Genetic and activity-dependent mechanisms underlying interneuron diversity

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Abstract | The proper construction of neural circuits requires the generation of diverse cell types, their distribution to defined regions, and their specific and appropriate wiring. A major objective in neurobiology has been to understand the molecular determinants that link neural birth to terminal specification and functional connectivity, a task that is especially daunting in the case of cortical interneurons. Considerable evidence supports the idea that an interplay of intrinsic and environmental signalling is crucial to the sequential steps of interneuron specification, including migration, selection of a settling position, morphogenesis and synaptogenesis. However, when and how these influences merge to support the appropriate terminal differentiation of different classes of interneurons remains uncertain. In this Review, we discuss a wealth of recent findings that have advanced our understanding of the developmental mechanisms that contribute to the diversification of interneurons and suggest areas of particular promise for further investigation.

Neural ensembles
Select groups of connected neurons within a network whose synchronous activity is highly correlated to aspects of particular behaviours.

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doi:10.1038/nrn.2017.30
Published online 6 Apr 2017

Since Ramón y Cajal's evocative discoveries in the 1880's, neuronal diversity has continued to excite investigators and driven them to generate hypotheses of how this cellular complexity is achieved. In the mammalian neocortex alone, years of amassed research have revealed a remarkable heterogeneity in cellular composition, organization and functional circuitry. There is no doubt that such neuronal complexity has evolved to support the wide range of sophisticated cortical computations, including the integration of sensory information and learning and memory, that ultimately results in the generation of executive planning and underlies most 'higher-order functions'.

At their root, such tasks rely on a precise network of connections between neurons, including both glutamatergic excitatory and GABAergic inhibitory cells. Within this division of labour, the excitatory neurons numerically provide the largest proportion of cortical cells. They are essential for long-range connections and for the integration of ascending and recurrent information across widely distributed brain regions. Inhibitory interneurons, despite representing the minority of cells, provide crucial control over information flow. Beyond ensuring that excitation remains in check, interneurons locally modulate the timing, shape and coordination of neural circuits[1,2]. Recent studies have provided a plethora of insights regarding their contributions to behaviourally relevant neural ensembles[3] and, conversely, indications that their dysfunction is a central cause of numerous neurological diseases[4,5]. Such exquisite control over cortical networks is only possible because of the extremely diverse properties of interneurons, which are reflected in their distinct morphology, synaptic specificity, and physiological and biochemical characteristics[6–10].

Although a full appreciation of interneuron diversity is far from complete, understanding how the unique properties of these cells are determined during development is under active investigation. To this end, the community is actively pursuing the roles of genetic programming and extrinsic cues in sculpting interneuron types. However, it is still uncertain how these influences merge to support the appropriate terminal differentiation and, particularly, the connectivity of different subtypes of cortical interneurons. At the heart of the issue, there remains a vigorous debate as to when particular subtypes are specified. Aided by a wealth of recent findings, in this Review, we discuss our present understanding of the developmental mechanisms that contribute to the diversity of cortical interneurons in rodents, with a focus on exploring the evidence for the role of activity in their specification. For a more complete consideration of early differentiation mechanisms and how they might influence progenitor specification, we refer the reader to a recent review (see REF. 11).

Cortical interneuron diversity

In the adult cortex, interneurons can be classified into major classes by the expression of neurochemical markers. Virtually all interneurons express at least one of four characteristic markers: the calcium-binding protein

parvalbumin (PV), the neuropeptides somatostatin (SST) and vasoactive intestinal peptide (VIP), and the large secreted signalling protein reelin (RELN) (FIG. 1). Together neurons expressing at least one of these markers account for almost all interneurons within the cortex[8,12]. Each of these classes can be further broken down into subclasses on the basis of various attributes, most notably synaptic specificity (FIG. 2). For example, the PV-expressing (PV^+) cortical interneurons can be divided into those that target the soma of excitatory cells, a morphology that earned them the name 'basket cells', and those that innervate the axon initial segment, referred to as 'chandelier cells' (REFS 13,14). SST-expressing (SST^+) cortical interneurons target dendrites. They are composed of 'Martinotti cells', which ramify the distal dendrites of excitatory neurons, and some recently recognized non-Martinotti cell subtypes that reside in all cortical layers[15]. Together, the PV^+ and SST^+ cortical interneurons make up most cortical interneurons (~70%) and are the best characterized in terms of their roles in cortical network functions (FIG. 2).

The VIP-expressing (VIP^+) cells and RELN-expressing ($RELN^+$) cells that do not express SST (hence, more precisely $RELN^+SST^-$), which comprise only 30% of all cortical interneurons, are proving to have intriguing properties. These include an important role for bipolar VIP^+ cortical interneurons in disinhibition[16] and a role for $RELN^+SST^-$ 'neurogliaform' cells in bulk-mediated slow GABA transmission[17] (FIG. 2). These properties, in combination with their afferent inputs and physiological attributes (such as their intrinsic physiology

