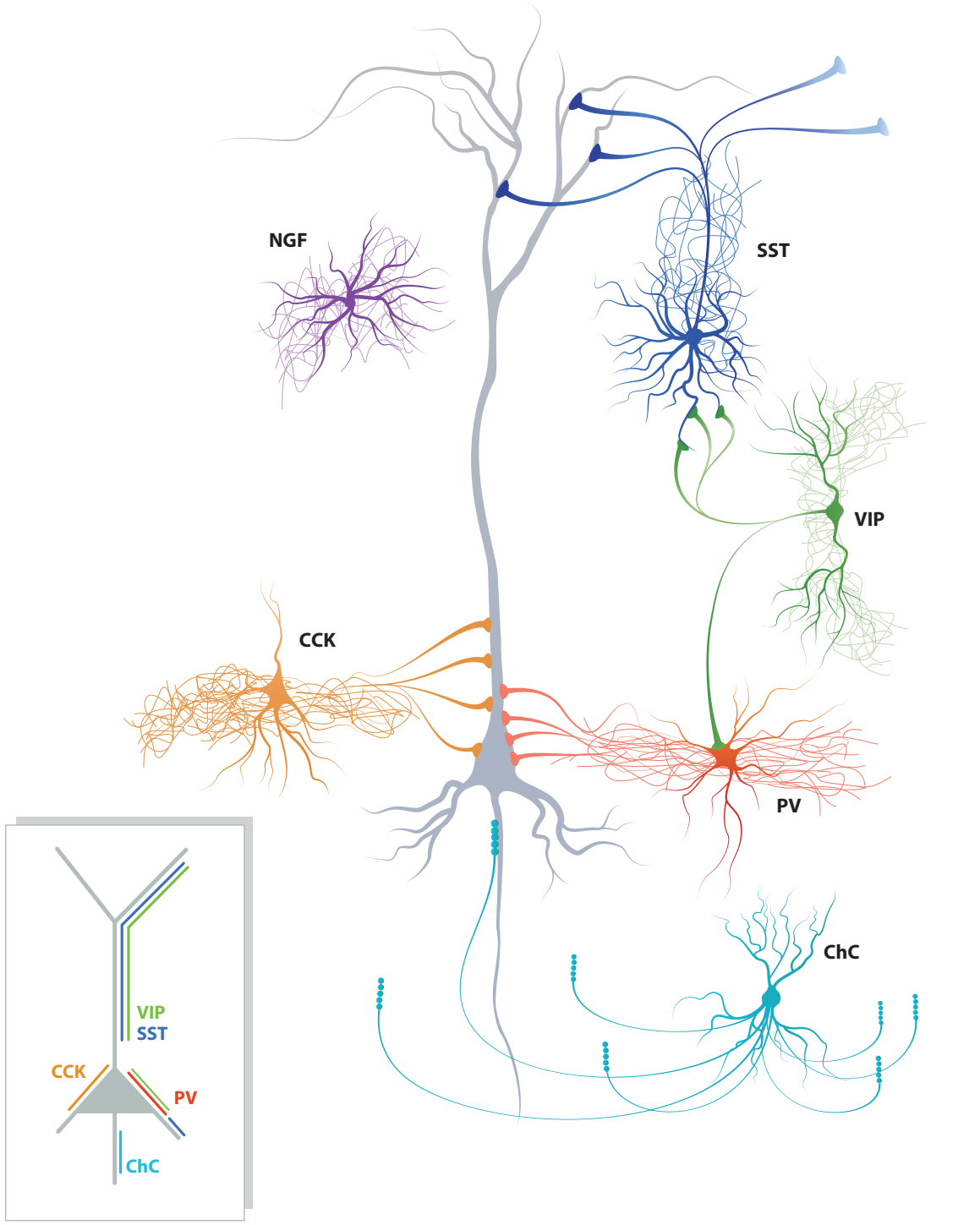
2. FUNCTION: FROM CARDINAL TYPES TO CIRCUIT MOTIFS

Before launching into a detailed examination of how existing experimental data support our configurational model of interneuron specification, it is worth reviewing the range of cortical circuits to which distinct classes of interneurons contribute. Cortical circuits are mainly composed of excitatory neurons, often with strongly recurrent connections and fewer inhibitory neurons that curb local excitations. The core function of inhibition is to provide balance by dynamically suppressing excitation to enable rich and rapid dynamics. Finely balanced excitation and inhibition have broad experimental support in cortical recordings (Froemke 2015, Haider et al. 2006, Okun & Lampl 2008, Wehr & Zador 2003), yet they present a puzzle. Why has such a diverse group of inhibitory neurons evolved, when ostensibly even a single neuron type could achieve balance? As a minority population (making up ∼20% of all cortical neurons), their sheer diversity points to the notion that synaptic inhibition is highly specialized, presumably to enhance the computational power of cortical circuits.

2.1. Diverse Family of Specialists for Controlling Excitation

Classic studies have identified a rich assortment of inhibitory neuron types through their morphology, expression of protein markers, coreleased neuromodulators, complement of ion channels, intrinsic firing patterns, and many other ways (Burkhalter 2008, Freund & Buzsaki 1996, Kubota & Kawaguchi 1994). Is there a computational role for this diversity? One answer to this puzzle may lie within the complexity of excitatory cells. Pyramidal neurons have large dendritic trees, with distinct domains (e.g., basal and apical dendrites) that receive different synaptic inputs and produce different types of electrogenic responses (e.g., slow calcium versus fast sodium spikes) along with separate plasticity rules (Spruston 2008). As a consequence, inhibitory inputs received on different portions of the dendritic tree will have rather different effects in how they modulate and control action potential generation (Lovett-Barron et al. 2012, Miles et al. 1996, Royer et al. 2012) (**Figure 1**). Interestingly, one method to categorize interneurons is based on their synaptic targeting since many varieties specialize in targeting distinct pyramidal cell domains or compartments. The resulting two main categories of interneurons are those that synapse on the soma and proximal dendrites of pyramidal cells—PV interneurons—and those that target distal dendrites—somatostatin (SST) interneurons. There are also specialists for targeting basal and apical dendrites as well as distinct varieties of soma-targeting basket cells, cholecystokinin (CCK), and PV-expressing interneurons (Freund 2003). A particularly unique subtype is the chandelier cell, which provides inhibition exclusively to the spike initiation zone of pyramidal cells (Lu et al. 2017, Somogyi 1977, Szentagothai 1975, Taniguchi et al. 2013). In addition, there are specialists that target other interneurons (Gulyas et al. 1996, Lee et al. 2013, Pfeffer et al. 2013, Pi et al. 2013), as well as those that have long-range projections, which are not, strictly speaking, interneurons (Jinno et al. 2007, Tamamaki & Tomioka 2010). Consequently, the inhibitory actions of interneurons depend in large part on their postsynaptic targeting.

Recent studies have used genetic strategies to target many of these classes on the basis of markers such as PV, SST, and vasoactive intestinal polypeptide (VIP) (Hippenmeyer et al. 2007,



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Distinct interneuron subtypes specialize in targeting different domains of pyramidal cells and each other. Different interneuron subtypes target distinct regions of the axo-somato-dendritic axes of pyramidal cells. Here we show a few major classes that differ not only in their targeting but also in their molecular markers, intrinsic properties, and morphology. Somatically targeting neurons can be classified into two large classes of parvalbumin (PV)- or cholecystokinin (CCK)-expressing basket cells. Chandelier cells (ChCs) target the axon initial segment. Somatostatin (SST) interneurons form synapses on dendrites, while vasoactive intestinal peptide (VIP)-expressing interneurons target mainly SST and, to a lesser degree, PV interneurons. Neurogliaform (NGF) cells use volume transmission to provide slow inhibition to superficial layers. Inset shows depiction of interneuron targeting to pyramidal cells.

