Abstracts

PRIMATE NEUROGENESIS SYMPOSIUM 19 – 20 May 2017, Varna, Bulgaria

Wieland Huttner

Max Planck Institute for Molecular Cell Biology
and Genetics, Dresden, Germany
E-mail: huttner@mpi-cbg.de

Neural stem and progenitor cells in neocortex development and evolution

Our group studies neural stem and progenitor cells in the context of the expansion of the neocortex in development and evolution. Two major classes of cortical stem/progenitor cells can be distinguished. First, stem/progenitor cells that reside in the ventricular zone (VZ), i.e. neuroepithelial cells, apical radial glia (aRG) and apical intermediate progenitors, collectively referred to as apical progenitors (APs). Second, stem/progenitor cells that reside in the subventricular zone (SVZ), i.e. basal radial glia (bRG) and basal intermediate progenitors, collectively referred to as basal progenitors (BPs). Neocortex expansion is thought to be linked to an increased abundance and proliferative capacity of BPs.

To gain insight into the genomic changes that underlie neocortex expansion, notably in humans, we have analyzed the transcriptomes of human vs. mouse VZ and SVZ, and of human vs. mouse aRG and bRG. This led to the identification of the human-specific gene *ARHGAP11B*

as a major player. Specifically, *ARHGAP11B* promotes the generation of BPs from aRG and the subsequent BP proliferation, thereby increasing BP abundance. Moreover, *ARHGAP11B* is able to induce folding of the embryonic mouse neocortex, which normally is smooth. The ability of *ARHGAP11B* to amplify BPs is based on a single C-to-G base substitution which creates a novel splice donor site; this leads to the removal of 55 nucleotides upon mRNA splicing, resulting in a reading frame shift and generating a human-specific 47-amino acid sequence that is thought to be key for BP amplification.

To compare neural stem cell division between human and great ape developing neocortex, we have performed live imaging using iPSC-derived 3D cerebral organoids. This revealed a specific lengthening of metaphase during AP mitosis in human as compared to chimpanzee and orangutan. The potential implications of this metaphase lengthening will be discussed.

Zaal Kokaia

Lund University Stem Cell Center, Lund, Sweden

E-mail: Zaal.Kokaia@med.lu.se

Reprogrammed cortical neurons for transplantation to the lesioned cortex

Dr. Kokaia's group is transplanting different types of neuroblasts generated either from human skin cell-derived induced pluripotent stem (iPS) cells or via direct conversion of skin cells to neurons (iN cells). Dr. Kokaia also works with endogenous neural stem cells, which participate in post-stroke recovery and can replace lost neurons. Exploration of mechanisms regulating this self-repair process, which was discovered by his team (Arvidsson et al., 2002, *Nature Medicine*), and definition of ways to modulate this response by which it could be developed into an efficient therapeutic tool is a major goal of their research. Dr. Kokaia also studies how inflammation associated with ischemic brain damage influences the generation and integration of neurons derived from stem cells and reprogrammed cells, and how the beneficial effects of innate and adaptive immune system should be promoted to improve functional recovery.

In recent years, the recovery phase of stroke attracted much of the attention of researchers and clinicians, and currently is considered as most suitable target for the stroke therapy. This is justified by the long-term therapeutic window and also intrinsic plasticity-based mechanism of recovery which is operating in the brain and represents suitable target of the therapy. These therapeutic approaches would be initiated, many hours, if not days and weeks after stroke onset, with the intention of improving neurological function and not necessarily reducing the burden of the ischemic lesion. The aim of such therapeutic strategy is to enhance and accelerate the spontaneously operating self-repair/recovery mechanism. Stem cells have the capacity to generate neurons and glia cells which are lost in neurodegenerative diseases including stroke. Cells from different sources have been tested for their ability to reconstruct the forebrain and improve function after transplantation in animals subjected to stroke. Somatic cells reprogrammed to induced pluripotent stem cells (iPSCs) improve behavioral recovery and give rise to neurons after implantation into stroke-injured adult rodent brain. We have recently

shown improved functional recovery after transplantation of human iPSC-derived cortical neuronal precursors in a rat model of cortical stroke (Tornerio et al., 2013, *Brain*). Grafted cells give rise to mature neurons that re-build the damaged tissue and send fibers to several host brain structures. However, the underlying mechanisms of this improved recovery are unclear and there is little evidence for neuronal replacement. Whether the grafted neurons receive direct synaptic inputs from the host brain and are functionally integrated into its neuronal circuitries is also still unknown.

