Lineage Tracing of Cortical Progenitors by Long-Term Live Imaging

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During development of the six-layered mammalian cortex, generation of the different excitatory pyramidal neuron subtypes proceeds sequentially with deep layer (DL) neurons being produced first, followed by upper layer (UL) neurons. Nevertheless, during mid-neurogenesis, both DL–and UL neuron types are produced simultaneously but their lineage relationship is yet unclear.

Aims

To investigate the lineage relationship of DL– and UL subtypes, we set out to visualize and monitor single progenitors in primary cortical cultures over time and determine DL–and UL marker expression of their progeny.

Methods

GFP expressing cells derived from dissociated cortices of E12.5 to E13.5 RosaGFP mice were mixed and cultured with an excess of isocronic wild-type cells. In an alternative approach progenitors were targeted by ex-utero electroporation to exclusively induce stable expression of fluorescent markers in progenitors and their offspring. Subsequently, cortical cultures containing single targeted progenitors among an excess of unlabeled cells were subjected to spinning disk confocal live imaging for five days. Cultures were fixed and the expression of Ctip2 (DL marker) and Satb2 (UL marker) was analyzed by immunohistochemistry to elucidate clonal composition.

Results

Contrary to culture of single isolated progenitors, our mixed cortical cultures yielded substantial amounts of UL type neurons in the time frame analyzed. Clonal size ranged from 2 up to 40 cells. The majority of clones contained neurons expressing either Ctip2 or Satb2. A proportion of clones included neurons that expressed no marker or were weakly positive for both, Ctip2 or Satb2. Interestingly, no two-cell clones consisting of both, a DL–and UL type neuron were found.

Conclusions

Long-term live imaging of primary cortical cultures containing single genetically labeled progenitors represents a valuable method to investigate cortical progenitor output. Our results suggest that individual progenitors preferentially produce either DL–or UL subtypes during mid-neurogenesis.

References


Transcriptional Control of Cortical Pyramidal Neuron Differentiation and Axonal Growth by Neurod 1/2/6

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Aims

The closely related neuronal bHLH transcription factors Neurod1, Neurod2 and Neurod6 are expressed by differentiating pyramidal neurons in the developing cerebral cortex and have long been suspected to regulate the maturation of these cells. Each of the three genes was genetically inactivated in mice, but studies of single-deficient animals failed to identify important functions in embryonic pyramidal neurons. High sequence similarity and overlapping expression patterns suggest functional redundancy. We used double and triple deficient mice to identify NeuroD-family dependent functions and downstream molecular mechanisms.

Methods

We bred Neurod2/6 double-deficient and Neurod1/2/6 triple deficient mice and analyzed cerebral cortex development with an emphasis on pyramidal neuron identity and neocortical connectivity. We use in situ hybridization to visualize and in utero electroporation to manipulate the expression of possible target genes in the cerebral cortex.

Results

Neurod2 and Neurod6 indeed share several hitherto unknown functions and compensate for each other’s loss. At least one of the two genes is necessary for: (1) the control of radial migration in a subset of pyramidal neurons; (2) area determination in the neocortex; and (3) the formation of fiber tracts connecting the neocortex to the striatum, to the thalamus, and to the contralateral hemisphere. In Neurod2/6 double-deficient mice, callosal axons form fasciculated fiber bundles that grow tangentially into the medial neocortex, but stall and defasciculate before reaching the ipsilateral cingulum or any midline associated structure. This new variant of callosal agenesis implies the presence of a so far identified axon guidance mechanism in the medial neocortex. We present EphrinA signaling as possible mechanism. Neurod1 shares additional functions with Neurod2 and Neurod6. At least one of the three genes is necessary for hippocampal pyramidal neuron differentiation and the prevention of developmental cell death in the medial cortex. While the simultaneous inactivation of Neurod1/2/6 results in the complete loss of archicortical pyramidal neurons, many neocortical pyramidal cells survive, migrate radially and settle in the cortical plate. However, terminal pyramidal neuron differentiation is incomplete and neocortical connectivity is dramatically reduced in triple-deficient mice.

Conclusion

NeuroD-family transcription factors cooperatively regulate pyramidal neuron differentiation, survival, migration, specification and axon growth in the developing cerebral cortex.

References


Defining the Role of Ciliary Proteins BBS (Bardet-Biedl Syndrome) in Neuroplasticity

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Bardet-Biedl syndrome (BBS) is a genetically heterogeneous, autosomal recessive disorder characterised by early-onset retinal degeneration, obesity, polydactyly, and renal malformation/dysfunction. It affects approximately 1:100,000 people in Northern Europe reaching 1:13,500 in more isolated populations. More than half of all individuals with BBS also experience developmental disabilities ranging from mild learning impairment to severe mental retardation independent of gene mutation. Many patients display obsessive/compulsive tendencies and a preference for fixed routines and 37% of children attending our national BBS clinics have autism spectrum disorder. Poor memory and cognition in Bardet-Biedl syndrome lead to inability to live independently and few are in employment (9%).
Neuronal Store Operated Calcium Entry as Novel Therapeutic Target for Treatment of Alzheimer’s Disease