Taniguchi et al. 2011). The use of optogenetic activators to manipulate these neurons has finally enabled the field to test many long-held ideas about the roles of subtype-specific inhibition. For instance, PV interneurons mediate the excitation–inhibition balance (Atallah et al. 2012, Lee et al. 2012, Moore & Wehr 2013, Wilson et al. 2012) and regulate the timing of principal cells (Cardin 2018, Royer et al. 2012). Whether the output of PV basket cells is dense and nonspecific (Karnani et al. 2014) or targeted to specific neuron types or ensembles is not yet resolved (Kvitsiani et al. 2013, Lee et al. 2014, Yoshimura & Callaway 2005). SST interneurons also impact local circuits in complex ways, providing lateral inhibition and supporting oscillations (Adesnik et al. 2012, Attinger et al. 2017, Gentet et al. 2012, Munoz et al. 2017, Nienborg et al. 2013, Veit et al. 2017). Since SST interneurons target dendrites, their major impact is likely to be on dendritic spikes (Palmer et al. 2012), and they can even be targeted to select dendritic branches (Cichon & Gan 2015, Stokes et al. 2014) yet are often not visible on spike action during behavior (Kvitsiani et al. 2013). PV and SST interneurons can also provide complementary control over sensory adaptation (Natan et al. 2015).

VIP interneurons preferentially target other interneurons (Lee et al. 2013, Pfeffer et al. 2013, Pi et al. 2013), mainly SST and a smaller fraction of PV interneurons, thereby providing disinhibitory control to principal neurons (Lee et al. 2013, Pi et al. 2013) and increasing response gain (Fu et al. 2014, Pi et al. 2013). Chandelier cells targeting the axon initial segment provide selective inhibition (Lu et al. 2017; but see Woodruff et al. 2010). We cannot do justice to the number and breadth of recent articles mapping the functional roles of cortical inhibitory neurons; others have reviewed these exciting studies more thoroughly (Cardin 2018, Feldmeyer et al. 2018, Khan et al. 2018, Lovett-Barron & Losonczy 2014, Lucas & Clem 2018, Naka & Adesnik 2016, Pelkey et al. 2017, Roux & Buzsaki 2015, Urban-Ciecko & Barth 2016, Wood et al. 2017, Yavorska & Wehr 2016), and our brief overview simply underscores the great excitement that these studies provide. The emerging complexity of inhibition is daunting and likely to increase given the strong neuromodulation abilities of interneurons (Chevy & Kepecs 2018, Urban-Ciecko et al. 2018), and whether and how they support canonical computations remain unknown (Harris & Mrsic-Flogel 2013, Miller 2016). Details aside, these findings support the long-held hypothesis that the diversity of interneurons reflects the division of labor between distinct interneuron types.

2.2. Interneurons Coordinate Cortical Neural Populations at Multiple Timescales: Balance, Rhythms, and Information Flow Control

At the level of networks, different interneuron subtypes participate in distinct cell-type-specific network motifs with defined computational functions. As the search for consistent motifs and their function continues, it is worth remarking that these motifs are embedded in much larger and well-connected cortical networks (**Figure 2**); hence, it may be overly simplistic to ascribe distinct functions without considering a fuller complement of connections. Certainly, the functional output of local circuit motifs must impact areas such as the thalamus and basal ganglia, with which they maintain long-range connections.

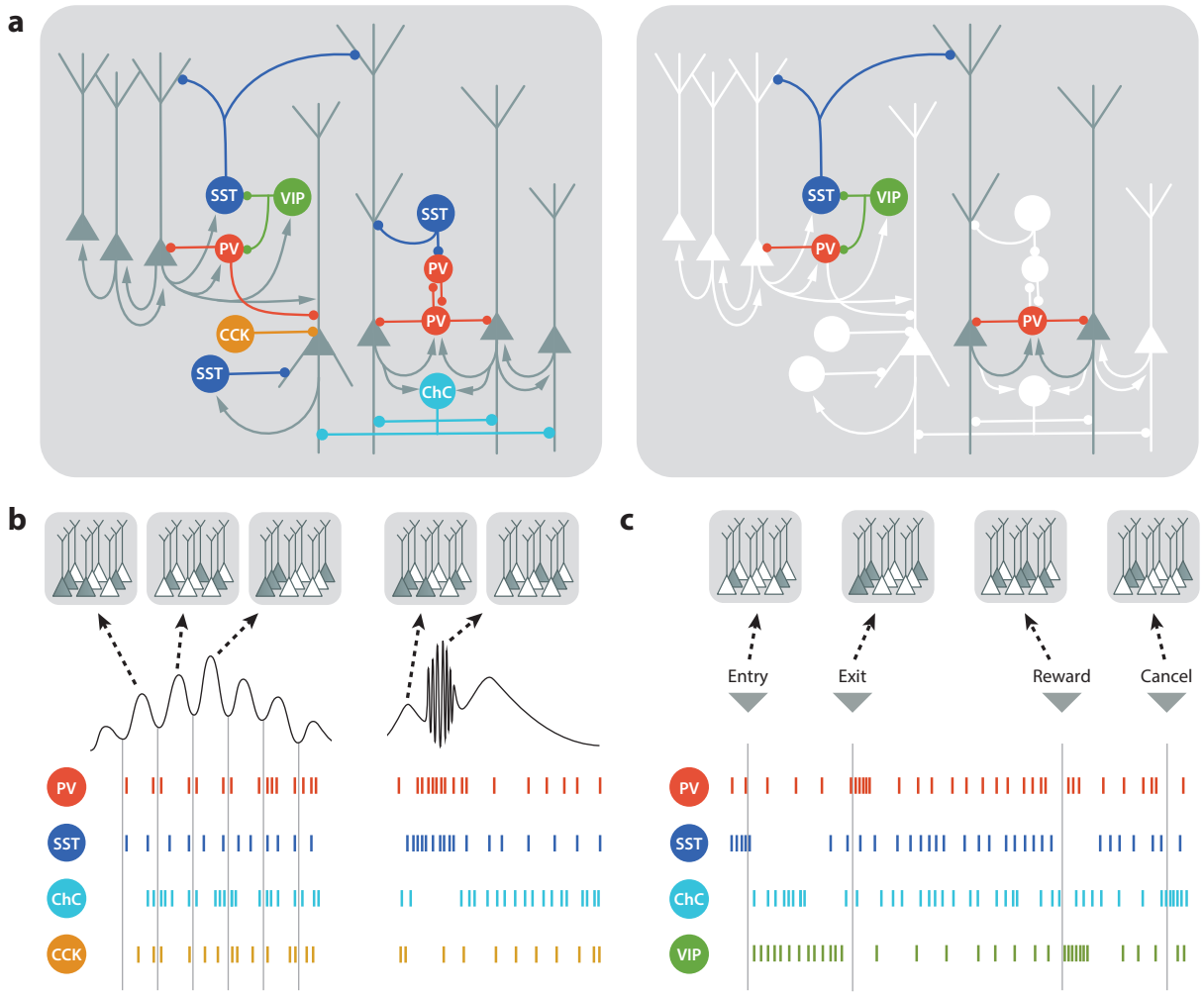


Figure 2

Diagram of cortical circuit motifs and interneuronal circuit control. (*a*) Cortical networks. These networks comprise complex, cell-type-specific circuits, and repeated circuit motifs based on interneuron connectivity are embedded within these. The left panel shows that most neurons are connected to multiple partners, obscuring clear patterns. The right panel shows two distinct circuit motifs centered around interneurons that may not be obvious when considered in the context of the cortical jungle. (*b*) Oscillatory control. The top panels show pyramidal cell ensembles that are controlled by interneurons. The middle trace shows an LFP, representing the network state in the hippocampus. The bottom panel shows the firing of four different interneuron types that can be described in reference to the LFP, with each subtype firing during different network states and with distinct phase relationships to each other and the LFPs. The timing of interneurons can control oscillations at timescales ranging from milliseconds to hundreds of milliseconds. (*c*) Flow control. The top panels show how distinct groups of pyramidal neurons are activated in response to interneuron control. The middle panel marks the timing of four behavioral events: entry, exit, reward, and cancel. The bottom panel shows that the firing of four different cortical interneuron subtypes can be described in reference to these events on the behavioral timescale of seconds. Interneurons may provide control in the information flow by gating, gain modulation, veto, and other circuit operations. Abbreviations: CCK, cholecystokinin; ChC, chandelier cell; LFP, local field potential; NGF, neurogliaform; PV, parvalbumin; SST, somatostatin; VIP, vasoactive intestinal polypeptide.