In our recent study, we used a rabies virus (RV)-based strategy to explore whether host cells can establish functional synaptic connections with transplanted cells. Two or five months after transplantation of modified It-NES cells in a rat stroke model, we injected the RV in the location of the graft. Expression of TVA receptor in the mature neurons (synapsin I+) generated from grafted cells makes them suitable for infection with the RV. The presence of Rabies-G glycoprotein in these cells allows the virus to infect the cells that connect to them by functional synapses. Therefore, grafted and infected cells will express nuclear GFP and cytoplasmic RFP while the ones connected to them will only present RFP in the cytoplasm.

Immunohistochemical analysis of injured and transplanted brains one week after the infection with RV revealed the presence of RFP+ neurons in different areas, some of them located far away from the implantation site.

Using electrophysiological recordings *in vivo* and optogenetics in brain slices recordings combined with patch-clamp we demonstrated, for the first time, that intracortical grafts of human iPSC-derived cortical neurons establish functional afferent synaptic connections with stroke-injured brain and respond to peripheral sensory stimulation. Further studies should evaluate how the reconstruction of stroke-lesioned network by grafted stem cell-derived neurons contribute to post-stroke recovery.

Gerd Kempermann

German Center for Neurodegenerative Diseases,
Dresden, Germany
E-mail: gerd.kempermann@tu-dresden.de

Humans as model for mice: how to assess functions dependent on adult neurogenesis

The group is interested in how adult-born neurons in the hippocampus contribute to brain function in health and disease. The main focus is on how activity-dependent regulation of adult hippocampal neurogenesis results in a ‘neurogenic reserve’ for the aging brain and contributes to individualization. The method spectrum ranges from molecular biology and genetics to behavioral analyses.

Gerd Kempermann & Alexander Garthe

Adult hippocampal neurogenesis takes place at a prominent location within the hippocampal circuitry and there is increasing evidence that adult-born neurons perform important functions. Although details have been difficult to unravel, a remarkably consistent picture emerges. First, adult-generated neurons appear to contribute to ‘be-

havioral pattern separation’ as a key functionality assigned to the dentate gyrus. Second, on the level of more complex functions, we have proposed that new neurons allow the flexible integration of new information into existing contexts. While adult neurogenesis itself cannot be measured in living humans, such specific functional outcomes can. We have developed (and continue to do so) a behavioral test that allows parallel investigations in humans and mice and is tailored to identify increasingly specific functional contributions that can be related to adult neurogenesis. Irrespective of whether, ultimately, such putatively causal link can indeed be made, these experiments have brought to light, how powerful bi-directional translation is in this context.

Magdalena Götz

Institute for Stem Cell Research, Helmholtz
Center, Munich, Germany
E-mail: magdalena.goetz@helmholtz-muenchen.de

Novel molecular mechanisms regulating cerebral cortex expansion

Our research aims at identifying the endogenous mechanisms of neurogenesis where and when they work and then implement these for neural repair after injury.

Our research has led to the discovery of a novel nuclear protein involved in cerebral cortex folding, Trnp1. However, the molecular mechanism of how Trnp1 exerts its function was entirely unknown. I will present unpublished data

about a surprising mechanism of how Trnp1 regulates proliferation of neural stem cells. I will then describe a novel centrosomal protein with a key role in subventricular zone formation by a novel mechanism. The phylogenetic relevance of both of these proteins for cerebral cortex evolution will be discussed.

Víctor Borrell

Institute of Neuroscience, San Juan
de Alicante, Spain
E-mail: vborrell@umh.es

Genetic control of cerebral cortex size and folding

My lab is focused on understanding the cellular and molecular mechanisms governing the expansion of the cerebral cortex observed across mammalian evolution. This evolutionary expansion of the cerebral cortex is recapitulated during development in higher mammals, when the embryonic cerebral cortex undergoes massive growth in surface area, and folds itself in stereotypic patterns. We are interested in the identification and analysis of the basic cellular and molecular mechanisms involved in the normal expansion and folding of the cerebral cortex in higher mammals. To this end, we combine genetic tools (in vitro and in vivo electroporation, viral vectors, transgenic and knock-out mice), experimental embryology, state-of-the-art imaging techniques and standard histological, cellular and molecular biology methods, using various species as experimental models, including mouse, ferret, cat, sheep, chick and snake. Currently, our efforts focus on understanding the role played by the different types of cortical progenitor cells in the tangential and radial expansion of the cerebral cortex, and in the formation of gyri at stereotypic locations in the cerebral cortex during development. Data from a transcriptomics screen in ferret is allowing us to begin testing the involvement and functional role of specific candidate genes, many of which appear mutated in human malformations of cortical development and folding. Our long-term goal is to understand how the size and folding of the cerebral cortex influence its function as a computing system, and thus how its abnormal development leads to severe cognitive impairment and intellectual disability in humans.

