



Determination of the connectivity of newborn neurons in mammalian olfactory circuits

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Abstract The mammalian olfactory bulb is a forebrain structure just one synapse downstream from the olfactory sensory neurons and performs the complex computations of sensory inputs. The formation of this sensory circuit is shaped through activity-dependent and cell-intrinsic mechanisms. Recent studies have revealed that cell-type specific connectivity and the organization of synapses in dendritic compartments are determined through cell-intrinsic programs already preset in progenitor cells. These progenitor programs give rise to subpopulations within a neuron type that have distinct synaptic organizations. The intrinsically determined formation of distinct synaptic organizations requires factors from contacting cells that match the cell-intrinsic programs. While certain genes control wiring within the newly generated neurons, other regulatory genes provide intercellular signals and are only expressed in neurons that will form contacts with the newly generated cells. Here, the olfactory system has provided a useful model circuit to reveal the factors regulating assembly of the highly structured connectivity in mammals.

Keywords Development · Synaptogenesis · Cell fate · Transcription factor · Mouse

Abbreviations

AON	Anterior olfactory cortex
EPL	External plexiform layer
ETC	External tufted cells
GC	Granule cell
GCL	Granule cell layer
MC	Mitral cell
MCL	Mitral cell layer
MTC	Middle tufted cells
OSN	Olfactory sensory neurons

Introduction

Two major findings have largely promoted the research on circuit wiring in the early olfactory system. The first one was the discovery of the molecular specification of olfactory sensory neurons (OSNs) with each sensory neuron only expressing one type of olfactory receptor [123, 159, 182]. All neurons that express one of the more than one thousand receptors selectively target one of roughly thousand discrete areas of the olfactory bulb called glomeruli in the bulb. We will focus here on the synaptic wiring within the main olfactory bulb (MOB). The second finding relevant to wiring in the MOB is that neurons continue to add throughout the postnatal life [13, 55, 97, 103].

Organization of the early olfactory system

The early olfactory system comprises the olfactory epithelium and the MOB (Fig. 1a). The olfactory

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epithelium harbors the OSNs that project to the MOB. OSNs project to neuropil structures in the MOB called glomeruli, where they make direct or indirect contacts with a number of interneurons and the MOB projection neurons. MOB projection neurons comprise mitral cells (MCs) and middle tufted cells (MTCs) that directly convey sensory information to the olfactory cortices. Olfactory cortex neurons are thus just two to three synapses away from the peripheral sensory neurons. The activity of mitral and middle tufted cells (MC/MTCs) is modulated by two major groups of interneurons (Fig. 1b): granule cell interneurons (GCs) and periglomerular interneurons. In the mouse, there are approximately 40,000 MCs and MTCs compared with 50,000 periglomerular interneurons and three million GCs. The MOB is organized in different layers, determined by the position of neuronal cell bodies or neuropil. The two MOB layers with the largest volume are the granule cell layer (GCL) close to the center of the MOB, where the cell bodies of GCs are located, and the external plexiform layer (EPL), a neuropil layer located between the mitral cell layer (MCL) and the glomeruli (Fig. 1b). The EPL mainly contains the synapses between GCs and the lateral dendrites of MCs and MTCs. A third glutamatergic cell-type exists in the form of external tufted cells (ETCs) that are located in the EPL just below the glomeruli. ETCs project primarily locally within the MOB and connect mirror glomeruli of OSNs expressing the same odor receptors [15, 96]. Other less numerous interneuron types contribute to circuit function in the EPL and GCL, such as short-axon cell interneurons [129]. Finally, neurons in the MOB receive massive excitatory top-down inputs from the anterior olfactory cortices that are transiently active in a brain-state-dependent manner [18, 138, 160].

This review will focus on the development of three abundant neuronal elements that constitute the MOB (Fig. 1b). They include MC/MTCs as the output neurons of the MOB, GCs as the main interneuron type, and top-down inputs from the olfactory cortex that provide central feedback to the MOB. For each of these circuit elements, we will initially highlight their anatomical organization and function and then describe their development, followed by a discussion of the currently known cell-intrinsic and activity-dependent wiring mechanisms. Despite major advances in the past decades, it will become clear that there are still large gaps in our knowledge on the assembly of this intensively studied mammalian model circuit.

Olfactory bulb projection neurons

Cell bodies of MCs form a narrow band between the GCL and the EPL (Fig. 1b). MCs have an apical dendrite that targets one glomerulus and several lateral dendrites that extend up to few millimeters long horizontally in the deeper half of the EPL. Each glomerulus receives apical dendrites from approximately 20 MCs [124]. MTCs are smaller than MCs and their cell bodies are located in the EPL. Again, each MTC has an apical dendrite that targets preferentially a single glomerulus, and lateral dendrites, which are shorter than those of MCs and extend into the superficial half of the EPL.

The apical dendrites of MCs and MTCs receive input from OSNs. The information is back-propagated along the lateral dendrites and integrates inhibition mediated through reciprocal synaptic contacts with GCs [194]. These reciprocal dendro-dendritic synapses contain glutamatergic excitatory output synapse cells emerging from MC/MTC dendrites and a GABAergic inhibitory synapse back from

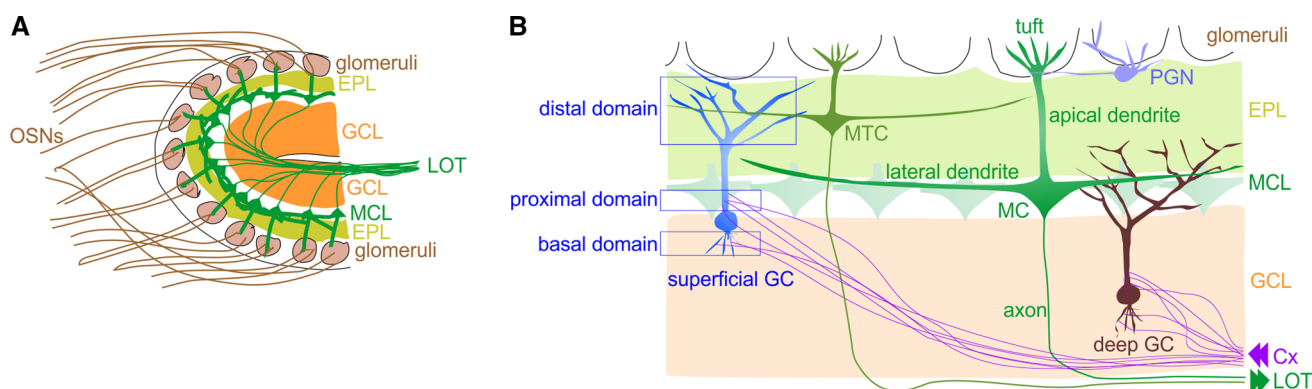


Fig. 1 Organization of the main olfactory bulb. **a** Axons of olfactory sensory neurons (OSNs) project to the glomeruli and form contacts with mitral cells (MC) and middle tufted cells (MTC) that then send their axons through the lateral olfactory tract (LOT) to higher brain regions. The three main layers of the bulb are the external plexiform layer (EPL), granule cell layer (GCL), and mitral cell layer (MCL).

b MCs and MTCs branch their lateral dendrites in the deep and superficial EPL, respectively. The two main interneuron types are periglomerular neurons (PGN) and granule cells (GC). The distal domain of GCs either branches in the deep or superficial EPL. The proximal and basal domains receive rich inputs from the olfactory cortices (Cx)

the GC onto the same MC/MTC dendrite [124]. Activity invading the lateral dendrites of MCs and MTCs elicits the lateral inhibition of other MCs and MTCs via dendro-dendritic connections through GCs [170, 198]. Lateral inhibition of MC/MTCs is thought to be involved in the odor discrimination and synchronization of rhythmic MC/MTC activity [107].

