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Understanding brain development – Indian researchers' past, present and growing contribution

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Abstract

The brain is the seat of all higher-order functions in the body. Brain development and the vast array of neurons and glia it produces is a baffling mystery to be studied. Neuroscientists using a vast number of model systems have been able to crack many of the nitty-gritty details using various model systems. One way has been to size down the problem by utilizing the power of genetics using simple model systems such as *Drosophila* to create a fundamental framework in order to unravel the basic principles of brain development. Scientists have used simpler organisms to uncover the fundamental principles of brain development and also to study the evo-devo angle to brain development. Complex circuitry has been unraveled in complex model systems, such as the mouse, to reveal the intricacies and regional specialization of brain function. This is an ever-growing field, and with newer genetic and molecular tools, together with several new centers of excellence, India's contribution to this fascinating field of study is continually rising. Here, I review the pioneering work done by Indian developmental neurobiologists in the past and their mounting contribution in the present.

Keywords

developmental neurobiology; India; Drosophila; mouse; chick; zebrafish; developing brain; neuron; glia

Introduction

The human brain has 86 billion neurons. Yes, this is indeed the right number and not the ballpark of 100billion as reported widely for several years (Azevedo *et al.*, 2009). As we seamlessly interact with the world around us, we forget that each of these neurons have to be generated from scratch from a common pool of cells called stem cells.

The building of the neural network consists of several steps. It begins with the proliferation of the stem cells followed by fate specification into neurons, neuronal migration and glia generation followed by synapse formation and connectivity to form functional circuitry. Indian developmental neurobiologists working in a range of model organisms have contributed immensely in unraveling many aspects of this fascinating journey of making a

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functional brain. Their work creates a framework for understanding the evolution of this intricate structure, its complex functions and mutations which cause neurodevelopmental disorders like autism, schizophrenia, epilepsy and seizures.

This review is dedicated to the study of contributions made by them in the past and present in this fascinating and rapidly advancing niche field of developmental neurobiology.

Development of the olfactory nervous system in *Drosophila melanogaster*

Work from the lab of Prof. Veronica Rodrigues

The first seeds for studying nervous system development in India were sown by scientist and leader Prof. Veronica Rodrigues who studied insect olfaction on a multitude of fronts. Utilizing the power of *Drosophila* genetics her lab initially at the Department of Biological Sciences at the Tata Institute of Fundamental Research (TIFR), Mumbai and later at the National Centre for Biological Sciences (NCBS), Bengaluru elegantly showed the regulation of multiple aspects of the development of the olfactory system. The various sensory modalities provide vital information to the brain regarding its surrounding environment. In order to create a faithful internal representation of the external world in the brain, the incoming sensory information must be organized accurately, and this involves one to one matching of the pre-synaptic inputs into postsynaptic outputs (Vadakkan 2015). A well-studied example of this organization comes from studying the olfactory sensory nervous system.

The initial work in Prof. Rodrigues lab was dedicated to characterizing the various cellular events during the development of the olfactory sensory organs, which are involved in the mechanism of sensing the odorant molecules. They characterized both the intrinsic and the extrinsic cues that lead to cell fate specification, positioning and ultimately the development of the olfactory sense organs (Ray *et al.*, 1995; Gupta *et al.*, 1998; Jhaveri *et al.*, 2000 and Sen *et al.*, 2003). This was then followed by an equally pioneering contribution by her and her collaborators towards the study of the brain regions where the olfactory information is coded.

The olfactory nervous system consists of a large number of highly specialized neuronal cell types (Vadakkan, 2015). The olfactory sense organs project from the antenna of the fly to distinct glomeruli in the antennal lobe (Fig. 1). The olfactory receptor neurons (ORNs) or the olfactory sensory neurons send their axons from the antennae to the antennal lobe and are associated with different types of glial cells. Glomeruli are densely packed neutrophils within the antennal lobe, composed of synaptic contacts between the incoming sensory neurons and lobe interneurons and act as functional units of odor coding. Each olfactory neuron has a selective expression of a single odorant receptor and connects synaptically to a single type of relay neuron in the primary olfactory central nervous system (CNS) region (Fig. 1). Using classic genetic markup studies like the MARCM (see Box for definitions) based clonal analysis and genetic ablation studies her lab has made some insightful contributions in the study of the formation of the olfactory nervous system.

In the first of the papers, her lab has outlined the precise events that occur during the development of the antennal lobe in the fruit fly. Work from her lab has shown that pattern formation in the olfactory lobe is a result of complex interaction between the sensory neurons, glial cells and the lobe interneurons (Jhaveri *et al.*, 2000). Neurons from the sense organs are organized into distinct fascicles by the glial cells (Fig. 1). A majority of the glial cells in the antennae arise from the lineage of the sensory precursors expressing the gene *Atonal.* Loss of *Atonal* disrupts the fasciculation of neurons by the glia. The olfactory afferents when they enter the brain, they remain at the periphery and do not show any arborisation, and even the glomeruli at this stage are not organized. It is the simultaneous entry of the sensory neurons along with the glia, which leads to the formation of distinct glomeruli in the olfactory system. Their work underscores the importance of how sensory neurons influence the development of the olfactory antennal lobe and pinpoints the coordination between peripheral and central nervous systems in generating the necessary olfactory behavior.

They further address crucial aspects of the glomeruli formation by studying the *Atonal* mutants where a class of sensory neurons called pioneer neurons are not formed. The *Atonal* lineage sensory neurons are the pioneer neurons that enter the glomeruli first and are necessary for the precise targeting of the other neurons into the lobe. In the absence of the *Atonal* neurons, other sensory neurons fail to enter the glomeruli initially and remain stalled at the periphery. Ultimately they enter the lobe but fail to reach the correct glomeruli target. The peripheral glial cells also fail to project appropriately, and the glomeruli interneurons fail to terminate in the glomeruli (Jhaveri and Rodrigues, 2002).

Finally, they pinpoint towards the guidance cues, which are crucial for the formation of the olfactory map. The Robo-Slit signaling is an evolutionarily conserved pathway involved in axon guidance of growing and migrating neurons. They described the involvement of this evolutionarily conserved Robo-Slit signaling as a critical component for the sensory neurons to be within defined sites within the glomeruli and also for the formation of tracts, which connect the two halves of the brain. The absence of Robo in the sensory neurons results in their aberrant targeting of the sensory neurons (Jhaveri *et al.*, 2004).

Her lab then shifted their attention towards addressing the developmental mechanisms playing out in the neuroblasts from which the various projection neurons and interneurons of the *Drosophila* brain are derived. Using classic MARCM clones, they demonstrate that the same neuroblasts first give birth to interneurons and later go onto produce projection neurons. These neuroblasts are specified by the cephalic gap gene *empty spiracles.* Loss of *empty spiracles* led to the loss of both interneurons and the projection neurons (Das *et al.*, 2008) whereas in a separate study neuroblasts from the mutant-Notch lineage led to the loss of interneurons with a concomitant increase in projection neuron numbers (Das *et al.*, 2010). Thus work from her lab shows the existence of distinct neuroblast types derived from specific lineages, which work simultaneously to produce the various neuronal cell types in the olfactory system.