While the impact of individual interneurons is proportional to the relevant GABA receptor time constants on the postsynaptic neurons, once we consider their network interactions, substantially longer times scales of coordination can be produced (Litwin-Kumar et al. 2016). Indeed, as implied by their name, the function of interneurons needs to be understood in the context of the local circuit where they coordinate nearby principal neurons (**Figure 2**). Distinct inhibitory neuron subtypes may enable richness in the possible dynamics within networks of principal neurons. For instance, different subtypes of interneurons have been proposed to serve as temporal specialists, coordinating principal neurons at different oscillation frequencies (Buzsaki 2002, Klausberger & Somogyi 2008). Optogenetic experiments have confirmed that the activation and inhibition of PV and SST neurons can produce different rhythms in cortical structures (Cardin et al. 2009, Royer et al. 2012, Sohal et al. 2009, Veit et al. 2017), and their genetic ablation in superficial layers produces cortical dysrhythmia (Takada et al. 2014).

Is recruitment of an interneuron subtype best understood with reference to a network state or a behavioral contingency? Recent observations suggest that some neurons are activated during specific behavioral events; hence, it is important to consider not only the state of the network but also behavioral contingencies when examining neural activity. For instance, researchers have found that prefrontal PV and a narrow spiking of SST interneurons show strong behavioral correlates (Kim et al. 2016, Kvitsiani et al. 2013, Lagler et al. 2016). For instance, in the medial prefrontal cortex, SST neurons uniformly suppressed their activity as mice entered the reward zone, whereas PV neurons were phasically activated (Kvitsiani et al. 2013). On the other hand, auditory cortex VIP interneurons were activated by both reward and punishment (Pi et al. 2013), similar to a subtype of layer 1 interneurons (Letzkus et al. 2011). The uniformity of behavioral responses suggests that these genetic markers broadly correspond to functional types as well. An additional implication of this homogeneous recruitment is that, despite the complex connectivity of cortical networks, specific circuit motifs may in fact be relevant if indeed neurons within these motifs are coactivated. For instance, coactivation of VIP neurons could produce a net disinhibitory signal.

These observations of the behavioral correlates of inhibitory neurons lead to the flow control hypothesis proposed in our previous review (Kepecs & Fishell 2014). According to this idea, the behavioral timescale of activation indicates that these interneurons exert control over the flow of information in the cortex by selectively gating distinct input channels, providing gain control or resetting activity, to match the requirements of ongoing behaviors. This hypothesis extends the relevant timescales of interneuron operation to the behavioral scale of seconds. Thus, while the postsynaptic impact of an individual interneuron is on the timescale of milliseconds, coordination across cortical networks produces longer timescales, and at behavioral timescales these operations may serve the needs of even larger interareal networks, producing flow control.

Are slower behavioral timescale representations generated largely locally or triggered by control signals received from outside of a local circuit? While at present there is no general answer, numerous recent articles point to the possibility that neuromodulatory control of interneurons can provide cell type– and circuit-specific control. Acetylcholine, a key neuromodulator throughout the brain, can profoundly transform cortical processing and enhance learning. Recent results reveal that acetylcholine can turn SST interneurons on or off based on their subtypes (Munoz et al. 2017); boost pyramidal-to-SST synapses, thus enhancing feedback inhibition (Urban-Ciecko et al. 2018); and recruit a subtype of layer 1 interneurons (Letzkus et al. 2011, Poorthuis et al. 2018). During behavior, reinforcers drive brief bursts of acetylcholine (Hangya et al. 2015) that, by transiently reconfiguring interneuron circuits, may support associative plasticity (Letzkus et al. 2015). Thus, interneurons may serve as fast conduits for neuromodulators in a cell type–specific manner (Alitto & Dan 2012, Ferezou et al. 2007, Kawaguchi & Kubota 1997). These new directions reveal

the contours of a canonical cortical microcircuit with distinct interneuron subtypes in critical positions to support cortical computations in a manner that is responsive to circuit demands.

3. CARDINAL, DEFINITIVE, AND STATE SPECIFICATION: DEVELOPMENTAL TRAJECTORIES OF INTERNEURON GENE EXPRESSION

We suggest that interneuron diversity is generated through nature, nurture, and circumstance: (*a*) Cardinal specification (nature) occurs when interneurons become postmitotic and defines their intrinsic properties; (*b*) definitive specification (nurture) relies on cues imposed during migration and at the settling position and determines local afferent and efferent connectivity; and (*c*) state specification (circumstance) transpires when some interneuron subtypes change their gene expression in the context-specific brain activity.

3.1. Cardinal Specification: The Developmental Emergence of Cardinal Interneuron Subtypes

Since the first TFs controlling interneuron specification were identified (reviewed in Rubenstein & Puelles 1994, Shimamura et al. 1995), it has been clear that particular genes play important roles in coordinating the specification of interneurons. It is also clear from fate-mapping experiments that interneuron type can be predicted based on where and when they were generated (Nery et al. 2002, Taniguchi et al. 2013, Wichterle et al. 2001, Xu et al. 2004) and that aspects of interneuron subclass identity become fixed upon interneuron progenitors becoming postmitotic (Mayer et al. 2018, Mi et al. 2018, Nery et al. 2002). Longitudinal whole-genome analyses using single-cell RNA-seq methods have provided considerable clarity regarding when interneuron subtype identities first emerge at a transcriptional level. Analysis by two different groups indicate that, while a small number of regionally expressed genes can be detected within the proliferative zones, subtype identities or even differences between progenitors giving rise to projections versus interneurons were not apparent (Mayer et al. 2018, Mi et al. 2018). By contrast, nearly coincident with interneurons becoming postmitotic, the four primary cardinal classes become evident, as discussed above (although the latter study suggested considerably more refined subtypes can be identified within these newborn populations).

These findings are consistent with the concept of cardinal identity (Kepecs & Fishell 2014), which describes the major interneuron classes based on development and function. An attractive feature of this nomenclature is that four major cardinal classes represent complementary, nonoverlapping groups that can be identified by their expression of specific neuromarkers: PV, SST, VIP, and Reelin (Rln) (reviewed in Miyoshi 2018). This last category is complicated, as these cells should be accurately referred to as Rln-positive/SST-negative to reflect that a subpopulation of SST interneurons also express Rln. As a result, we have now replaced Rln with inhibitor of DNA binding 2 (Id2) or lysosomal-associated membrane protein family member 5 (Lamp5), both of which provide less ambiguous markers for this fourth category (Mayer et al. 2018). In addition, recent analysis suggests that a number of smaller categories of cardinal interneuron types exist, denoted by TH, SNCG, Meis2, and Igfbp6 (cf. Mayer et al. 2018 with Tasic et al. 2016). Therefore, at present there appears to be about four major and multiple additional minor cardinal types, although it seems likely that this number will be revised upward as the breadth of subtypes is further refined. For simplicity, we mainly refer to the four best-understood cardinal classes and denote these based on major marker genes that have largely nonoverlapping expression, thereby enabling simple genetic experimental strategies. With increased resolution, we expect that there

might be improved means of identifying these subgroups, but this is unlikely to change the core contention that few interneuron cardinal classes exist postmitotically.

While the absolute number of cardinal classes and their associated subtypes remains a matter of debate, it appears that in some cortical regions as many as 90% of all interneurons are derived from one of the four largest cardinal classes (Kim et al. 2017, Rudy et al. 2011). This estimate comes with the clear caveat that both the percentage composed by these four cardinal classes and their relative contributions in specific areas will vary widely across the cortex. Thus, while clearly a simplification, these four categories reflect the four major subdivisions of interneurons, even when considered across the cortex, hippocampus, and striatum (Saunders et al. 2018). On the other hand, as discussed below, evidence suggests that further interneuron subdivisions arise during migration and settling within the cortex and that some of these may represent further definitive classes that are genetically determined upon becoming postmitotic (Mayer et al. 2018).

3.2. Definitive Specification: Migration and Settling of Interneurons and Postmitotic Control of Interneuron Identity

The remodeling of interneurons from a cardinal to definitive identity likely depends upon extrinsic cues. These presumably can be supplied from any local source but likely occur either during migration or upon settling. We hypothesize that migration pathways are generic, so, as has recently been hypothesized (Lim et al. 2018), these are likely segregated into the two major pathways within the marginal zone or subventricular zone, respectively. By contrast, cues within layers or areal territories that interneurons are exposed to post-settling could be both much more diverse and specialized in refining interneurons to their specific local environments.