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Memory loss in Alzheimer’s disease (AD) results from “synaptic failure”. Mushroom dendritic spine structures are essential for memory storage and the loss of mushroom spines may explain memory defects in aging and AD. To understand the basis for memory loss in AD we performed a series of mechanistic studies of hippocampal synaptic spines in mouse models of familial AD (FAD). In our experiments we used presenilin 1 (PS1) M146V knockin (PS1KI) and APPKI models of FAD. In the course of these studies we discovered an existence of spine maintenance pathway that is mediated by neuronal store-operated Ca2+ entry (nSOC) in postsynaptic spines (Sun et al, 2014). We established that nSOC pathway plays a key role in stability of mushroom spines by constitutively activating synaptic CaMKII kinase. We further demonstrated that synaptic nSOC is controlled by stromal interaction molecule 2 (STIM2) and that STIM2-nSOC-CaMKII pathway is compromised in PS1KI and APPKI neurons, in aging neurons and in sporadic AD brains due to downregulation of STIM2 protein (Sun et al, 2014; Zhang et al 2015). Moreover, we have demonstrated that expression of STIM2 protein rescues synaptic nSOC and mushroom spine loss in PS1KI and APPKI hippocampal neurons (Sun et al, 2014; Zhang et al 2015) and protects mushroom spines from amyloid synaptotoxicity (Popugaeva et al, 2015). These studies suggested that STIM2-nSOC pathway is a potentially important AD therapeutic target, and that activators and positive modulators of this pathway may have a utility for treatment of synaptic loss and memory decline in aging and AD.

References


The 2.2-Angstrom Resolution Crystal Structure of the Carboxy-terminal Region of Ataxin-3

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Memory loss in Alzheimer’s disease (AD) results from “synaptic failure”. Mushroom dendritic spine structures are essential for memory storage and the loss of mushroom spines may explain memory defects in aging and AD. To understand the basis for memory loss in AD we performed a series of mechanistic studies of hippocampal synaptic spines in mouse models of familial AD (FAD). In our experiments we used presenilin 1 (PS1) M146V knockin (PS1KI) and APPKI models of FAD.

In the course of these studies we discovered an existence of spine maintenance pathway that is mediated by neuronal store-operated Ca\(^{2+}\) entry (nSOC) in postsynaptic spines (Sun et al., 2014). We established that nSOC pathway plays a key role in stability of mushroom spines by constitutively activating synaptic CaMII kinase. We further demonstrated that synaptic nSOC is controlled by stromal interaction molecule 2 (STIM2) and that STIM2-nSOC-CaMKII pathway is compromised in PS1KI and APPKI neurons, in aging neurons and in sporadic AD brains due to downregulation of STIM2 protein (Sun et al., 2014; Zhang et al. 2015). Moreover, we have demonstrated that expression of STIM2 protein rescues synaptic nSOC and mushroom spine loss in PS1KI and APPKI hippocampal neurons (Sun et al., 2014; Zhang et al. 2015) and protects mushroom spines from amyloid synaptotoxicity (Popugaeva et al., 2015). These studies suggested that STIM2-nSOC pathway is a potentially important AD therapeutic target, and that activators and positive modulators of this pathway may have a utility for treatment of synaptic loss and memory decline in aging and AD.

References


SHAPING THE DENDRITIC TREE: THE ROLE OF ARHGAP33 IN NEOCORTICAL DEVELOPMENT AND DISEASE

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Neuropsychiatric developmental disorders, such as autism spectrum disorders (ASD) and schizophrenia, are typically characterized by alterations in social behavior. At a cellular level, these conditions have been linked to aberrant development and maturation of the dendritic tree. We show here that the Cdc42 GTPase-activating multidaptor protein, NOMA-GAP/ARHGAP33 regulates autism-like social behavior in the mouse as well as the formation of the dendritic tree and its maturation into a synaptically-connected branched structure (1, 2). ARHGAP33-deficient mice show cortical thinning associated with poorly branched dendritic trees. We demonstrate that inhibition of the small GTP-binding protein Cdc42 in postmitotic neurons is necessary for activation of the actin-binding protein cofilin and hence for proper branching of the neuronal dendritic tree during development of the mammalian neocortex. Furthermore, we demonstrate that these early steps are dependent on ARHGAP33, which acts as the main GTPase-activating protein for Cdc42 during neocortical development.

ARHGAP33 also has critical functions in the maturation of dendritic spines and in synapse formation in the neocortex. Surprisingly, we show that these later developmental functions are independent of its Cdc42 GAP activity. However we show that ARHGAP33 also directly interacts with several members of the MAGUK family of scaffold proteins and that, in mature neurons, it concentrates at the postsynaptic site. Furthermore we show that ARHGAP33 regulates the phosphorylation and subcellular localization of the MAGUK family protein and major postsynaptic component, PSD-95 as well as surface expression of the AMPA receptor, thereby providing a molecular basis for autism-like social behavior in the absence of ARHGAP33.
References


The Suppressors of Cytokine Signalling Soc6 and Soc7 Are Essential for Cortical Layering and Reelin Signalling

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Mutations of the reelin gene cause severe defects in cerebral cortex development and profound intellectual impairment. While many aspects of the reelin signalling pathway have been identified, the molecular and ultimate cellular consequences of reelin signalling remain unknown. Specifically, it is unclear if termination of reelin signalling is as important for normal cortical neuron migration as activation of reelin signalling.

Aims

The aim of this project was to determine the role of members of the suppressors of cytokine signalling (SOCS) family of negative regulators of cell signalling in reelin signalling and cortical layer formation.

