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Review article

Time Windows of Interneuron Development: Implications to Our Understanding of the Aetiology and Treatment of Schizophrenia

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Abstract: Schizophrenia is a devastating neuropsychiatric disorder widely believed to arise from defects during brain development. Indeed, dysfunction in the formation and function of GABAergic cortical interneurons has been implicated as a central pathogenic mechanism in this, and other, neurodevelopmental disorders. Understanding the coordination and timing of interneuron development including the complex processes of specification, proliferation, migration and their incorporation into finely tuned cortical networks is therefore essential in determining their role in neurodevelopmental disease. Studies using mouse models have highlighted the functional relevance of transcription factor networks and common signalling pathways in interneuron development but have faced challenges in identifying clear time windows where these factors are essential. Here we discuss recent developments highlighting critical time frames in the specification and migration of cortical interneurons and the impact of aberrant development to aetiology and treatments of schizophrenia.

Keywords: interneurons; schizophrenia; neurodevelopment; cortex; parvalbumin; Shh; Nkx2.1.

1. Introduction

The cerebral cortex is primarily comprised of two distinct neuronal populations namely excitatory neurons and GABAergic inhibitory interneurons which constitute approximately 80% and 20% of the

neuronal population, respectively [1,2]. A fine balance between these two neuronal types is crucial for proper function of the complex brain networks. Dysfunction in GABAergic cortical interneurons has been implicated in several developmental disorders including schizophrenia, epilepsy, autism spectrum disorder and Down syndrome [3–5]. Now widely regarded as one of the main pathologies associated with schizophrenia, interneurons were brought to the forefront of research when post mortem schizophrenic brains were found to have decreased levels of GABA [6]. As discussed further below, numerous findings since then have also highlighted a subtype specific reduction in interneurons in the post mortem schizophrenic brain [7] and underpin the GABAergic/interneuron hypothesis of disease progression [8,9].

The remarkable diversity of interneurons accounts for the complexity of the inhibition required for correct brain function; however, this complexity also makes the study of interneurons difficult and leaves many unanswered questions in the field. Interneurons differ from other locally derived cortical excitatory neurons in that they migrate large distances from their place of origin in the subpallium. To reach their target sites interneurons first undertake tangential migration into the neocortex and then switch to a radial migration phase to find their correct neocortical layer. In contrast, other neuronal cell types will undergo only a comparatively short migration along radial glial scaffolds to their target neocortical layer. This intricate migration combined with a complex specification process opens many avenues through which deficits can disrupt their development. The major hypothesis in schizophrenia, to date, suggests that its underlying pathology arises from defects during neuronal development (Reviewed in [10]). Indeed, more and more research implicates early developmental insult with irreversible dysfunction in adulthood as the likely mode of pathogenesis. If we are to understand the origins and identify rational treatment regimes for this disorder it is therefore essential to identify critical periods during embryonic and postnatal interneuron development.

There are a wealth of studies addressing the specification and subsequent expression profiles of cortical interneurons as they migrate to the cortex, however, the field has lacked a clear understanding of the consequences of dysfunction at early development stages and their implications in the disease process. Understanding the coordination of interneuron specification, proliferation, migration, integration and specialisation is essential to determine their role in neurodevelopmental disease and provide insight toward novel therapeutic approaches. To date, studies have used mouse models to investigate the functional relevance of transcription factors and common signalling pathways in interneuron development but have not defined clear time windows where these molecules are critical. Here, we discuss recent developments in the study of the specification and migration of cortical interneurons and the impact of aberrant function during early developmental processes toward neuropsychiatric outcomes.

2. Classification of Cortical Interneurons

Different interneuron subpopulations show extraordinary diversity with distinct morphological, physiological and transcriptional profiles (see Figure 1) [11–13]. After initially being described over 100 years ago by Ramon y Cajal, interneurons were distinguished based on their morphology due to

their short axons compared to excitatory neurons with little regard for their physiological properties [14]. There exists a large amount of inconsistency in classifying interneurons with some researchers referring to interneuron subclass based on morphology and cell type and others referring to their expression of marker proteins. Though their classification has been far from consistent over the last few decades, interneurons were traditionally sub-divided into three largely non overlapping groups based on their expression of calcium binding proteins Parvalbumin (PV), Calretinin (CR) and the neuropeptide Somatostatin (SST) [12,14,15]. Recently, however, this classification standard has changed with Lee and colleagues (2010) reporting that serotonin 5-hydroxytryptamine 3A receptor (5HT3aR) is expressed in the vast majority of interneurons that do not express PV or SST, therefore defining three major subtypes [16]. Each interneuron subtype has a unique function in controlling inhibition and excitation which is dependent on the position of the interneuron within the cortical circuit and its chemical profile. To control both inhibition and excitation of cortical networks interneurons regulate projection neurons in addition to other interneurons. It is important to consider that interneurons in each subtype have different morphology, intrinsic properties and can be further sub-divided into different classifications [17]. However, despite this, interneurons expressing similar markers will often have similar functions, origins and positions in the brain. PV expressing interneurons are mostly characterized as fast-spiking neurons essential for gamma oscillations, while SST+ interneurons have been established as low-threshold interneurons with SST+ Martinotti cells reported to fire at theta frequency [18], capable of sending low-threshold calcium spikes. Fast spiking interneurons have a unique function in feed forward inhibition in the brain due to their early response and hence expand the dynamic range of interneuron activity [19]. SST expressing interneurons in the fourth layer of the cortex act specifically in a disinhibiting context [20], this is contrasted by SST interneurons in layers 2/3 of the cortex whose primary function is to inhibit pyramidal neurons. Takada *et al*. 2014 reported that alterations to the distribution and/or subtype of interneurons leads to disorganized oscillation and lack of control [21]. Furthermore, many studies have suggested that the specific loss of PV interneurons without alterations to the total number of interneurons per se may be a primary factor in schizophrenia [7,22,23].

These largely non-overlapping subtypes can be further sub-divided on the basis of expressing additional interneuron markers with each having distinctive functional roles. Amongst these sub-divisions, the 5HT3aR expressing interneurons are particularly heterogeneous. For example, vasoactive intestinal polypeptide (VIP) is co-expressed in 40% of interneurons that express 5HT3aR with their primary function being the inhibition of other interneurons [17,24]. Reelin (RELN) is also co-expressed with the majority of 5HT3aR interneurons that do not express VIP. These RELN positive cells make up the majority of neuroglia interneurons in cortical layer I [17]. Unlike VIP and RELN, neuropeptide Y (NPY) is not only expressed in 5HT3aR but also in other interneuron types and is responsible for the slow GABAergic inhibition of pyramidal cells [24].

