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DEVELOPMENT of CORTICAL NEURONAL NETWORKS

MYST Family Histone Acetyltransferases in Brain Development and Cognitive Disorders

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Embryonic development is the process of one cell proliferating and differentiating into the diverse cell types of the body. The different cell types possess identical genetic material, but differ in their gene expression. DNA-binding transcription factors regulate cellular phenotype, but they typically act in more than one cell lineage, such that changes to the chromatin are essential in restricting their function to the appropriate subsets of their target genes. The organisation of DNA and histones into chromatin governs the conformation of DNA and the transcriptional status of genes. Post-translational modifications of histones regulate chromatin conformation. Histone acetylation correlates with the euchromatin state and active transcription. The MYST (MOZ, Ybf2/Sas3, Sas2, TIP60) family histone acetyltransferases have important functions in human and mouse brain development. The five MYST family members comprise about one third of all histone lysine (K) acetyltransferases (KAT) in the mammalian genome.

Our work on MYST family histone acetyltransferases has elucidated their molecular functions.[1-5] We reported the role of KAT6B (QKF) in brain in 2000[6] and, in 2011, showed that the loss of one allele of the human KAT6B gene causes cognitive disorders.[7,8] Similarly, mutations of the human KAT6A (MOZ) gene were identified as the cause of a cognitive disorder.[9,10] New data on three MYST family members in brain development will be presented.

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The Role of Protein Kinases (MARK1, ADCK1, HUNK1) in Cortical Development

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The neocortex is a highly organized structure, responsible for the integration of sensory signals, the processing of motor coordination and determining personality. Disruption to cortical layer formation may be an underlying factor behind various neurodevelopmental disorders, including intellectual disability, schizophrenia and ataxia disorders. There are a lot of transcription factors, that involve in signaling, that can influence to the post-mitotic fate acquisition, cortical positioning and neuronal efferents in neurons of the cerebral cortex. Mutations in genes encoding for proteins that regulate neuronal cell fate can lead to aberrances in neuronal connectivity. Protein phosphorylation affects 30% of the human proteome and has effects on protein function, structure, localization and activity. Therefore, we have investigated the role of protein kinases (MARK1, ADCK1, HUNK1) in establishment of neuronal cell fate during corticogenesis. Our preliminary data shows that down-regulation effects of MARK1, ADCK1, HUNK1 in the mouse brain results in delay in cortical neuronal migration, disruption in positioning and strange neuronal morphology.

Post-Transcriptional Regulators Controling Cortical Neuron Migration, Outgrowth and Connectivity

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miRNAs intervene between the transcriptome and the proteome at all stages of brain development. Many essential miRNAs are themselves subject to post-transcriptional regulation. For example, in the developing mouse cerebral cortex the RNA precursor for miR-128 is present in progenitor cells and in migrating neurons even though accumulation of 22 nt mature miR-128 is restricted to postmitotic neurons in the cortical plate. Precise temporal control of miR-128 activity is essential for its functions in the regulation of dendritic outgrowth and excitability of upper layer neurons in the cortex.

Using *in utero* electroporation, we showed that inhibition of miR-128 in nascent upper layer neurons leads to over-migration with inappropriate bunching near the marginal zone. Premature miR-128 expression has the opposite effect: migrating neurons display aberrant morphology and impaired migration. The affected neurons also display reduced dendritic complexity and altered electrophysiological properties. Phf6, a gene mutated in the cognitive disorder Börjeson-Forssman-Lehmann syndrome, is an important regulatory target for miR-128. Restoring Phf6 expression counteracts the deleterious effect of miR-128 on neuronal migration, outgrowth and activity. These results place miR-128 upstream of Phf6 in a pathway vital for cortical lamination as well as for the development of neuronal morphology and intrinsic excitability.