Dendritic refinement is amongst the final steps in the making of a functional neural network in the brain. Accurate synaptic contacts made by the dendrites ensure the right connectivity

between the neurons in the different regions of the brain. To understand how dendritic refinements underscore neural connectivity they first identified a wide field serotonergic neuron in the antennal lobe of *Drosophila* brain called the CSDn. These neurons undergo remodeling during metamorphosis leading to lesser number of dendrites in adult flies. Cell-autonomous neural firing, possibly mediated by the Wnt signaling, plays a vital role in this refinement. Reduction in excitability or loss of Wingless *(Drosophila* Wnt1) and knockdown of NMDAR receptors all affect the process of refinement negatively (Singh *et al*., 2010). Thus using a central neuron in the *Drosophila* brain, they were able to elegantly demonstrate the role of neuronal firing, Wnt signaling and NMDAR in dendritic form, remodeling and refinement.

Thus her lab's contributions span a broad understanding of the olfactory nervous system development in fine details, ranging from the specification of neurons and glia and their role, to axon outgrowth and dendritic arborisation.

Work from the lab of Prof. VijayRaghavan

A significant contribution has come from the lab of the ace development geneticist and neurobiologist in the country Prof. Vijayraghavan. He has also been a steadfast collaborator with Prof. Veronica Rodrigues in establishing developmental biology in the country and employing *Drosophila* as an elegant model system to ask and answer fundamental questions for understanding development and behavior.

Serotonin a key monoamine neurotransmitter has varied roles in behavior in fruit fly behavior like sleep, circadian rhythm, aggression, learning and memory (Yuan *et al*., 2005, Yuan *et al*., 2006, Sitaraman *et al*., 2008, Alekseyenko *et al*., 2010 and Johnson *et al*., 2011). Structural organization during development often determines emergent function in adult life. They highlight this aspect in a work on CSDn serotonergic neurons in the *Drosophila.* Serotonergic neurons have diffuse neuromodulatory function in that a few neurons modulate multiple brain circuits. The specificity is then dependent on the one to one differential innervation patterns carried out at the very end of the dendritic terminals between the various neurons. Impinging on the fact that for a large number of sensory, projection and interneurons there exist a single serotonergic neuron in the *Drosophila* brain makes it an ideal system to study the role of serotonin in modulating olfactory behavior in the fly. In Singh *et al*., 2013 his lab has demonstrated that Ephrin and Ephrin receptor signaling between the sensory neurons and serotonergic neuron determines the pattern of arborisation in some glomeruli. Disrupting this signaling leads to arborisation defects in that repulsive high Ephrin expressing glomeruli restrict the growth of CSDn terminals during development. Finally, they correlated this alteration in the fine dendritic structure with a tangible behavioral outcome of pheromone-mediated response to mating. cVA, a male pheromone, transferred to females from males during courtship renders them less attractive to males during subsequent encounters. They first demonstrated that CSDn modulates this behavior in flies and blocking neurotransmitter release from CSDn led to males exhibiting increased courtship to females treated with cVA. In Eph hypomorphs with increased dendritic innervations, the males showed increased sensitivity to cVA leading to decreased courtship.

This study establishes a clear structure-function relationship and highlights the importance of how structure determines behavioral function.

Cell-intrinsic determinants acting within progenitors or stem cells play a significant role in timing neurogenesis and deciding neuronal lineage. His lab focused on two kinds of neuroblasts in the developing fly brain. One expressing the gene *orthodenticle* leads to the specification of wide field interneurons that innervate the central complex and has multiple roles including controlling fly movement.

The other kind of neuroblast does not express *orthodenticle* and leads to the specification of olfactory interneurons. Deletion of *orthodenticle* from the neuroblasts leads to a complete and total switch in the molecular properties, structure, and functional innervation such that the entire central complex neuron lineage transforms into the olfactory projection neuron lineage (Sen *et al.*, 2014). This suggests that cellintrinsic mechanisms particularly transcription factors which bind crucial gene regulatory elements and dictate gene expression are instructive determinants of neuronal lineage.

Interestingly the mammalian homologue of orthodenticle Otx1 is similarly functional in the progenitors and controls cortical size by regulation of progenitor proliferation (Simeone, 1998). Thus fundamental mechanisms chalked out in *Drosophila* highlight conserved roles for genes in complex mammalian brains.

These studies using *Drosophila* as a model system have yielded in-depth insights into the working of a simple brain and paved the way for studying complex organisms as can been in the spectacular examples as below.

Nervous system development in mammalian systems

The cerebral cortex is the seat of sensory perception, decision-making, language, learning and memory. The cerebral cortex is a complex brain structure that processes higher functions in distinct regions. Elucidating the mechanisms that generate the cerebral cortex remains a significant challenge facing modern biological research. The staggering complexity of the cortex makes it a difficult task. The cerebral cortex is a mammal-specific structure. In order to study the cerebral cortex, the mouse serves as an excellent model for studying mammalian brain development notably higher order centers like the hippocampus and the six-layered neocortex.

Work from the lab of Prof. Shubha Tole

Prof. Shubha Tole's lab in DBS, TIFR, Mumbai has been focused on unraveling multiple aspects of the mammalian nervous system development and has put India's contribution at the forefront in this niche field. In the sections below I highlight the seminal work done in her lab in furthering the understanding of forebrain development. The first section highlights the genetic control exerted by the multifunctional transcription factor LHX2 in making the brain. The second section highlights the identification of novel migratory streams in the forebrain and their importance in brain development.

***LHX2, a transcription factor that regulates multiple aspects of forebrain development* - Lhx2 *as a cortical selector gene* —**During nervous system development, cell fate choice is brought about by establishing specific gene regulatory networks, which define a distinct cell type (Guillemot, 2007). The gene regulatory networks which consist of transcription factors, the corresponding regulatory elements they bind and the chromatin modifiers are critical determinants of cell fate and are key modules for evolutionary and diseases mechanisms (Nord *et al.*, 2015).

One such transcription factor is LHX2, which belongs to the LIM-homeodomain family of transcription factors. The homeodomain binds to AT-rich motifs in the genome (Berger *et al.*, 2008), and the LIM domain interacts with other partners to form multimeric complexes (Chou and Tole 2018). Her lab has published several impactful articles on the role of LHX2 in regulating multiple aspects of cortical development. A landmark study published in Science in 2008, from her lab and that of Prof Edwin Monuki at University of Chicago, Irvine, established LHX2's role as a central molecule in specifying the cerebral cortex (Mangale *et al.*, 2008).

The developing dorsal telencephalon gives rise to the cerebral cortex (Fig. 2). This neuroepithelium consists of a developing cortical primordium ensconced in between two potent signaling centers at the two ends namely the hem and the antihem which are non-cortical tissue. In *Lhx2's* absence, the cortical primordium was totally shrunken and instead taken over by the expansion of the hem and the antihem. (Fig. 2). Thus LHX2 specifies the boundary between cortical and non-cortical tissue. Using elegant genetic mosaic mutants in which some cells were positive for *Lhx2* and some were negative, she and her collaborator Prof. Edwin Monuki were able to demonstrate elegantly, *Lhx2* to be a cortical selector gene. Cells which express LHX2 cluster together and take on a cortical fate and cells which do not express LHX2 take on non-cortical fate, i.e. hem and antihem. Thus in these genetic mosaics, they were able to create multiple ectopic hems and antihems (non LHX2 expressing tissue) adjacent to cortical neuroepithelium (LHX2 expressing tissue) (Fig. 2).