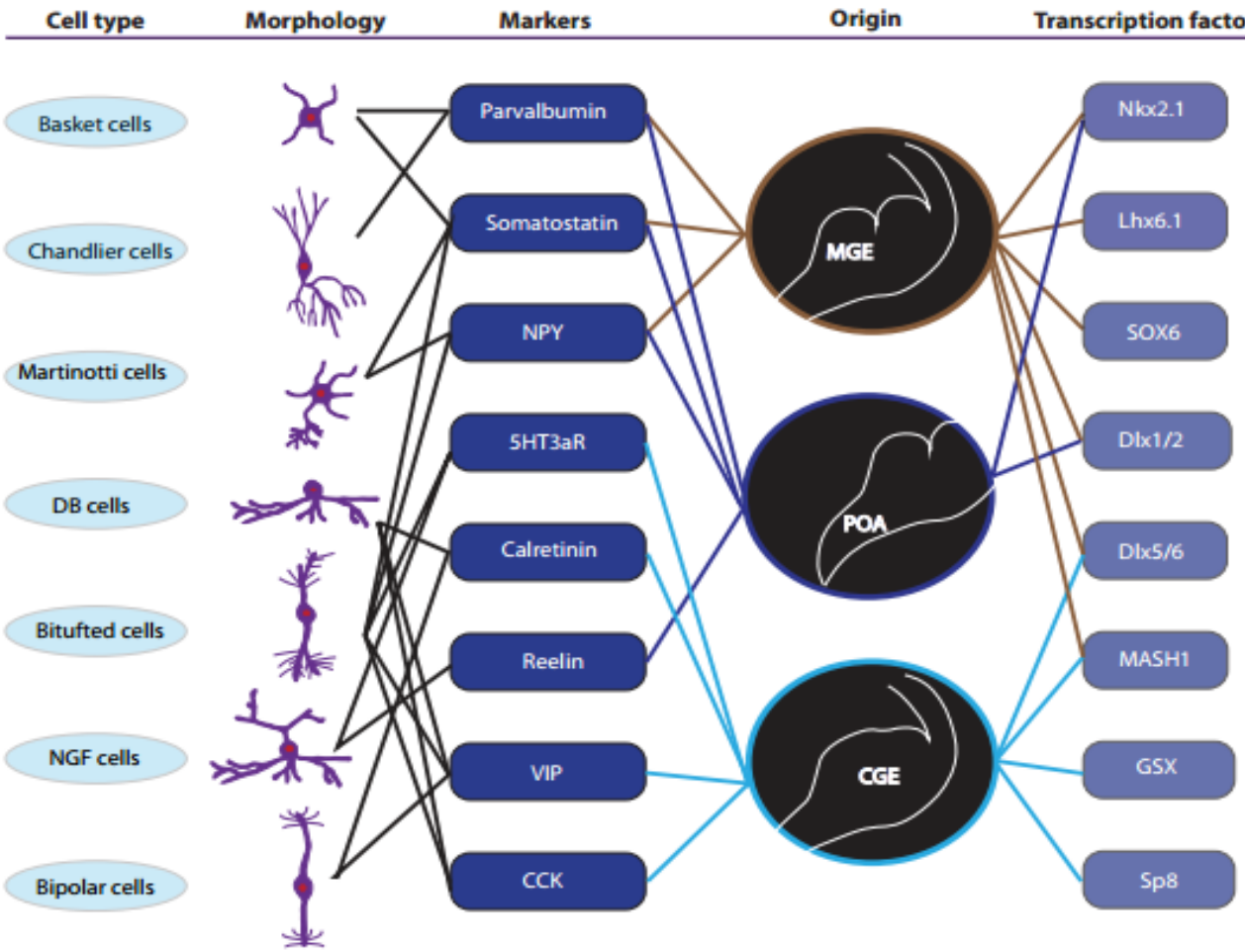

Figure 1. Schematic illustration of the complex identity of interneurons. Cortical interneuron function can be classified based on their cell type, morphology, marker expression, origin and transcriptional profile. DB, Double bouquet; NGF, Neurogliaform; NPY, Neuropeptide Y; VIP, Vasointestinal peptide; CCK, Cholecystokinin; 5HT3aR, 5-hydroxytryptamine 3a receptor.

3. Interneurons in other Regions of the Brain

Interneurons are very diverse with populations found in all areas throughout the central nervous system, including the cortex, striatum, hippocampus, thalamus, cerebellum, hypothalamus, olfactory bulb and spinal cord. Interneurons in different areas have vastly different characteristics reflective of the area in which they are located. This is due to the neurochemical environment of each area of the brain as well as the function of the interneuron within the region. When studying the role of interneurons it is important to consider that within the different regions of the brain interneurons will arise from different proliferative zones and ultimately develop through different signalling and transcriptional mechanisms. Interneurons often have different morphologies between regions of the brain: interneurons of the striatum often appear projection-like which accounts for their need to

function over large distances while cortical interneurons have short axon morphology and function within local circuits. Furthermore, the interneuron marker expressed by certain cell types is also dependent on the area of the brain. For example, in the hippocampus SST is expressed in Oriens Lacunosum-Moleculare cells however in cortical interneurons SST is predominately expressed in Martinotti cells.

4. Generation of Cortical Interneurons

Cortical interneurons are born in the ganglionic eminences and the Preoptic Area (PoA) in the ventral telencephalon of the developing rodent brain. These ganglionic eminences can be subdivided into various regions depending on their physical location, including the Lateral Ganglionic Eminence (LGE), Medial Ganglionic Eminence (MGE) and Caudal Ganglionic Eminence (CGE) (See Figure 2). The area in which an interneuron is born plays an important role in dictating the fate and function of that interneuron. For example, an interneuron born in the MGE will incur an MGE-like fate and likely give rise to a PV or SST phenotype. Interneurons born in the CGE will likely express 5HT3aR and have a different transcriptional profile than MGE derived interneurons. Though the LGE has been associated with some interneuron populations (i.e. olfactory bulb and striatal interneurons), it has not been found to produce any cortical interneuron populations in mice. Indeed, most fate mapping studies in mice suggest that the vast majority (approximately 70%) of cortical interneurons derived from the MGE [25].