We now show that posttranscriptional mechanisms leverage miR-128 activity at a second level, the regulation of select miR-128 mRNA targets by the miR-128 host gene product ARPP21. We used iCLIP to show that ARPP21 is an RNA-binding protein that recognizes uridine-rich sequences with exquisite sensitivity for 3'UTRs. Surprisingly, ARPP21 antagonizes miR-128 activity by co-regulating a subset of miR-128 target mRNAs enriched for neurodevelopmental functions, including Phf6. In contrast to miR-128, we show that ARPP21 acts as a positive post-transcriptional regulator, at least in part through interaction with the eukaryotic translation initiation complex eIF4F. This molecular antagonism is also reflected in inverse activities during dendritogenesis: miR-128 overexpression or knockdown of ARPP21 reduces dendritic complexity; ectopic ARPP21 leads to an increase. The regulatory interaction between ARPP21 and miR-128 is a unique example of convergent function by two products of a single gene.

Sip1 Loss Affects Cortical Neurons Migration

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Sip1 is a transcription factor that plays essential role in neocortex development. It controls expression of a wide number of genes that regulate various aspects of neuronal development. Heterozygous mutations of Sip1 gene in humans was shown to cause Mowat-Wilson syndrome, which is characterized by multiple severe neurodevelopmental defects such as intellectual disability, microcephaly, epilepsy, Hirschsprung's disease, delayed growth and motor development, agenesis of corpus callosum and craniofacial abnormalities. Previous studies have addressed the cellular defects underlying the agenesis of the corpus callosum present in a proportion of patients and the characteristic craniofacial defects. Intellectual disability, however, is usually associated with defects in the formation and maturation of the dendritic tree. We have therefore analyzed the role of Sip1 during this process in the mouse using floxed Sip1 mice and *in utero* electroporation of a Cre expressing construct. Our preliminary data shows that loss of Sip1 in the mouse results in delay in cortical neuronal migration, abnormal dendritic arbor formation and maturation. We are currently addressing the cellular and signaling defects underlying this defect.

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The Activity of the Infragranular Layers Modulates the Immature Patterns of Activity in the Developing Barrel Cortex

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The first postnatal week of somatosensory cortex development is characterized by the expression of the unique immature patterns of activity - early gamma oscillations (EGOs) and spindle-bursts (SBs). Besides the difference in dominant frequency (gamma frequency for EGO and alpha/beta frequency range for SB), those immature oscillations are also characterized by different types of sensory stimulation and functional properties. While single sensory input stimulation evokes EGO, that synchronizes the activity in topographically aligned thalamic and cortical neurons, the SB is resulted from the simultaneous activation of the multiple sensory inputs and supports the horizontal synchronization between cortical columns in the developing barrel cortex. Here, we demonstrate that beta frequency component, characterizing SBs, is also observed during EGOs. We have found irregular skipping of the EGOs cycles that resulted in the dropping of the dominant frequency from gamma to the beta frequency band. Moreover, those episodes of skipped gamma cycles were associated with an increase in the infragranular layer activity. Blockade of the infragranular layer activity by the local injection of the GABAa agonist resulted in recovering of the gamma rhythmicity and disappearance of the dropping out of gamma cycles in the granular layer of active cortical column. Evoked SB activity was also modified by the local inhibition of the infragranular layer activity. While multiple sensory stimulations evoked SB in multiple barrels, EGO was also observed in the barrel with suppressed infragranular layer activity. Altogether, these observations demonstrated the infragranular layer modulation of the EGO and SB. Moreover, the present result suggested the hypothesis of the cortical origin of the SB pattern of activity observed during the early postnatal period of barrel system development.