Granule cell interneurons

GCs are the most abundant cells in the MOB. GCs are axon-less inhibitory interneurons that have a basal and an apical dendrite (Fig. 1b). Their apical dendrite is composed of an unbranched segment emerging from the soma followed by a branched segment. The apical dendrite can be divided into proximal and distal synaptic domains. The distal domain consists of the branched segment of the apical dendrite and is covered with spines containing reciprocal dendro-dendritic synapses as described above. The basal dendrite (basal domain) and the proximal domain of the unbranched apical dendrite receive glutamatergic input from the axon collaterals of projection neurons of the MOB and olfactory cortex [8, 34, 104, 125]. Besides glutamatergic input synapses, GCs receive GABA_AR-containing inhibitory synaptic inputs that originate, among others, from short-axon cells [42, 144].

Periglomerular cells

The second most abundant interneuron type in the MOB is the highly heterogeneous group of periglomerular cells [145]. Periglomerular cells divide into several subtypes based on their expression profile of marker proteins, such as tyrosine hydroxylase or calcium-binding proteins as calbindin, calretinin, or [83, 129]. Periglomerular cells project their dendrites to a single glomerulus and they ramify their dendrites in a smaller portion of glomeruli, where periglomerular neurons receive synaptic input from axons of OSNs; yet, some periglomerular cells extend their dendrites to multiple glomeruli. In addition, certain periglomerular neurons have dendro-dendritic synapses with MC/MTC dendrites [127, 163]. While some periglomerular cells are axon-less [84], the majority has axons that extend over several glomeruli [150].

Feedback projections from the anterior olfactory cortices

The MOB does not simply function as a feed-forward circuit to propagate sensory information from the nose to the olfactory cortex. The MOB receives dense input from the anterior olfactory nucleus (AON) and tenia tecta, piriform cortex, nucleus of the horizontal limb of the diagonal

band, locus coeruleus, and raphe nucleus [38]. The centrifugal inputs to the MOB can be classified into two broad groups. One group releases neuromodulators, such as noradrenaline, serotonin, or acetylcholine [38]. The other group of projections is glutamatergic and originates from the AON and to a lesser extent from other olfactory cortices. The AON can be divided into pars principalis and the pars externa [24]. The pars externa forms an outer shell of cells that partially surrounds pars principalis. Pars externa receives ipsilateral projections from the MOB [110] and projects back through the anterior commissure to the contralateral MOB with a dorso-ventral [24] and rostro-caudal topography [197]. Pars principalis centrifugal projections are both ipsi- and contralateral and more diffuse [24]. There is additional segregation towards the targeting of projections to different MOB layers: While most parts of the pars principalis and the pars externa project to the GCL of the MOB, the ventro-posterior aspect of the pars principalis also projects to the glomerular and EPL [24]. In the GCL, cortical feedback projections directly excite the GCs and produce feed-forward inhibition through deep short-axon cells [19].

Development and emerging connectivity of olfactory bulb neurons

The MOB develops from the ventral pallium of the rostral telencephalon [69, 102, 175]. The development of the mouse MOB starts at E9–E10.5, although its structure cannot be detected macroscopically as a protrusion of the rostral telencephalon until E11.5–12.5 [56]. At E12.5, the MOB divides into a ventricular zone formed by neuro-epithelial cells aligned in radial orientation and a thinner mantle zone (Fig. 2). These neuro-epithelial cells proliferate and give rise to radial glia cells and to the post-mitotic future projection neurons that then form the superficial mantle zone [7]. Both neuro-epithelial and radial glia cells can be considered neuronal stem cells [85, 154, 183].

Targeting of olfactory sensory axons to the olfactory bulb

OSNs project their axons to the MOB where they are thought to primarily target the apical dendrites of glutamatergic MOB neurons, i.e., external tufted cells and MC/MTCs, as well as dendrites of periglomerular neurons in the glomeruli. Mammalian odor receptors form a large family of seven transmembrane G-protein-coupled odor receptor that plays an instructive role in targeting of OSN axons to form the glomerular map [15, 123, 189]. During the embryonic development, OSNs expressing the same

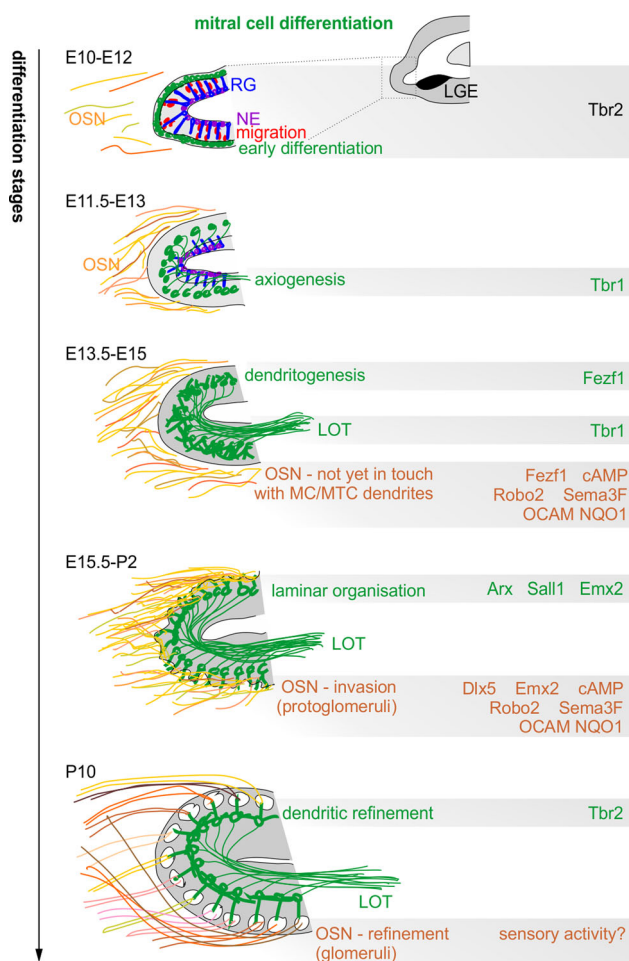


Fig. 2 Mitral cell differentiation and main olfactory bulb formation. The major steps of MC/MTC development from generation, migration to dendritic and axonal growth are shown over time at different embryonic (E10–E15.5) and postnatal days (P2–P10) in mice. MC/MTC differentiation is set in relation to the development of olfactory sensory neurons (OSN) and MOB layers. Exemplary factors for the corresponding differentiation steps are indicated on the *right*

receptor converge their axons in a specific pair of glomeruli at stereotypic location in the MOB [62, 122, 126, 177], thereby generating an olfactory map that is found at the level of the glomeruli, but dissipates further downstream.

Early differentiation of olfactory bulb projection neurons

In rodents, MCs are mainly born between embryonic days E9–E13 (Fig. 2). While the early born (E9–E10) MCs integrate preferentially dorso-medially in the rostral MOB, later-born (E11–E12) MCs integrate ventro-laterally in the caudal region [64]. The generation of MTCs peaks around E15 [17, 57, 58, 64]. The MC development can be divided roughly into several stages according to their morphological differentiation: first, a ‘post-neurogenesis’ stage after

MCs have been generated in the ventricular zone and migrate radially to reach the intermediate zone. In the intermediate zone, MCs undergo a tangential reorientation of their soma and start to form axons projecting to the piriform cortex that initiate the lateral olfactory tract (LOT) [17].