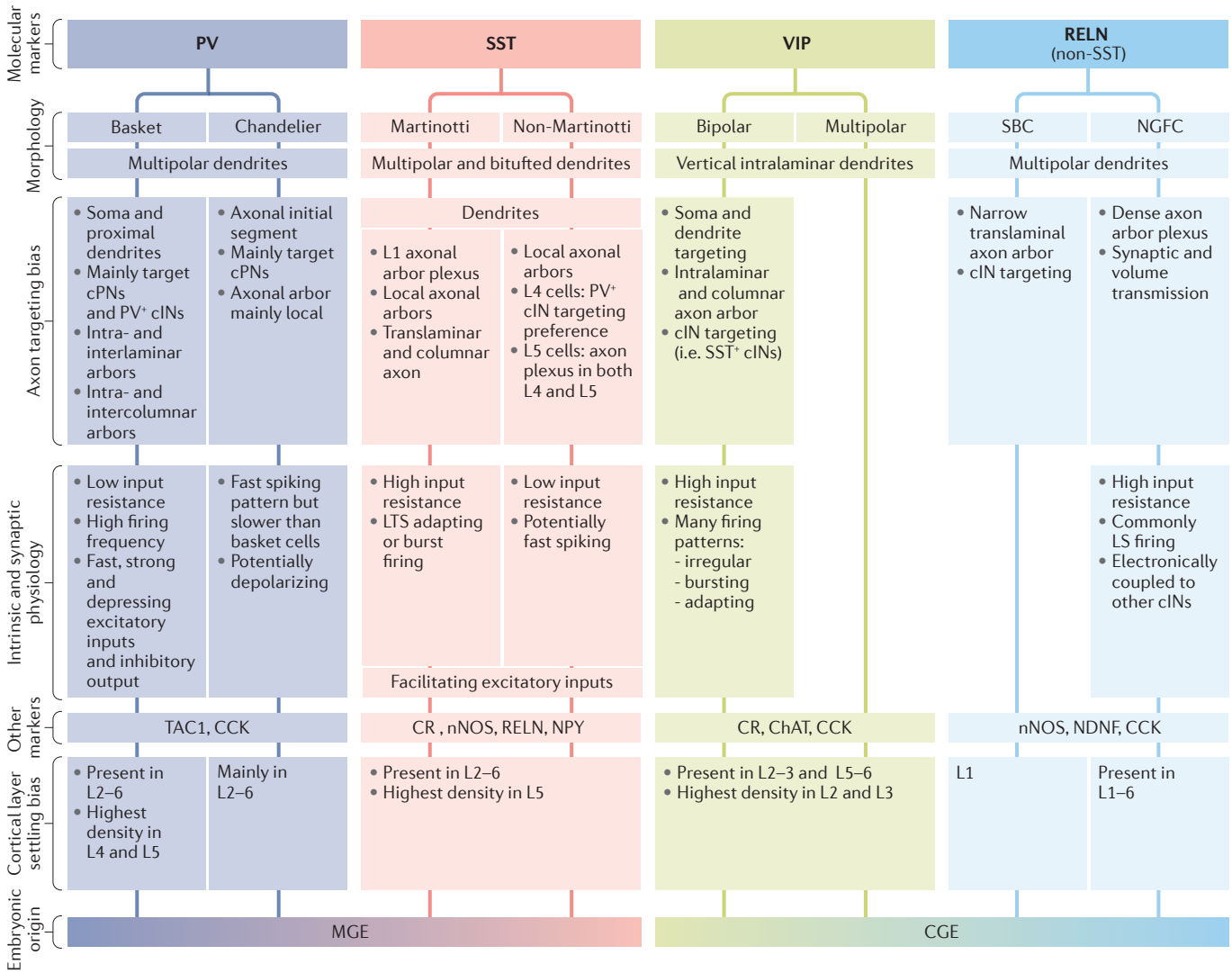

Figure 1 | **Cortical interneuron diversity.** There are four major classes of interneurons marked by their expression of parvalbumin (PV), somatostatin (SST), vasoactive intestinal peptide (VIP) and reelin (RELN) (the latter interneurons being known as $RELN^+SST^-$ cells, to differentiate them from a population of interneurons that express both SST and RELN). These classes are then further broken down into subclasses according to their morphology, axon synaptic specificity, physiological properties, cortical layer settling position, marker expression and location of origin. CCK, cholecystokinin; CGE, caudal ganglionic eminence; ChAT, choline acetyltransferase; cINs, cortical interneurons; cPNs, cortical pyramidal neurons; CR, calretinin; L1, layer 1; LS, late-spiking; LTS, low-threshold spiking; MGE, medial ganglionic eminence; NDNF, neuron-derived neurotrophic factor; NGFC; neurogliaform cell; nNOS, neuronal nitric oxide synthase; NPY, neuropeptide Y; SBC; small basket cell; TAC1, tachykinin 1.

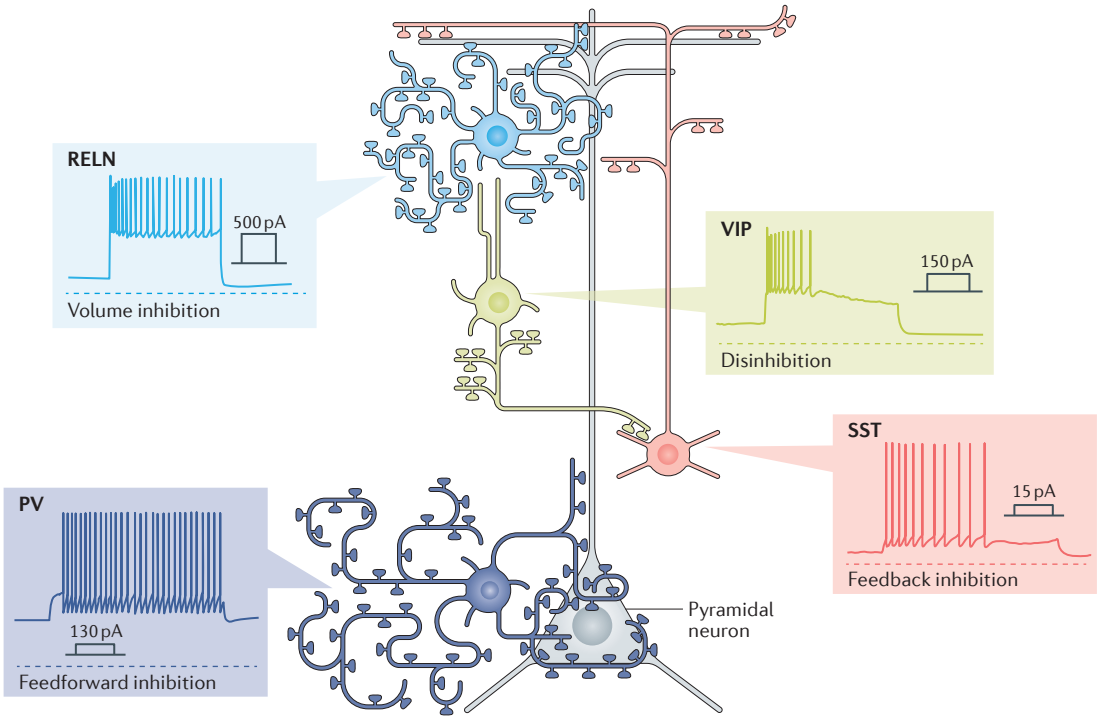

Figure 2 | **Connectivity of the four main classes of cortical interneurons.** The four main classes of cortical interneurons have distinct synaptic targeting biases onto neighbouring excitatory pyramidal neurons and engage in common circuit motifs. Parvalbumin (PV)-expressing basket cells are fast spiking and target the somatic compartment, engaging in feedforward inhibition from the thalamus onto the pyramidal cell. Somatostatin (SST)-expressing Martinotti cells can be burst spiking, target the distal dendrites and engage in feedback inhibition. Reelin (RELN)-expressing ($RELN^+SST^-$) cells are late spiking and either directly or by proximity target the distal dendrites. Vasoactive intestinal peptide (VIP)-expressing neurons can be irregular spiking, target the dendrites of SST^+ cells and thus participate in disinhibition.

and neuromodulatory control), provide an impressive breadth of possibilities in terms of their abilities to functionally modulate cortical excitatory networks.