Structural Plasticity of Neural Stem Cells in Mammalian Brain Development

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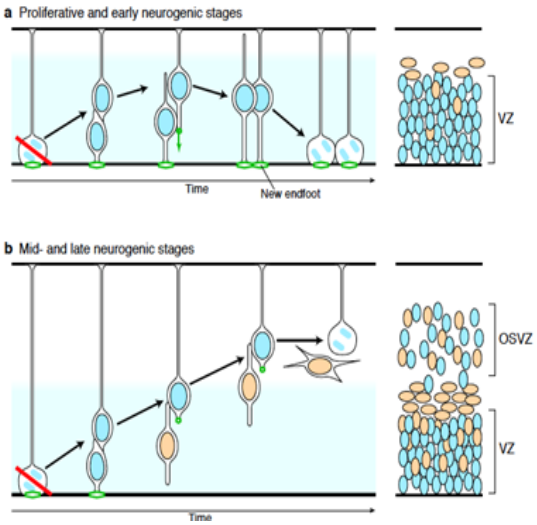
Neural stem cells called radial glia maintain epithelial structure during cortical development. The prevailing view claims that during the proliferative stage in early development, symmetric divisions of radial glia require tight regulation of spindle orientation, the perturbation of which causes precocious neurogenesis and apoptosis. By contrast, in the subsequent neurogenesis, oblique spindle orientation induces the translocation of radial glia by loss of the apical endfoot to form outer radial glia, a hallmark of gyrencephalic development. Here, we show a high regenerative ability of proliferative radial glia, which confer epithelial robustness against perturbations such as spindle misorientation, despite the conventional view. Regenerating endfeet bear ectopic adherens junctions at their leading edge. This ability, however, declines during neurogenesis and allows the formation of outer radial glia. Our study reveals thus a temporally changing cryptic property, which initially ensures symmetric divisions of radial glia and subsequently provides a basis for brain size expansion in mammals.



Transcriptional Control of Neuronal Differentiation in the Telencephalon

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I will discuss a project that addresses the transcriptional mechanisms that control cell fate and/or differentiation of telencephalic neurons – either cortical projection neurons or cortical interneurons. The talk will include *in vivo* genetic evidence for the function of a transcription factor, and potentially will include epigenetic investigations that address how the transcription factor functions at the genomic level.

Axo-Axonic Synapses at the Axon Initial Segment: a Homeostatic Plasticity Hub

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GABAergic interneurons are chiefly responsible for controlling the activity of local circuits in the cortex. However, the rules that govern the wiring of GABAergic interneurons are not well understood. Chandelier cells (ChCs) are a type of GABAergic interneuron that control the output of hundreds of neighbouring pyramidal cells through axo-axonic synapses which target the axon initial segment (AIS). Despite their importance in modulating circuit activity, our knowledge of the development and function of axo-axonic synapses remains elusive. Here, we investigated the role of activity in the formation and plasticity of ChC synapses. *In vivo* imaging of ChCs during development uncovered a narrow window (P12-P18) over which axons arborized and formed connections. We found that increases in the activity of either pyramidal cells or individual ChCs during this temporal window resulted in a reversible decrease in axo-axonic connections. Voltage imaging of GABAergic transmission at the AIS showed that axo-axonic synapses were depolarising during this period. In parallel, we also saw changes in the structure of the AIS, which matched a decrease in pyramidal cell excitability. Identical manipulations of network activity in older mice (P40-P46), when ChC synapses are inhibitory, resulted in an increase in axo-axonic synapses. We propose that the direction of ChC plasticity follows homeostatic rules that depend on the polarity of axo-axonic synapses.

Sensory-Evoked Movements Shape Cortical Responses in Neonatal Rats

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The behavior of neonatal rodents is characterized by spontaneous and sensory-evoked twitches and generalized startles. These movements in neonatal rodent share similar features with physiological reflexes in fetuses and neonatal human babies. Given that tactile stimulus evokes startle-response, we hypothesized that global reafferentation from startle would activate large areas in S1 cortex resulting in apparently non-topographic maps. Another relevant to this issue question addressed in the present study was how the motor response affects cortical response at the topographic location. We provide evidence that S1 maps in neonates are topographic and that sensory-evoked startle produces widespread delayed S1 activation. This startle-mediated activation by reafferentation is characterized by a significantly longer latency than the direct sensory-evoked topographic response. While the direct sensory-evoked topographic responses persisted during repetitive stimulation, startle-induced late response disappeared in an all-or-none manner upon habituation to repetitive stimuli. Surprisingly, we also found that at the topographic location, startles reduced the late part of the ongoing sensory-evoked response. Thus, motor responses significantly shape cortical sensory responses in neonatal rats resulting in an apparent loss of topography but also inhibiting the response at the topographic location.