Development of axons of projection neurons to the cortices

Axons of MCs and MTCs project to the olfactory cortices and ventral striatum through the LOT. In addition, MCs and MTCs form axon collaterals throughout the MOB. We will focus here on the better characterized projections to the olfactory cortices. MOB projection neurons start to form axons around E11.5–E13 [102, 188]. Their axonal growth cones advance through the ventrolateral part of the telencephalic vesicle. While the LOT has not developed much further, some isolated fibers have reached quite remote locations from the prospective MOB. These early axons reside in a space largely devoid of cell bodies and they extend caudally along the ventrolateral part of the telencephalic vesicle. At E15, a number of fibers form a compact bundle, corresponding to the primordial LOT that is formed by E16 and its axons ultimately reach the olfactory cortical zone [102, 188]. The axons grow further caudally at E17. The extension of axonal projections from the MOB to the cortex occurs before the arrival of the OSN axons into the MOB, and thus, the growth of the LOT is likely regulated by factors intrinsic to the MOB projection neurons and cues in their targets, the future olfactory cortices. In agreement with this observation, the cross-sectional diameter of the LOT is not affected by occlusion [16].

Axon collaterals from the LOT grow in size with earlier innervation of the AON, anterior and posterior piriform cortex, and entorhinal cortex at birth, while the olfactory tubercle of the ventral striatum is innervated only between P1 to P7 [188]. MCs project their axons preferentially via the dorsal parts of the LOT to the cortices, whereas MTCs have a preference to project through the ventral parts of the LOT [128].

MCs and MTCs appear to serve as parallel pathways for processing of sensory information [45, 60], and their projection patterns differ. The complete reconstructions of MCs revealed that a single neuron usually branches and innervates the AON, the piriform cortices, olfactory tubercle, amygdala, and lateral entorhinal cortex, while MTCs project mostly to a sub-region in the anterior olfactory cortex [60, 173]. In other sensory modalities, such as touch, audition, and taste, adjacent neurons often respond to similar sensory stimuli and project their axons to the same cortical targets, creating a topographic sensory

map between them [148, 164, 191]. The overall diffuse projection patterns in the cortices are somewhat surprising given the strong order further upstream at the level of OSNs. For instance, MCs that receive input from the same glomerulus make connections in different regions of the olfactory cortices [117]. Hence, in the piriform cortex, the projections of MCs connected to the same glomerulus appear no different from the MCs innervated by different glomeruli [46, 173]. The highly divergent innervation of the cortex may serve to integrate odor information originating from multiple receptors.

Dendritic development of olfactory bulb projection neurons

After projection neurons have started to extend axons, the differentiating MC/MTCs re-orient toward the surface of the MOB following the arrival of the OSN axons [102]. The reorientation from a tangential to radial orientation is influenced by the presence of the olfactory epithelium [102]. After adopting a radial position, MCs develop multiple and widespread primary apical dendrites [17, 58]. OSN axons that penetrate into the MOB then form contacts with the apical dendrites. These sites of axonal in-growth at the surface of the MOB form proto-glomeruli that then progressively transform to glomeruli [17]. Subsequently, the multiple apical dendrites are pruned and MCs assume their typical mature morphology with one primary apical dendrite and several secondary dendrites extended in the EPL parallel to the MCL [17, 55, 65, 106]. During the refinement process, the somata of MCs align to form the MCL, while most MTC nuclei are scattered in the EPL. MCs and MTCs also differ in their lateral dendrites in the EPL [65, 125]. While the early born MCs extend their secondary lateral dendrites in the deep layers of EPL, the lateral dendrites of later-born MTCs develop in the superficial layer of EPL [66].

External tufted cells

Another type of neuron in the MOB is external tufted cells (ETCs) that are less characterized with respect to their morphologic development. ETCs are usually glutamatergic, but recent studies support that some are GABAergic as well [178]. ETCs form glutamatergic synapses with the apical dendrites of MCs [47, 169] and interneurons, such as periglomerular cells and short-axon cells [53, 169]. ETCs also mediate di-synaptic excitation between OSNs and the apical tufts of MCs [47, 130, 178, 185]. ETCs extend axon collaterals just below the MCL and make latero-medial connections in the MOB with ‘mirror’ glomeruli in the contralateral part of the MOB that receives inputs from OSNs expressing the same olfactory receptor [15].

Generation and migration of granule cell interneurons

MOB interneurons are generated in the subventricular zone. After leaving the subventricular zone, they migrate through the rostral migratory stream (Fig. 2), where cell divisions are still observed in particular early in the postnatal period [50]. Although the majority of these interneurons are born between E18–P5, a pioneering population is generated already in the lateral ganglionic eminence between E12.5–E14.5 [56]. Other interneuron types are primarily generated in the embryo. The generation of tyrosine hydroxylase-positive neurons of the glomerular layer peaks at E12.5 and calbindin-positive interneurons at E15.5 [12]. Only some GCs express marker proteins. The most superficial GCs express trophoblast glycoprotein (also named 5T4) that is an antagonist in the Wnt/ β -catenin signaling pathway [67]. Calretinin-positive GCs are also located in the superficial parts of the GCL. Whereas the number of 5T4-expressing GCs remains constant throughout the animal’s life, the number of calretinin-positive GCs further increases postnatally [12]. Postnatal-born GCs are generated from the populations of neuronal stem cells in the lateral wall of the lateral ventricles. Recent data support the view that the different interneuron populations are generated from distinct stem-cell populations that are turned on and off at different stages of development and in postnatal life [44].

Wiring of granule cell interneurons

The development of adult-born GCs can be defined according to morphological criteria as stages 1–5 [149]. The ‘migration phase’ (stage 1) describes the neuroblasts of GCs in the rostral migratory stream (Fig. 3). Following tangential migration through the rostral migratory stream, new GCs disperse radially into the GCL [98]. During the following ‘phase of the initial dendrite outgrowth’ (stage 2), new GCs reach the GCL and begin to extend their first neurites that are followed by a ‘dendrite extension phase’ (stage 3) that is roughly 10 days after their last cell division when GCs penetrate the EPL with their apical dendrite and form the first synaptic inputs [26, 70, 72, 192]. About 2 weeks after their birth, new GCs undergo a ‘phase of branching of their apical dendrite’ (stage 4) in the EPL and develop further synaptic inputs [26, 70, 72, 192]. At this stage, few spines and synaptic sites have evolved in the distal domain [149]. From around the third week after the birth of GCs on, they experience a ‘terminal differentiation phase’ (stage 5) during which the distal domain achieves full spine densities [149]. During this final phase of maturation, new GCs acquire fast-action potential firing [26] and form most of the reciprocal dendro-dendritic synapses