The number of known interneuron subtypes continues to expand with emerging technological advances[102], and already the diversity of interneurons within the cortex rivals the 26 subtypes of interneurons that have been described within the CA1 region of the hippocampus[18] (for more on interneuron diversity, see REFS 8,19).

Models of interneuron development

Interneuron diversity emerges during embryogenesis and continues to be further elaborated throughout postnatal stages (FIG. 3). Most cortical interneurons are born within one of three embryonically distinct proliferative regions that line the ventricles — the medial ganglionic eminence (MGE), the caudal ganglionic eminence (CGE) and the lateral ganglionic eminence[20–22] — with a smaller cohort of cortical interneurons arising from the primary optic area[23] within the ventral telencephalon (which is also known as the subpallium). Genetic loss-of-function studies and recent lineage analyses indicate that multipotent progenitors sequentially produce distinct cortical interneuron types in a manner that is possibly influenced by highly dynamic morphogen cues within the progenitor zone[14,21,24–35]. After becoming postmitotic, interneurons undergo a prolonged migratory period spanning the last third of embryogenesis, during which sequential cohorts of interneurons invade the cortex (as well as other brain regions) and disperse radially to integrate within the nascent laminar layers. Upon reaching their target lamina, they mature into distinctive morphologies, form contacts with both local and long-range inputs, extend elaborate axons to precisely select their local synaptic targets and express specific neurochemical markers (FIG. 3a). It is only at this point that cortical interneuron subtypes become distinguishable from each other. The disconnect between when interneurons are born and when they acquire their mature characteristics leaves open the issue of whether they are specified at birth as progenitors or only after they have reached their regional settling position. Hence, we propose two competing models to explain when and how interneuron diversity is specified.

Progenitor specification hypothesis. The progenitor specification hypothesis suggests that interneuron identity is established approximately at birth through environmental cues that shape intrinsic progenitor identity. The most extreme version of this model posits that, upon generation, interneurons are bestowed with a covertly encoded blueprint that allows them to follow a precise differentiation and maturation programme to develop into a specific subtype (FIG. 3c). Supporting this hypothesis, PV^+ chandelier cells were found to originate from a spatially restricted pool of progenitors that are born relatively late in embryogenesis[13].

Ganglionic eminence
The name given to any one of the three transient embryonic proliferative zones that line the floor of the lateral ventricles. These zones give rise to almost all inhibitory projection neurons and interneurons that populate the cortex and basal ganglia.

Multipotent progenitors
Proliferative cells that have the potential to give rise to distinct cell types on the basis of differences in developmental stage, spatial position, environmental cues and mode of division.