One of the most prominent features of the human brain is the fabulous size of the cerebral cortex and its intricate folding, both of which emerge during development. Cortical size is determined by the balance between progenitor cell self-renewal and neurogenesis. Cortical folding depends on the abundance of a particular type of basal progenitor, basal Radial Glia Cells (bRGCs), which abundantly populate a unique germinal layer, the Outer Subventricular Zone (OSVZ). Here I will discuss recent findings from my laboratory revealing novel cellular and genetic mechanisms that regulate cortical expansion and folding. In particular, we have identified an unprecedented mechanism of cortical development that underlies the embryonic emergence of the OSVZ. During a brief developmental period, apical progenitors in the ventricular zone generate a burst of bRGCs that become founders of the OSVZ. After closure of this period and for the remaining development, progenitors in the OSVZ follow a lineage completely independent from the other germinal layers. This brief time window is confined by the dynamic temporal regulation of genes key for bRGC formation, which hence determine the emergence of the OSVZ. Once the OSVZ is formed, the cortex folds in highly stereotyped patterns. We have identified unique transcriptional signatures along cortical germinal layers that map the prospective location of folds and fissures, including genes mutated in human cortical malformations. This map reflects mosaic expression patterns across the developing gyrencephalic cortex of ferret and human, but not the lissencephalic mouse, and may contribute to define cortical folds in gyrencephalic species.

Colette Dehay

Stem Cell and Brain Research Institute,
University of Lyon, France
E-mail: colette.dehay@inserm.fr

Proliferation, lineage characteristics and migration properties of primate cortical neurons

My team is studying the cellular and molecular mechanisms of cortical development. We are one of the few world-wide teams that is bridging the gap between work in rodents and human developmental neuroscience by studying primate development. One of our significant contributions is the demonstration of the role of the G1 phase of the cell-cycle in areal specification (Dehay et al., *Nature*, 1993; Lukaszewicz et al., *Neuron*, 2005; Dehay and Kennedy, *Nat Rev Neurosci*, 2007; Pilaz et al., *PNAS*, 2009). We have established extensive know-how in manipulation of gene expression on ex vivo embryonic cortex organotypic slices in combination with state of the art facilities for live-imaging of cortical precursors in rodents and primates. Using RNA-Seq and miRNA-Seq, we are currently investigating the molecular underpinnings of the distinctive properties of the primate cortex germinal zones and of the high diversity of cortical precursors (Betizeau et al., *Neuron* 2013).

The expansion and complexification of the cerebral cortex that characterize the primate are largely linked to the emergence of the Outer Sub Ventricular Zone (OSVZ), a uniquely structured germinal zone that harbors a higher diversity of progenitors than observed in non-primate species. We report the distinct lineage properties and cell-cycle kinetics of deep and superficial neuron progenitors in the non

human primate visual cortex. Supragranular neuron (SG) progenitors (generated by the OSVZ) are characterized by a shortening of G1 and S phases compared with infragranular (IG) neuron progenitors. miRNA-seq analysis performed at the time of generation of SG neurons, revealed that newly evolved, primate-specific miRNAs uniquely characterize distinct compartments of the OSVZ in the macaque monkey. A high proportion of these differentially expressed primate specific miRNAs target cell-cycle control genes and more specifically the G1/S transition regulators in SG neuron progenitors. This evolutionary innovation in wiring of the gene networks that targets the G1 /S transition has contributed to the dramatic expansion of the OSVZ, considered to underlie the complexity of the human and non human primate cerebral cortex (reviewed in Dehay et al., *Neuron*, 2015). Right after cell-cycle exit, we observed that IG and SG postmitotic neurons exhibit distinct behavioral signatures during radial migration to the cortical plate, pointing to a coordination between migration and proliferation. Compared to IG neurons, SG neurons are characterized by increased migration velocity and global membrane dynamics as well as a change in vesicular traffic directionality. We observed that p27^{kip1} is upregulated in radially migrating SG neurons of area 17 and influences both vesicular traffic and migration velocity.

Barbara Treutlein

Max Planck Institute for Evolutionary
Anthropology, Leipzig, Germany
E-mail: barbara_treutlein@eva.mpg.de

Dissecting human and chimpanzee cerebral organoids using single-cell transcriptomics

Every cell has a story to tell. New technologies to sequence RNA and DNA from individual cells are providing unprecedented insights into the biology of complex tissues. We use single cell genomic and transcriptomic data to reconstruct differentiation pathways, lineage hierarchies, and tissue heterogeneity in human organs and organoids, mouse brains and lungs, amphibian appendages, and any other system we find fascinating.

Our primary goal is to understand the mechanisms controlling cell fate decisions during development and regeneration, with a particular interest in illuminating uniquely human biology.