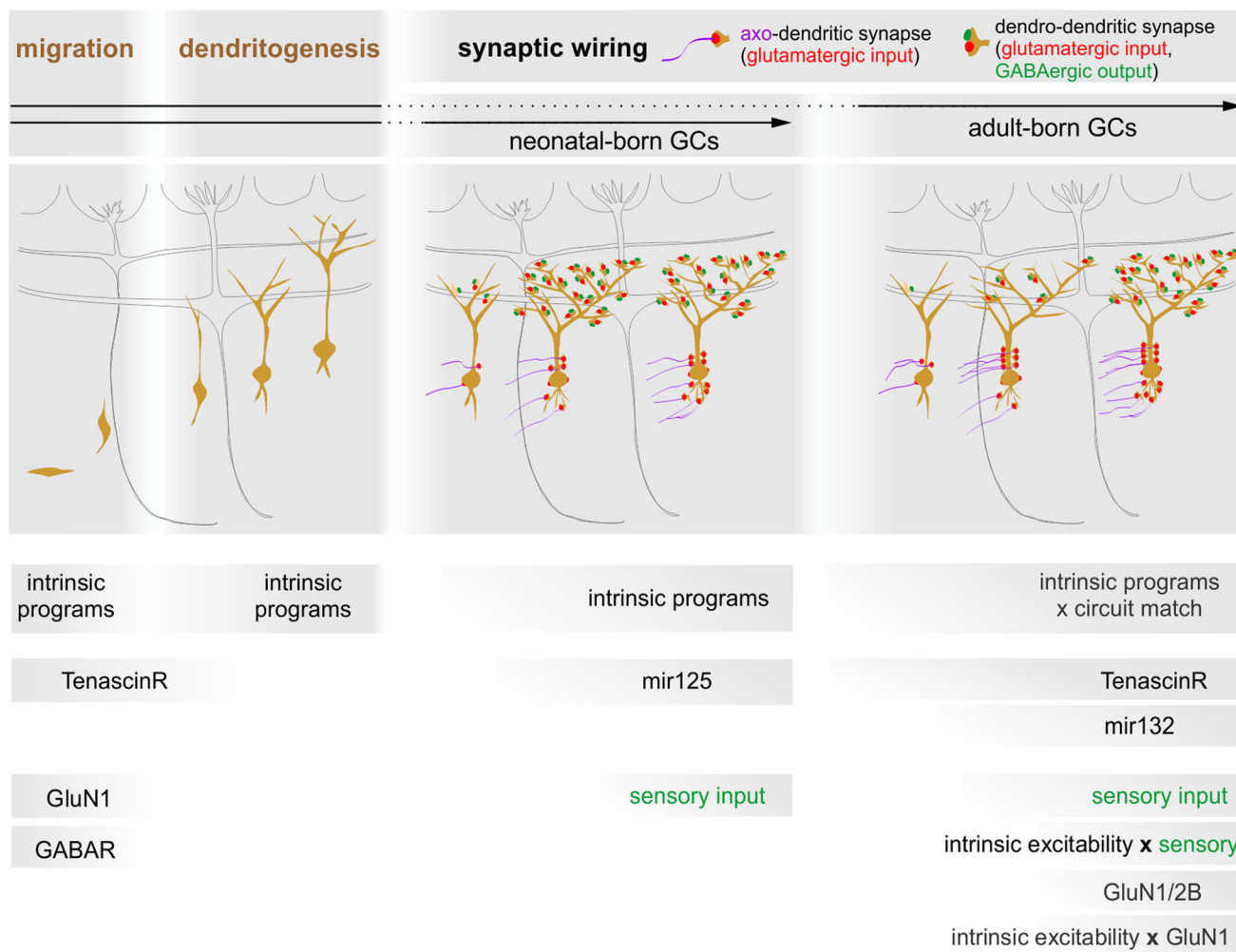


Fig. 3 Early steps in the differentiation and later synaptic wiring of granule cell interneurons. *Left* the early steps of neonatal- and adult-born GC addition follow several stages with switching from tangential to radial migration with subsequent extension of the main dendritic arbors that is followed by branch formation in the distal domain of the apical dendrite. *Middle* during later stages, neonatal-born GCs develop glutamatergic input in the different dendritic domains. Inhibitory output synapses develop in the distal domain parallel to

proximal inputs. *Right* adult-born GCs develop distal input and output synapses only after the proximal glutamatergic input synapses near the soma. General factors have been identified for the different steps of GC maturation shown in the environmental factors mostly related to activity-dependent processes; cell-intrinsic programs likely determined already at the precursor stage. In addition, exemplary molecular factors are indicated

in the distal domain [72, 192]. Synaptic development is mostly complete by 4 weeks after the generation of adult-born GCs [26, 72, 119, 149, 192].

Differences exist in the synaptic wiring of neonatal- and adult-born GCs. Adult-born GCs first develop input synapses in their proximal dendritic domain, before developing most of their reciprocal output synapses [72, 144, 192] and prior to acquiring the full ability to fire action potentials [26], i.e., they ‘listen’ before they can ‘speak’. This sequential pattern of the synaptic development of adult-born GCs sharply contrasts with the maturation of GCs generated in neonates. GCs added to the neonatal MOB develop reciprocal output synapses on the distal domain earlier and simultaneously with input

synapses in the proximal domain [72]. The transition from ‘neonatal’ to ‘adult’ synaptic wiring patterns occurs in a stepwise fashion [158]. GCs born at postnatal day (P)20 have already switched to an ‘adult’ pattern of synaptic development in the proximal, but not in the distal domain. In contrast to the proximal domain, the switch to an ‘adult’ synaptic development occurred later in the distal domain. The complete ‘adult’ pattern is observed only in GCs generated at P40. Thus, the delayed distal development of output synapses only appears after the neurons already form the high densities of synaptic inputs to the proximal domain [158]. The stepwise transition from ‘neonatal’ to ‘adult’ synaptic development could thus present independent processes in these two major dendritic compartments.

Development of the anterior olfactory nucleus

The AON provides the highest density of glutamatergic top-down projections to the MOB [24]. Neurons in the AON are generated between E15–E21 [14] with a caudal-to-rostral gradient of neurogenesis (as also seen in the piriform cortex) and superficial-to-deep gradient (contrary to the ‘inside–out’ order of other cortices) [14, 32]. A dense cell layer emerges by E19 that directly merges with large numbers of immature cells at its diffuse deep boundary. At the time when mice and rats are born, a deep boundary is recognized in its caudal parts and adult morphology is obtained by P15. The patterns of axonal in-growth from the MOB follow the sequence of cell proliferation. Contralateral projections through the anterior commissure from pars externa to the MOB develop earlier than those from pars principalis. In addition, the contralateral projections of pars principalis develop first from caudal regions; with rostral regions of the pars principalis following few days later [165]. Finally, myelination in the anterior commissure emerges between P11 and P14 [23]. The further postnatal growth of the AON is region-dependent between P10 and P60 [21]. While lateral aspects of pars principalis show a little expansion, the medial aspects of pars principalis and externa display substantial growth up to P30 and subsequently reduce their total brain volume. Medial and lateral aspects of the AON may serve different functions, as, for instance, the medial aspect of pars principalis in rats has a preference to target the deep GCL [24]. These different projection patterns may support functional segregation of AON sub-regions.

Transcriptional control of circuit assembly and synaptic wiring

The multifaceted roles of developmental transcription factors

A number of transcriptional regulators have been identified to be involved in the formation of the MOB (Fig. 2). A comprehensive overview of their actions is provided in a recent review [39]. Transcription factors can control the developing wiring diagram of the MOB in two broad ways. They can act in the new neuron, where they are expressed to regulate the positioning of the cell bodies, the growth of dendrites and axons or synaptogenesis. Other transcription factors are expressed in the cells with which the new neurons will form contacts, but not the new neuron itself, and influence trans-synaptically the wiring of the new neuron. A second common pattern seems to be so far that none of the identified single transcription factor acts exclusively on a single step in the differentiation of new neurons.