Methods

The methods used included mice that were single or double deficient for Socs6 and/or Socs7, histological analysis, immunofluorescence, BrdU birth dating, telencephalon explant and cortical neuron migration cultures, protein affinity purification, immunoprecipitation, immunoblotting or mass-spectrometry, isothermal titration calorimetry and functional mutation.

Results

We discovered that combined loss of the suppressors of cytokine signalling, SOCS6 and SOCS7, recapitulated the cortical layer inversion seen in mice lacking reelin and led to a dramatic increase in the reelin signalling molecule disabled (DAB1) in the cortex. The SRC homology domains of SOCS6 and SOCS7 bound DAB1 ex vivo. Mutation DAB1Y300F greatly diminished binding and protected from degradation by SOCS6. Phosphorylated DAB1 was elevated in cortical neurons in the absence of SOCS6 and SOCS7.

Conclusions

We concluded that constitutive activation of reelin signalling was equally detrimental as lack of activation. We hypothesise that by terminating reelin signalling, SOCS6 and SOCS7 may allow new cycles of reelin signalling to occur and that these may be essential for cortical neuron migration.

A Dynamic Unfolded Protein Response Controls Cortical Neurogenesis

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Establishing Neuronal Identity in the Cerebral Cortex

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The functional integrity of the brain system relies on the precisely coordinated production of diverse neurons and their placement along the three-dimensional axis. Specifically in the cerebral cortex, progenitor cells produce distinct neuronal subtypes in a stereotypical order and establish a six-layer structure, which are further modified into functional areas. A prevailing view concerning the neurogenesis of the neocortex is that, neural stem cells undergo successive rounds of asymmetric cell divisions to produce the principal layer subtypes: preplate, deep-layer, and upper-layer neurons, through a progressive restriction in cell competence. Consistent with this view, we previously showed that foxg1, a forkhead transcription factor expressed in the telencephalon, plays a central role in establishing early gene network and switching neurogenesis from preplate cells to deep-layer neurons. However, our recent studies have also indicated that the specification and integration of neocortical neurons may rely on communication between distinct cell types, in addition to intrinsic transcriptional regulation. Here, I would like to present our findings on the mechanisms by which neocortical subtype identities establish in the neocortex, by manipulating gene expression and number of neurogenesis in the developing mouse cortex. Our results indicate that neocortical progenitors integrate both intrinsic and extrinsic cues to generate distinct layer neurons, a system which ultimately balances the production of neocortical subtypes during development and possibly evolution.

References

Despite an apparently similar laminar and cell-type organization, neocortical areas have distinct features in terms of molecular identity, morphology and long-range connectivity of residing projection neurons, leading ultimately to area-specific circuits. Despite its well-defined anatomical character and functional significance, the molecular mechanisms by which neuronal subtypes are specified within cortical layers and across domains as well as their precise assembly into distinct functional neocortical areas, remains largely unknown. Since neocortical areas are first pre-patterned by a set of transcription factors expressed in gradients during development and then acquire a distinct function postnatally (sensory-input versus motor-output), a complex interplay between intrinsic and extrinsic activity-dependent mechanisms might exist during formation of area-specific circuits. This talk will focus on how factors expressed in distinct prospective areas and layers control the ratio and distribution of projection neuron subtypes (intracortical versus subcortical) and how these factors modulate activity-dependent mechanisms in the motor and somatosensory postnatal cortex. Together with epigenetic modifications, we propose that the great variety of projection neurons in the mammalian cerebral cortex is not only due to the existence of genetic programs directing the development of each single neuronal subtype, but also to mechanisms that modify and refine after birth the processes specifying major projection neuron classes. Overall, our data contribute in unraveling some of the developmental mechanisms of how diverse populations of cortical projection neurons are coordinated into high-functional territories and how they interact during assembly of cortical circuits into distinct functional areas.

References


Behavioral Characterization of SATB 1 (+/-) Heterozygous Mice

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Satb1 (special AT-rich sequence binding protein 1) gene encodes a matrix protein that regulates chromatin structure and gene expression. In the present study, Satb1 heterozygous (+/-) mice were investigated to unravel the functional role of Satb1 in the brain.
**Materials and Methods**

The heterozygous knockout mutant of the Satb1 gene has been generated using the Cre-loxP system. Thirty two male (8 WT/8 SATB1 (+/-)) and female (8 WT/8 SATB1 (+/-)) mice at an age of two months were used. A battery of sensorimotor tasks was performed to assess coordination, climbing, locomotion and orienting reactions. In each test, the time was measured with the criterion of 120 s to complete. The mice were subjected to: walking initiation, bridges (1, 2 and 4 cm), wire suspension, turning in an alley, turning on an inclined screen. Spontaneous horizontal and vertical (rearing) locomotor activities and average speed were measured during 5 min using an Infrared Actimeter (Actitrack, Panlab, Barcelona, Spain). For assessment short-term memory in passive avoidance tests the Shuttle Boxes LE918 (Panlab, Barcelona, Spain) were used. During the acquisition/conditioning phase the animal is placed in the white compartment. When the animal innately crosses to the black compartment it receives a mild foot shock. During the test phase the animal is again placed in the white compartment and the latency of the entrance to the black compartment is evaluated. Startle response and prepulse inhibition of acoustic startle responses were measured by the Start and Fear Combined system (Panlab, Barcelona, Spain). Statistical analysis was performed using Satistica 10.0 software.