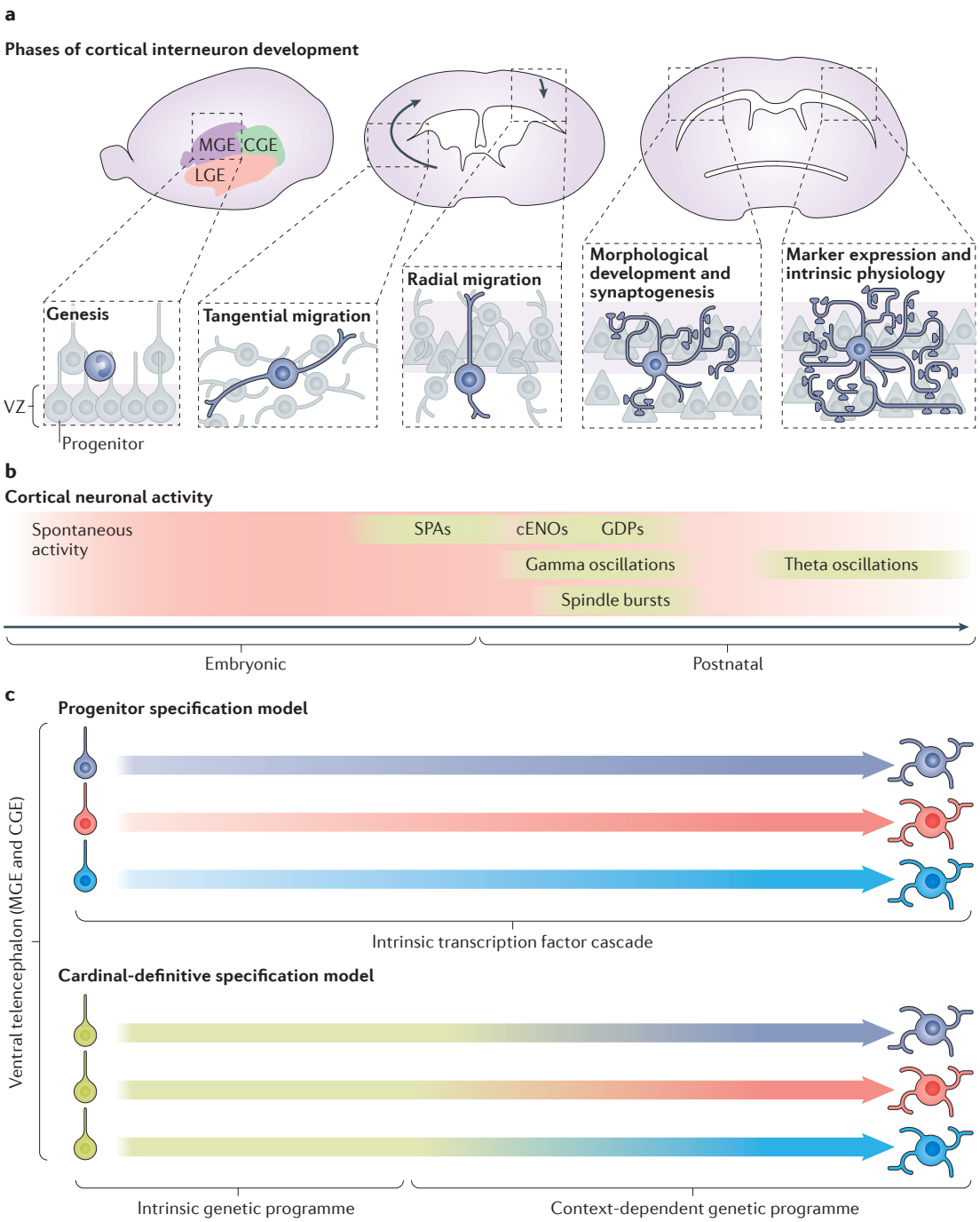

Figure 3 | **Models of cortical interneuron development. a** | During embryogenesis, cortical interneurons are generated from the medial ganglionic eminence (MGE) and the caudal ganglionic eminence (CGE). They then undergo a long tangential migration to the cortex, followed by radial migration into the developing cortical layers. During postnatal development, they reach a settling position within a laminar layer and establish their distinct morphology and synaptic contacts. The expression of particular neuronal markers and physiological attributes are acquired in parallel. **b** | Throughout development, neurons are dependent on activity for proliferation, their selection of settling position, and their morphological and synaptic development. This activity could be derived from a wealth of dynamic cortical network activities such as cortical early network oscillations (cENOs), giant depolarizing potentials (GDPs), spindle bursts and gamma oscillations. The schematic illustrates the stages at which each form of cortical activity is likely to influence cortical interneuron development. The embryonic and postnatal distinction shown in part **b** applies to all schematics within this figure. **c** | The progenitor specification model posits that early in neurogenesis distinct progenitors prescribe the fate of a given subtype of interneuron covertly through an intrinsic genetic cascade. The cardinal-definitive specification model, on the other hand, speculates that progenitors bestow a basic and potentially uniform intrinsic programme that is then further modified and supplemented by later context-specific changes that are induced through genetic regulation to inform the fate of an interneuron. LGE, lateral ganglionic eminence; SPAs, synchronous plateau assemblies; VZ, ventricular zone.

This model suggests that cell type-specific genetic programmes are established at birth and progressively instruct the development of distinctive subtypes within the four broad cortical interneuron classes. Indeed, the maturation of MGE-derived cortical interneurons is characterized by a temporal progression of transcription factor expression. *Nkx2.1* is expressed transiently within progenitors[36,37]. This is followed by a more prolonged postmitotic sequential expression of *Lhx6* (REFS 38–40), *Sox6* (REFS 41,42) and *Sip1* (REFS 43,44) as they migrate, and then of *Satb1* (REFS 45,46) as they settle into cortical lamina and develop their mature physiological and synaptic properties. Interestingly, the functions of these transcription factors are seemingly both overlapping and pleiotropic: conditional ablation of the genes that encode these transcription factors within cortical interneurons affects a range of properties within MGE-derived interneurons (including all PV^+ and SST^+ subtypes), such as settling position, proper marker expression and intrinsic physiology[38,39,41–43,45,46]. However, the question of whether these transcription factors fundamentally alter subtype identity or function generically to ensure that the progressive steps in maturation (including the tangential migration, radial migration and marker expression) occur in all MGE-derived cortical interneuron types is inherently difficult to assess because the mature PV^+ and SST^+ subtype characteristics fail to develop in these mutants.

Implicit to the progenitor specification hypothesis is the expectation that we will ultimately identify specific genetic factors or distinct genetic cascades that direct the specification of different subtypes. What then does the lack of evidence for such specificity in the expression and actions of known transcription factors indicate? One possibility is that, simply owing to technical limitations, we have yet to identify the crucial factors that are required for the specification of discrete subtypes. Alternatively, the factors that have already been identified may indeed be sufficient to specify distinct subtypes but function through a combinatorial mechanism that has yet to be fully deciphered. Some evidence supports the existence of just such an intrinsic mechanism. For instance, the loss of some specific transcription factors results in distinct effects on SST^+ and PV^+ type identities[39,46,47]. This suggests that either their functions are distinguished by acting on different epigenetic landscapes or by forming functionally distinct transcriptional complexes. In either case, this model predicts that these transcription factors can differentially direct the specification of distinct subtypes through intrinsic cell type-specific programmes. For example, *Lhx6* hypomorphic mutant mice[39] and *Satb1* conditional mutant[46] mice display a more pronounced requirement for these genes in SST^+ cortical interneurons than in PV^+ cortical interneuron subtypes. Specifically, a higher level of *Lhx6* and *Satb1* may be required within SST^+ cortical interneurons to drive the expression of SST and/or to elaborate subtype specific synaptic features[39,45]. Similarly, the PV^+ subtypes that are less dependent on *Lhx6* and *Satb1* may instead require additional factors for their differentiation.

Although the specifics of such mechanisms remain to be determined, such nuance in the relative dependence on specific transcription factors indicates that methods beyond simple loss-of-function analysis will be required to form a complete understanding of cortical interneuron specification.

Progressive specification hypothesis. Another possibility is that the genetic information necessary to shape the identity of particular interneuron subtypes is only acquired postmitotically, later in development. The progressive specification model, like the progenitor specification model, posits that interneurons are restricted into a general class at birth. However, in this progressive specification scenario, the determinants of cortical interneuron subtype identity are established relatively late through the interaction with their cortical environment. This hypothesis is consistent with a two-step model we previously proposed suggesting that early genetic programming establishes an interneuron 'cardinal' identity, which is then later refined into a 'definitive' identity, possibly through an activity-mediated mechanism[3] (FIG. 3c).

In line with this idea, upon concluding their migration to the cortex, interneurons are known to begin to express genes that regulate aspects of their maturation (such as potassium–chloride cotransporter 2 (*Kcc2*; also known as *Slc12a5*)) and the transcription factors *Satb1* and *Mef2c*), and some of these genes and/or their protein products are regulated by calcium signalling in response to neuronal depolarization[46,48,49]. Although multiple cortical interneuron types express these genes, activity-mediated regulation provides a mechanism by which the expression and function of a gene could be precisely timed and customized to the particular context of a cell.

Also in favour of the importance of environmental cues, recent work suggests that the most subtype-specific features of cortical interneurons — including settling position, morphology, synapse specificity, and afferent and efferent connectivity — rely on activity impinging upon interneurons during maturation[48,50–52]. The question remains as to whether these influences deterministically relegate cortical interneuron fate by acting as permissive cues that initiate the timing of a previously established intrinsic genetic programme or by acting as instructive cues that initiative new and distinct gene expression. To explore this emerging area, we focus attention for the remainder of this Review on recent findings examining the role of activity in the definitive specification of cortical interneurons.

Neuronal activity and development

Brain development is highly sensitive to input from the environment, but neuronal activity is observed even before sensory circuits are formed. Early in embryogenesis, uncorrelated electrical and chemical activities are observed in neurons and progenitors, and these events are known to aid in proliferation[53–56], migration[57,58] and axon guidance[59,60]. Later, spontaneous network activity is observed across many developmental systems in the retina, cochlea, spinal cord, hippocampus and neocortex[61–63].