The first element of the main olfactory system is the OSNs that terminate in the glomeruli. Glomeruli are grouped into dorso-ventral domains of the MOB. Olfactory receptors’ identity in the olfactory epithelium correlates with the axon location along the dorso-ventral axis in the MOB [78, 118, 176, 204]. The dorsal domain is formed first with subsequent expansion of the glomerular map towards the ventral surface of the MOB [176]. The two zones can be divided also based on the expression pattern of specific markers the olfactory epithelium with the dorsal zone expressing, for instance, NQO1 and O-MACS and the ventral zone OCAM [48, 78, 137, 184, 203]. Sets of repulsive ligands/receptors, such as Slits/Robo2 and Sema3F/Nrp2, appear to participate in OSN projection along the dorsal–ventral axis [132, 135, 176], while OSN axon targeting to glomeruli in the anterior–posterior axis seems to be controlled by olfactory receptor-derived cAMP signals [63, 166]. Each type of olfactory receptor generates a unique level of cAMP. The levels of cAMP appear to define the expression levels of guidance molecules and determine the anterior–posterior topography of axonal projections to the MOB [29, 63]. OSNs producing high levels of cAMP project their axons to the posterior MOB, whereas those producing low levels target the anterior MOB [63].

Some molecular factors also have a dual role in the developmental regulation of the olfactory epithelium and in the MOB circuit formation. For instance, in the developing telencephalon, the *Paired family of homeobox* transcription factor 6 (Pax6) is expressed in the pallium and involved in the formation of the olfactory epithelium, the MOB, and the olfactory cortex [27, 30, 174]. Pax6 homozygous mutant mice do not develop MOBs [37]. Conditional deletions of the PAX6 gene in GCs interfere with the generation of subsets of GCs [79, 208]. Although Pax6 mRNA transcription is widespread along the ventricular walls, Pax6 protein is restricted to the dorsal aspect [36]. The restriction of PAX6 expression to the dorsal ventricular zone is regulated post-transcriptionally in the neuronal stem cells by micro-RNA 7a (miR-7a), which is expressed in a Pax6-opposing ventro-dorsal gradient [36]. The *Genomic screened homeobox* (Gsx) transcription factors Gsx1 and Gsx2 are involved in patterning the ventral forebrain. Gsx2 expression in the lateral ganglionic eminence appears to repress the expression of dorsal genes, such as Pax6, and regulates the quiescent, undifferentiated state of neuronal stem cells [115, 147]. Gsx2 deletion impairs interneuron migration to the MOB [27, 179, 186, 206]. Homozygous Gsx1/2 double mutants display more severe disruptions than Gsx2 mutant alone, resulting in near complete loss of MOB interneurons [205].

Among the *Distalless* (Dlx) transcription factors, Dlx1, Dlx2, Dlx5, and Dlx6 are expressed in differentiating

GABAergic neurons [143]. Dlx1/2-positive precursors give rise to dopaminergic-, calbindin-, and calretinin-expressing interneurons in the glomerular layer and parvalbumin-positive interneurons in the EPL [3, 12, 80, 93]. While Dlx1 and Dlx2 are nearly absent in the embryonic MOB, their mRNAs are expressed abundantly in the lateral ventricle, RMS, and MOB in the postnatal and adult glomerular and GCL [20, 25]. Dlx1 and Dlx2 expression precedes that of Dlx5 and Dlx6 during neuronal differentiation, and Dlx5 or Dlx6 becomes only significantly expressed after cells leave the adult subventricular zone [41]. Thus, the DLX5/6 expression is relatively low in the RMS and increases following their arrival to the MOB [20]. In the adult MOB, Dlx5 and Dlx6 are detected in the deeper GCL and the glomerular layer [3]. While no gross abnormalities have been detected in the forebrain of Dlx1 mutant mice [5], the disruption of Dlx2 results in the loss of dopaminergic neurons [155]. Dlx2 overexpression has the opposite effect [20]. A more pronounced phenotype was observed for double Dlx1/Dlx2 mutant mice [5, 25, 99, 101]. These double mutants die within a few hours after birth, and in these double mutants, GABAergic and dopaminergic neurons are lost following impaired migration and maturation. Dlx5 mutants produce a global defect in the differentiation of MOB interneurons with smaller MOB and a disorganized MCL [92, 100]. Importantly, Dlx5 mutants lack axonal connections between OSNs and the MOB, suggesting that this transcription factor is involved in axonal targeting [92, 100].

A number of transcription factors not only control interneuron development, but their deletion also lead to gross changes in the MCL during circuit assembly. For instance, mice deficient of another transcription factor subfamily, *Aristaless-related homeobox* (Arx) die in the neonatal period, and their MCL is small and disorganized [77, 200]. Another class of transcription factors is *Emx homeobox genes* (Emx1 and Emx2). Emx1 and Emx2 are expressed in the subventricular zone of the MOB at E15. Both Emx1 and Emx2 are observed in the olfactory epithelium and MCL at E18 and after birth [105]. Emx1 continues to be expressed in the adult [131]. While, the deletion of Emx1 had negligible effects alone on adult brain morphology, Emx2 mutant mice die early on, do not develop proper projections of sensory neurons to the MOB, and eventually display small MOB with a disorganized MCL [40, 199]. Similar to Dlx 5 mutants, sensory neurons of Emx2 knock-out mice do not develop proper projection patterns to the MOB. Double mutants of Emx1/2 display thin and disorganized mitral cell, external plexiform, and glomerular layers [40, 199]. Different effects are observed for collapsin response mediator protein 4 mutants with alterations in the length of the apical dendrite of MCs and consequently altered MOB activity [180, 181]. In addition,

another molecule identified through genetic screens (TARSH) appears to affect dendritic complexity of MCs [28].

In the developing MOB, the *T-box gene 1* (*Tbr1*) is expressed in projection neurons throughout their differentiation beginning with their final-cell divisions [25, 114, 155]. The deletion of *Tbr1* impairs the growth of their axons and, hence, the formation of the LOT [25]. *Tbr2* is highly expressed in many MCs around E14 and in adulthood [121]. The deletions of *Tbr1* and *Tbr2* result in fewer MCs and MTCs to an extent that the MCL is undetectable [6, 25, 167]. The selective inactivation of *Tbr2* in MCs and MTCs at the late embryonic development leads to a compensatory increase in the expression of *Tbr1* and switches vesicular glutamate transporter expression from subtype 1–2 [121]. Selective inactivation of *Tbr2* in MCs and MTCs results in the impaired formation of dendro-dendritic synapses between the MCs and MTCs and inhibitory interneurons with altered responses of projection neurons to odors [121]. Hence, *Tbr2* appears to be required for establishing a functional MOB circuit and excitatory-inhibitory balance. *Tbr2* could thus provide an interesting entry point to dissect neural circuit assembly and the regulation of the excitatory-inhibitory circuit balance.

The *Neurogenin 1* (*Ngn1*) transcription factor is involved in the migration of olfactory interneurons in part via the induction of Prokineticin 2 secretion that attracts new MOB neurons [207]. In particular, in *Ngn1* mutants, a large fraction of dopaminergic cells do not reach the glomerular layer and instead remain in the GCL [207]. In *Ngn1* and *Ngn2* double mutants, the MOB is barely detectable with substantial reduction in MCs and MTCs during embryogenesis [168]. The *Sal-Like genes* *Sall1* and *Sall3* are expressed in both the developing olfactory epithelium and MOB [139, 140] with neuronal precursors and differentiating cells expressing them at E13.5 [51, 52]. From E17.5 to adulthood, both factors are expressed in the MCL, GCL, and glomerular layer. *Sall1* is expressed preferentially in the MCL [51, 52, 54]. The disruption of *Sall1* reduces the size of the MOB and results in a disorganized laminar organization of MCs, followed by the perturbed migration of interneurons [51, 133]. Finally, the *Fezf1* gene (*Forebrain Embryonic Zinc Finger 1*) transcription factor is expressed in the OSNs, but not in MOB neurons [171]. In *Fezf1*-deficient mice, OSNs do not develop axonal projections to the MOB [59, 190]. The MOB is smaller and shows abnormal layer formation with a broader MCL and the polarity of the MCs is disorganized [59, 190]. In addition, the migration of interneuron progenitors through the RMS is altered and periglomerular neurons are absent. The actions of *Fezf1* in MC formation and interneuron development in the MOB are, however, most likely not cell-intrinsic, as *Fezf1* is not expressed in

these progenitors [59]. The deletion of another transcription factor involved in the pathogenesis of Rett syndrome, *Mecp2* results in impaired axonal growth, but no gross abnormalities in dendritogenesis of MCs [142].