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Flurothyl-Induced Seizure and Spreading Depression in a Cortical Barrel Column

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Spreading depression (SD) is a slowly propagating wave of collective neuronal depolarization, which causes depolarization block of action potentials and transient suppression of cortical activity. SD often accompanies epileptic discharge and terminates it. While SD slowly spreads in cortex not only horizontally but also vertically, how vertical spread of SD affects seizures across cortical layers remains unknown. The purpose of this work was to study the spatial and temporal dynamics of SD and epileptic activity in all layers of the barrel cortex of the rat Wistar.

Wistar rats from postnatal days P40–60 were used in electrophysiological experiments. We used 16-channel linear silicone probes to record LFP in DC mode and MUA from all layers of a cortical barrel column in urethane-anaesthesized head restrained rats. Position of electrodes in different layers was identified from depth-profile of the PW-evoked sensory response, short latency MUA and SD profile. Concomitant piezo-recordings of the hindlimb movements were used to monitor motor seizure manifestations. To induce seizure activity, 0.1 ml of flurothyl in a plastic muzzle was placed on the nose of an animal for 90 s.

Inhalation of flurothyl evoked generalized tonic-clonic seizures lasting for 1-2 min. In a half of seizures, SD was observed. SD started in L2/3 or L4 at variable time points during seizure, and spread vertically at a velocity of 2-3 mm/min. In a half of seizures with SD, SD propagated through the entire cortical depth terminating in white matter. In another half of cases with SD, SD propagated only partially terminating at the L4 /5 border. In the cases without SD, seizures were characterized by synchronized population firing across all cortical layers through the entire time course of seizure. When SD occurred, epileptic activity in layers recruited by SD was transiently silenced during SD. Vertical spread of SD and recovery from SD were characterized by dynamic states, during which epileptic activity was entirely restricted to the layers below SD front, whereas layers recruited by SD were silent and only displayed passive sources of population spikes in deep layers.

Thus, SD often occurs and strongly impacts population cross-layer dynamics during flurothyl-induced seizures in the cortical barrel column. On a technical note, we also found that slow negative LFP shifts during SD are eliminated during highpass filtering using conventional EEG filter settings.

Regulation of Neural Development Genes by Histone Acetylation

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During development, neural progenitors give rise to the cellular diversity of the brain and nervous system. These cells persist into adulthood in certain areas as neural stem cells (NSCs), capable of contributing to neural networks in adults. Despite their requirement for development and continued replenishment of the cells that comprise certain adult neural networks, the factors and mechanisms that regulate progenitors and NSCs *in vivo*, remain poorly understood.

The MYST family histone acetyltransferase, KAT6B (MYST4, QKF) plays essential roles in the developing cortex, as well as in maintaining self-renewal and cell fate specification in adult NSCs of the subventricular zone (SVZ), with a progressive decrease in Kat6b gene activity observed as NSCs undergo differentiation. Accordingly, mutations in KAT6B are seen to underlie at least two distinct but related intellectual disability disorders in humans. Genitopatellar syndrome and the Say-Barber-Biesecker-Young-Simpson variant of Ohdo syndrome result from heterozygous mutations in KAT6B. However, the mechanisms by which KAT6B impairment results in intellectual disability remain unknown.

Here, we demonstrate that KAT6B directs NSC proliferation and differentiation *in vivo* and *in vitro*. Cultured Kat6b-/- NSCs show impaired proliferation and reduced neurogenesis and neurite outgrowth. Conversely, Kat6b overexpression enhances NSC proliferation and neuronal differentiation *in vitro*. We show that KAT6B regulates key NSC pluripotency and neurogenesis pathways and identify KAT6B as a novel regulator of a discreet set of transcription factors family proteins, both during corticogenesis and in adult NSCs.