3.2.1. Extrinsic local cues from cells encountered during migration and settling shape subtype identity. Although interneuron diversity is apparent upon cell-cycle exit, during their subsequent migration and integration, further subtype diversity emerges. Moreover, there is considerable evidence showing that the interactions experienced by an interneuron during its migration and settling within the cortex shape its morphology and, by proxy, its connectivity. Multiple recent examples suggest that interneurons adapt in accordance with their proximal partners and local environmental cues. The earliest indications came from the Kriegstein and Polleux laboratories (Bortone & Polleux 2009, Wang & Kriegstein 2009), demonstrating that GABA affects proliferation and migration. More recently, emerging evidence suggests that local cues influences morphology, connectivity, and cell death (De Marco Garcia et al. 2011, 2015; Dehorter et al. 2015; Denaxa et al. 2018; Lodato et al. 2011; Priya et al. 2018; Tomassy et al. 2014; Tuncdemir et al. 2015; Wamsley & Fishell 2017; Wong et al. 2018).

The most direct test for the roles of local cues has come from transplant experiments where migrating cortical interneurons were transplanted either heterochronically or heterotopically (Lim et al. 2018, Lodato et al. 2011, Quattrocolo et al. 2017). As has been amply documented, interneurons from the MGE migrate dorsally into the cortex using two distinct streams, one that transits near the marginal zone and a deeper one positioned beneath the cortical plate within the subventricular zone (SVZ) (reviewed in Marin 2013; Marin & Rubenstein 2001, 2003). While it has long been speculated that different populations utilize these two streams to access the cortex, it remains unclear which specific subtypes are found within each stream. Through a clever isolation of the two streams, the Marin laboratory (Lim et al. 2018) was able to demonstrate that interneurons within each stream express genes associated with particular SST and PV interneuron populations. Within the marginal zone, they found a population of Martinotti neurons that project translaminarly, while those that migrate within the SVZ have the molecular character

associated with laminar-restricted populations. By transplanting these populations back to the divergence point of the two migratory streams in vitro, they were able to show that, although not absolute, the neurons prefer migrating back into the stream where they were previously located. Nonetheless, whether those that select a different migratory path upon transplantation assume a different fate has not been explored. Furthermore, when cortical versus hippocampal interneurons are heterotopically transplanted just prior to settling, they can adapt appropriately to each other's environment (Quattrocolo et al. 2017).

3.2.2. Local cues impact interneuron development at multiple stages. The growing evidence that local cues can instructively or passively affect interneuron development raises the question about the identity of population(s) providing such cues (reviewed in Cossart 2011, Kanold & Luhmann 2010, Luhmann et al. 2014). Within migrating populations, recent evidence has implicated a role for both of the primary neurogenic populations: the Cajal Retzius and subplate cells that reside above and below the developing cortical plate, respectively (Kanold & Luhmann 2010). While the precise interactions between migrating interneurons and these primary neurogenic populations are still poorly characterized, multiple groups have reported synaptic connections to both (De Marco Garcia et al. 2015, Luhmann et al. 2014, Quattrocolo & Maccaferri 2013, Tuncdemir et al. 2016). In addition, the extracellular protein Rln, which is selectively expressed by Cajal Retzius cells and known to be essential for proper pyramidal cell migration (Frotscher 1998), has also been implicated in the radial migration of interneurons (Pla et al. 2006). Furthermore, cellular interactions between interneurons and various neuronal and nonneuronal populations have been recently demonstrated (De Marco Garcia et al. 2015, Lodato et al. 2011, Thion et al. 2018). Regarding the interactions of interneurons with pyramidal cells, work from the Arlotta and McBain laboratories (Lodato et al. 2011, Webster et al. 2019) has demonstrated that the laminar position of interneurons matches their presumptive partners. Moreover, even when positioned ectopically, MGE-derived populations can be induced to colocalize with deep-layer pyramidal cells (Lodato et al. 2011). Similarly, recent findings support a role for nonneuronal cells, including astrocytes, oligodendrocytes, microglia, and endothelial cells in influencing the migration and synapse formation of developing interneurons (Tan et al. 2016, Thion et al. 2018, Tomassy et al. 2014). In addition, mounting evidence suggests that diencephalic and telencephalic afferents influence morphogenesis, synapse formation, and cell death (De Marco Garcia et al. 2015, Denaxa et al. 2018, Luhmann & Khazipov 2018, Minlebaev et al. 2011, Priya et al. 2018, Wong et al. 2018). The intricacies of interactions between each of these cell populations and interneurons require further investigation. Indeed, there is no question that studying their contributions and their accompanying molecular signals will transform our understanding of how interneurons achieve their exquisite patterns of morphology and connectivity.

3.3. State Specification: To What Extent Do Interneurons Adjust Their Function in Accordance with Local Circumstance?

Increasingly powerful methods for analyzing the transcriptional profiles within specific interneuronal adult subclasses have provided clear evidence that particular subtypes exist as discrete transcriptional states (Hodge et al. 2018, Tasic et al. 2018). Indeed, recent work comparing the numbers of pyramidal versus interneuronal cell types and their associated transcriptional states indicates that they are remarkably similar across regions of the cortex that are functionally quite divergent. Tasic, Yao, and colleagues (Tasic et al. 2018) found that pyramidal neurons in the visual and anterior lateral motor cortex of mice can be transcriptionally divided into approximately 56 subtypes. However, their local flavor varies in the range of hundreds of genes, which could allow

paralog subtypes to be distinguished across these regions. The same group found that, in contrast, interneurons can be divided into approximately 61 types, whose gene expression profiles across these two cortical areas vary at most by 8–12 genes, and in a majority of types by none at all. At face value, this seems to indicate that interneurons in the adult mouse are unitary, distinct, and conserved across cortical regions. Importantly, single-cell work, by its nature, captures cells at a particular point in time. What appears to be precise and immutable may prove to represent cell states rather than types once methods are available to track gene expression across time. Hence, a subset of these 61 types may ultimately be shown to represent a smaller number of types that can transit between different gene expression states. In this regard, the authors create what they call a constellation plot, which demonstrates commonalities in gene expression between interneurons that collectively arise from the CGE or MGE. This, at least provisionally, provides support for the idea that some interneuron types may be able to undergo state changes in vivo based on circumstance (Dehorter et al. 2017). In addition, a number of recent examples demonstrate that interneurons adapt their morphology and intrinsic properties in accordance with the networks in which they are embedded. For instance, studies from both the Caroni and Marin laboratories (Dehorter et al. 2015; Donato et al. 2013, 2015) indicate that PV interneurons adjust their molecular profile and firing patterns based on local engagement. Thus, cortical interneurons can undergo lasting but reversible changes in transcriptional states based on local circuit demands.

Transcriptomic analysis alone may also erroneously group together distinct interneuron types if few genes are differentially expressed. Connectivity, which is key to the function of an interneuron (or in fact any neuron), can be strongly influenced by individual genes/pathways (e.g., semaphorins). Indeed, recent work shows that specific synaptic proteins can direct the connectivity of specific interneuronal subtypes (Favuzzi et al. 2019). Work from the Huang laboratory (Paul et al. 2017) provides strong evidence that what appear to be identical programs within transcriptionally similar interneuronal subclasses actually result from their RNA profiles being examined too superficially. The Huang laboratory performed very deep single-cell RNA-seq on five distinct interneuron groups defined using intersectional genetics. This work reveals an enormous diversity between interneuron subtypes that the shallower but broader sequencing fails to detect. A particularly appealing aspect of this work is the identification of transcriptional signatures that shape the input–output structure of particular interneuron populations. For instance, they suggest a role for peroxisome proliferator-activated receptor gamma coactivator 1-alpha in the organization of efferent synapses of PV basket cells. Furthermore, coordinated gene expression exists within particular subtypes with regard to a variety of protein categories, including channels, cell adhesion molecules, neurotransmitters and modulators, second messenger pathways, neuropeptides, and vascular release mechanisms. This may leave room for remarkable regional diversity that is only detectable when low-expression messenger RNAs (mRNAs) are fully considered. In support of the idea that transcriptional similarity may not indicate functional homology, a recent study from the Tolias lab (Scala et al. 2019) demonstrates that layer IV SST interneurons in the visual versus somatosensory cortex are both physiologically and morphologically distinct, despite sharing similar transcriptional programs. Whether this reflects an insufficient sensitivity for the detection of low levels of gene expression masking transcriptional variance or that the dynamic range of single-cell RNA-seq methods is too limited to detect quantitative differences remains to be determined.