Tangential migration
A mode of migration used by newly generated inhibitory interneurons that originate within the eminences. These interneurons migrate from the ventricular progenitor zones into the overlying mantle and disperse to populate various brain structures. Tangentially migrating interneurons from the medial and caudal ganglionic eminences populate cortical and subcortical structures, whereas those from the lateral ganglionic eminence populate the olfactory bulb.

Radial migration
Radial glia-guided migration used by both interneuron and projection neurons primarily to position themselves within the cortical plate.

Calcium transients
Transient increases in the level of intracellular calcium generally within a 1–5 Hz frequency. These are typically optically recorded within the cell soma through fluorescence calcium indicators; however, dendritic and axonal recordings have also been recorded.

Coincidence detection
A mechanism whereby the coordinate timing of presynaptic and postsynaptic stimuli translates temporal and spatial differences in arriving synaptic transmission into changes in the probability of action potential generation and/or synaptic plasticity (including Hebbian strengthening, long-term potentiation and long-term depression) within the target neuron.

Cortical early network oscillations
An early rhythmic pattern of synchronized increases in membrane potential among large groups of neurons recorded *in vitro* in neocortical slices. The oscillations are dependent on glutamate, and those recorded *in vitro* are slower and of higher voltages than early gamma oscillations observed *in vivo*.

Giant depolarizing potentials
A form of synchronized neuronal depolarization patterns that supplant cortical early network oscillations during development and that are dependent on the actions of excitatory GABA. Each event is shorter than an early network oscillation but similar in magnitude.

Spindle bursts
Oscillatory events observed *in vivo* in the neonatal cortex. They are produced by the synchronized depolarization of a small localized group of neurons and can be evoked by sensory stimuli. Spindle bursts are slower events compared with early gamma oscillations.

Early gamma oscillations
One of the premier oscillatory events observed *in vivo* in the neonatal cortex. They are brief synchronized events evoked spontaneously and by feedforward excitation from the thalamus. Early gamma oscillations are transient events during the first postnatal week that are replaced by adult gamma oscillations dependent on parvalbumin-expressing interneuron inhibition.

These later activity patterns are vital to the maturation of their circuits. Typically, they are recorded as synchronous calcium transients that rely on temporary cell-intrinsic or non-synaptic properties (such as gap junctions) that are present for a select time during development and guide circuit organization[63,64].

As mentioned above, whether these early activities have an instructive or permissive role in shaping neuronal development has remained a persistent question in neuroscience. Historically, the question is framed as to whether activity directly influences the connectivity of cells within a circuit by influencing competition for synaptic space upon a given target cell through a process of coincidence detection (that is, through Hebbian plasticity[65]) or indirectly by activating a latent intrinsic developmental plan[66]. How activity influences the whole identity of cortical interneuron subtypes remains particularly unclear.

If activity is instructive in cortical interneuron subtype determination, there exists a wealth of early network activity patterns that could encode differentiation signals (FIG. 3b). This prompts the question of whether activity exerts its effects on interneuron development simply through depolarization or whether the pattern of excitation is crucial to the response. Within the first postnatal week alone, cortical interneuron activity is initiated by events that are mediated through gap junctions, glutamate and GABA, as well as through (although less well studied) serotonin, dopamine and acetylcholine. Is the depolarization mediated by any of these neurotransmitters and their associated afferent axons equivalent? The probable differences in their stimulation frequency and strength, the localization of their obligate postsynaptic receptors, whether they function ionotropically or metabotropically, the second messenger signalling employed and whether they induce specific genetic programmes mean that there is no shortage of mechanisms by which different modes of excitation could prove qualitatively different and instructive. On the other hand, activity may simply act to initiate differentiation through depolarization, and different modes of excitation may be interchangeable. Below, we discuss some of the key findings that have revealed a link between neuronal activity and activity-dependent modalities of cortical interneuron development.

Early cortical activity patterns. During the first postnatal weeks, interneurons settle and integrate into the nascent cortex, where they finally develop the characteristics that define their identity. This period of cortical interneuron maturation is accompanied by rapid progressive changes in the types of neuronal and network activity (FIG. 3b). Cortical early network oscillations dominate the first few postnatal days, until they are replaced by giant depolarizing potentials. These two forms of network activity rely on glutamate and the excitatory action of GABA release, respectively[67–69]. Likewise, *in vivo* recordings during the first postnatal week reveal slow oscillations that are sparsely marked by spindle bursts and early gamma oscillations that evolve into theta rhythms and fast oscillations by the end of the first month[70–72]. Indeed, interneurons contribute to and are engaged in these early activity patterns (for more information, see REFS 73–75). But do these different forms of activity contribute to cortical interneuron specification?

Activity-dependent development. Different interneuron subtypes have distinct requirements for activity during critical windows in development to permit the proper elaboration of their mature properties. Dampening the excitability of MGE-derived and/or CGE-derived neurons in a cell-autonomous manner has revealed that activity is required during radial migration, programmed cell death, morphological and synaptic development, as well as for the expression of some neuromodulatory markers (such as neuropeptide Y and PV)[48,50,76–78] (TABLE 1). For example, overexpression of inward rectifier potassium channel *Kir2.1* (also known as *Kcnj2*) can suppress excitability by making the cell more hyperpolarized[50,79]. This limits calcium entry and as such has been widely used to examine the role of activity during development. During radial migration (which takes place between postnatal days 1–3), cell-autonomous overexpression of *Kir2.1* results in the ectopic settling of cells expressing VIP and calretinin (CR^+) (VIP^+CR^+ cells) and $RELN^+SST^-$ cells within the cortical lamina. Performing the same manipulation selectively a few days later stunts the morphogenesis of $RELN^+SST^-$ and CR^+ cells, but not of VIP^+CR^- cells[50]. During the second postnatal week, preliminary findings suggest that *Kir2.1* expression selectively within PV^+ or SST^+ neurons results in a reduction in the density of their axonal synaptic outputs (B.W. and G.F., unpublished observations).

Interestingly, between these first two postnatal weeks there is a window of cortical interneuron cell death that peaks at postnatal day 7 and subsides by day 10 (REF. 80). This developmental winnowing of cortical interneurons has been detected through increases in cleaved caspase 3-positive cells and through a decrease in the density of all GABAergic cells during this time period[80]. It remains unclear whether this developmental cell death in cortical interneurons is intrinsically programmed or whether there is a 'population-autonomous' mechanism whereby cortical interneurons compete for commonly required survival signals[80]. Moreover, there is preliminary evidence that activity has a subtype-specific role (R. Priya, A. A. Buylla and G.F., personal communication) in the regulation of cortical interneuron cell death, indicating that it will take more effort to understand how these competing observations fit together.