The Sonic Hedgehog (Shh) signalling pathway has been identified as a key factor in interneuron specification and development. In the rodent brain, the morphogen Shh is secreted from the floor plate of the neural tube along the entire rostral/caudal axis between embryonic day E8.5–E9.0. This initial expression later induces *Shh* expression in the PoA region and mantle zone of the MGE, where post-mitotic MGE neurons are located [26–28]. Though reduced, *Shh* will continue to be expressed throughout development and in some cell types even into adulthood [27]. The most well characterized Shh signalling pathway is mediated via a Gli dependent mechanism in which Shh binds to the inhibitory cell surface receptor Patched to release another cell surface receptor Smoothened from a state of inhibition. Disinhibition of Smoothened then leads to activation of the Gli transcription factors that relocate to the nucleus. Within the MGE, activation of Gli family proteins leads to the up regulation and maintenance of the early NK homeobox transcription factor *Nkx2.1*, as well as providing positive feedback to maintain expression of *Shh* itself [27,29].

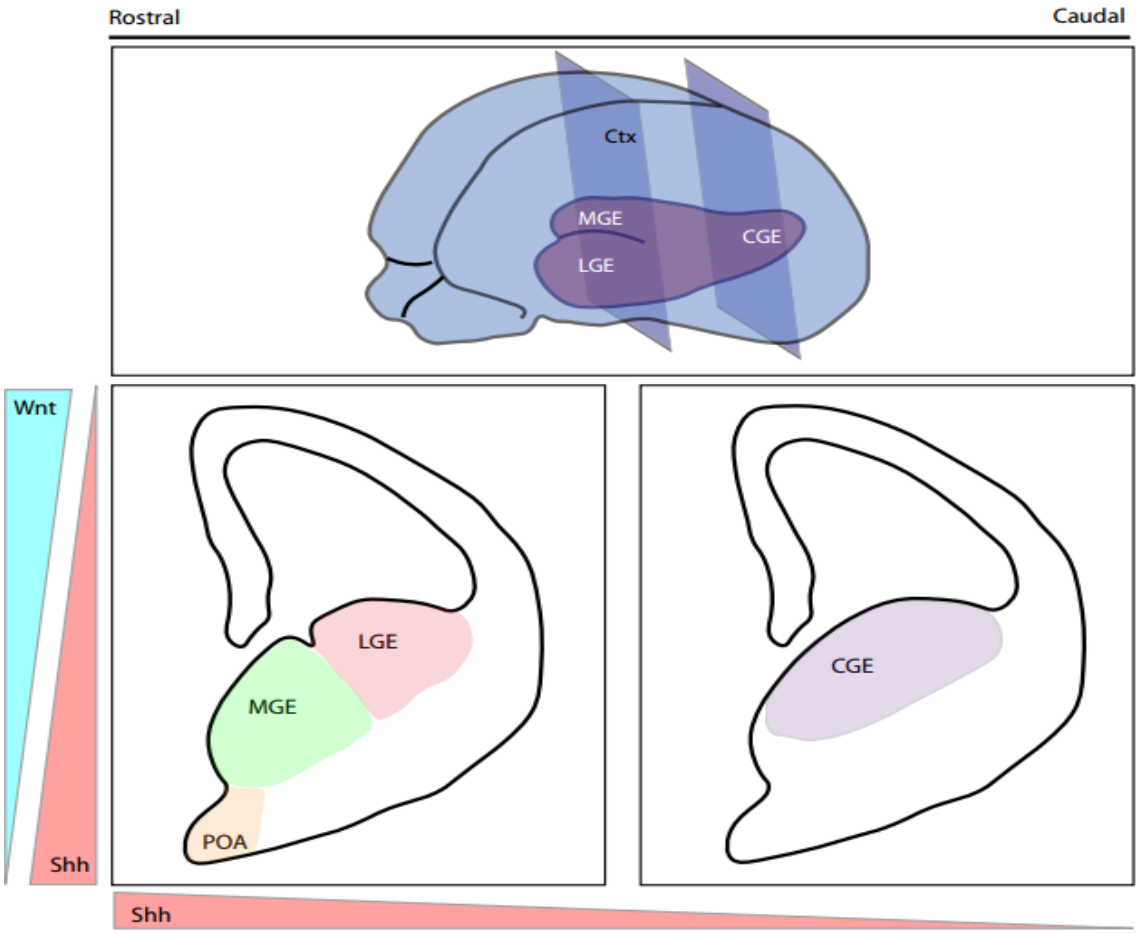

Figure 2. Schematic illustration of the Ventral Telencephalon region in the developing mouse brain. Interneurons are born in the ganglionic eminences in the ventral telencephalon of the developing rodent brain, specifically the medial (MGE), lateral (LGE) and Caudal (CGE), and to a lesser extent the Preoptic Area (PoA). Shh and Wnt signalling are important pathways in the development of interneurons and are expressed in high to low gradients throughout the developing brain. This gradient is important in establishing different classes of interneurons from different ganglionic regions.

5. Sonic Hedgehog Signalling Induces Cortical Interneuron Formation

Despite Shh signalling being generally well characterized elsewhere during embryogenesis, its expression and function within the ganglionic eminences is complicated, and to date, incompletely understood. Shh signalling has a critical role in the initial patterning of the ganglionic eminences, as highlighted in the *FoxG1-Cre:Smoothened*$^{fl/fl}$ line where Smoothened is deficient from the telencephalic neural precursor population from E9.5 [30]. In these mice the ganglionic eminences fail to undergo initial expansion and patterning, emphasizing a critical role of Shh in the development of all cortical interneurons. A reduction in *Shh* after the formation of the ganglionic eminences at E12.5

has also been documented to lead to a reduction in the SST+ and PV+ expressing interneurons [31–33]. However, in this case the altered interneuron landscape occurs without affecting the total number of interneurons in the cell cycle (S-phase), and with only minimal effect on CGE derived interneurons [33]. These findings correspond well with the expression gradient of *Shh* along the rostral/caudal axis and within the PoA and MGE. Interestingly, Xu *et al*. 2010 showed that overexpression of *Shh* in the MGE resulted in low-threshold SST subtype interneurons being favoured at the expense of fast spiking PV+ cells [34]. This is fitting with the data since the SST population of interneurons predominantly arise from cells located more rostrally than those giving rise to PV+ interneurons. These findings suggest that although some cell types are favoured with either an increase or decrease of *Shh* expression, that Shh signalling is crucial for all interneuron subtypes and that a delicate balance of Shh activity is essential for proper interneuron development and cell identity. This data therefore identifies 3 core roles for Shh in interneuron development: (1) contribution to initial patterning and specification of the ganglionic eminences in early development (E9–E12.5), (2) expansion of progenitors in the ventral telencephalon (E12.5), and (3) maintaining expression of *Nkx2.1* within the MGE (E12.5 onwards) [34].