**Results**

Sensorimotor tests showed no impairment in locomotor activity of Satb1 (+/-) male and female mice. The four tested groups completed all the sensorimotor tasks. However in the locomotor activity test Satb1 (+/-) mice demonstrated an increased locomotion compared with WT mice. Distance moved in the central zone by male and female Satb1 (+/-) mice was significantly higher compared with WT mice. Significant increase in number of rearing in Satb1(+/-) female mice were showed. There were no significant differences due to genotype in the startle response and prepulse inhibition test. There was no difference in latency of the entrance to the black compartment between conditioning and test phase in passive avoidance task in Satb1 (+/-) male and female mice which indicates about deficit in retention of memory for an aversive experience in Satb1 (+/-) compared with WT mice.

**Conclusions**

Taken together, our findings suggest that Satb1 (+/-) presented behavioral alterations compared to their wildtype littermates, such as: hyperlocomotion and deficit in learning capacity.

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**Postnatal Dlx1&2 Functions in Cortical Interneuron Development**

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The postnatal functions of the Dlx1&2 transcription factors in cortical interneurons (CINs) are unknown. Here, using conditional Dlx1, Dlx2 and Dlx1&2 knockouts (CKOs), we defined their roles in specific CINs. The CKOs had dendritic, synaptic and survival defects, affecting even PV+ CINs. We provide evidence that DLX2 directly drives GAD1, GAD2 and VGat expression, and show that mutants had reduced mIPSC amplitude. Furthermore, the mutants formed fewer GABAergic synapses on excitatory neurons and had reduced mIPSC frequency. Furthermore, Dlx1/2 CKO had hypoplastic dendrites, fewer excitatory synapses, and reduced excitatory input. We provide evidence that some of these phenotypes were due to reduced expression of GRIN2B (a subunit of the NMDA receptor), a high confidence Autism gene. Thus, Dlx1&2 direct coordinate key components of CIN postnatal development by promoting their excitability, inhibitory functions and survival.
Post-Translational Control of Vesicular Release by NO

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Nitric oxide (NO) signalling is implicated in several neurodegenerative diseases through induction of high NO release. However, its exact contribution to degeneration remains elusive due to the complexity of downstream nitrergic modifications. High levels of NO can induce post-translational modifications which are associated with neuronal degeneration. NO reacts with superoxide anions to form cytotoxic peroxynitrite which in turn leads to 3-Nitrotyrosination with largely detrimental changes in protein function. Additionally, NO signalling alters protein function through S-nitrosylation. To date, little is known as to what extent these post-translational modifications contribute to or exacerbate neuronal dysfunction. We use glutamatergic synapses as a model system to identify novel nitrergic signalling pathways to correlate protein modifications with functional changes.

The Drosophila neuromuscular junction was used to characterise nitrergic effects employing electrophysiological methods. Two-electrode-voltage-clamp (TEVC) analyses were carried out in HL-3 solution using sharp electrodes (20-30MΩ). Data denote mean±SEM (n-number) with *p<0.05 indicating statistical significance (t-test, ANOVA). Evoked excitatory junctional currents (eEJC) amplitudes and quantal content (QC) were strongly reduced following NO exposure for >40min (eEJC: Ctrl: 119±7nA (22) vs NO: 62±8nA* (14); QC: Ctrl: 189±12 vs NO: 104±12*) suggesting a reduction in presynaptic release. Cumulative postsynaptic current analysis (500ms 50Hz train) further showed a reduced number of release-ready vesicles following NO exposure (Ctrl: 276±21 (22) vs NO: 108±19* (14)). Fluctuation analysis estimating the number of available release sites further confirmed a strong reduction under NO conditions. The above NO effects were detected following inhibition of the soluble guanylyl cyclase (sGC) and absent in the presence of N-ethylmaleimide which prevents the formation of S-nitrosothiols suggesting that NO modulates release via post-translational modifications. This interpretation is also supported by the findings that nitric oxide synthase (NOS) KO NMJs showed a strongly enhanced synaptic release and larger available vesicle pools. Importantly, enhancing presynaptic S-Nitroso glutathione reductase (GSNOR) or glutamate-cysteine ligase (GCLC) enzyme activities, by overexpression (OE), prevented the above nitricergic effects. Both pathways favour denitrosylation by reducing S-nitrosothiols and elevating cellular glutathione, respectively.

Together, our data suggest that NO can modify synaptic signalling possibly via inducing post-translational protein modifications. This data interpretation is supported by the notion that sGC inhibition is ineffective but modulation of neuronal nitrosylation pathways impacts on synaptic physiology implying presynaptic actions of NO in a sGC-independent manner. The data extends our understanding of NO signalling, potentially leading to the identification of putative targets disease.

Acknowledgements

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References


Dynamic Control of Neural Progenitor Fates in the Developing Neocortex

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Our laboratory studies neurogenesis, the process whereby neural progenitors generate neurons of the cerebral cortex during embryonic development. Our long-term objective is to help broaden our fundamental understanding of how the brain is built, how stem cells behave, and the etiology of neurodevelopmental diseases. The lab employs genetic,
BECOMING A NEW NEURON IN THE CEREBRAL CORTEX

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During neocortical development, excitatory neurons are born in the ventricular zone and migrate to the cortex, where they form the circuits that underlie mammalian skilled processing abilities. While the genetic programs that specify distinct subtypes of neurons within the neocortex are increasingly understood, how neuronal identity is dynamically acquired upon progenitor division is largely unknown. Identifying these primordial transcriptional processes is critical to understand how progenitor behavior is coupled to neuronal fate, and to provide mechanical insights into postmitotic neuron plasticity. Here, we will discuss recent findings from our laboratory on the mitotic and early-postmitotic biology of progenitors and their daughter cells, and how they inform neuronal specification and circuit assembly in the developing neocortex.