Interestingly, adjacent to every ectopic hem, an ectopic hippocampus formed thereby leading to the formation of multiple hippocampi instead of one. This study provided evidence for the first time the "organizer function" of the hem for specifying hippocampus. This organizer function is akin to the historic "Spemann's organizer" in making the body axis. (Grove E.A, 2008). This study answered some of the fundamental questions in brain development of that of cortical selector gene function, how the hippocampus is made, the role of "signaling organizers" in patterning brain and perhaps also showed a method of genetically inserting multiple hems in the growing brain. Her lab has followed up this landmark study recently by showing Lhx2's interaction with two other master regulators in the developing cortex namely FOXG1 and PAX6 in suppressing hem and restricting it to the midline (Godbole *et al.*, 2017 and Godbole *et al.*, 2018).

Lhx2 and the neuron glia cell fate switch—In the vertebrate nervous system, neural stem cells generate neurons first and then glia. This timing is regulated by the interplay of transcription factors and environmental cues (Miller and Gauthier 2007). A key question in

the neurogenic to gliogenic switch is: What restricts progenitors from making glia during the neurogenic period?

Notch signaling has an instructive role in the process of generating glia. Notch activates NFIA, which in turn activates glial specific genes in instructing the progenitors to take on a glial fate (Deneen *et al.*, 2006 and Namihira *et al.*, 2009). However, Notch signaling is active even during the neurogenic period. Her lab showed a crucial link in understanding this evolutionarily conserved process in the CNS system. LHX2 promotes neurogenesis and inhibits gliogenesis in the developing hippocampus (Fig. 3). Removal of LHX2 during the neurogenic period led to premature glia production at the expense of neurogenesis. Overexpression of LHX2 during the gliogenic period was sufficient to prolong neurogenesis late into development, at the cost of reduced glial cell numbers. LHX2 was able to induce neurogenesis even in the presence of activated Notch signaling or its downstream effector NFIA, which is both necessary and sufficient for astrogliogenesis. Thus LHX2 acts as a molecular brake, that prevents precocious glia production and ensures adequate generation of neuronal numbers before the glial are made (Subramanium *et al.*, 2011).

Recent work from her lab has further unraveled molecular aspects of this process. We identified a gene regulatory network with a bi-directional control in regulating the neuron-glia cell fate switch. Firstly we identified DMRT5 to be a novel regulator of neurogenesis in the hippocampus. DMRT5 expression was regulated by LHX2 and vice versa. Thus the two neurogenic genes had reciprocal control over each other's expression in the developing hippocampus. LHX2 was able to substitute for the loss of DMRT5 and vice versa. Loss of LHX2 or DMRT5 lead to premature glia production and overexpression suppressed gliogenesis while promoting neurogenesis. Finally, each transcription factor had opposing effects on the expression of established neurogenic genes namely Neurog2 and Pax6. DMRT5 decreases the expression of *Neurog2* and *Pax6*, and our work shows that LHX2 is required to maintain their expression levels (Muralidharan *et al.*, 2017b). Our study has deciphered a complex gene regulatory network with bidirectional control of a fundamental feature of the central nervous system development, the regulation of the production of neurons versus astroglia in the developing hippocampus (Fig. 4).

Molecular mechanism of Lhx2 action—With her lab having characterized so many different roles for LHX2 the significant next step was to decipher the molecular mechanisms by which LHX2 regulates these processes. Transcription factors exert their molecular phenotypes by regulating the expression of the downstream target genes to whose regulatory regions they bind to, and their mechanism of action is in turn governed by the chromatin complexes they are part of.

Thus to understand the molecular underpinnings of LHX2 action I worked towards identifying the downstream targets of LHX2 and its interacting molecular partners. This culminated in us deciphering a novel role for Lhx2 in the regulation of neuronal subtype specification in the neocortex (Muralidharan *et al.*, 2017a). The neocortex is the crowning glory of brain evolution. It has evolved an unparalleled cellular diversity to allow the brain to perform complex social behaviours. The diversity of neocortical cell types, their local and

long-range cortical circuitry and their potent functional properties make the study of their development, evolution and function a topic of great interest still.

Projection neurons are excitatory neurons in the neocortex, which connect the cortex to its various targets. They can be broadly classified into ones that connect within the cortex called intra-cortical or ones, which connect outside the cortex or corticofugal neurons. The corticofugal neurons are of two major subtypes (Molyneaux *et al.*, 2005). The corticothalamic – these are long-range neurons which connect the cortex to a region called the thalamus to modulate the incoming sensory information. The sub-cerebral projection neurons project to targets below the brain and include the corticospinal motor neurons which project to the spinal cord and affect movement. Our study, which began by identification of the various downstream targets of LHX2 in the developing neocortical primordium discovered LHX2 to be bound to the enhancers of transcription factors *Fezf2* and *Sox11.* These two transcription factors are crucial determinants for the specification of the sub-cerebral projection neurons.

Upon LHX2 removal in the postnatal brain, there's an increase in the numbers of sub-cerebral projection neurons at the expense of cortico-thalamic neurons. This suggested that LHX2 is critical in controlling the numbers of the different sub-classes of projection neurons in the brain with varied functions.

We correlated this postnatal increase in the number of one class of neurons with an upregulated expression of Fezf2 and Sox11 during embryonic development. Furthermore, our mass spectrometry data revealed that LHX2 is part of the Nuclear remodelling complex (NuRD), which it recruits to the enhancers to suppress the expression of its target genes namely *Fezf2* and *Sox11* by altering the epigenetic signature (Fig. 5). Very few studies in the field of neocortical development bring such compelling insight into understanding mechanistic details of neuronal subtype specification. This study, which begins from deciphering the targets genes, their epigenetic status finally connects the workings in the nucleus to the functional outcome of altered neuronal numbers.

Uncovering novel migratory streams in the developing brain—The amygdala controls species-specific behavior including, flight-fight responses feeding and reproduction and also forming and processing emotion-associated memories and responses. The amygdala is an ancient structure and is seen from the amphibians, reptiles to mammals whereas the neocortex is the newest part of the cortex to evolve. Her much-acclaimed paper in the prestigious journal Nature neuroscience discovered a novel migratory stream called the caudal amygdaloid stream (CAS), which originated not from the amygdala but the domain where neocortical cells originate. Thus her seminal work presented evidence for an evolutionary, developmental and mechanistic link between the amygdala and the neocortex (Remedios *et al.*, 2007). Her group in a few years again published in Nature Neuroscience a similar novel migratory stream, which contributes projection neurons to the pheromone sensing accessory olfactory bulb (AOB) (Huilgol *et al.*, 2013).