At the earliest stages of interneuron specification, Shh initiates a signalling cascade that leads to the up regulation of *Gli1* and *Gli3* transcripts, which further lead to expression of the transcription factor *Nkx2.1* within the MGE and PoA. The role of Nkx2.1 in the MGE has been previously well described [35], here we will briefly discuss more recent findings. The expression of *Nkx2.1* is crucial at an early time point to generate a downstream transcriptional response which leads to the specification of different interneuron subclasses. As the loss of *Nkx2.1* results in no changes to CGE derived interneurons this further suggests that *Nkx2.1* is preferentially required by the MGE and the PoA [33]. *Sox6* and *Lhx6* act downstream of *Nkx2.1* and have a key role in the differentiation and migration of MGE derived interneurons. In *Sox6* knockout mice MGE derived neurons are mispositioned and fail to express PV and SST correctly [36]. Interestingly, while this altered expression profile and mispositioning phenotype is also observed in *Lhx6* knockout mice, the MGE derived cells are pushed toward a CGE like cell fate in these mice. Given the dual defects on interneuron positioning and specification, this raises the question of whether the role of specification has downstream effects on migration or whether *Lhx6* acts upon multiple developmental processes independently. After initially deriving from the floor plate and the PoA, *Shh* expression is later driven in the MGE by *Nkx2.1* and the *Lhx* transcription factor family via a positive feedback loop. While *Lhx6* and its homolog *Lhx8* have largely redundant functions in regulating interneuron development, when removed together, *Lhx6;Lhx8* double knockout mice lack *Shh* expression in early MGE derived neurons (E11.5) [27].

Despite its initial role acting under the Gli-dependent pathway, Shh has also been found to have Gli-independent actions in maintaining *Nkx2.1* expression at later developmental stages (i.e. after E12.5). Gulacsi and Anderson (2006) found that removal of *Shh* in a *Gli3* null background was necessary to inhibit *Nkx2.1* expression. While the initial patterning of the MGE relies on the Gli

dependent pathway, progenitor expansion and patterning maintenance also require a Gli independent pathway [35]. Furthermore, Shh's role in maintaining *Nkx2.1* expression in the absence of Gli may be indirect. Gulacsi & Anderson (2006) put forward the hypothesis that Shh may regulate *Nkx2.1* in the absence of *Gli* by repressing the inhibitory effect of Wnt or BMP. To date there is little research looking into the effects of Shh in maintaining *Nkx.2.1* in a Gli independent manner. It will be important for the field to determine the significance of this interaction and how this could be potentially impacting on neurodevelopmental disorders.

Despite numerous advances in our understanding of the complex relationship between transcription factor networks and interneuron fate, the transcription profiles of interneuron subtypes and the consequences of abnormal specification are poorly understood. Unfortunately due to the redundant nature of these transcription factors and lack of knowledge of their roles at variable time points of interneuron development it is difficult to gauge the true effect of an abnormal transcription profile in the formation of physiological traits associated with schizophrenia.

6. Additional Signalling Pathways Involved in Cortical Interneuron Development

Although Shh arguably plays the most important role in patterning the ganglionic eminences, the Wnt, FGF and BMP signalling pathways have also been implicated in coordinating cortical interneuron development. In many cases these signalling pathways interact in a reciprocal manner, with the activation of one pathway inhibiting the activity of the other [37]. For example, the bone morphogenetic protein (BMP) family represses the expression of Shh via a negative feedback loop in tissues outside the brain [38] and represses Shh induced interneuron production in cortical explants [39]. BMPs also play essential roles in refining the expression patterns of *Shh* within the ventral midline prior to induction of *Nkx2.1* in the ganglionic eminences [40]. After initial specification within the MGE interneurons migrate into the cortical layers. Upon reaching the cortex BMPs also play important roles in interneuron subtype specification [41–43]. Indeed, while mice lacking the BMP receptors *BMPR1a* and *BMPR1b* have a similar degree of GABAergic neurons overall, they have reduced PV+ expressing interneurons compared to controls.

Fibroblast growth factor (FGF) signalling is also essential for expansion and patterning of the MGE, with most evidence suggesting that FGFs act as effectors of Shh signalling. In the absence of FGF receptors (i.e. *Fgfr1; Fgfr2* double knockout mice) the MGE fails to express *Nkx2.1* albeit that *Shh* and *Gli1* expression is normal [44]. This morphogenic cascade is further supported by the finding that *Fgf8* expression is dysregulated in both *Gli3* and *Shh* knockout mice [38,45].