These findings have led to a growing consensus that different interneuron types have variable dependencies on early neuronal activities for the emergence of key aspects of their differentiation (TABLE 1). It should be noted that these activity manipulations quantitatively disrupt activity irrespective of the specific source or effects of various patterned activities. Hence, there may be considerably more subtlety in the influences of activity upon development than these findings have revealed. For example, the possibility that the regional differences in afferent and efferent connectivity of interneurons of the same subtype could be shaped qualitatively by different activity-dependent interactions remains an attractive possibility.

Table 1 | **Activity-dependent development of cortical interneurons**

Manipulations	Outcome	Activity-regulated genes identified	Refs
Neurogenesis			
• Gap-junction blockers • ATP-blockers • Intracellular Ca^{2+} blockers	Reduced proliferation	Unknown	53,54
• Embryonic monocular enucleation • Nicotinic acetylcholine receptor blockers	Increased neurogenesis	Unknown	56
Migration			
Blockade of excitatory $GABA_A$Rs. AMPARs and NMDARs and L-type calcium channels	Slowed motility within cortex	Unknown	48
Kcc2 overexpression	Stopped migration in a voltage-sensitive manner	Unknown	48
Radial migration and establishment of settling position			
Kir2.1 overexpression	Ectopic settling of CGE cINs in deeper cortical layers	*Npas1*, *Dlx1*, *Elmo1*	50
Establishment of morphology and synaptogenesis			
Kir2.1 overexpression	• Reduced axon length and complexity in $RELN^+$ and CR^+ CGE cINs • Reduced dendritic complexity in $RELN^+$ and CR^+ CGE cINs • Decreased output of presynaptic structures in PV^+ and SST^+ neurons	Unknown	50
• NMDAR cKO in CGE cINs • Whisker plucking • Visual deprivation	• Reduced axon and dendritic length in $RELN^+$ CGE cINs • Decreased output of presynaptic structures in PV^+ neurons • Decreased excitatory input onto SST^+ neurons (visual deprivation) • Reduced inhibitory inputs onto VIP^+ neurons (visual deprivation)	• *Elmo1* ($RELN^+SST^-$) • *Npas4*, *Nptx2* (SST^+) • *Igf1* (VIP^+)	81,82, 96,97
Establishment of intrinsic physiology and synaptic maturation			
• Whisker plucking • TTX monocular injection • Chronic cortical muscimol injection • *Kir2.1* overexpression • GABA cKO in PV^+ cINs	• Decreased output of presynaptic structures from PV^+ neurons (all manipulations) • Reduced output of presynaptic structures in SST^+ neurons (*Kir2.1* overexpression) • Action potential firing properties of PV^+ neurons altered (Whisker plucking, *Kir2.1* overexpression and muscimol injection)	*Gad1*, *Gad2*, *Etv1* (PV^+)	52, 76–78, 82

AMPARs, AMPA receptors; CGE, caudal ganglion eminence; cINs, cortical interneurons; cKO, conditional knockout; CR, calretinin; *Elmo1*, engulfment and cell motility protein 1; *Etv1*, ETS translocation variant 1; $GABA_A$Rs, GABA type A receptors; *Gad1*, glutamate decarboxylase 1; *Igf1*, insulin-like growth factor 1; *Kcc2*, potassium–chloride cotransporter 2 ; NMDARs, NMDA receptors; *Npas1*, neuronal PAS domain-containing protein 1; *Nptx2*, neuronal pentraxin 2; PV, parvalbumin; RELN, reelin; SST, somatostatin; TTX, tetrodotoxin; VIP, vasoactive intestinal peptide.

Critical windows
Distinct time frames within the perinatal period when particular transient activity-dependent developmental events have a lasting impact on the functional behaviour of particular classes of neurons or neuronal ensembles.

Activity-dependent mechanisms

In the somatosensory cortex, differences between the effects of thalamic and cortical input onto $RELN^+SST^-$ neurogliaform cells demonstrate that the specific source of excitation can differentially regulate interneuron maturation. Attenuating sensory input through chronic whisker removal or by inhibiting vesicular release in thalamic axons prevents the $RELN^+SST^-$ neurogliaform cells from developing normal morphologies. However, the equivalent interference with cortical excitatory input has no obvious effect on the maturation of these same cells[81]. Similarly, experiments examining PV^+ basket cells illustrate a developmental dependence on thalamic and sensory input for synaptic development. Whisker removal up until the second postnatal week or tetrodotoxin injection into an eye reduces the number of perisomatic 'basket' synaptic structures, and the physiological properties of their inhibitory currents resemble more those of immature PV^+ cells within the somatosensory cortex[82] or visual cortex, respectively[77]. Therefore, glutamatergic thalamic afferents have powerful and selective effects on the morphology and synaptic development of at least some cortical interneuron subtypes. How then do these activities mechanistically influence cortical interneuron differentiation?

NMDA receptor-mediated transmission. In line with the selective effect of thalamic activity outlined above, optogenetic stimulation of early postnatal thalamic afferents onto $RELN^+SST^-$ cells indicated a preferential contribution of NMDA receptor (NMDAR)-mediated currents to differentiation[81] (FIG. 4). Indeed, conditional knock out of *Nr1* (which encodes an obligate subunit of the NMDAR) or *Nr2b* (which encodes a subunit that permits calcium passage through receptor pore) within $RELN^+SST^-$ cortical interneurons phenocopies the abnormalities in morphology that are observed upon blockade of thalamic afferents[81]. NMDARs have long