Olfaction or the sense of smell is essential for a variety of behaviours like feeding, mating, fear and aggression. In the rodents the olfactory system is bipartite – the main olfactory

system, which is responsible for the sense of smell, and the accessory olfactory system which is vital for pheromone-based sensing (Halpern and Marcos, 2003). The AOB consists of the posterior aspect and the anterior aspect. Seminal work from her lab identified a developmental basis for the functional subdivisions of the anterior and the posterior AOB and showed that the two subdivisions differ vastly in their developmental trajectories. Projection neurons of the aAOB originate locally whereas the projection neurons of the pAOB come long distance traversing the entire length of the telencephalic surface. They described a very unusual trajectory for the migration of neurons from one far end of the brain to the pAOB. Further, they demonstrated evolutionary conservation of this migration in the frog, *Xenopus.*

Her lab has made some unparalleled discoveries in elucidating the mechanisms of development of structures such the hippocampus, amygdala, olfactory bulb and the neocortex which are the centres for learning, memory and emotion in the brain. Also, put India's contribution firmly on the international map in the field of cutting edge molecular developmental neurobiology. Her work on understanding developing nervous system has created a framework to understand what goes wrong in neurodevelopmental disorders like schizophrenia, autism and epilepsy.

Work from the lab of Prof Shyamala Mani

Development of the cerebellum—Prof. Shymala Mani's lab initially at the National Centre for Brain Research at Manesar and later at the Centre for Neuroscience, indian Institute of Science, Bengaluru, worked towards understanding the development of the cerebellum. The cerebellum is the region in the hindbrain, which receives information from the sensory systems, spinal cord, and other parts of the brain and coordinates motor movements. It contains the second main cortex in the brain and is comprised of 50% of the neurons in the brain. As with all regions in the brain, the granule neurons of the cerebellum are produced from the mitotic progenitors adjacent to the ventricle.

Her lab's work has characterised the role of centrosome positioning and mitotic spindle control in the progenitor and the newly postmitotic neuron (Mishra *et al.*, 2008). The position of the centrosome during final mitosis is crucial for marking the point of emergence of the future axon. Work from her lab has shown that GAP-43, an actin-binding protein regulates progenitor proliferation and cell cycle exit. It is also required for the centrosome positioning and generates the neuronal polarity in the cerebellar granule neurons. In the absence of GAP-43, the cell cycle was extended, and the mitotic spindles were disorganised. This disorganisation, which affected the centrosome positioning, led to migration defects in the newly postmitotic granule neurons.

In a follow- up study they have also developed a novel micro-patterning assay set up to spatially segregate the extrinsic cues received by the polarising granule cells from the intrinsic cues. They used this assay to determine the effect of external vs internal mechanisms used by the granule cells to orient their centrosomes to orient axon extension. From this elegant setup, they determined that GAP43 coordinates both the extrinsic and intrinsic cues to orient the centrosome to polarise the cerebellar granule neurons (Gupta *et al.*, 2010).

In a cross-species study between mouse and humans, her lab studied the role of a critical signalling pathway in development, the Shh pathway. They analysed the expression pattern of Shh, its receptor patched, smoothened, and its effectors belonging to the Gli family of transcription factors from both human and mouse developing cerebellum and also from medulloblastomas which show abnormal cell proliferation in the cerebellum to highlight cross-species differences in the regulation of the Shh pathway (Haldipur *et al.*, 2012).

Maternal care and fetal development—Fetal development is strongly linked to maternal nutrition. Her lab established a mouse model to understand the critical role of maternal nutrition in fetal development. Mothers fed with low protein diet resulted in offsprings with motor deficits. These animals also showed defects in the proliferation of progenitors and reduced thickness of external granule cell layer in the cerebellum. Thus this study established a correlation with a low-protein diet and the development of the cerebellum (Ranade *et al.*, 2012).

Her lab has also shown how preterm delivery affects the developmental program of the cerebellum as rapid cerebellar development takes place during the last trimester of the gestation. Pre-term delivery leads to decreased thickness and increased density of neurons in the different regions of the cerebellum. Thus the cerebellar development is compromised because of pre-term delivery (Haldipur *et al.*, 2011).

The last decade or so has seen many new scientists venture into the fascinating field of developmental neurobiology utilizing newer model systems in India. In the following sections I define the contributions of these upcoming labs to showcase the current and rising contribution of Indian researchers in the field of developmental neuroscience.

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Work from the lab of Prof. Jonaki Sen

By comparing brain development in different species, one can gain significant insights into the origin of the mammalian neocortex and also obtain valuable information on evolutionary changes that might have occurred into reaching the pinnacle of evolution- the human neocortex. Comparative and genetic analyses contribute towards understanding the rules of cortical development and shed light into uncovering the deeper workings of the mammalian and primate brains (Molnar *et al.*, 2006).

Prof. Jonaki Sen's lab at the Department of Biological Sciences at Indian Institute of Technology (IIT) Kanpur is working in two model systems namely the chick and the mouse to understand comparative nervous system development. Recent work in her lab has uncovered conserved gene expression patterns in the hippocampus of the two model systems thereby revealing structural homology between avian and mammalian hippocampus. This reinforces the idea that the basic principles of development are well conserved in evolution. This study also revealed the origin, production, survival, migration and differentiation of the chicken hippocampal neurons. Besides delineating conserved expression patterns, this work also identified molecules showing differences in gene expression pattern between the chick and the mouse hippocampus. These are yet uncharacterized genes whose role in hippocampal fate specification if any is not yet known. These could potentially pinpoint

towards interesting differences in the hippocampal development between avian and the mammalian brain (Gupta *et al.*, 2012).

The roof plate is a signaling center in the forebrain, which invaginates to form the Choroid plexus and the cortical hem. Further the choroid plexus and the cortical hem pattern the different regions in the developing forebrain. In a recent paper in Development, her lab shows that the retinoic acid signaling in the chick forebrain is both necessary and sufficient for inducing roof plate characteristics in the developing forebrain unlike the mouse developing roof plate. The absence of retinoic acid leads to holoprosencephaly-like phenotype (Gupta and Sen 2015). The findings indicate a novel role for retinoic acid signaling in chick forebrain development.

Neuronal migration is one of the critical steps in making a functional neural network. It is the method by which neurons travel from their birthplace to their final position in the brain. It is a ubiquitous feature of development that brings neurons into appropriate spatial regions and facilitates their interaction via synapse formation. Abnormal neuronal migration leads to structural disorders of the nervous system like epilepsy developmental delay, mental retardation and seizures (Kanatani *et al.*, 2005).

Very recent work from her lab has elucidated a novel role for BMP signaling in neuronal migration of upper layer cortical neurons. Upper layer cortical neurons are the ones that project intra-cortically and consist of the callosal projection neurons, which connect the two hemispheres of the brain. Inhibition of BMP signaling leads to defective migration in the neurons, which did not reach their specified location in the radial column. Further, the inhibition resulted in neurons being disorganized and misaligned in the upper layer with reduced dendritic branching. The above defects were because of misregulation of both the canonical and non-canonical BMP signaling pathway (Saxena *et al.*, 2018).