Genetic Interaction of Neurod1/2/6 and WWP1/2/Mir140 Pathways

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Cortical expansion during evolution involved a significant increase in neuron number and the generation of new neuron types that formed new layers, extending the three-layered reptilian paleocortex into the six-layered mammalian neocortex (Dugas-Ford and Ragsdale, 2015). The developmental mechanisms that regulate such an evolutionary expansion and complexification of the cerebral cortex remain not fully uncharted.

Differences in cerebral cortex size and composition are thought to result from variations in the lineage of neural progenitor cells during development (De Juan Romero and Borrell, 2015, Fish et al., 2008, Kriegstein et al., 2006). In developing cortex neurons can be generated from radial glial cells (Pax6+) directly and indirectly via intermediate progenitor cells (Tbr2+). In humans and other primates with a very large cerebral cortex, their abundant neurogenesis involves the massive generation of intermediate progenitor cells (IPs) (Tbr2+) forming an exceptionally sized subventricular Zone (SVZ) (Hansen et al., 2010, Smart et al., 2002). Neuronal development is a multistep process, regulated by a myriad of signaling cascades involving protein ubiquitination and regulation of gene expression using transcription factors. Recently, we have reported that ubiquitin ligases Wwp1/2 are necessary for correct laminar positioning of developing neurons. Additionally, we demonstrated that NeuroD1/2/6 transcription factor family is indispensable for neuronal development.

Interestingly, both mouse models exhibit defects to Tbr2 expression during neurogenesis.

In NeuroD1/2/6 TKO we discovered excessive numbers of Tbr2-positive cells and SVZ expansion. The IPs residing in the SVZ transiently express Tbr2 which in consumptive divisions give rise to immature neurons. NeuroD genes which inactivate Tbr2 in young neurons. Another process that requires NeuroD factors is neuronal radial migration of young neurons. We discovered that NeuroD TKO neurons display severe migration arrest, fail to initiate radial migration and reside in the SVZ.

In Wwp1/2/miR140 TKD neurons we observe similar prolonged Tbr2 retention and migration initiation failure. Additionally, we noted a marked decrease in the fraction of TKD neurons positive for upper-layer neuron markers, indicative of neuronal differentiation defects (Ambrozkiewicz et al., 2018).

These data indicate that Tbr2 expression might contribute to regulation of neuronal migration and differentiation. For this reason, to mimic the prolonged defective Tbr2 expression in TKO and TKD neurons, we expressed Tbr2 in cortical progenitors by means of *in utero* electroporation (IUE). We observed a striking arrest of cells in the intermediate zone that were not capable to migrate into the cortical plate (CP) anymore.

To then investigate if the effects of increased Tbr2 dosage prevail until adulthood or are transient, we also fixed the electroporated brains at P9. Again, we observed a croup of cells arrested, not entering into the CP.

Further, to study if restoring Tbr2 level in TKO, we rescued Tbr2 expression using sh-RNA in TKO neurons. Strikingly, cells migrated into the CP correctly without any signs of arrest.

These data indicates that NeuroD1/2/6 and WWP1/2/miR140 are necessary for correct neuronal differentiation and migration during developing. Tbr2 is a potential common downstream target in both cascades that control migration.

Mechanisms of Neuronal Subtype Specification and Integration in the Cerebral Cortex

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The cerebral cortex holds a remarkable capacity to integrate multimodal sensory information and generate coordinated outputs that underlies higher cognitive functions. While the localization of cortical areas responsible for modality-specific information processing has been mapped over a century ago, how each area is assembled to serve its unique function remains largely elusive. We recently identified the earliest molecular program that preselects projection neuron types in the neocortex to confer their unique hodological properties, an initial step in the formation of modality-specific circuits. We further established a genetic labeling system to assess the integration dynamics of temporal cohorts of newborn neurons. *In vitro* culture of these temporal precursors recapitulated *in vivo* cortical development, implying the critical impact of intrinsic regulation in regional identity establishment. I will further discuss the underlying mechanisms involved in these processes.