DYNAMIC CONTROL OF NEURAL STEM CELLS

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During brain development, neural stem cells gradually change their competency, giving rise to various types of neurons first and glial cells later. It is thus very important to maintain neural stem cells until the final stage of development to generate a full diversity of cell types. The basic helix-loop-helix (bHLH) factor Hes1 plays an important role in maintenance of neural stem cells by repressing proneural gene expression. We found that the Hes1 expression oscillates by negative feedback, and that this oscillation is important for proliferation of neural stem cells, as sustained Hes1 expression inhibits proliferation of these cells. Hes1 oscillation drives the cyclic expression of proneural factors such as Ascl1/Mash1. During neuronal differentiation, Hes1 expression disappears and proneural factor expression becomes sustained. By contrast, during astrocyte differentiation, Hes1 expression becomes dominant while proneural factor expression disappears. These results suggest that the multipotency is a state controlled by multiple oscillating fate-determination factors such as Hes1 and Ascl1/Mash1, and that one of them becomes dominant during fate choice. We further showed by optogenetic approach that sustained expression of Ascl1/Mash1 promotes neuronal differentiation, whereas oscillatory expression of Ascl1/Mash1 activates proliferation of neural stem cells, suggesting that the expression dynamics is important for the function of fate-determination factors. We also found that the Notch ligand Delta-like1 (DLL1), a downstream of Ascl1/Mash1 and Hes1, is expressed in an oscillatory manner, and that this oscillation is important for Hes1 oscillation and proliferation of neural stem cells. These results indicate that the oscillatory expression of these factors in neural stem cells is essential for neural development.

EVOLUTION OF CORTICAL DEVELOPMENT

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The cerebral cortex appears in stem amniotes and evolved in divergent manner in the two main amniote branches, namely the synapsids, that include premammals and mammals, and the sauropsids, now represented by reptiles and birds. Progress in our understanding of cortical neurogenesis, neuronal migration and layer formation allow to define common principles that are therefore presumably homologous and inherited from stem amniotes. On the other hand, critical features of mammalian cortex are absent in sauropsids and evolved after divergence of the two main radiations. Chief among those is the multilaminar organization of the mammalian cortex and its propensity to increase its surface by folding. Careful studies of human genetic disorders of cortical development and of animal models allow us to formulate mechanisms that can be tested using modern genetic and cellular technology. An integrated understanding of cortical development and evolution no longer seems an unattainable goal.

CORTICAL EXPANSION IN THE DEVELOPMENT OF COMPLEX MAMMALIAN BRAINS

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Introduction

Rapid expansion of brain size and complexity is a hallmark of mammalian evolution. The rodent dorsal brain, which is typically lissencephalic, forms a single primary germinal zone, the ventricular zone (VZ), which faces the ventricle on the apical side during development. In the VZ, self-renewing neural progenitors called radial glia undergo interkinetic nuclear movement and divide asymmetrically at the apical surface to give rise to a pair of daughter cells of distinct fates: another radial glial cell and an intermediate progenitor that divides once to generate a few neurons at the adjacent subventricular zone (SVZ). During the development of the complex brain, such as in ferret or primate, however, huge numbers of neurons are generated in the formation of the complex organization of the folded cortical structure. In such gyrencephalic brains, a new germinal zone, the outer SVZ (OSVZ), is formed during neurogenesis, and is thought to play important roles in the expansion of the neuronal population and formation of gyrencephaly.

Results and discussion

To gain a better understanding of the processes by which the OSVZ is formed from the VZ, we have used the ferret brain as a model of the complex brain in studies using long-term time-lapse imaging of brain slices, lineage analysis, and genetic perturbations (based on CRISPR/Cas9). We found that the cerebral cortex develops in a similar manner to the ganglionic eminence (the ventral side of the telencephalon) in ferret, unlike in rodent models. We discuss recent results from our group in light of this model of OSVZ formation.

References


DISTINCT EPIGENETIC FUNCTIONS OF SOX2 IN SELF-RENEWAL AND DIFFERENTIATION

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Introduction

The HMG-box transcription factor SOX2 is ubiquitously expressed in multipotent neural stem-precursor cells (NPCs) and supports their self-renewal. SOX2 expression is also required for the onset of neurogenesis in the central and peripheral nervous systems. However, the exact SOX2 function and its molecular mechanism are poorly understood.

Methods/Results

We have uncovered two distinct epigenetic mechanisms of Sox2 function in neural stem cell self-renewal and differentiation. Using human embryonic stem cell-derived NPC, we found that Sox2 promotes proliferation of neural stem cells via LIN28/let-7 pathway. Specifically, Sox2 promotes histone acetylation at Lin28 promoter thus maintaining Lin28 expression in NPCs, which is sufficient to maintain NPCs proliferation in the absence of Sox2 (1). Historically, SOX2 has been regarded as a transcription factor that opposes neurogenesis, and SOX2 repression must be relieved to allow initiation of transcription of the proneural gene NeuroD1. However, we found that SOX2 function is required to generate neurons both in central and peripheral nervous systems (2, 3). Mechanistically, we demonstrated that Sox2 primes epigenetic landscape in NPCs enabling proper gene activation during the onset of neurogenesis. Namely, Sox2 binds to bivalently marked promoters of poised proneural genes where SOX2 functions to maintain the bivalent chromatin state by preventing excessive PRC2 activity (3).