Molecular determinants of granule cell wiring

Some molecules, including micro-RNAs, were examined for their involvement in the later stages of GC wiring. Some of these molecules seem to be involved in the wiring of adult-born, but not the early generated GCs. For instance, although miR-125 is absent in neonatal-born neurons [2], it was found to regulate morphogenesis and control functional integration of adult-born neurons. Furthermore, miR-132 regulates the morphological development and synaptic integration of neonatal-born GCs. Its downregulation and overexpression directly correlates with morphological complexity and spine numbers [146]. Not unexpectedly, extracellular cues also play a role. The extracellular matrix glycoprotein Tenascin-R is produced in the GCL of the MOB and its expression increases during the postnatal development [33]. Time-lapse video imaging and morphological analyses revealed that Tenascin-R deletion impairs radial migration in the adult, but not in the developing MOB. Tenascin-R deletion also reduces the spine development of adult-, but not neonatal-born GCs. At present, it is, therefore, difficult to tell whether a given molecule is required at a specific stage of maturation or whether the observed effects on dendritic growth are, for instance, due to indirect effects of that molecule at the earlier steps of differentiation.

Outlook on molecular determinants in MOB wiring

No single transcription factor has been identified that selectively acts on one aspect of circuit assembly. The relative scarcity of identified transcription factors acting on the synaptic wiring and functional connectivity that occurs at later stages of neuronal differentiation is due to the focus of the existing studies mostly on the earlier steps of differentiation and large-scale changes in MOB cellular morphology. Studies particularly examining the development of the synaptic connectivity and functional maturation are needed to understand the impact of the transcriptional machinery on the connectivity. In particular, it will be interesting to understand how actions of factors coming from the new neurons themselves or from future synaptically connected partners, orchestrate the developing wiring diagram. In addition, it appears important to note here that most of the published data on mutants that regulate MC morphology are based on the incomplete reconstructions of dendritic arbors. More complex aspects in the regulation of MC morphology and connectivity thus still need to be elaborated.

Neuronal activity and the refinement of synaptic connectivity

Global effects of sensory activity in the development of the olfactory bulb circuit

Circuit assembly of the MOB is also regulated by neuronal activity in the early postnatal period. Most of our knowledge comes from experiments using sensory deprivation through unilateral naris occlusion usually starting after birth [22]. Early naris closure induces a cascade of changes, with cell-death as one of the final events. Within hours after the onset of sensory deprivation, protein synthesis is down-regulated in all the layers of the neonatal, but not the adult MOB [82]. Activity-dependent changes are not necessarily uniform. After the perinatal onset of deprivation, the volume of the GCL decreases first around P8 followed by shrinkage around P12 of the external plexiform layer [43]. Conflicting reports exist on sensory deprivation-induced changes in the size and number of glomeruli [113, 161]. Sensory deprivation consistently decreases the soma size of MCs, while the number of MCs does not seem to substantially change [16, 43, 112, 113].

Activity-dependent mitral cell development

Contrary to the pronounced effects on MC morphology caused by transcription factor deletions, the majority of MCs show relatively 'normal' gross dendritic morphology when they differentiate in olfactory bulbs deprived of sensory input [112, 113]. In addition, whereas the dendrites of GCs in non-deprived animals are highly plastic as observed by long-term *in vivo* imaging, the dendrites of MCs in adult mice displayed very few changes, either in the normal conditions or after sensory deprivation [120]. Deprivation, however, markedly affects the reciprocal synapses between MCs and GCs, as described in the following sections [22]. Naris closure leads to an initial depression of spontaneous activity in MCs [49]. Together, existing data suggest that synaptic connectivity between MCs and GCs can be regulated by olfactory inputs, but that gross dendritic morphology of MCs is not dependent on sensory inputs. Yet, another study found that lesioning the olfactory epithelium during the early postnatal development can modify the growth of the apical dendrite of MC/MTCs towards glomeruli [31]. In the absence of OSNs, MC/MTCs emit several dendrites into the EPL, but they do not form dendritic tufts [31]. Such lesioning of OSNs may, however, also affect the activity-independent, developmental signaling between OSNs and MCs, as described in the studies on the deletion of transcription factors.

MCs and MTCs initially have multiple dendrites that project into several glomeruli [109]. Within 10 days after birth, however, the vast majority of MCs and MTCs go through a refinement process, such that they will display a single apical dendrite that branches into a single glomerulus. Unilateral naris closure delayed the refinement of MC/MTCs, such that the refinement period is prolonged by a few days. However, by P14, the refinement is complete, and even with olfactory deprivation, most MCs have a single apical dendrite projecting into a single glomerulus [109]. This suggests that spontaneous activity (independent from OSN inputs) may be sufficient for the refinement of MCs. To address this issue, it may be necessary to block neuronal activity directly in MCs and MTCs and to analyze their survival and morphology. The investigation of how olfactory inputs regulate the morphology and connectivity of MCs will require high-resolution studies tracking MC dendrites and synapses over time.

Activity-dependent development of granule cells

Compared with MC/MTCs, sensory activity heavily influences the survival of adult-born GCs and their synaptic connectivity. When neonatal- or adult-born GCs are subject to sensory deprivation during the critical period, they display fewer spines [162] and excitatory synapses [73, 75]. Loss of reciprocal output synapses triggered by sensory deprivation is prominent only during the synaptic development, but not seen when sensory deprivation is performed after the synaptic development is completed [73]. In addition, there is a critical period during which the survival of new neurons is influenced through sensory input manipulations [195], which parallels the developmental stage at which neurons have a high degree of plasticity. For instance, long-term potentiation can be induced in adult-born neurons during the early stages of their maturation, but not after this period [134]. It is an interesting question how different forms of activity-dependent plasticity relate to each other to eventually shape-specific synaptic organization patterns in newly added GCs. In addition, recent studies highlight that the importance of activity-dependent transcriptional regulators, such as *Npas4*, regulates other transcription factors and experience-dependent dendritic spine development of GCs [95, 202]. In addition, the aforementioned *5T4* regulates sensory input-dependent development of a subset of superficial GCs [201]. Here, links may be provided between sensory input-dependent development and the intrinsic molecular machinery set by precursor programs.