3.3.1. Evidence that local cues influence circuitry. Given that the 61 transcriptomic interneuron types across the cortex are largely generated from possibly as few as four cardinal classes, their cardinal identity must be a strong determinant for how positional cues are interpreted. Specifically, extrinsic environmental signals must be precisely linked to the appropriate intrinsic response. While the details of how this is accomplished remain obscure, recent studies

have provided ample evidence that such cues do exist. A beautiful example of this is that CA3 mossy fibers can form drastically different synapses onto hippocampal interneurons versus pyramidal neurons (Maccaferri et al. 1998) [the process depends on neurexin/neuroligin interactions that undergo cell-specific splicing, as shown by the Scheiffele laboratory (Mauger et al. 2016, Nguyen et al. 2016, Schreiner et al. 2014, Traunmuller et al. 2016)]. This suggests that retrograde signals from target cells can inform presynaptic axons to target them specifically and selectively.

3.3.2. Potential local cues that affect local circuitry. What types of mechanism could be envisioned to direct such processes on a broader scale? Neuronal activity–coupled gene expression provides one obvious source (Hong et al. 2008, Mardinly et al. 2016, Spiegel et al. 2014; reviewed in Wamsley & Fishell 2017) but could be complemented by similar mechanisms that direct cell type–specific translation, localization, or splicing. For instance, recent work from the Fishell laboratory demonstrates that RbFox1, an RNA-binding protein, is differentially required for axonal targeting and synapse formation in PV versus SST cortical interneuronal populations, respectively (Wamsley et al. 2018). The question of how local cues are coupled with intracellular signaling to selectively direct specific transcriptional, translational, splicing, and trafficking events will no doubt help elucidate how local circuitry within interneuron populations is established. Indeed, a promising answer as to how a common genetic trajectory could result in appropriately tailored local connectivity may lie in the many mechanisms that allow for the differential mRNA utilization through local translation and alternative splicing. Work from the Scheiffele laboratory (reviewed in Furlanis & Scheiffele 2018) based on extremely deep single-cell sequencing (on the order of 1.25 million reads per cell) has revealed specific subtypes based on their patterns of alternative splicing as well as their expression of transcripts encoding RNA-binding proteins. As the biological importance of these findings comes to light, it may prove that the differential splicing or selective trafficking and subcellular translation of specific mRNAs produce much of the local information needed to account for regional differences in connectivity. Nonetheless, how such differential utilization of mRNAs is coupled to local events remains a daunting and largely unanswered question.

4. A NEW MODEL FOR INTERNEURON SPECIFICATION

4.1. Transcriptional Codes and the Generation of Interneuron Type Diversity

Based on the diverse observations considered above, there are clearly numerous mechanisms that contribute to the generation of interneuron diversity. Although these extend beyond transcription, it seems clear that the earliest and most profound influence on their identity is the intrinsic genetic program imparted upon them on becoming postmitotic. How then does the transcriptional program of interneurons unfold across development? Differential gene expression can be regulated by TFs, which act to promote or suppress the transcription of RNA. Transcriptional regulation is typically mediated by combinations of TFs at specific moments, such that a small number of factors can result in a correspondingly larger number of neuron-specific transcriptional programs. The prevailing model is that each interneuron type can be defined by a combinatorial code of TFs that, through complex interactions, results in stable gene expression networks (Flames et al. 2007, Gelman et al. 2012). Combinatorial codes are imagined to collectively specify a cell's fate, like a unique barcode. Such a model suggests that a precise combination of TFs cooperatively activate a gene program that determines a neuron's identity. Alternatively, and perhaps more reasonably, a combination of expressed TFs may act individually to bestow particular properties on a neuron such as its firing properties, morphology, or connectivity. This latter idea has been championed in the terminal selector model of neuronal identity (Hobert 2016), which has been

beautifully described and documented in the nematode. The Hobert group (Deneris & Hobert 2014, Kratsios & Hobert 2018, Patel & Hobert 2017) has demonstrated through exquisite genetic analysis that so-called bottom-up codes select for particular emergent features in particular neurons. As a wealth of data have demonstrated, features such as neurotransmitter identity, and also ion channels and synaptic organizers, are regulated by assemblies of genes that are coregulated by common TFs. Accumulating evidence has begun to support the idea that terminal selection motifs exist in cortical interneurons (Paul et al. 2017), which is to say that at the working end of specification a combinatorial code of terminal selectors, each of which imbue interneurons with specific properties (e.g., neurotransmitter function or firing properties), is acting. Nonetheless, we suggest that the top-down GRNs that transcriptionally act to direct the expression of terminal selectors may be better described by a configurational code through attractor dynamics rather than a static combinatorial code.

4.2. Experimental Examination of the Requirement for Transcription Factors in the Specification of Interneurons

The strongest single determinant of interneuron cell fate identified to date is *Nkx2.1*. The removal of this single TF results in a class switching from MGE-derived PV and SST cardinal classes to CGE-derived VIP and Id2 cardinal classes. As its expression is extinguished within cortical interneurons when they become postmitotic, does it act by altering lineage fate decisions? Probably not, given that the progenitors that produce interneurons can also give rise to neurons across the telencephalon (Harwell et al. 2015, Mayer et al. 2015). Constitutive loss of *Nkx2.1* gene function results in a marked reduction in proliferation accompanied by the MGE taking on a lateral ganglionic eminence identity (Sussel et al. 1999). However, the impact of *Nkx2.1* on fate appears to correspond with its function as interneurons exit the cell cycle. Conditional removal of *Nkx2.1* coincident with progenitors becoming postmitotic results in a switch of cardinal class fate (Butt et al. 2008). Indeed, all other TFs that have been shown to selectively affect particular interneuron subtypes are only expressed postmitotically.

The precise outcome of removal of TFs depends on the developmental stage at which they are removed. The most common result of the removal of key factors such as *Lhx6* (Fragkouli et al. 2009, Liodis et al. 2007), *Sox6* (Azim et al. 2009, Batista-Brito et al. 2009), *Satb1* (Close et al. 2012, Denaxa et al. 2012), or *Prox1* (Miyoshi et al. 2015, Rubin & Kessaris 2013) is a decrease (but not elimination) in the net numbers of the population expressing the factor. Moreover, as noted above, the result is highly time dependent, with early removal having considerably more severe consequences than late removal (Batista-Brito et al. 2009). For instance, while the loss of *Satb1* embryonically results in the loss of both SST and PV cortical interneurons, postnatal removal has no obvious effect on the PV population and only reduces the expression of SST itself within the SST interneuron population (Close et al. 2012). Similarly, while the embryonic loss of *Prox1* severely impacts the bipolar VIP population through the loss of calretinin expression and dendritic truncation, postnatal removal has similar but much less severe consequences (Miyoshi et al. 2015).

What then can be inferred about the role of TFs in the production of specific interneuron subtypes? Two general rules can be extrapolated from these findings: Early removal, particularly within newly postmitotic populations, tends to have more severe consequences than late removal, and the loss of particular factors tends to reduce the absolute number of particular interneuronal populations rather than ablate them wholesale.

Complementing loss-of-function analysis are gain-of-function efforts. Many groups have examined the ability of combinations of TFs to direct progenitors to specific interneuronal subtypes (Au et al. 2013, DeBoer & Anderson 2017, Petros et al. 2013, Yang et al. 2017) as well as a variety of other neuronal identities (Andersson et al. 2006, Davis-Dusenbery et al. 2014, Panman et al. 2011,

Wichterle et al. 2002). Within induced pluripotent stem cells that have been directed to assume ventral telencephalic identities, the controlled expression of many of these same factors, including Nkx2.1, Lhx6, and Pou3F4, selectively enriches the production of specific interneuronal subtypes (Au et al. 2013). The advent of increasingly sophisticated three-dimensional culture methods to produce telencephalic cerebroids, combined with the controlled gain-of-function expression of such TFs, holds the promise of producing human interneuron subtypes on demand (Birey et al. 2017, Eiraku et al. 2008, Lancaster et al. 2013, Quadrato et al. 2017). So, with further efforts, will be able to identify combinations of TFs that specify interneuronal subtypes? Recent findings from the Baldwin laboratory (Tsunemoto et al. 2018) offer hope that this may be possible. In this work, they identify 76 combinations of TFs that can neuralize fibroblasts in a manner akin to how Yamanaka factors transform somatic cells into stem cells (Yamanaka 2008). However, in both these cases the TFs are likely acting as attractors rather than as combinatorial determinants. Specifically, as beautifully shown by Jaenisch and colleagues (Buganim et al. 2012, Shu et al. 2013), somatic cells forced to express Yamanaka factors rapidly induce other determinants that are needed for somatic cells to progress into stem cells. Hence, reprogramming occurs through the activation of a transcriptional program, transitioning a cell into a different attractor state, and can be driven by a few TFs alone.