Discussion

To our knowledge, this is the first example of a lineage-specific transcription factor that maintains the bivalent state at the promoters of poised genes, thus coordinating the onset of a developmental (neurogenic) program and consequently ensuring a robust and appropriate terminal (neuronal) differentiation process.

Conclusion

We propose that SOX2 sets a permissive epigenetic state in neural stem cells, thus enabling proper activation of the neuronal differentiation program under neurogenic cues.

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**RODENTS AND HUMANS ARE ABLE TO DISCRIMINATE THE ODOUR OF L-LACTATE**

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Olfactory receptors (Olfrs) are seven transmembrane G-protein coupled receptors that are able to sense odorants. Rodents express over 1000 olfactory receptors while in humans only less than 400 of these genes are active. Some of the olfactory receptors are expressed not only in olfactory epithelium but in other tissues. One such receptor is mouse Olfr78 which has a rat and human orthologs (Orl59, OR51E2, correspondingly). Olfr78 has been implicated in various physiological processes due to its ectopic expression outside the olfactory system in other parts of the brain (brainstem, area postrema, nucleus tractus solitarius) and peripheral tissues (carotid body, prostate gland, kidney). It is activated by short chain fatty amino acids acetate and propionate, but also by L-lactate. Given that Olfr78 is specifically expressed in olfactory sensory neurons, we hypothesized that both rodents and humans should be able to smell lactate. To test this hypothesis we performed olfactory discrimination tasks with mice, rats and humans.

Our results show that adult male C57Bl/6 mice are able to distinguish L-lactate (1M, pH=7.4) in olfactory habituation-dishabituation and discrimination tasks. Mice spend twice as much time sniffing L-lactate compared to water, and equal amounts of time sniffing peppermint extract. Upon multiple exposures, mice habituate to the smell of L-lactate, however, they clearly discriminate the next introduced odour as measured by increased sniffing time.

We also used an operant-conditioning task reinforced by food to test the ability of rats to distinguish L-lactate (1M, pH=7.4). Rats were kept to a diet to maintain 85% of their free body weight and trained to find a food reward guided by the smell of either almond (positive control) or L-lactate. Rats were offered three sponges in an arena, where only one was marked with an odorant and contained a food reward. Success in finding a food reward orienting on the smell was measured by the time elapsed until food retrieval. Both almond and L-lactate odour were equally effective as operant stimuli as rats spent the same amount of time to find and retrieve the food reward upon exposure and this latency to food retrieval decreased over trials for both odours. We observed no difference in accuracy (number of incorrect pokes into sponge probes) over all trials between almond and L-lactate.

In order to test whether humans can sense L-lactate we recruited young adult volunteers for an odorant discrimination task. Participants were asked to indicate a presence of smell in four jars containing liquids (1M acetate, propionate, pyruvate and L-lactate adjusted to pH 7.4). Distilled water served as a negative control. 99% of participants indicated the presence of smell for a solution containing propionate, 97% and 95% detected an odour in solutions of pyruvate or acetate, respectively. 93% of participants detected an odour in a solution containing L-lactate. All the participants were asked to rate all samples in dimensions of taste and odour. 34% of participants identified the smell of L-lactate as sweet and 19% described it as sour/acidic.

Altogether, our data demonstrate that both rodents and humans are able to sense L-lactate, most likely via Olfr78 (or its ortholog). The biological significance of L-lactate detection by olfaction requires further investigation.

**CALCINEURIN INHIBITION ATTENUATES FUNCTION OF THE NEURONAL POTASSIUM-CHLORIDE COTRANSPORTER**

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**Aims**

Calcineurin inhibitors, cyclosporine and tacrolimus, are broadly used for immunosuppression after organ transplants but may cause serious neurologic side effects such as tremor, ataxia, or seizures suggesting that these drugs may induce hyperexcitability of neocortical neurons. Calcineurin has been implicated in the regulation of neuronal excitability and cellular chloride homeostasis. In this context, we tested the hypothesis that calcineurin inhibitors interfere with the function of major neuronal cation-chloride cotransporter KCC2.
Methods

To study effects of calcineurin inhibition on the KCC2 activity cyclosporine was applied in vivo or in rat brain slices. The evaluations were performed using immunofluorescence, immunoblotting and co-immunoprecipitation, quantitative PCR, and electrophysiological measurements with sharp microelectrode.

Results

Calcineurin and KCC2 co-localized in rat neocortical neurons by immunofluorescence and interacted by co-immunoprecipitation. Short term administration of cyclosporine to rats (25 mg/kg for 1 to 4h) resulted in increased tyrosine phosphorylation of KCC2 suggesting inhibition of its activity. In line with this, intracellular recordings of chloride homeostasis after iontophoretic CI- loading revealed strong cyclosporine-induced prolongation of the CI- extrusion time (+3.4s, p<0.05) which was compatible with KCC2 blockade. Chronic administration of cyclosporine to rats (5 to 25 mg/kg for 14 days) drastically reduced the level of activating KCC2 phosphorylation at S940 (-65%, p<0.05). In addition, expression of the KCC2-inhibiting SPAK kinase was significantly increased upon chronic cyclosporine treatment (+59%, p<0.05).