Glutamate receptors are the major source of excitatory drive to GCs and regulate various processes from migration to survival. Among glutamate receptors, NMDA receptors provided some unexpected insights on the developmental

regulation of interneuron wiring [70, 71, 94, 151, 152]. During the development, NMDA receptors are composed mostly of GluN1 and GluN2B subunits. In hippocampal pyramidal neurons, the deletion of GluN2B promotes the functional maturation of glutamatergic synapses, suggesting that the normal function of GluN2B-containing NMDA receptors is to set a brake on synapse maturation (e.g., [1]). In contrast, single-cell deletion of GluN2B in adult-born GC prevented the maturation of glutamatergic synaptic input [70]. This severe impairment in synaptic development was associated with a decreased response to novel odors and eventually led to the death of GluN2B-deficient adult-born GCs. The effect of GluN2B on GC survival is subunit specific, as it is not be rescued by GluN2A, the subunit conferring mature properties to NMDA receptor function. Thus, contrary to their function in glutamatergic neurons, GluN2B-containing NMDA receptors promote synapse activation in adult-born interneurons. The same synapse promoting actions are found for NMDA receptors in interneurons that are connected to the above-described glutamatergic pyramidal neurons in the developing hippocampus [71]. These findings suggest a homeostatic model that should keep circuit activity stable within a certain range. While increasing circuit activity that recruits NMDA receptors limits the sensitivity of glutamatergic neurons to excitatory inputs through setting a brake on synapse maturation, NMDA receptors promote the recruitment of inhibitory neurons to dampen circuit activity. It may also suggest that inhibitory interneurons and principal neurons utilize different molecular machineries and synaptic mechanisms to mediate these opposing effects on synapse maturation.

The effects of activity on the survival of GCs are not uniform to all GCs. GCs can be divided into deep and superficial GCs based on their dendritic branching in the EPL (Fig. 1b). The functional differences between deep and superficial GCs also extend to neuronal survival. Whereas neonatal-born GCs often reside in the superficial GCL, adult-born neurons tend to localize within the deep GCL [68, 90]. Although most superficial and neonatal-born GCs survive for long periods throughout the animal's life [68, 90], some recent studies suggest that most adult-born neurons are turned over and thus continuously replaced [68, 149]. However, two long-term studies found that cell death in adult-born GCs is largely limited to the first month after neuron birth [90, 193]. The question of long-term turnover of adult-born GCs warrants further clarification. Adult-born GCs that survive throughout more than half of the lifespan of rodents maintain synaptic densities similar to the ones that they had acquired a month after their birth [75]. In contrast, mature neonatal-born neurons continue to change net synaptic densities in some of their dendritic domains over the same period of time [75], raising the

possibility that particularly neonatal-born GCs are suited to adapt to changing environments throughout the animals' life. Whether long-term synaptic re-arrangement and the differences in negative selection over months are set in distinct molecular programs in 'neonatal' and 'adult'-born GCs, need to be determined out in future studies.

Synapse formation of GCs occurs mostly in the third and fourth weeks of the development [26, 72, 192]. This period coincides with a time window during which the survival of GCs is most sensitive to sensory deprivation and reduced by half in a deprived bulb [149, 195]. The effects of olfactory deprivation on the synaptic development are complex: Adult-born GCs that survive after sensory deprivation display an increased density of proximal input synapses in the unbranched apical dendrite [73]. This observation suggests that neurons may compensate for the absence of sensory input by receiving additional excitatory drive arriving mostly from the olfactory cortices, which may elevate their activity level above the threshold required for survival. Olfactory learning increases spine densities in the basal and proximal domains of adult-born GCs and promotes remodeling of both excitatory and inhibitory inputs selectively in these domains [91]. Glutamatergic top-down inputs from the olfactory cortex innervating these domains become active during postprandial rest and are thought to promote the elimination of newly added GCs [81]. These observations suggest that different sources of input and potentially also their interplay on nascent synapses regulate the stable integration of new neurons in the adult olfactory system. It may need to be considered here that it is not clear whether the effects of sensory deprivation and enrichment on GC survival are due to direct inputs from MCs and MTCs. Pioneering descriptions [35, 187] speculated that top-down projections from AON to MOB 'may permit more central structures to coordinate olfactory processing with ongoing behavior and endocrine or nutritional requirements' [35]. For instance, food odors evoked higher MC/MTC firing responses in hungry rats than satiated animals [141]. Top-down projections have been implicated in state-dependent modulation of olfactory bulb activity [76, 141, 172, 187, 196]. These and other studies had, however, not addressed how AON top-down inputs modify odor processing in this or other behaviors, including social interaction. A recent study revealed that the neuropeptide Oxytocin acting in the AON is critical for olfactory sampling and recognition of conspecifics [136]. Oxytocin increases transiently the excitability of AON regular-firing neurons and the synaptic drive in the AON network [136]. AON excitation through Oxytocin propagates through top-down projections to increase glutamatergic synaptic input to GCs. In vivo, we also found putative GC units that display transient increases in their firing rate following

Oxytocin receptor activation in the AON. Oxytocin in the AON increase the activity of GCs, and thus indirectly provides inhibition to MC/MTCs. Interestingly, similar to GC apoptosis during postprandial sleep [81], single-social interactions that activate AON top-down projections appear to promote death of adult-born GCs that are just in the process of wiring (Oettl, Ravi, Huber, Kelsch, unpublished observations). The elimination of new adult-born GCs was maximal after Oxytocin release was triggered during that social interaction. Yet, contrary to postprandial sleep, the GC elimination following social interaction appeared to occur at a more delayed time scale; possibly arguing for a slow process in adaptive circuit modifications rather than a function in relatively short-lived social memory.

The relationship between intrinsic excitability and synapse formation adds to the fascinating complexity of MOB assembly. Recent experiments indicate that raising the intrinsic excitability of adult-born GCs by expression of a voltage-gated bacterial sodium channel does not affect the synapse formation or eventual synapse densities [73], but promotes the survival of adult-born GCs [94]. Interestingly, increased intrinsic excitability blocks sensory deprivation-triggered synaptic changes. GCs that express a virally delivered bacterial sodium channel develop a normal organization of glutamatergic input synapses when these GCs are added to a bulb deprived of sensory input [73]. Somewhat surprisingly, dampening the excitability of new GCs by overexpressing a potassium channel does not affect the synapse numbers of the surviving neurons [94]. Taken together with the finding that olfactory deprivation decreases the survival of adult-born GCs [195], these observations support that synaptogenesis in adult-born GCs is sensitive to changes in synaptic input and suggest that both survival and synaptic development are driven by a multiple sources of excitation with distinct effects.

Beyond cortical top-down and sensory bottom-up inputs that seem to play opposing roles, neuromodulatory input, such as acetylcholine, noradrenaline, or neuropeptides, exert their actions. For instance, cholinergic stimulation causes sustained depolarizations in GCs [153] and enhances the survival of new GCs [111] and may be responsible for the reported enhanced survival of adult-born neurons when olfactory tasks involved increased attention levels [4]. Hence, phasic excitation provided by synaptic input from MCs and MTCs is only one of the many determinants of survival and wiring of new GCs in the circuits.

In summary, the existing data point to a fascinating interaction of intrinsic precursor programs that determine the cells' synaptic properties and intrinsic excitability, and prepare them to respond in particular ways to circuit

activity that in itself can have multiple actions. This arising picture supports the potential of the MOB in elucidating the interplay of genetic determination and neuronal activity in the assembly of the circuit and later plasticity through continuous synaptic reorganization or neuronal addition.

Sensory activity in the development of the anterior olfactory nucleus

Relatively few studies exist on the activity-dependent wiring of AON neurons. Sensory deprivation for the first 30 days of the postnatal life results in a smaller ipsilateral AON, while few changes are observed on the contralateral side and do not appear to affect either overall laminar growth or the dendrite formation of pyramidal cells [9, 10, 21]. While inter-hemispheric AON projections exist from birth [108], substantial postnatal maturation still appears to occur. At a behavioral level, up to P12, conditioned memories are stored unilaterally [86, 88]. After P12, memory retrieval with odors works also on the contralateral side following unilateral conditioning presumably due to the maturation of the anterior commissure. Severing the anterior commissure resulted in a return to unilateral olfactory learning [89] with the anterior aspects of the AON being required for learning [87]. Future studies may reveal the genes and cellular processes that control the connectivity and synaptic wiring within the AON and the regulatory mechanisms that govern the innervation of interneurons in the MOB. With respect to GC wiring, it might be particularly interesting to understand the regulation of different synaptic input densities on GCs and their downstream consequences on GC output synapses.