Work from the lab of Dr. James Jackson

Dr. James Jackson’s lab at the Rajiv Gandhi Centre for Biotechnology, Trivandrum, has characterized the role of signaling molecule Wnt5a and the transcription factor Tlx3 in the development of the cerebellum. Work from his lab has shown that loss of Wnt5a in the cerebellum leads to hypoplasia, which is a result of reduced proliferation of progenitors. Overall there’s reduction in both excitatory and inhibitory neurons in the cerebellum and further the neurons display poorly branched dendritic arbors. Molecularly Wnt5a regulates key proliferative genes in a beta-catenin independent manner in the cerebellar cells (Subashini *et al.*, 2017). In a separate study they define a novel role for a transcription factor Tlx3 that is crucial for regulation of genes involved in synaptic connection and neuronal migration in the developing cerebellum. Its expression is specific to the posterior lobes of the developing cerebellum and is induced by Pax6 (Divya *et al.*, 2016). They thus describe the interaction between Pax6-Tlx3 specifically in the developing cerebellum and its deregulation could possibly lead to neurodevelopmental disorder like autism.

The vast numbers of neurons are all born from a common pool of stem cells- the progenitors in the ventricular zone. These neurons are generated in a timely fashion with the synthesis of deep layers of neurons first, followed by a switch in the progenitors to start producing upper

layer neurons later in development. Thus the progenitors are progressively fate-restricted in their neurogenic potential as development proceeds (Molyneaux *et al.*, 2005). Time and again identification of newer progenitors with distinct molecular signatures with exceptions to the above rule shed immense light into the temporal dynamics of neurogenesis in the brain. Notable examples of progenitors with distinct characteristics include the Cux2 positive progenitors, which give, rise to exclusively the upper layers and are thus fate-restricted but are found to exist in the ventricular zone even when the deep layers are generated (Franco *et al.*, 2012).

Dr James Jackson's lab has identified similarly a newer class of progenitors in the ventricular zone under the control of the Notch signaling pathway. Hes1 is a transcription factor under the direct control of Notch canonical signaling pathway. Notch dependent activation of Hes1 is essential for the maintenance and proliferation of radial glia cells in the ventricular zone (Ohtsuka *et al.*, 1999). These are rapidly dividing progenitors, which undergo asymmetric divisions to give rise to neurons. In their recent paper in Cerebral cortex, Dr James Jackson and colleagues have shown the existence of a Notch-independent Hes1 expressing neural stem cells which are slow dividing and precede the Notch-dependent Hes1 expressing radial glia (Dhanesh *et al.*, 2016). Perhaps these Notch-independent Hes1 stem cells mark the prospective radial glia and represent a crucial step in the transition of neuroepithelial precursors into radial glia progenitors generating neurons.

Two other labs deserve mention in this review for their upcoming papers in this field. Among the many extraordinary features of the developing nervous system is the ability of the growing axons of neurons to find the right synaptic partners in the complex embryonic brain terrain leading to the formation of the accurate neural circuitry. This process is performed by growth cones, which are specialized structures at the extending tip of axons. Growth cones are highly motile structures, which sense the extracellular environment to determine the direction of growth and then guide the axons in that direction. Directional motility of growing axons is achieved by localized remodeling of the actin cytoskeleton in the growth cone (Gomez and Letourneau, 2014).

Prof Aurnab Ghose's lab at the Indian Institute of Science Education and Research (IISER) Pune had identified an unanticipated role for Formin2 in this process of growth cone-mediated axon extension. Formin2 is an actin-binding protein, which is involved in actin-cytoskeleton assembly and reorganization (Vizcarra *et al.*, 2011). It mediates spindle positioning and meiosis by generating contractile actin networks. Their study shows that Formin2 is highly expressed in the chick spinal cord tissue. Further investigation revealed it to be localized in the filopodial actin bundles in neuronal growth cones. Loss of formin2 led to decrease in growth cone area and filopodial numbers and length thus affecting filopodia dynamics. Formin2 performs its function by regulating the focal adhesion stability and depleting it from the developing spinal cord affects the crossing of the midline by commissural neurons (Sahasrabudhe *et al.*, 2016).

D. Vatsala Thirumalai at NCBS, Bengaluru utilizes the zebrafish as a model system. The embryonic and larval stages of the zebrafish are transparent thus allowing for direct visualization of the development of internal organs like the brain. Neuronal gap junctions are

channels on neuronal membranes made of protein subunits which allow for passage of chemical messengers across synapses and thus allow for functional neural circuitry to be formed. Her lab has described the distribution and expression of the gap junction protein Connexin 35 whose mammalian orthologue Connexin 36, is widely expressed in the interneurons of the mammalian central nervous system (Jabeen and Thirumalai, 2013). Future work could potentially delineate the role of Connexin 35 in regulating chemical synaptogenesis during development.

Further by utilizing the zebrafish as a model system, her lab has been able to study voltage currents from a single Purkinje neuron when the animal is in an awake state, which is very difficult to do so from a conscious rodent. Purkinje neurons in the cerebellum are some of the largest neurons in the brain with intricate and elaborate dendritic arborisations. They are inhibitory and are the sole output neurons for motor coordination in the cerebellum. Their electrophysiological activity is thus a very important measure of their function of motor coordination and motor learning. They noted the existence of two kinds of firings (bistability) in these neurons 1) steady or tonic firing 2) short bursts of firing. When the membrane potential was depolarized, they observed tonic bursting and short burst during hyperpolarized membrane potential. The switch between the two states was spontaneous and also triggered by currents.

Activation of the AMPARs was enough to trigger the bursts. The Purkinje neurons received strong excitatory synaptic inputs from the climbing fibres and the excitatory postsynaptic current triggers bursts in the Purkinje neurons. Inhibition of the ESPSs using antagonists abolished bursts firing. Interestingly this bistability was observed developmentally soon after the fate specification of the Purkinje cell and persisted till late larval stages of the fish development (Sengupta and Thirumalai, 2015).

Conclusions

Landmarks in neural development include progenitor proliferation, birth and differentiation of neurons from the progenitors, glial cell production, migration of immature neurons from the place of their birth to their final positions of function, axonal and dendritic outgrowth from the neurons, guidance of the growth cone towards postsynaptic partners and the generation of synapses to form a complete functional circuitry. Indian developmental neurobiologists have contributed immensely to studying the various steps delineated above in a range of useful model systems.

An area in which Indian scientists are yet to make a foray is the study of human brain development. Elucidating the molecular mechanisms of human neocortex development and evolution is essential for our understanding of species-specific differences in cognitive capacities and our susceptibility to neurodevelopmental disorders. This is a great challenge as the paucity of adequate fetal brain samples, and the associated ethical concerns is a huge bottle-neck. Modeling human brain development in a dish using iPSCs holds great promises in the future. The field is rapidly advancing with newer and robust 2D culture and 3D cerebral organoid models of the human brain (reviewed in Brown *et al.*, 2018). It would be interesting to see Indian developmental neurobiologists entering and making their mark

internationally in this cutting-edge and burgeoning field of iPSCs and related technology to advance our understanding of human-specific developmental mechanisms in both health and disease.

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Abbreviations used in this paper

AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazo-lepropionic acid receptor
BMP	bone morphogenetic proteins
CNS	central nervous system
CSDn	serotonin-immunoreactive deutocerebral neuron
cVA	cis-vaccenyl acetate
ESPS	excitatory postsynaptic potential
GAP43	growth associated protein 43
MARCM	mosaic analysis with a repressible cell marker
NMDAR	N-methyl-D-aspartate receptor
ORN	olfactory receptor neuron
Shh	Sonic hedgehog
Wnt	wingless and int-1.