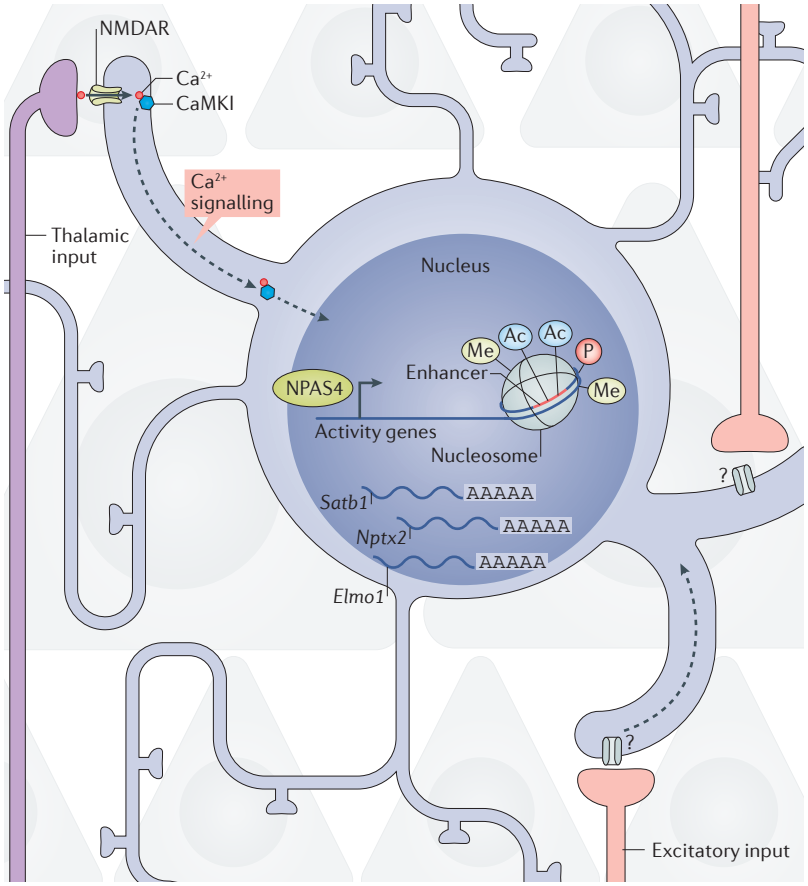

Figure 4 | **Activity-dependent mechanisms in developing cortical interneurons.** Specialized NMDA-containing synapses can support the proper morphological and synaptic development of select interneurons. The schematic illustrates some of the pathways by which NMDA receptor (NMDAR) and other unknown receptor signalling might be linked to cell subtype-specific gene expression. NMDAR activation followed by an influx of Ca^{2+} into the dendrite may elicit Ca^{2+} signalling (potentially through calcium/calmodulin-dependent protein kinase type I (CaMKI)) to the nucleus. In turn, upon reaching the nucleus, this may activate changes in epigenetic signatures (such as histone and enhancer modification) and gene expression (affecting genes such as neuronal PAS domain-containing protein 4 (*Npas4*), *Satb1*, neuronal pentraxin 2 (*Nptx2*) and engulfment and cell motility protein 1 (*Elmo1*)) that have been linked to morphological and synaptic development. As the field continues to expand, it is expected that new and distinct mechanisms will emerge beyond the few listed here. Ac, acetylation; Me, methylation; P, phosphorylation.

been central to activity-dependent processes and classic Hebbian connectivity, as their activation requires both presynaptic glutamate and postsynaptic depolarization. Consequently, their variable expression during development and within select synapses presents a viable mechanism by which NMDARs may provide an instructive signal in specific cells or at specific synapses[83,84]. However, similar manipulations of NMDARs in pyramidal neuron populations had no obvious effect on synaptic development[85] or connectivity[86], suggesting that the situation in $RELN^+SST^-$ interneurons may prove unique.

Immediate early genes
(IEGs). A set of genes, the expression of which is induced within minutes to hours following neuronal depolarization.

Induction of calcium signalling. It is intriguing to hypothesize that specific afferent connections could be privileged such that their activation results in distinctive and salient signalling to the nucleus. On the basis of work in other cell types, it seems probable that calcium entry represents a central conduit through which such activity is translated into discrete intracellular signals to elicit differential gene expression within interneurons (for reviews, see REFS 87–98). The calcium permeability of NMDARs represents only one of the many routes through which the levels of intracellular calcium can be altered by activity. The most touted alternative is the voltage-gated L-type calcium channel, which has been implicated by recent work in mature excitatory neurons as being central in the coupling of activity to transcription through activation of calcium/calmodulin-dependent protein kinase type II (CaMKII)[90]. Other possible routes by which calcium levels could be altered by activity include calcium-permeable homomeric AMPA receptors and second messenger-mediated release from intracellular stores. Certainly, much remains to be learned about the precise mechanisms by which depolarization leads to increased levels of intracellular calcium within developing interneurons.

Equally poorly understood are the diverse second messengers (including the CaMK family, calmodulin, calcineurin and the mitogen-activated protein kinase (MAPK) family) that act as the effectors of calcium signalling in the nucleus. Given the lack of CaMKII and, according to one report, of calcineurin in interneurons[91,92], the precise mechanism by which calcium signals reach the nucleus in developing interneurons remains an open question. Recent work implicates CaMKI as being capable of acting as a substitute for CaMKII by acting as a calmodulin shuttle for calcium into the nucleus of interneurons[93] (FIG. 4). Regardless, given the wealth of potential second messenger pathways that could be differentially engaged, the route of calcium entry is likely to result in marked differences in genetic changes within the recipient interneuron[94,95]. To this end, recent work has begun to explore activity-mediated gene responses in interneurons.

Activity-regulated transcription. Upon receipt of an excitatory stimulus, developing cortical interneurons and excitatory neurons activate similar immediate early response transcription factors (also known as immediate early genes (IEGs)), including *Fos*, early growth response protein 1 (*Egr1*), and neuronal PAS domain-containing protein 4 (*Npas4*)[96]. However, it seems that the late response genes (LRGs) that act downstream of these IEGs differ. Using a sensory deprivation paradigm in which animals were reared in darkness and then acutely exposed to light, it was found that discrete sets of LRGs are selectively induced within immature excitatory, SST^+, VIP^+ and PV^+ neurons within the cortex and differentially affect synaptic development[96,97]. For example, insulin-like growth factor 1 (*Igf1*) is strongly upregulated selectively within VIP^+ cortical interneurons and acts cell autonomously to increase inhibitory synapses upon VIP^+ cells[97]. Moreover, some IEGs, such as *Npas4*, induce LRGs that have opposing actions on synaptic development within cortical interneurons versus excitatory neurons. Excitatory cells induce an *Npas4*-dependent set of genes (including brain-derived neurotrophic factor (*Bdnf*)) that increase the number

of afferent inhibitory synapses upon them. By contrast, *Npas4* induction within SST+ cortical interneurons provokes a set of genes (including potassium voltage-gated channel subfamily A member 1 (*Kcna1*) and FERM and PDZ domain-containing protein 3 (*Frmpd3*)) that produce an increase in the density of the excitatory synapses that innervate them[96] (FIG. 4).