4.3. Combinatorial Becomes Configurational Transcription Code

Taken together, combinations of TFs can initialize but not realize cell fates. The loss of specific factors does not result in the loss of specific interneuron subtypes (i.e., redundancy), and specific TFs function in the specification of often highly diverse interneuron subtypes (i.e., iterative). The apparent redundancy on one hand and the iterative nature of TFs on the other suggest that the idea of a fixed combinatorial code for the specification of interneurons needs to be reconsidered. As a first pass, combinatorial codes provide a good guide in explaining the early specification of progenitor domains (e.g., *Nkx2.1*) and the selection of particular mature features (e.g., terminal selection). However, with regard to general specification, combinatorial codes lack the robustness to reliably produce cell types.

If a combinatorial code was all there was, cell identities, as with the special case of Nkx2.1, should be destabilized by the loss of single factors. The lack of robustness in such a system warrants an alternative model. We suggest an attractor transcriptional model of specification that provides a more realistic model for how interneuronal or, more generally, any neuronal identity is established and maintained. Central to such a scheme is the dynamic nature of a highly connected transcriptional network by which cell identities are generated. Combinatorial codes are considered static in their specification of cell identities, implying that they can be classified by the expression of a particular set of TFs across time. When the gene expression within interneurons was examined across developmental timepoints, gene expression proved to be highly dynamic (**Figure 3**). In fact, it turned out to be computationally difficult to align gene expression within particular cells across time. Recent computational methods such as canonical categorical association (Butler et al. 2018) provide a way to align shared variance among particular cell types longitudinally, but the conserved gene expression profile is difficult to detect and impossible if only TFs are considered. For instance, a variety of TF expression patterns are observed, including early and transient expression (*Nkx2.1*, *Dlx2*) as well as dynamic (*Lhx6*, *Zeb2*) and static (*Satb1*, *Mef2c*) expression. Hence, rather than being specified by particular combinatorial codes, the gene expression profile during interneuron development is highly dynamic and more consistent with the model classically suggested by Waddington's landscape (Trapnell 2015). Note that, at early stages of specification, the attractor model can be approximated by a combinatorial model: The cardinal interneuronal divisions are determined by a low number of TFs in a manner akin to the

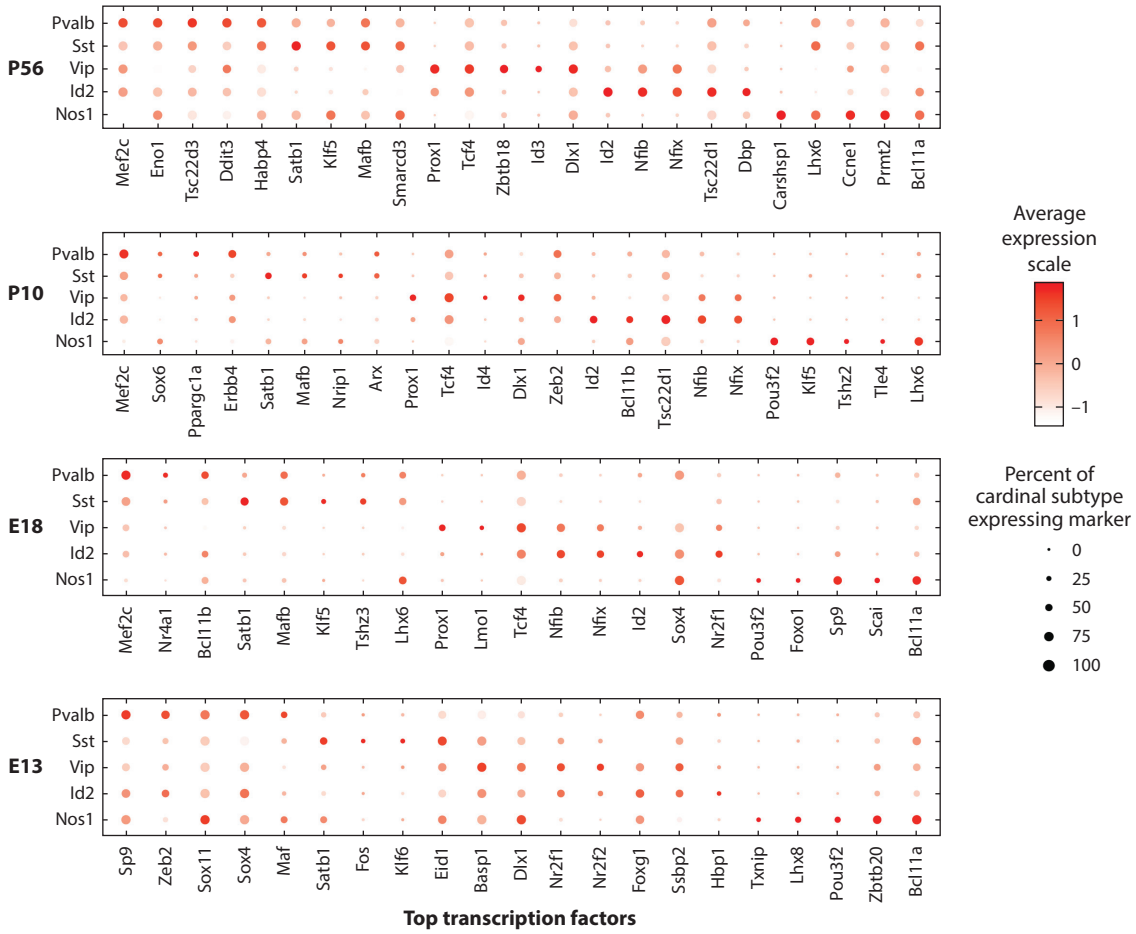


Figure 3

Survey of transcription factor (TF) expression across development showing the expression trajectory of the top TFs across four sequential time points within each of the four cardinal GABAergic interneuron classes, as well as the Nos1-expressing GABAergic projection neuron type. Note that, with a few exceptions, the expression of each of these factors is highly dynamic and evolves across development in a manner consistent with the emergence of attractor dynamics underlying the maturation of each interneuron subtype. Abbreviations: E, embryonic day; P, postnatal day.

action of Yamanaka factors. As refinement progresses, the seeding cardinal factors induce gene networks defined by multiple configurational codes (see **Figures 3–5**). Recognizing this explains why the best Cre lines for targeting specific cell types, including interneurons, take advantage of fortuitous genes that encode for proteins other than TFs, such as PV, SST, and VIP. The answer seems clear: The selection of cell fate relies on configurational rather than combinatorial codes.