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Keyword definitions

Antennal lobe
It is the primary structure in the *Drosophila* brain that relays odor information from the fly antennae to higher order brain centres. It is a neuropil center and receives inputs from the olfactory receptor/sensory neurons from antennae.

Antihem
Signaling center in the mammalian developing cortical primordium that releases signaling molecules and patterns the adjacent cortex. It is present at the very lateral end of the developing cortical primordium. Cerebelleum - It is part of the hindbrain in mammals and receives information from sensory systems, spinal cord and other regions of the brain and coordinates motor movements.

Cerebral cortex
It is the outer most region of the forebrain and region where all higher order functions like thinking, memory, learning etc are processed. It is

Choroid plexus
It consists of ependymal cells, which produce cerebrospinal fluid in the brain.

Climbing fibres
It is a series of neuronal projections from medulla oblongata to the cerebellum and form synapse with cerebellar neurons.

Commissural neuron
Neurons whose axon cross to the opposite side of the brain or spinal cord.

Cortical primordium
Embryonic neuroepithelial tissue which gives rise to the cerebral cortex.

Filopodia
Structures in a growth cone, which appear as cylindrical extensions protruding outside of the edge of the growth cone.

Glomeruli
Cluster of nerve endings in the antennal lobe where the sensory neurons synapse with projection neurons and interneurons.

Granule neurons
These are neurons of the cerebellum and account for the majority of neurons in the human brain.

Hem
Signaling center in the mammalian developing cortical primordium that releases signaling molecules and patterns the adjacent cortex into the hippocampus.

It is present at the very medial end of the developing cortical primordium.

Hippocampus
It is a medial structure in the brain devoted to memory formation and consolidation. It is archicortex- i.e. phylogentically the oldest region in the brain’s cortex.

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Holoprosencephaly
Neurodevelopmental disorder caused by the failure of the embryonic brain to divide into two lobes of the cerebral hemispheres.

Hypoplasia
Incomplete development of tissue due to reduced cell numbers.

Interneurons
They are primarily inhibitory neurons in the brain and spinal cord and function to create neural circuits by communicating between the various neurons.

MARCM analysis
Mosaic analysis with a repressible cell marker. It is a genetics technique used to create individually labelled cells in *Drosophila.* MARCM clones can be used to study mutant phenotypes in wild type cells.

Membrane potential of a neuron
A resting neuron has a voltage across its membrane called as resting membrane potential.

Neuroblast
Stem cells of the developing fly brain.

Neocortex
Evolutionarily the most advanced part of the brain and centre for processing higher order functions like thinking, language decision making and consciousness. It is six layered and is hugely expanded in primates and humans.

Neocortical layers
The six layers of the neocortex consisting of distinct neuronal subtypes with unique morphological characteristics, expressing a unique combination of transcription factors and ultimately serving different functions.

Projection neurons
Neurons which extend axons from the neuronal cell body in the CNS to more distant regions of the CNS.

Purkinje neurons
GABAergic neurons in the cerebellum whose electrophysiological activity determines motor coordination.

Selector gene
Gene that governs the fate of group of cells.

Ventricular zone
transient embryonic zone containing neural stem cells or progenitors which rise to the different neurons and glia in the central nervous system.

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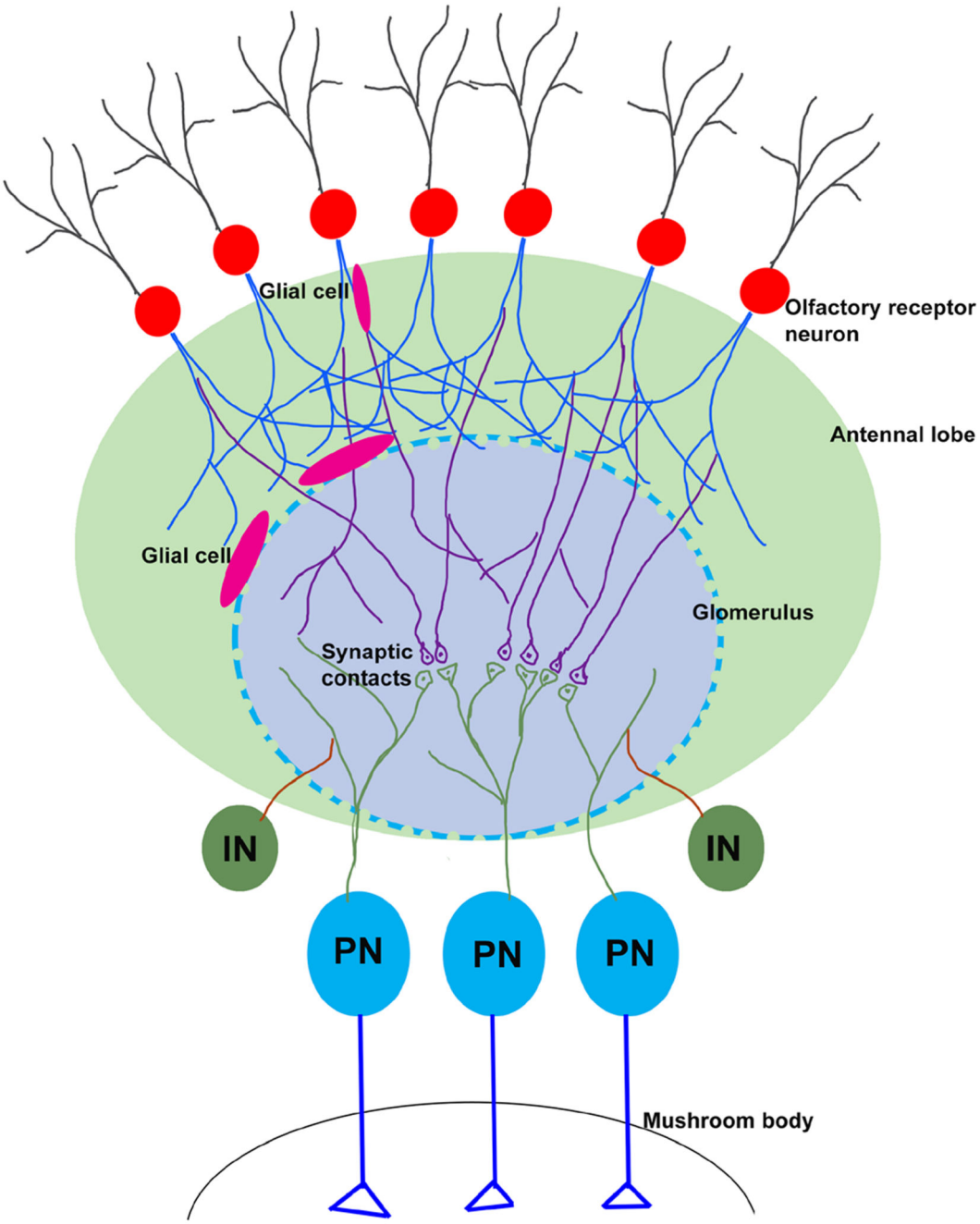

Fig. 1. Schematic of the *Drosophila* olfactory nervous system.