Wnt signalling pathways are conventionally categorized as either canonical or non-canonical depending on whether β-catenin is the primary target of the cascade. In canonical Wnt signalling, activation of Wnt ligands stabilizes cytoplasmic β-catenin which would otherwise be degraded by GSK3β. This stabilization leads to a build-up of β-catenin in the cytoplasm and initiates its transportation to the nucleus where it interacts with transcriptional co-regulators to promote target

gene expression. Wnt signalling has well defined roles in promoting neuronal identity, proliferation and migration [46–49] and has been shown to negatively regulate cell fate and neuronal identity in the subpallium [50]. More recently, canonical Wnt signalling has been shown to directly mediate interneuron cell cycle progression. Using *Nkx2.1Cre;* β-*cat*$^{fl/fl}$ mice and a range of *ex vivo* assays in which the Wnt signalling pathway has been modulated, Gulacsi & Anderson (2008) found that loss of canonical Wnt signalling lead to proliferation defects within the MGE [51]. The Wnt/β-catenin pathway has also been shown to promote expression of the transcription factor *Sox5* to control interneuron proliferation within the spinal cord [52]. However, whether this molecular pathway is also at play in the subpallium remains to be explored. Recent findings in the olfactory bulb have also shown that non-canonical Wnt signalling is important for interneuron development in other regions of the brain. Pino *et al*. (2011) identified a role for Wnt5a (associated with non-canonical Wnt signalling) but not Wnt3a (associated with canonical Wnt signalling) in neurite formation and morphology of olfactory bulb interneurons *in vitro* [53]. While the role for non-canonical Wnt signalling in cortical interneuron development remains undefined the field would benefit immensely from detailed analysis of receptors specific to this pathway. Indeed, modulation of receptors specific to the canonical pathway would also be helpful in determining which defects arising from β-catenin deficiency are specific to Wnt signalling.

Though the signalling pathways described above are known to interact with one another, the exact mechanisms and significance of these interactions are incompletely understood. To date, most investigations have studied these pathways independently, which may give an incomplete understanding of how these complex signalling pathways interact.

7. Tangential Migration of Cortical Interneurons

Interneurons begin migration from the ganglionic eminences at approximately E11.5 in response to chemoattractant and chemorepellent cues from the cortex and striatum. This migration will continue throughout embryonic development until the formation of new neurons within the ganglionic eminences has ceased. Unlike other neuronal cell types, interneurons exit the cell cycle before migration, with *Lhx6* being expressed after undergoing cell cycle exit. Failure to exit the cell cycle before migration results in aberrant migration and ectopic positioning of postnatal interneurons within the cortex. For example, Vidaki and colleagues (2012) showed that a reduction of *Rac-1* in progenitor interneurons perturbed G1 cell-cycle progression thus resulting in a subsequent loss of MGE derived interneurons in the cortex [54]. However, reduction of *Rac-1* after the onset of interneuron migration had no effect on the cell cycle and showed little to no effect on the interneuron landscape in older mice.

To reach their appropriate position within the cortex interneurons must first navigate away from the ganglionic eminences and into the dorsal pallium. Invasion of the cortex is highly ordered and occurs through two major tangential migration pathways. The first neurons to exit the ganglionic

eminences are primarily biased toward PV+ fate and migrate around the striatum and into the Marginal Zone (MZ). In contrast, the neurons born at later time-points consist of a mixed population of interneurons that migrate through the subventricular zone (SVZ) (see Figure 3A). The first stage of migration out of the ganglionic eminences is primarily controlled by chemorepulsion signals that were originally described as axonal guidance cues. The class 3-semaphorins (Sema3A and Sema3F) are expressed by the striatum and interact with the cell surface neuropilin receptors (NRP1 and NRP2) which are expressed on migrating interneurons. Due to the presence of chemorepulsive signals interneurons expressing the neuropilin receptors migrate around the striatum in a distinctive path. Indeed, deficiency of the neuropilin receptors leads to aberrant interneuron migration with an increase number of interneurons localising to the striatum [55]. Notably, the interneurons that target the striatum during normal developmental contexts lack expression of the neuropilin receptors [56]. This semaphorin/neuropilin axis therefore represents part of the mechanism for sorting different classes of interneurons to their appropriate locations, similar to their roles in other neuronal types [57–59]. Hernández-Miranda *et al.* (2011) showed that the roundabout 1 receptor (Robo1) is also important for guiding newly formed interneurons out of the ganglionic eminences and along the tangential migration paths [60]. Robo1 is a transmembrane receptor that recognises secreted chemorepulsive signals from the Slit family. *Robo1* is expressed by migrating interneurons within the ganglionic eminences and the *Slit* ligands are expressed in complementary pattern within the ventricular zone of the cortex [61]. However, rather than controlling migration along the tangential paths in the cortex, the Slit/Robo signalling pathway also helps to keep interneurons away from the striatum [55,60]. The overlap in phenotypes of the Slit/Robo and semaphorin/neuropilin knockout mice may be partly explained by the finding that Robo1 can physically interact with neuropilin [60], perhaps suggesting that the two pathways functionally interact. The Eph/Ephrin signalling pathway also plays important roles in promoting the initial phases of interneuron migrations away from the ganglionic eminences. *EphrinB3* and *EphA4* are reciprocally expressed in MGE and POA derived interneurons and through forward/reverse signalling are involved in sorting these different classes toward different cortical migratory streams [62]. *EphrinA5* is also expressed in the ventricular zone of the MGE and interactions between EphrinA5 and EphA4R are required to push interneurons away from the ventricular zone [63]. The aristaless related homeobox transcription factor *ARX* has also been suggested to have an important role in promoting early interneuron migration. *ARX* knockout mice display an aberrant accumulation of interneurons in the proliferative zones of both the MGE and the CGE which results in the loss of cortical interneurons at later stages [64]. However, as interneuron progenitors fail to exit the cell cycle in *ARX* deficient mice, its role in migration as opposed to specification is unclear.

Cortical interneurons that navigate around the striatum to enter the pallium then migrate further dorsally under control of chemoattractive cues. The epidermal growth factor family member neuregulin-1 (*Nrg-1*) is expressed in the cortex and has been suggested to act as an attraction signal for interneurons expressing the tyrosine kinase family receptor *ErbB4* [65]. However, the exact role

of neuregulins in interneuron development is still open to debate. Contrary to the notion that neuregulins act as chemoattractive cues, data recently reported by Li *et al*. (2012) suggest that neuregulin may instead act to inhibit the tangential migration of interneurons [66]. In the latter example the authors found minimal overlap between interneurons and neuregulin expression domains in wild-type mice but found significant colocalisation of interneurons in these expression domains in *ErbB4* knockout mice. The chemokine receptors CXCR4 and CXCR7, which interact with the secreted ligand SDF-1, have also been found to have a role in tangential migration within the cortex [67]. Thus, in *CXCR4* and *CXCR7* knockout mice there is a 25% reduction in *Lhx6* expressing interneurons in the lateral regions of the neocortex [68].