How then do single transcription factors (the IEGs) activate distinct gene programmes in different cell types? The answer is likely to lie in differences in both their transcriptional profiles and chromatin conformations. Indeed, neuronal activity induces pronounced changes in the activation and repression marks across the genome[96,98,99], which have significant effects on the transcriptional and post-transcriptional processes within the nucleus. For instance, NPAS4 differentially targets discrete genes in excitatory versus inhibitory cells. This is achieved through the association of NPAS4 with distinct genetic elements in each cell type, as indicated by comparing the variations of active chromatin marks that are induced in these cell types[96]. Within excitatory neurons, membrane depolarization induces the accumulation of activation marks (such as acetylation of histone 3 lysine 27 (H3K27ac)) at *cis*-elements associated with *Bdnf,* whereas, in inhibitory neurons, gene regulatory elements that are associated with RAS-related and oestrogen-regulated growth inhibitor (*Rerg*) become preferentially activated[96]. Therefore, variations in the accessibility of specific enhancers and/or promoters could allow a single transcription factor to stimulate expression of distinct genes within excitatory versus inhibitory cells.

Of note, a growing list of activity-mediated genes have been identified, including the transcription factors *Dlx1* and *Satb1,* engulfment and cell motility protein 1 (*Elmo1*), neuronal pentraxin 2 (*Nptx2*) and *Igf1,* and seem to be recruited within particular interneuron subtypes at specific developmental stages (TABLE 1). For instance, *Dlx1* and two downstream genes regulated by its expression[100], *Npas1* and *Elmo1,* are activity regulated and involved in cortical interneuron migration[50]. Indeed, the migratory defect in CR+ and RELN+SST− cortical interneurons that is caused by attenuation of activity could be rescued by overexpression of *Elmo1* (REF. 50). Likewise, preliminary findings suggest that a later step in cortical interneuron development, the morphological and synaptic maturation of SST+ interneurons, depends on the expression of *Satb1* (REF. 101) (B.W. and G.F., unpublished observations). Overexpression of *Satb1* in SST+ progenitors results in the precocious adoption of a mature morphology by these cells[46]. Furthermore, as discussed above, the differential LRGs deployed in inhibitory neurons (such as *Nptx2* within SST+ interneurons and *Igf1* within VIP+ interneurons versus *Bdnf* induction within excitatory neurons) specifically target synaptic development such that circuit homeostasis is maintained following excitatory stimulation[96,97]. Lastly, recent evidence has implicated the activity-dependent expression of the transcriptional activator ETS translocation variant 1 (*Etv1*; also known as *Er81*) in the modulation of the physiological properties of PV+ fast-spiking cortical interneurons[52]. Taken together, activity seems to act to selectively induce the expression of genes that modulate cortical interneuron character during different developmental stages. Although these studies have contributed to our understanding of how molecular machinery and activity cooperate in the maturation of specific cortical interneuron properties, they have yet to indicate that activity directly instructs the specification of cortical interneuron identity.

Discussion

The tight correlation between birthdate and cell type has been used to argue for early specification of cortical interneuron identity (the progenitor specification model), whereas the delayed acquisition of subtype character is compatible with a late procurement of definitive cortical interneuron identity (the progressive specification model). Although these two models of specification provide convenient opposing hypotheses to explain when cortical interneuron diversity is established, the relative contributions of each of their mechanisms remain unclear. Realistically, a sharp separation of the intrinsic genetic and environmental cues that shape interneuron subtype selection is artificial, and there is clear evidence that both interact along the entire development axis in the production of particular cortical interneuron subtypes. However, the relative contributions of developmental predisposition and environmental specialization will require considerable effort to be sorted out. Performing longitudinal profiles of gene expression within specific interneuron subtypes of the same class across development, coupled with loss-of-function experiments of intrinsic determinants and/or qualitative environmental cues, should aid considerably our understanding of how discrete subtype characters emerge.

Looking forward, we have an unparalleled access to molecular techniques that may afford a deeper understanding of the relationship between cortical interneuron development and diversity. Indeed, recent progress in single-cell sequencing has provided a basis to redefine subtypes of cortical interneurons that are matched to their genetic state[102,103]. One can imagine that using this technique across developmental stages, coupled with systematic genetic knockout models, will provide a finer-grained view of how molecular signatures (that is, gene networks) translate functionally into the specification of specific subtypes.

However, a true understanding of cortical interneuron diversity requires a combination of analyses at both a genetic and an epigenetic level. The time has come to look beyond transcription and to incorporate richer genetic regulation using a multitude of approaches including querying chromatin structure and activation, alternative splicing and RNA regulation, and translational controls. For instance, our inability to identify specific transcription factor cascades that are restricted to distinct interneuron subtypes could indicate that the genetic and functional complexity of cortical interneurons may be defined by their underlying genomic and epigenetic diversity. In adulthood, it has been shown that SST+, PV+, VIP+ and excitatory cells contain thousands of distinct sites at which their chromatin structure and DNA methylation

signatures differ within gene regulatory regions[104]. Do these differences relate to how cortical interneuron diversity and function are established during development? Second, another mechanism by which neurons considerably expand their transcriptional profile throughout development is alternative splicing. Alternative splicing can change the expression levels of a given mRNA transcript and alter protein–protein interactions and/or the localization of their protein products. Several alternative splicing factors (including polypyrimidine tract-binding protein 1 (PTBP1), PTBP2, RNA-binding protein FOX1 homologue 1 (RBFOX1), RBFOX2, and the RNA-binding proteins NOVA1 and NOVA2) coordinate this cellular diversification at progenitor and postmitotic stages through modulation of their genetic programme and induction via activity-dependent mechanisms[105]. Although some broad differences in RNA spliced transcripts have been characterized, the field is just beginning to map alternative transcript profiles back to particular splicing factors and, further, to characterize these profiles in individual neuronal subtypes as oppose to whole tissues. The rapid expansion of these investigations and the improvement of single-cell RNA sequencing to gain access to individual-cell RNA splice profiles will probably lead to a new appreciation of the genetic variations underlying the diversification of neurons. Understanding these processes more thoroughly not only promises to provide us with a more complete picture of how interneuron fate is specified but is also likely to clarify how interneurons maintain such exquisitely specific roles in the mature cortex.

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Acknowledgements
The authors thank X. Jaglin, T. Petros and the remaining members of the Fishell laboratory for their support, thoughtful discussions and critical reading on the manuscript. The authors apologize to colleagues whose research was not cited owing to the broad scope and space limitations of this Review. The Fishell laboratory is supported by grants from the US National Institutes of Health (NIH) (5R01NS081287, 5P01NS074972) and the Simons Foundation (274578, 383356). B.W. was partially supported by the NIH training program at New York University (NYU) for Molecular, Cellular, and Translational Neuroscience training grant (5T32NS086750).

Competing interests statement
The authors declare no competing interests.