4.4. Attractor Dynamics of Transcriptional Program Lead to Configurational Codes

What rules govern the cellular programs that generate cardinal classes from inhibitory neuron progenitors? Cellular differentiation and commitment to a subtype is orchestrated by the dynamics of GRNs, which coordinate a diverse set of requisite cellular processes. Based on extensive work

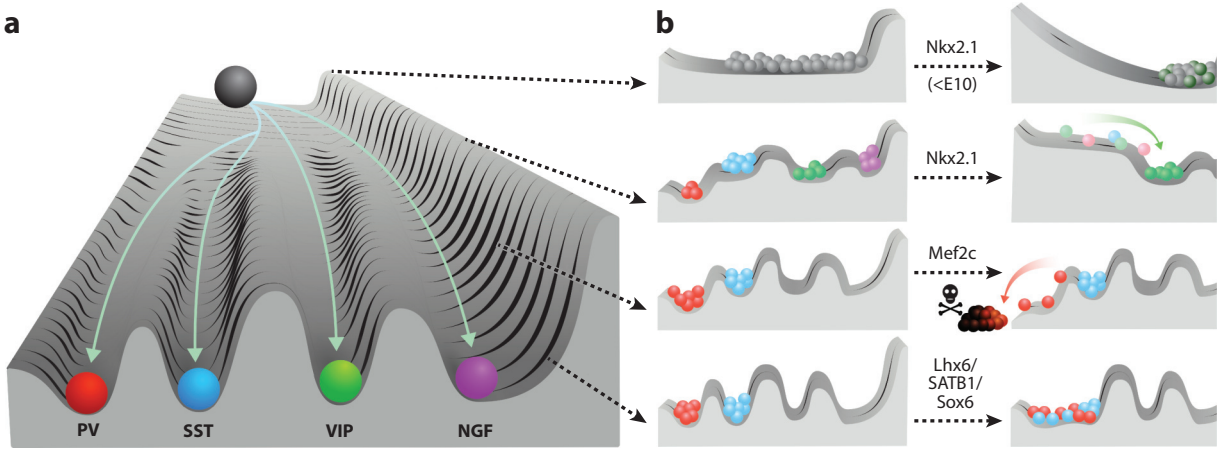


Figure 4

The developmental landscape reflects attractor dynamics. (*a*) Diagram showing the landscape of development as the energy function of an attractor gene regulatory network, with cells rolling down through bifurcating valleys. At the bottom, the basins of attraction provide robustness to external perturbations and confer distinct stability properties, depending on the height of the energy barrier between interneuron subtypes. (*b*) Schematic showing how distinct transcription factor manipulations generate distinct development landscapes, reducing barriers between attractor states and/or making them unstable. Abbreviations: NGF, nerve growth factor; PV, parvalbumin; SST, somatostatin; VIP, vasoactive intestinal polypeptide.

in other biological systems, we propose a computational framework in which interneuron subtypes represent stable states in the attractor dynamics of transcriptional networks during development.

The origin of these ideas goes back to Conrad Waddington's metaphor for cellular development as an epigenetic landscape (Slack 2002) (**Figure 4**). The notion is that the development process, starting from an initial pluripotent state through a succession of different phenotypes and ending in a range of committed phenotypes, can be viewed as a rugged valley. Uncommitted cells at the top roll downhill like balls, following distinct valleys (trajectories), encountering inflection points (decisions), and ending at the bottom, representing a commitment to a terminal state. Visually, it is obvious that as the ridges confine the identity of each cell type they also create basins of attraction, which makes the process robust to external perturbations.

This conceptual picture can be backed up by a rich mathematical theory of dynamical systems, translating Waddington's concepts of chreods (canalized paths of development) to trajectories and homeorhesis (the tendency to return to a path) to attractor states. Stuart Kauffman originally suggested that the network of TFs underlying development could be modeled as a Boolean network, whose attractor states correspond to cell types. In Boolean networks, each node represents a gene. Based on inputs to each node, the gene can be activated (on, 1) or inhibited (off, 0). TFs govern these interactions, with each interaction representing an edge. The activity state of all genes defines the network state. Such Boolean networks have been used to explain the dynamics of numerous biological systems, including *Drosophila* development and cancer (Huang et al. 2009, Koulakov & Lazebnik 2012, Manu et al. 2009). An interesting side point for a neuroscientist is that these networks have a very similar mathematical formalism to Hopfield neural networks in which attractors represent neural activity patterns, each encoding a memory (Hopfield 1982).

If the dynamics of these networks are dissipative and ultimately form local minima, then the arising stable attractor states will correspond to cell identity. The potential energy of these systems then becomes analogous to Waddington's epigenetic landscape. The temporal evolution of cell states can then be described by the gradient of this potential energy function, formally known as

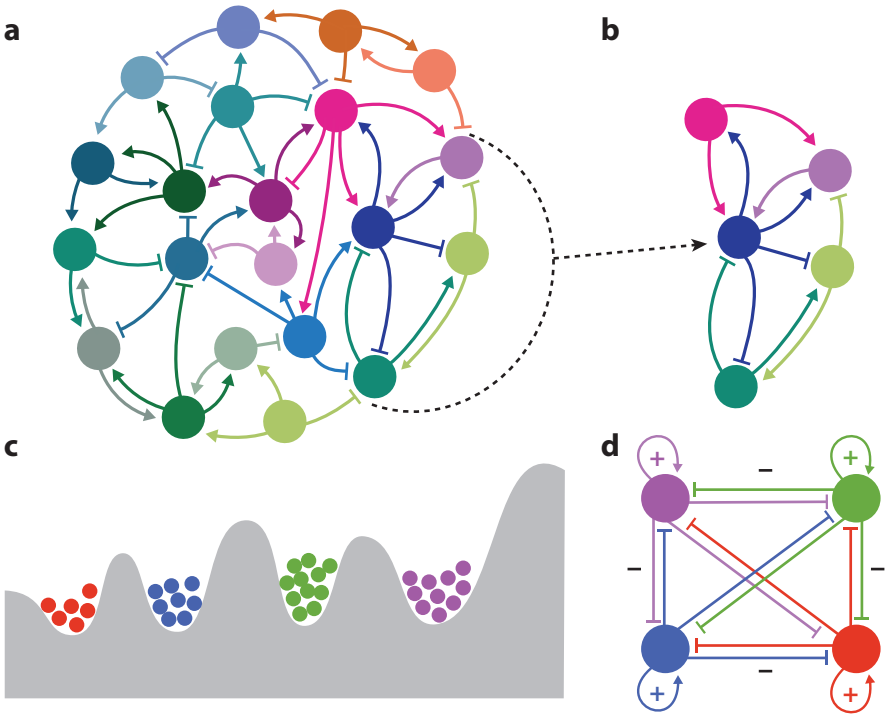


Figure 5

Genetic regulatory network motifs may create cell-type attractors. (*a*) In this hypothetical topology of a complex genetic regulatory network, transcription factors (TFs) promote or suppress each other's expression. (*b*) The complexity of this network's topology may be reduced by identifying motifs such as clusters of TFs that promote each other's expression while suppressing other clusters. (*c*) This topology can give rise to multi-stable dynamics with valleys representing individual cell states and balls the final state of individual interneurons. (*d*) Reduced network topology reflects positive feedback loops within clusters of TFs and cross-inhibition to other clusters, with each cluster corresponding to a configurational code for a specific cell type.

a Lyapunov function, reflecting the slopes of these landscapes. Note that genetic networks, due to their dissipative nature and complex topology, may prevent determination of a defined energy function. Nevertheless, even when the requirements are not met (e.g., specific network topology and dynamics of individual nodes), there are ways to decompose the dynamics into so called quasi-Lyapunov functions (Kirschner & Tsygvintsev 2009). In this case, the quasi-Lyapunov function governs the approach to attractor states, with a remainder term corresponding to dynamics along the attractor. Hence, the dynamics of cell states correspond to a landscape, such as the one illustrated in **Figure 4*a***, where distinct basins of attraction define distinct cell types. It is visually intuitive in such systems that the final states are robust to small deviations and external perturbations.

The dynamics of cell fate decisions can also be studied in a formal way by applying bifurcation theory to describe the evolution of new cell states (**Figure 5**). This analysis can then be used to understand how different parameters controlling the shape of the landscape induce different bifurcation types with distinct features. For instance, the landscape in **Figure 4*a*** illustrates initially monostable states smoothly splitting into two new stable states, which might define a supercritical pitchfork bifurcation in formal terms.

4.5. Transcriptional Dynamics, Configuration Codes, and the Generation of Cell Type Attractors

Can we infer the dynamics of cell states and how they give rise to distinct subtypes from transcriptomic data? Genetic regulatory networks tend to be very complex and, at present, not sufficiently constrained by data. While differential coexpression of genes could be a signature of the underlying attractor dynamics, this measure is highly susceptible to the normalization required to eliminate batch confounds in sequencing data. There are other obvious technical challenges since each cell is assessed in isolation, hence technical and biological noise sources can be confounding. Indeed, inferring regulatory networks in general is fraught with challenges. Nevertheless, there may be specific network motifs that can help us to understand the dynamics of even complex networks with uncertainties about the precise topology. For instance, networks where some clusters of TFs tend to mutually activate each other (positive feedback) while repressing other clusters (negative feedback) can generate multistability. Once a few TFs become dominant, they are self-reinforcing and inhibit the rest of the network, creating stable states. At present, it is unknown whether TF networks can be reduced to this conceptual model and to what degree master regulators (well-connected hubs) can be inferred from the network topology alone. If this model is correct, then the only way to force cells into a new basin of attraction is to destabilize previously stable states.