8. Radial Migration and Laminar Distribution of the Interneurons in the Cortex

Once interneurons reach the developing neocortex, they change from long distance tangential migration to radial migration before settling in their final resting place where they integrate into local cortical circuits. Interneurons sort into deep and superficial layers in an inside out fashion with the first-born MGE derived interneurons occupying the deeper layers and later CGE derived interneurons occupying more superficial layers (see Figure 3B). There is a large amount of evidence to suggest that the final position of interneurons is dictated at their time of birth and that interneurons will ultimately localise to the same cortical layer as projection neurons born at the same time. While the mechanisms controlling this colocalisation remain unknown it has been suggested that interneuron radial migration may be arrested in response to interactions with projection neurons born on the same day in neurogenesis [69].

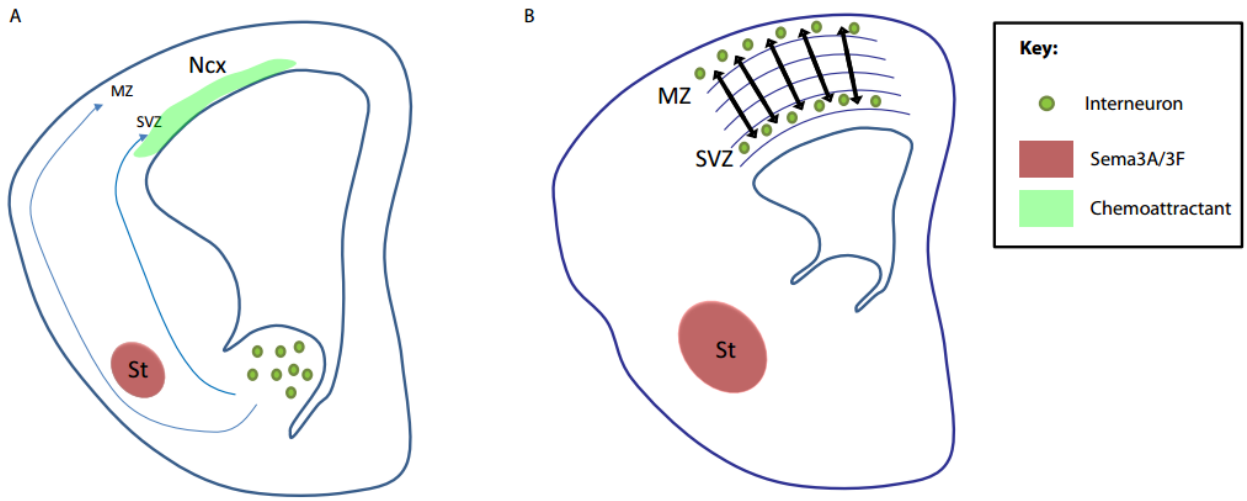

Figure 3. Migration of cortical interneurons. (A) Cortical interneurons born in the ganglionic eminences will migrate via two spatially distinct pathways to the neocortex. Early migrating cells will migrate from the MGE and into the marginal zone (MZ) and later migrating cells will

migrate via the subventricular zone (SVZ). Repulsive cues, specifically Sema3A and Sema3F, are expressed in the striatum to aid in directing cortical migration away from the striatum and towards the neocortex. Nrg-1 also acts as a morphogenic factor to direct migration to the neocortex. (B) Interneurons switch from tangential migration to radial migration once they reach the neocortex. Radial migration will allow interneurons to distribute throughout the cortex into their ultimate laminar position. Early migrating interneurons will occupy the deeper layers of the cortex whereas later migrating interneurons will occupy the more superficial layers.

Correct distribution of interneurons throughout the cortical layers is essential for their ultimate function and the formation of cortical microcircuits. In comparison to the well-described migration route of pyramidal neurons along radial glial scaffolds interneurons have been found to migrate independently of glia and in response to different molecular cues [70–72]. While SDF-1 is a positive regulator of tangential migration within the cortex, its down regulation may also play an important role in promoting the switch to radial interneuron migration at later stages of development. Thus, the inhibition of SDF-1/CXCR signalling in slice cultures after interneurons have already navigated into the cortex promoted a rapid switch from tangential to radial migration [73]. Defects in radial migration have also been shown in Lhx6 deficient mice. In these mice interneurons failed to occupy the middle neocortex, and instead occupy the very deep or superficial layers [74,75]. Interestingly, this deficiency was somewhat reversed by the addition of downstream proteins CXCR7 and ARX at E13.5, identifying this as a critical period for interneuron radial migration.

9. The GABAergic/Interneuron Hypothesis of Schizophrenia

Although the aetiology of schizophrenia is not well understood, there is general consensus within the field that disrupted connectivity is a central component in its pathology [76]. Recent studies have shown that neuronal migration, neurite formation and disruption of synapses are key influences in the formation of clinical symptoms [77,78]. These findings provide strong support to the notion that the pathophysiology may be due to underlying disturbances in brain formation. Indeed, further support of a neurodevelopmental origin has come from the absence of neurodegenerative defects in post-mortem schizophrenia brain samples [79].

The link between interneuron deficiency and schizophrenia was first established in the 1970's by Perry and colleagues [6] who reported a loss of the inhibitory neurotransmitter GABA in post mortem brain samples. In the 40 years since this first description, cortical interneuron defects have been consistently found in post-mortem tissue samples of schizophrenia patients. At the structural level, a common finding has been aberrant interneuron density and altered cellular distribution within multiple brain areas including the cingulate cortex [80], hippocampus [81,82], orbital cortex [83], prefrontal cortex [84–87], fusiform cortex [88] and planum temporale [89]. However, while altered positioning of neurons within the adult schizophrenic brain has been taken to reflect earlier defects in

neuronal migration, whether these anatomical defects arise from aberrant developmental processes has been hard to ascertain due to an inability to analyse brain structure in the prodromal stage of the disease.