Precursor programs in the formation of neuronal connectivity

In the following section, we will focus on some recently discovered aspects of cell-intrinsic features of newly added GCs and the interactions of these intrinsic features with brain environment. Insights into the factors that are either activity-dependent or already determined in neuronal precursors, may help to build a framework to then detail the molecular machinery underlying the formation of synaptic connectivity patterns. Recent studies revealed an unexpected level of determination of synaptic wiring features in precursor programs: features that had previously been thought to be controlled exclusively by activity-dependent mechanisms in mammals [74, 116, 158]. In particular, GC wiring has helped to identify synaptic wiring principles and to attribute them to activity-dependent processes or the early determination in precursor programs, respectively (Fig. 3).

Dendritic targeting of granule cells

The lateral dendrites of MC/MTCs, which form dendrodendritic synapses with GCs, are located in the deep and superficial EPL, respectively (Fig. 1b). Most GCs ramify distal dendritic branches only in one location within the EPL, not in both [74, 125]. This phenomenon turned out to be genetically predetermined in neuronal progenitors as demonstrated by the fate-mapping and transplantation studies [74, 116]. Recent studies support that sonic hedgehog family and related transcription factor signaling contributes to the preferential generation of GCs with deep or superficial dendritic targeting [61, 79]. Hence, MCs and MTCs that constitute functionally independent microcircuits [45, 60] are targeted by the distinct populations of GCs through their dendrites in mammals. This microcircuit-specific targeting of new neurons in the adult brain is consistent with the proto-map model of circuit assembly [156] and raises the possibility to genetically engineer stem cells to generate specific neuronal types to replace those lost to disease or injury.

Synaptic wiring of granule cells

As detailed in the previous sections, GCs display different patterns of synaptic development depending on whether they are generated in the neonatal or adult brain [72, 144, 192] (Fig. 3). It has been an open question whether GCs adopt the synaptic input patterns typical of the host circuit, in which they integrate. Alternatively, the differences in the synaptic input patterns in neonatal- and adult-born GCs may be an intrinsic property determined already in their neuronal progenitors. If so, progenitors proliferating at different times in the animal's life may generate GCs that do not revert their synaptic input patterns when challenged by hetero-chronic grafting. Indeed, recent grafting experiments revealed that the synaptic input patterns are determined to a surprising extent already in their progenitor cells [158]. The specification of subventricular zone progenitors thus does not only dictate the neurotransmitter phenotype and the position of the neurons in different layers [50, 116, 157], but is also linked to the formation of characteristic synaptic input patterns. In adult-born GCs, the spine densities increase in the distal domain before synapses mature with a substantial delay to proximal inputs [11, 72, 192]. These proximal synapses form and become functional early on. The temporal dissociation in domain-specific synapse maturation persists after hetero-chronic grafting of adult progenitors [158]. Furthermore, neonatal precursors differentiating in the adult retain their characteristic early increase in synapse density in the distal domain, while those synapses continue to form late in iso-chronically grafted adult-born GCs. Together, these

findings reveal that the circuit itself permits for variable wiring patterns as GCs derived from neonatal and adult progenitors retained their respective 'neonatal' and 'adult' wiring patterns upon grafting to the adult host. Grafted GC precursors were mostly Mash-1-positive fast-amplifying cells. Future studies may clarify whether these properties are already determined at even earlier stages, such as the slowly dividing stem cells.

GCs derived from neonatal and adult progenitors also differ in their timing in the migration and initial outgrowth of their dendrites. The differences in the proportion of GCs taking position in the MOB and the extent of dendritic branch development do, however, not anymore differ between grafted GCs origination from neonatal- and adult-born GCs when the synaptogenesis starts. Different synapse patterns then evolve when the progenitors differentiate in a hetero-chronic environment. It is, therefore, possible that the early differences in migration or dendrite formation will, through yet unknown mechanisms, still influence the eventual synaptic patterns of GCs. These effects at multiple steps of GC differentiation resemble observations for genetic manipulations of transcription factors in development.

The synaptic connectivity patterns can, however, only be formed in matched circuits. This was supported when grafting adult GC progenitors into the neonatal subventricular zone [158]. While the new GCs display normal migration and dendrite formation as well as the initial synapse formation, they ended up with low synapse densities in the proximal and distal domains towards the end of their maturation. The densities neither match neonatal- nor adult-born GCs, suggesting that cell-intrinsic programs require factors from contacting cells and that these extrinsic factors are only present at certain developmental stages in the MOB. The basal domain, however, develops the normal densities of input synapses, and the formed distal and proximal synapses become functional. In addition, the hetero-chronically grafted GCs survive for at least 2 months. These latter observations support that different aspects in the maturation are at least partially independently regulated. In line with the interactions of precursor programs and brain environment, certain molecules are upregulated in the adult MOB (Tenascin-R, mir-125) and may provide adult-born GC compared with neonatal-born GC with unique intercellular signals that are at work when the new neurons wire in the respective circuit. The surprising extent of determination at precursor cell stage of cell-type-specific connectivity and formation of characteristic organizations of input and output synapses may provide a framework to further identify the underlying transcriptional programs.

Summary and outlook

In summary, recent studies have revealed a relatively good understanding of the basic order of circuit assembly with some of the influencing factors being identified. However, the later steps in the differentiation of new neurons, i.e., formation of specific synaptic connectivity patterns within the circuit, need further elaboration. While more is presently known about the synaptic wiring of the interneurons, other aspects in the formation of MOB connectivity are still largely unknown. Much needs to be studied to understand the interaction between the projection neurons and interneurons in the MOB.

A first picture has emerged for the broad factors determining the synaptic makeup of interneuron types. Here, recent work has revealed a strong genetic component in the formation of specific synaptic organizations within cells and wiring pattern among those cells, yet the exact molecular pathways still need to be elaborated. The impact of the unexpected extent of intrinsic determination of cell-type specific wiring and formation of synaptic organizations warrants further study to understand the logic of circuit assembly and harness them for neuronal repair strategies providing targeted repair with controlled synaptic connectivity patterns.

In addition, the exact interplay of this intrinsic determination with activity-dependent processes is just beginning to be understood. Here, interrogating the connections between MCs/MTCs and the major interneuron type GCs through conditional genetics in combination with synaptic and circuit physiology and reconstruction of genetically labeled synapse types may provide a strategy to understand the influence of the surrounding neurons on the maturation of synaptically connected cells. The actions of trophic and activity-dependent effects are expected to overlap on these developmental processes. With relevance to activity-dependent processes, bottom-up olfactory inputs and central top-down inputs may differentially influence the refinement of the connectivity among the different neuron-types.

As this review indicated, no molecules seem to act only on one discrete cell-type or the stage of differentiation. As evident from the influence of OSNs on MC/MTC differentiation, trans-synaptic changes in developing neurons following the manipulation of specific molecules in one cell-type can be of critical importance to the developmental behavior in cells that form synapses with them. It might, therefore, be of particular interest to study the interactions of cell-type-specific manipulations on contacting cells to understand the logic of circuit assembly and the contribution of cell-intrinsic determination already present at

precursor stage and still active at the late stages when the synaptic connectivity is formed.

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