Conclusions

In summary, our data suggest that calcineurin inhibition using cyclosporine attenuates KCC2 function in acute and chronic settings. Our data have clinical implications for immunosuppressive therapy using calcineurin inhibitors.

Lack of Diap3 Relaxes the Spindle Checkpoint Causing the Loss of Neural Progenitors and Microcephaly

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The diaphanous homologue Diap3 (also referred to as mDia2, DIAPH3 in humans) is a major regulator of actin dynamics. Loss of Diap3 has been constantly associated with cytokinesis failure ascribed to impaired accumulation of F-actin in the cleavage furrow. We provide evidence that Diap3 is required prior to cell fission to ensure the accurate segregation of chromosomes. In mice, inactivation of the Diap3 gene causes a massive loss of neural progenitor cells with subsequent reduction in the number of intermediate progenitors and neurons, and ultimately results in microcephaly. Mechanistically, we show that Diap3 co-localises with chromosomal passenger complex (CPC) proteins at the kinetochore–mitotic spindle interface, and interacts physically with BubR1 and Survivin, components of the spindle assembly checkpoint (SAC) and CPC respectively. Diap3-deficient neural progenitor cells have decreased levels of BubR1, and fail to properly distribute CPC proteins, or activate the SAC. Hence, they bypass the mitotic arrest and embark on anaphase in spite of incorrect chromosome segregation which causes their apoptotic death. These findings identify Diap3 as an important guard of cortical progenitors, shed new light on the mechanisms of action of Diaphanous formins during cell division, and add insights into the pathobiology of primary microcephaly.

Sip 1-Mutation Causes a Disturbance in Activity of NMDA- and AMPA-Receptors of Neurons in Cerebral Cortex

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It is known that a mutation of Sip1 gene leads to disruption of normal development and as consequence to distort functioning of cortex [1] and hippocampus [1] of the brain. Sip1 mutation physiologically expresses in dysregulation of neurogenesis [2], an increases the risk of developing of epilepsy [3], and mental retardation [4]. The aim of this research is to analyze effect of the mutation in transcription factor Sip1 on receptor-mediated component of calcium signaling in cortical neurons.

Cells were loaded by calcium-sensitive probe Fura-2 for registration of cytosolic calcium levels. Fluorescence detection was performed with help of an image analysis system «Cell observer» (Carl Zeiss, Germany), on the basis of inverted motorized microscope Axiovert 200M. As the object of research we used primary cultures of cells from cortex and brainstem of S1P1 mutant mice (p1-p2) [5,6]. Experiments were performed on 8th day of culturing of neurons, when culture has well-developed neural network. Neurons distinguished from astroglia by presence of $\text{Ca}^{2+}$ response on depolarization by KCl application. The main measuring parameter was amplitude of $\text{Ca}^{2+}$-response of neurons on short-term application of agonists of ionotropic glutamate receptors.

Sequential application of activators of NMDA- (fig. 1a) and AMPA-receptors (fig. 1b) causes a transient increase in cytosolic $\text{Ca}^{2+}$ in both neurons from wild-type mice and in cells of homo- and heterozygous mutant Sip1 mice. In this case, the amplitude of the $\text{Ca}^{2+}$-signal on NMDA and FW is significantly lower in neurons of Sip1$^{fl/fl}$ mice than in neurons of Sip1$^{fl/wt}$ and wild type mice (Sip1$^{wt/wt}$). In neurons derived from brainstem of homozygous mutant Sip1 mice (control for the cortex) amplitude of $\text{Ca}^{2+}$-response to NMDA and FW are almost the same (not shown) as in neurons of wild type mice and heterozygozite because this part of the brain is not damage by directed mutagenesis.

Diagrams of dependence of dose that was built for activators of NMDA- and AMPA-receptors shown that Sip1$^{fl/fl}$-neurons characterize by considerable resistance (fig. 1c and d), and the EC 50 value is 425 and 188 nM, respectively. Whereas for heterozygous mutant neurons (Sip1$^{fl/wt}$) characterize on the contrary by increase of sensitivity to NMDA activators (83 nM) and AMPA receptors (8.4 nM), relative to wild-type neurons whose EC50 for NMDA and FW is 96 and 18 nM, respectively.

Thereby Sip1 mutation changes the activity of basic ionotropic glutamate receptors of cortical neurons but does not change in brainstem. Besides in neurons of homozygotes these changes express in common signal insufficiency, and contrariwise in neurons of heterozygotes was shown the increasing sensitivity to NMDA- and AMPA-receptors activators. These changes in receptors activity can lead to faster activation of neuronal pathways, excitotoxicity and induction of cell death processes under moderate activity (short-time hypoxia) on physiological level [6].

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Multiplex Approach in Depressive Disorders Research


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Aims

Depressive disorders, with a lifetime prevalence of over 15%, is a great burden for both patients and the society. Depressive disorders is also increasingly implicated in a wide range of other medical conditions. As yet a reliable (biological) diagnostic marker for depressive disorders does not exist. Nevertheless, an increasing body of evidence indicates that the underlying neurobiology of depression likely involves a complex interplay of various factors. Thus far, research into single biomarkers for depression was not very successful. Arguably, a combination of biomarkers reflecting the various neurobiological disturbances in depression might enable a more specific and selective diagnosis of depressive disorder.