Altered expression of genes involved in interneuron development and function are also correlated with clinical symptoms. Several molecules within the GABA signalling pathway including the enzymes responsible for synthesising GABA, GAD67 and GAD65 (encoded by the genes *GAD1* and *GAD2*, respectively*)*, and the GABA transporter, *GAT1*, are often deficient in schizophrenia brain samples [7,23,90,100]. The GABA receptor subunits *GABRA1-2, GABRA4-6,* and *GABRD* that are present on excitatory neurons and responsible for post synaptic neurotransmission are also reported to be aberrantly expressed in several schizophrenia studies [80,101–104]. Contrary to expectation, however, in some cases the expression of GABA receptors is increased, which may arise as a compensatory mechanism for reduced interneuron density or aberrant function [80,100,105–108]. An increase in expression has also been noted for *ErbB4* and its ligand *Nrg-1* [105–108]. Perhaps the strongest evidence of an interneuron deficiency playing a role in disease progression comes from deficient expression of interneuron markers such as *PV, SOM, NPY, CB, RELN* and *cholecystokinin* (*CCK)* [7,102–104,109–112]. Moreover, many of these studies also report a subtype specific reduction of interneuron markers in schizophrenia patients compared to controls [112].

At the genetic level, Straub and colleagues (2007) have shown that a single-nucleotide polymorphism (SNP) haplotype within the promoter region of *GAD1* correlates well with reduced gene expression in several schizophrenia-based families [113]. The down regulation of *GAD1* and the 5HT3aR interneuron marker *RELN* have also been shown to occur as a response to epigenetic changes in their promoter elements [114–119]. The *Nrg-1* locus has strong linkage with schizophrenia with various non-coding polymorphisms and haplotypes identified within and around the promoter region of the gene [106]. Although not discussed in detail here, brain derived neurotrophic factor (*BDNF*) plays important roles in interneuron development and establishing cortical circuits during adolescence [120,121]. Notably, a Val66Met coding variant in *BDNF* has been associated with psychiatric disorders and reduced cortical and hippocampal volumes in a number of studies [122,123]. Recent whole exome sequencing and Genome Wide Association Studies (GWAS) on large schizophrenia cohorts further strengthen the genetic link between interneuron deficiency and disease pathogenesis. For example, from the analysis of 623 schizophrenia trios Fromer *et al.* (2014) revealed missense mutations in several genes known to play critical roles in interneuron development, including *Lhx6, Gli2, Gli3 and ErbB2* [124]. Moreover, GWAS studies have found linkage to genes and molecular pathways involved in interneuron development, including *Nrg-1, ErbB4* and genes within the Wnt signalling pathway [125,126]. The overall outcome of these genome wide studies is the realisation that although schizophrenia has a large genetic component, no single gene defect can explain the origin of the disease. Rather, the most likely explanation of clinical onset is synergistic effects of genetic and environmental insults acting on similar molecular pathways during critical stages of brain development.

With growing evidence of the GABAergic/Interneuron hypothesis, many questions are raised in regard to the contribution of interneuron deficiencies with dysfunctions in the glutamatergic and dopaminergic neurotransmitter pathways that have been identified in patient cohorts. Direct interplay between the GABAergic and hypoglutamatergic hypotheses has been addressed in mice by the specific removal of N-methyl-D-aspartic acid receptors (*NMDAR*) in GABAergic interneurons [127]. In strong support of interneuron deficiencies aligning with the glutamatergic dysfunction these mice presented with molecular, behavioural and physiological defects akin to those seen in the human condition [127]. Direct interactions between aberrant cortical inhibition and dopamine signalling have also been proposed, however, how this link is functionally established is highly debated. For a detailed account of the possible interactions between GABAergic deficiency and the hyperdopaminergic basis of schizophrenia we refer readers to a recent review dedicated to this topic [8]. One possible link is cortical disinihibition leading to increased dopamine in the nucleus accumbens as found when NMDAR antagonists were infused in to the prefrontal cortex of mice [128]. Considering the role of interneurons in modulating many neuronal networks it is likely that deficiency of interneurons during development may also contribute to the decreased glutamatergic and increased dopaminergic basis of disease progression.

10. Mouse Models of Schizophrenia

In order to understand the neurobiological basis and the causal relationship between interneuron deficiency and disease pathogenesis several mouse models of schizophrenia risk factors have been developed. Despite the genetic heterogeneity of the disorder, many of these mouse models have demonstrated interneuropathies (reviewed in [129]) and behavioural phenotypes representing the human condition. *GAD67* knockout mice are perinatal lethal but removal of this gene in specific populations of interneurons during postnatal and embryonic stages of development has been shown to induce behavioural and anatomical defects reminiscent to those in schizophrenia [9,130,131]. Moreover, behavioural changes as a result of amphetamine dosage further suggest that mice lacking *GAD67* in interneurons have altered dopaminergic signalling and therefore provide a direct link between the two neurotransmitter systems [131]. As GAD67 is essential for synthesis of GABA, removal of this enzyme in adult mice also leads to schizophrenia-like molecular and behavioural dysfunctions [131,132]. Taken together, these studies further suggest that the deficiency of *GAD67* identified in human cohorts is likely to contribute to at least part of schizophrenia behavioural deficits.

Complete removal of ErbB4 in mice leads to a 30% reduction of PV-positive interneurons and a corresponding decrease in gamma oscillations that are essential for brain connectivity [133]. Consistent with a cell autonomous role, removal of ErbB4 specifically from fast spiking interneurons also lead to cortical excitability, disruption of gamma oscillations and schizophrenia-like behavioural deficits [134].

One of the most replicated risk factors for schizophrenia is disrupted in Schizophrenia 1 (*DISC1*) that was first identified in a large Scottish family who had a breakpoint in this gene that occurred

through a balanced chromosomal translocation [135]. To recapitulate this mutation several mouse models have been generated to either remove or knock down the *DISC1* gene in a cell type specific manner. Notably, removal of *DISC1* from MGE at E13.5 has been shown to lead to a loss of cortical interneurons in adult mice that arises from aberrant tangential migration. How *DISC1* modulates interneuron migration and whether this defect replicates the schizophrenia-like behavioural defects of *DISC1* knockout mice remains to be explored [136].