Modeling cardinal classes and their associated subtypes as network attractors can explain a swath of existing data. For instance, within the spinal cord, overexpression of a number of different *Hox* genes can alter motor neuron identity at limb levels because their activation can force the system to a new basin of attraction. However, the removal of individual *Hox* genes has little effect because other TFs contribute to maintain the attractor state identity (Jung et al. 2014). More generally, this model explains how large and complex transcriptional networks actually have a low number of stable states and many relevant nodes beyond the key transcriptional factors previously implicated. These factors in turn help weigh and reinforce cell identities. The cluster of TFs that define a cell type can be viewed as a configuration code, with their respective roles in the GRN defining the degree to which individual TFs are necessary. In this context, the difference between a combinatorial and configurational code may seem semantic. However, configurational codes, unlike combinatorial codes, are dynamic and evolve over time. In our configurational model, with the exception of the major cardinal classes, the energy functions separating different cell types during early postmitotic development are shallow, and thus it is easier to move between different states. Upon attaining their settling positions within the cortex, the valleys separating specific subtypes may become deeper, making it more difficult to perturb or switch between subtypes. Finally, in mature interneurons, configurational dynamic changes still occur in a limited way within local minima. These changes represent cell states, which may be described as flat valleys (so-called line attractors) and result in dynamic cell states instead of fixed-point attractors. In **Figure 4**, we outline existing loss-of-function data demonstrating how the timing of TF removal in interneurons differentially results in changes in the configurational landscape. Working out the details of this model will necessitate further TF manipulations during development and careful computational analysis. Through such studies, it may be possible to directly infer or refute the notion that configurational TF codes produce cell types as attractor states. In particular, experimental data are needed that explore the transcriptomic consequences resulting from the temporal-specific removal of TFs, both individually and in combination.

In summary, we propose that such experiments will support a model in which interneurons converge upon particular stable identities through a process of gradient descent, as envisioned by Waddington (Slack 2002) and recently inferred computationally (Schiebinger et al. 2019).

5. EVOLUTIONARY ORIGINS AND DIVERSIFICATION OF INTERNEURONS

The evolution of the neocortex is notable for its expansions in neuronal numbers and areas. However, recent work suggests that interneuron subtypes are surprisingly ancient (Tosches et al. 2018). This at least superficially contradicts the prediction of Ramón y Cajal (1966, p. 480), who suggested that cortical evolution (and the emergence of intelligence) was accompanied by a "prodigious abundance and unaccustomed wealth of the so-called neurons with short axons." Indeed, it is easy to understand why the great anatomist would have expected that increases in interneuron diversity would be required to accommodate enhanced circuit complexity. How then do cortical cell types compare across species? Traditionally, comparative studies have relied largely on morphology and associated low-dimensional features. A more rigorous comparison can now be achieved by examining large-scale molecular characteristics using single-cell RNA-seq techniques. As a result, recently available transcriptomic techniques have brought a revolution in the identification of homologous neuron types based on molecular expression patterns, opening a new chapter in research on the evolution of neuronal cell types.

5.1. Evolution of the Telencephalon in Reptiles Versus Mammals

Despite being highly divergent from mammals, reptiles do possess a primitive cortex. Comparative analysis of cell types in turtles and lizards versus mammals has provided the first inkling of the evolutionarily conserved aspects of cortical architecture between these species (Striedter 1997). Despite the large structural differences between turtles and mammals, both are composed of the same fundamental glutamatergic excitatory and GABAergic inhibitory populations, which further share strikingly similar physiological properties (Laclef & Metin 2018, Metin et al. 2007, Puelles 2017). For instance, while the telencephalon is dominated by the dorsal ventricular ridge (DVR) in reptiles, the pallium occupies the dorsal aspect of the brain of mammals (Striedter 1997). Beyond this, however, strong differences in structural homology have made comparisons across these species difficult. Indeed, historically the DVR was considered homologous to the cortex, but it is now generally considered as a part of the basal ganglia, claustrum or, amygdala (Butler et al. 2011). Instead, within reptiles, the dorsal pallium appears to be relegated to a small portion of the dorsomedial wall and composed by archicortex that is most similar to the hippocampus, while the ventral pallium is considered most similar to the paleo- or entorhinal cortex (Striedter 2016).

5.2. Origin of Inhibitory Interneurons

In the first effort to compare cell types within the pallium at a molecular level, striking similarities were found among the inhibitory interneuronal subtypes, with clear homologies existing across the four major cardinal classes (PV, SST, VIP, and Lamp5/Id2) (Tosches et al. 2018). Notably, however, while the generalized patterns of gene expression across interneurons were conserved, basic subtypes such as Martinotti cells and basket cells were lacking. Similarly, while a VIP-like population could be detected, this population lacked expression of VIP per se. By contrast, differences in the pallium were considerably more marked, with the turtle brain looking as if it resembles an expanded ventral pallium, albeit with evidence of being quite divergent from those seen in mammals. While it is rather early to speculate about the broader significance of these findings, a few points seem evident. First, interneuron diversity is clearly very old. Turtles diverged from mammals approximately 320 million years ago, and yet these cell types have been maintained in surprisingly conserved form despite the dramatic phylogenetic differences across vertebrates and the truly massive evolutionary distance. Second, the conservation of interneurons in the face of

strongly divergent glutamatergic populations suggests that, despite the mismatch across species in excitatory versus inhibitory subtypes, the latter maintain their basic subclasses.

The comparison of more closely related species such as mouse and humans showed similar conservation of interneurons but also some marked differences. While most interneuron subtypes have close homologs, the expression of particular genes varies. Perhaps the most striking difference is an expansion of CGE cell types, which is consistent with the corresponding enlargement of superficial cortex, again suggesting an evolutionary matching between inhibitory interneurons and principal cells (Hodge et al. 2018). One tantalizing recent discovery is the identification of a novel dendritically targeting population of layer 1 cells in humans. These neurons are dubbed Rosehip cells due to their characteristic somal morphology (Boldog et al. 2018). These cells express CCK and have the general morphology of neurogliaform interneurons but possess larger boutons that are even more densely distributed than those seen in basket cells. On the other hand, at least two layer 1 interneuron subtypes have been observed in both mice and humans and share physiology, genetic markers, and even rapid neuromodulation (Poorthuis et al. 2018).

The gene networks functioning within interneurons are clearly ancient, as evident by the shared use of the same terminal selector genes in worms and mammals (Hobert 2016). It also seems likely that similar attractor dynamics are central to the evolution of interneurons within vertebrate species, at least at the level of cardinal classes. Nonetheless, while interneurons appear strongly conserved, they clearly vary across species in both numbers and the specific expression of particular genes. Evolution adds a fascinating context to explore how the relative changes in excitatory and inhibitory cellular interactions are shaped through speciation. Delving into the developmental strategies by which the interactions between these cell types have adapted to optimize circuits in vertebrates as divergent as reptiles, rodents, and primates will no doubt reveal further surprises. Given our speculation that definitive and state specification are dependent on local cues, the differential matching of principal interneuron populations across species is likely to strongly impact subclass identity. In particular, the huge variation in cortical size across mammals (Kelava et al. 2013) raises questions as to how seemingly ancient programs underlying the fundamental interneuron classes have adapted to the morphogenetic reshaping of the pallial regions.

6. CONCLUSIONS

In the past five years, the field has undergone a breathtaking revolution in the understanding of the transcriptional architecture of interneurons. New techniques have yielded remarkable insights into how these cells emerge through development and across species. This work dovetails with the great successes in the previous decade in which the embryonic origins of interneurons and their contributions to canonical functional circuits have been revealed. With the increased precision with which interneurons can be targeted and manipulated in functional studies, we foresee a transformation in our understanding of how interneurons coordinate neural populations within the cortex, including their balance and rhythms, as well as how they control information flow on behavioral timescales. We expect that these insights will further emphasize the centrality of inhibitory control to cortical computation as well as lead to new ways of correcting the emblematic inhibitory dysfunctions associated with various neuropsychiatric disorders (Lewis 2014, Marin 2012, Skene et al. 2018).

DISCLOSURE STATEMENT

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