Olfactory receptor neurons (ORNs) are of different types based on the odorant receptor they express. The ORNs send out axon terminals to different glomeruli. This process is aided by peripheral glia. ORNs expressing a kind of odorant receptor converge on the same glomerulus in the antennal lobe. The ORNs synapse with postsynaptic terminals of projection neurons and interneurons in the glomerulus. Projection neurons then pass on the olfactory information to neurons of the mushroom body and lateral horn (higher order centers for information processing in Drosophila). (Modified from Vadakkan 2015).

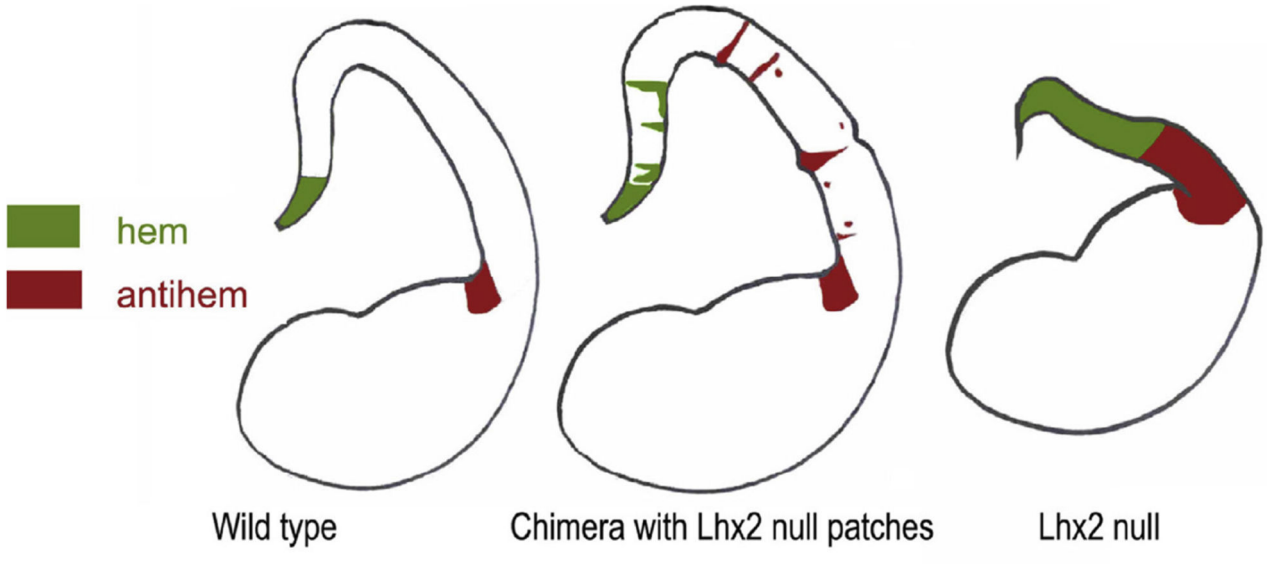

Fig. 2. The cerebral cortex arises from the dorsal telencephalon, neuroepithelial tissue ensconced between the hem (green) and antihem (red) located at polar ends of the cortical tissue.

In Lhx2 null embryos (far right), the entire cortex is taken over by the hem and antihem. In Lhx2 chimaeras multiple ectopic hems and antihems are made. Adjacent to each ectopic hem a hippocampus was organized leading to the formation of multiple hippocampi in one brain hemisphere (Reproduced with permission from Mangale et al., 2008).

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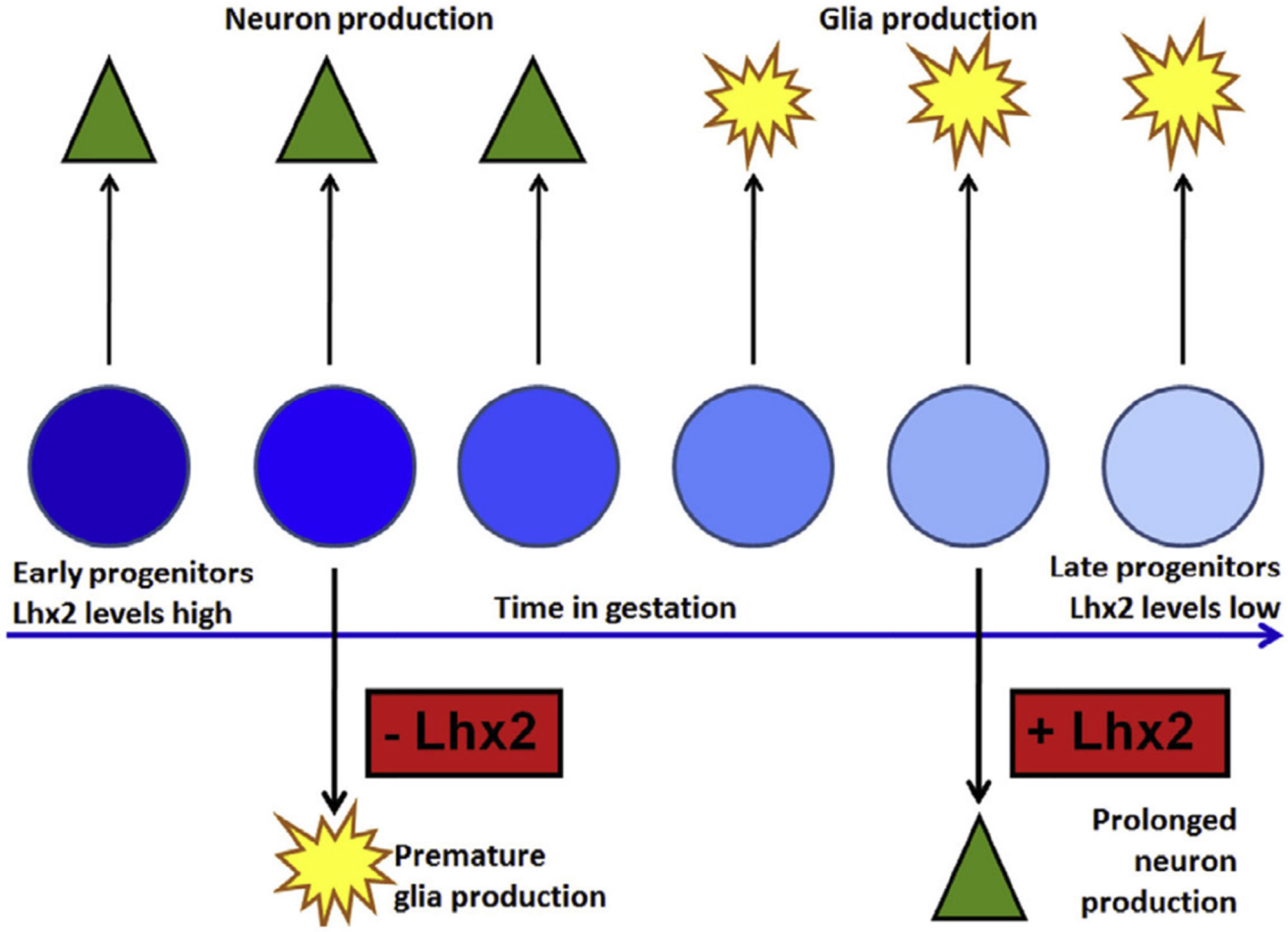

Fig. 3. Neuron-glia cell fate switch in the developing hippocampus.