Interneuron deficiencies and dysfunction have also been reported in many of the well-described environmentally induced mouse models of schizophrenia. The gestational MAM model in which the anti-mitotic and anti-proliferative agent MAM is administered to gestational day 17 rats recapitulates many of the anatomical, behavioural and processing defects observed in schizophrenia [137]. The MAM model leads to a specific loss in PV+ interneurons in the cortex which results in a loss of function in oscillation activation [138] and has also been linked to hyperactivity of ventral tegmental dopaminergic neurons [139]. Prenatal maternal immune activation (MIA) has been implicated as a risk factor for schizophrenia and MIA induced in rodents at various gestational ages precipitates behavioural, cognitive and pharmacological abnormalities in late adolescence [140,141]. MIA in rodents leads to GABAergic dysfunction similar to that defined in humans, with decreased GABA, decreased *GAD1* and increased levels of GABA receptor subunits [141–145]. The MIA models have also been used to address gene and environmental interactions in schizophrenia pathophysiology. In DISC1 mutant mice that have no behavioural abnormalities the sub-clinical application of the MIA agent poly (I:C) leads to the manifestation of schizophrenia-like phenotypes [146,147]. Stress is also a reported risk factor for schizophrenia and chronic stress induced postnatally in rats leads to altered brain development and behavioural deficits in late adolescence [148]. Rodent pups isolated from the age of weaning have reduced PV and calbindin immunoreactive neurons in the hippocampus and prefrontal cortex [149,150]. In addition, RELN expression was also decreased in the prefrontal cortex of rats raised in isolation with the peak stage of deficiency correlating with the onset of behavioural defects [151]. Stress has also been shown to synergise with MIA to induce schizophrenia-like deficiencies [152]. In mice with sub-clinical doses of poly (I:C) during gestation, peripubertal stress also leads to reduced GAD67 in several brain regions [143].

Earlier in this review we discussed several mouse models that provide insight to the molecular mechanisms controlling interneuron development. Many of these models have also been used to model schizophrenia. For example, *GAD67, Nrg-1* and *ErbB4* are known susceptibility genes for schizophrenia [126,153–155] and mouse models removing these genes have been explored widely. Notably, many of these studies not only highlight the critical roles of these genes in interneuron development but also in interneuron function. Deficiency of *Shh* and *Nkx2.1* in the MGE clearly lead to interneuron defects. However, given the lethality of mice lacking these genes their roles in behaviours associated with the human condition have been harder to address [156,157].

11. Implications of Cortical Interneurons in the Aetiology and Treatment of Schizophrenia

This review has so far highlighted the importance of critical time periods during embryonic interneuron development. In this section we now discuss the correlation of these critical times with human cortical development and the translational relevance of this understanding toward innovative and improved therapeutics in schizophrenia. It is widely accepted that schizophrenia is likely to arise from genetic and environmental insults during embryonic and post-natal development. Whether schizophrenia, which has an average age of onset between 16–25 years [158], could arise from aberrant interneuron development therefore stands as an important question. The specification and migration of interneurons, as discussed earlier, is largely completed in the early postnatal period. However, the final maturation of cortical networks is only achieved much later in adolescence. In humans the brain reaches maturation in the early 20's and this is recapitulated in mice where interneurons continue to mature up until the end of adolescence [159,160]. During adolescence the brain undergoes a period of rapid synaptic pruning and cortical thinning [159,161,162] which coincides with a time of strong emotional states and increased stress [159,160]. These cortical rearrangements extend the period of interneuron development from early embryogenesis through until late adolescence and define a large time window in which environmental insults can also occur. This protracted developmental period is also likely to account for the late manifestation of behavioural deficits in early adulthood.

As the diagnosis of schizophrenia is only made after the time window of interneuron development has closed, this poses a particular challenge for therapeutic methods targeted at replacing or fixing interneuron deficiency. The identification of early diagnostic markers and predictive testing is therefore essential if we are to provide innovative opportunities to alter the disease course during the critical time window. Nonetheless, it may also be possible to reopen artificial time windows as discussed below. Although it was once thought that neurogenesis ceases at birth, it is now well accepted that the adult brain contains a limited number neural stem cell progenitors that provide a population of renewable neurons throughout life. Pharmacological manipulation to enhance neurogenesis in the brain is not a new concept, however, has to date not been explored in the context of schizophrenia [163,164]. Although speculative, the notion of expanding the population of interneurons from resident neural stem cells could be perceived as one way of opening an artificial time window in adulthood. To achieve such a feat it would be necessary to reinstate a similar signalling environment as that experienced in the embryonic ganglionic eminences within the adult brain. Another possible means of therapy may be derived from increasing connectivity or functionality of the remaining interneuron populations within the schizophrenic brain. This could perhaps be achieved by increasing the number of functional synapses or via the up regulation of genes controlling interneuron function. In the latter case, demethylation agents such as histone deacetylase (HDAC) inhibitors may be of use in restoring gene function in cases where epigenetics are at play [118,119,165]. Indeed, support of such an approach may be taken from the

use of the atypical antipsychotic drug clozapine that, in addition to its other affects, has recently been shown to induce DNA demethylation [165].

Interneuron transplantation may also be seen as another method of overcoming the challenges faced by a closed developmental time window. Taking advantage of the unique ability of immature interneurons to undergo long distance tangential migration, interneuron transplantation into the adult striatum, cortex and hippocampus has been gainfully exploited to replace interneurons in animal models. Initially described by Wichterle and colleagues (1999), interneurons were derived from embryonic MGE and injected into adult mice [166]. Remarkably, interneurons were able to migrate through the adult cortex and disperse into the appropriate layers. One of the earliest examples of interneuron transplantation was in the MAM model of schizophrenia, in which MAM treated rats were injected with MGE derived cells. After migrating to the cortex these cells were able to differentiate and form functional synapses with existing pyramidal neurons to rescue deficits in GABAergic signalling. Furthermore, this also rescued aberrant dopamine signalling associated with these mice [167]. This technique has since been performed on several mouse models of psychosis and other neurodevelopmental disorders (reviewed in [168]) and provides hope of a cellular based therapy for neuropsychiatric disorders. This exciting method thereby introduces a new window of plasticity into the otherwise developed brain and may potentially be harnessed for the long-term treatment of interneuron deficiency in schizophrenia.

12. Conclusion and Summary

The loss of interneurons in the adult brain has been implicated in several developmental disorders with a clear link to psychosis. Due to their complex developmental progression, loss of interneurons or interneuron dysfunction can result from insults to many molecular pathways at almost any stage of brain development. Understanding critical stages at which different molecular pathways are required during development and the outcomes of insults at differing time points are imperative in understanding the roles of interneurons in neurodevelopmental disorders and psychiatric illness. Such information is likely to provide insight toward innovative therapies and preventative medicines for this highly prevalent disorder.

Conflict of Interest

The authors do not have any conflict of interest to disclose.

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