Aim of the study was to investigate levels of the following substances in the serum of patients with depressive disorders and healthy subjects: β-endorphin, orexin A, cortisol, melatonin, insulin-like growth factor 1 (IGF-1), interferon γ (INF-γ), interleukin 1β (IL-1β), interleukin 3 (IL-3), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α).

Materials and methods

78 patients (46 patients with depressive episode (ICD-10: F32), 32 patients with recurrent depressive disorder (ICD-10: F33)) were included in the study. Control group consisted of 71 physically and mental healthy persons. β-endorphin, orexin A, cortisol, melatonin, IGF-1, INFγ, IL-1β, IL-3, IL-6, TNFα were measured in the serum using Luminex xMAP® technology. Concentrations of investigated substances were measured by MAGPIX apparatus (Merck Millipore). Statistical analyses were performed using the SPSS software, release 20.0, for Windows. Results were expressed as median (25% quartile — 75% quartile). Between-group differences were evaluated using the Mann - Whitney U-test. p values less than 0.05 were considered as significant.

Results

Our study showed significantly higher concentration of cortisol in the group of patients with depressive disorders (214.17 (155.51 – 315.19) ng/ml) compare to control group (168.48 (121.61 – 263.34) ng/ml), (p=0.037). We found no difference between groups in concentration of neuropeptides and hormones, such as β-endorphin, orexin A, melatonin and IGF-1. Concentration of β-endorphin in control group was 252.28 (136.90 – 366.84) pg/ml, in group of depressive patients concentration was 259.26 (204.06 – 314.47) pg/ml, (p=0.436); concentration of orexin A in control group was 614.13 (379.25 – 891.34) pg/ml, in group of depressive patients concentration was 614.13 (379.25 – 891.34) pg/ml, (p=0.436); concentration of IGF-1 in control group was 8.75 (5.90 – 20.75) ng/ml, in group of depressive patients concentration was 6.26 (4.52 – 13.57) ng/ml, (p=0.101); concentration of melatonin in control group was 46.15 (27.55 – 77.86) pg/ml, in group of depressive patients concentration was 66.30 (32.59 – 132.59) pg/ml, (p=0.071). No difference between groups in concentration of cytokines INF-γ, IL-1b, IL-3 and TNF-α except level of IL-6 was observed. Concentration of IL-6 in the group of depressive patients was significantly higher (3.79 (2.90 – 5.63) pg/ml) compare to control group (3.22 (2.67 – 4.02) pg/ml) (p=0.002).
Conclusion

These results confirm the hypothesis of hypothalamic-pituitary-adrenal (HPA)-axis’s dysregulation and inflammation in the pathogenesis of depression and the ability to use the studied parameters as biomarkers (M. Furtado, M.A. Katzman., 2015). Dysregulation of the HPA-axis, one of the body’s major stress systems, has been frequently implicated in the etiology of depressive disorders. According to literature dates, patients with depressive disorders may have elevated cortisol levels in plasma. Over the last two decades, new developments in psychiatric research have led to the hypothesis that inflammatory processes and neural-immune interactions are involved in the pathogenesis of major depression and that these might underlie some of the frequently observed serotonergic and adrenocortical correlates of depression. The cytokine hypothesis of depression implies that pro-inflammatory cytokines, which act as neuro-modulators, represent key factors in the (central) mediation of the behavioural, neuroendocrine and neurochemical features of depressive disorders (O. Schiepers, M. Wichers and M. Maes., 2005). The central action of cytokines may also account for the HPA-axis hyperactivity frequently observed in depressive disorders, because pro-inflammatory cytokines can cause HPA-axis hyperactivity by disturbing the negative feedback inhibition of circulating corticosteroids on the HPA axis.

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References


In Vivo knockdown of Basal Forebrain p75 Neurotrophin Receptor Stimulates the Cholinergic Septo-Hippocampal System in Mature Animals

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We sought to investigate whether knockdown of p75 Neurotrophin Receptor (p75NTR) expression in cholinergic neurons of the basal forebrain would enhance the Cholinergic System and enhance Septo-Hippocampal function (as predicted by p75 knockout models). To measure cholinergic status we measured choline acetyltransferase (ChAT) activity in hippocampi of mature animals. Antisense oligonucleotides (oligos) targeting p75NTR were infused into the medial septal area of mature rats continuously for 4 weeks. In all rats, the cannula outlet was placed equidistant between the left and right sides of the Vertical Diagonal Band of Broca (VDB). We tested phosphorothioate (PS), morpholino (Mo) and gapmer (mixed PS/RNA) oligos. Gapmer antisense infusions of 7.5 μg/day and 22 μg/day decreased septal p75NTR mRNA by 34% and 48%, respectively. The same infusions increased hippocampal ChAT activity by 41% and 55%. Increased hippocampal ChAT activity correlated strongly with septal p75NTR downregulation in individual rats. Infusions of PS and Mo antisense oligos did not downregulate p75NTR mRNA or stimulate ChAT activity. These results demonstrate that p75NTR can dynamically regulate hippocampal ChAT activity in the mature central nervous system. They also reveal the different efficacies of three diverse antisense oligo chemistries, when infused intra-cerebrally. Of the three types, gapmer oligos worked best. We subsequently repeated the gapmer antisense and nonsense infusions and tested Spatial Navigation at the end of the infusion period. We used the Barnes Maze to test spatial navigation. There was a significant improvement in criterion attainment in p75-downregulated rats, consistent with enhanced cholinergic drive to the hippocampus.

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