Neurogenesis precedes gliogenesis across the central nervous system. Lhx2 removal from neurogenic progenitors results in precocious production of glia and overexpression extends the neurogenic period and leads to prolonged neuronal production at the expense of glia (From Subramanian et al. 2011, author summary).

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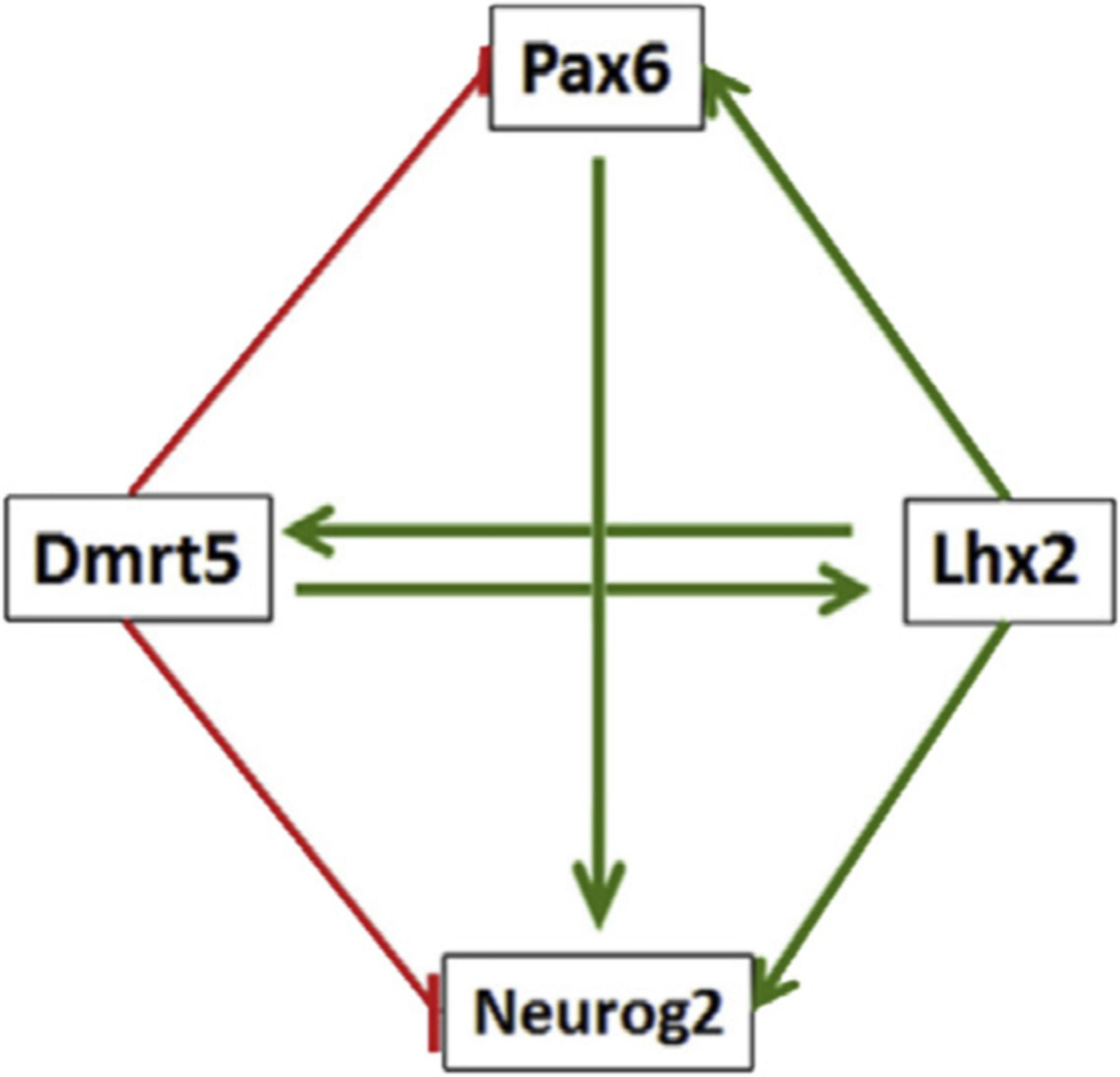

Fig. 4. A complex bi-directional gene regulatory network

functional in the developing hippocampus regulating the neuron-glia cell fate switch involving neurogenic genes namely LHX2, PAX6, NEUROG2 and DMRT5 (From Muralidharan et al., 2017b).

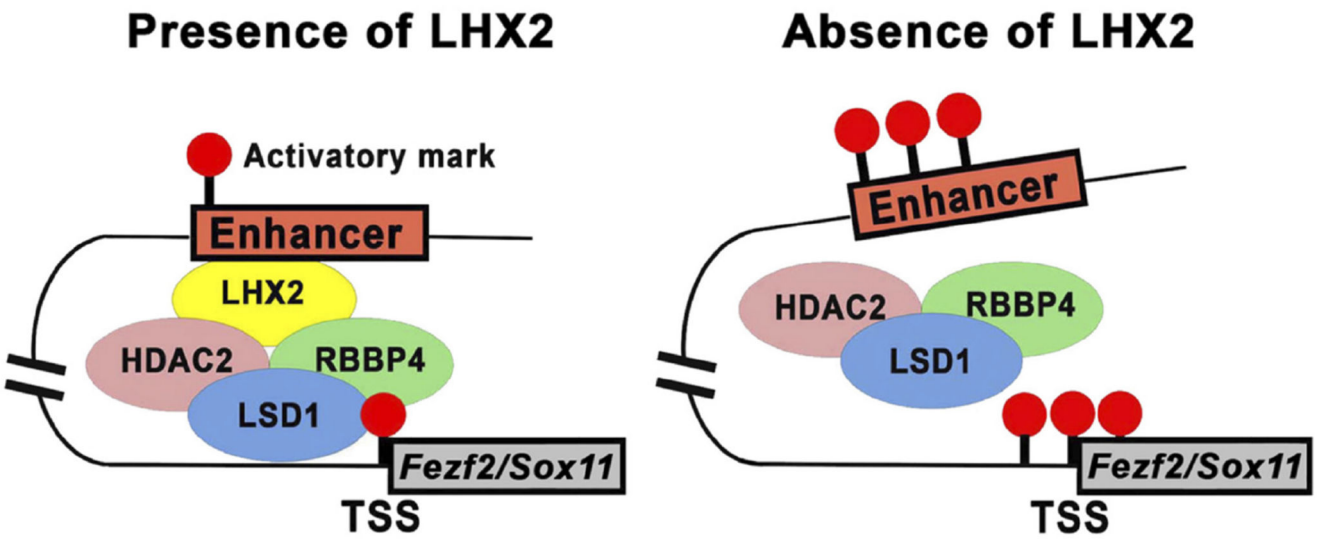

Fig. 5. Molecular mechanism of LHX2 action in regulating neuronal subtype identity.

LHX2 binds to its target genes Fezf2 and Sox11 and recruits components of the NuRD complex LSD1 and HDAC2, which associate with the transcription start site and the LHX2 binding site leading to decrease in active marks. In the absence of LHX2, the active marks are enriched leading to upregulation of its target genes. (From Muralidharan et al., 2017a).