J. Gray Camp¹, Sabina Kanton¹, Felipe Mora-Bermudez², Farhath Badsha², Marta Florio², Ben Vernot¹, Svante Pääbo¹, Wieland Huttner², Barbara Treutlein^{1,2,3}

Cerebral organoids have emerged as powerful models of human brain development, and offer the potential to study uniquely human brain evolution. However, the extent to

which cerebral organoid systems recapitulate fetal gene expression networks remains unclear. Here we use single-cell RNA sequencing (scRNA-seq) to dissect and compare cell composition and progenitor-to-neuron lineage relationships in human and chimpanzee cerebral organoids and fetal human neocortex. We find that human and chimpanzee organoid cortical cells use gene expression programs remarkably similar to those of the fetal tissue in order to organize into cerebral cortex-like regions. We identify genes that are differentially expressed in human progenitors and neurons relative to chimpanzee, and highlight modern human genetic changes that can be studied in organoid cultures.

¹Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

²Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany

³Technical University Munich, Munich, Germany

Tran Tuoc

Department of Anatomy, University Medical
Center, Göttingen, Germany
E-mail: tran.tuoc@med.uni-goettingen.de

Epigenetic and chromatin regulation in cortical progenitor amplification

Chromatin remodeling complexes are crucial in gene expression regulation via controlling chromatin dynamics. Proper function of these factors play critical roles in neural develop-

ment, including the establishment and maintenance of neural fates and their functionality. Recent human exome sequencing studies have revealed that mutation of genes encoding for these

factors is linked to wide range of neurodevelopmental disorders. My lab is focused on understanding chromatin regulatory mechanisms in neural development and how alterations to these mechanisms can cause such brain disorders.

The cerebral cortex comprises cellular laminae that provides the structural basis of sensorimotor processing and intellectual functionality in mammals. During embryogenesis, neural progenitor cells (NPCs) in germinal areas give rise to neurons that make up the mammalian neocortex. Different NPCs, including apical (APs) and basal progenitors (BPs), can be distinguished based on cell morphology, polarity, ability to generate cell lineage, and their mitosis site. Among cortical progenitor subtypes, BPs are essential for cortical neurogenesis

and cortical expansion.

Recently, numerous genome-wide gene expression investigations have aimed at elucidating the evolutionary and developmental phenomenon of cortical progenitor amplification and neocortical expansion. Nonetheless, epigenetic mechanisms assumed to coordinate the expression or repression of batteries of genes in progenitor amplification during evolution, are largely unknown.

In my talk, I will discuss recent data in understanding of chromatin remodeling during cortical neurogenesis. I will highlight roles of chromatin remodeling SWI/SNF (BAF) complexes and their epigenetic cofactors in genesis of basal progenitors.

Fiona Doetsch

University of Basel, Switzerland

E-mail: fiona.doetsch@unibas.ch

Stem cell quiescence and activation in the adult brain

Research in the laboratory of Fiona Doetsch focuses on the intrinsic and extrinsic regulation of stem cells in the adult mammalian brain. Using novel approaches to purify stem cells directly from their *in vivo* niche, they are defining the *in vivo* heterogeneity and potential of adult neural stem cells, and the gene regulatory networks underlying stem cell quiescence and activation. In addition, they are investigating how different compartments of the niche dynamically influence the behavior of adult neural stem cells and their progeny. Fiona Doetsch obtained her B.Sc. at McGill University in Montreal, Canada and her Ph.D. at Rockefeller University in New York City, USA. She then moved to Harvard University, where she was a Junior Fellow of the Society of Fellows and a Fellow at the Radcliffe Institute for Advanced Studies before joining Columbia University as faculty in 2003. In 2014, she moved to the Biozentrum, University of Basel in Switzerland. Among several honours, she has been awarded the David and Lucile Packard Fellowship for Science and Engineering, the Irma T. Hirschl Scholar Award and the Harold and Golden Lampert Award for Excellence in Basic Science Research.

Stem cells in different organs are important for tissue homeostasis and repair after injury. Specialized niches support the life-long maintenance and function of adult stem cells. In the adult brain, stem cells give rise to new neurons that functionally integrate into restricted brain areas. Adult neural stem cells share common features, including glial identity in different species. However the sites of neuronal integration, as well as the numbers of new neurons generated, differ across phylogeny. The largest germinal niche in the adult mouse brain is the ventricular-subventricular zone (V-SVZ) adjacent to the lateral ventricles. Quiescent V-SVZ adult neural stem cells integrate intrinsic and extrinsic signals to become activated and generate progeny. However little is known about the niche signals that mediate their behavior. I will present our recent findings about the functional and molecular properties of quiescent and activated adult neural stem cells as well as unique features of the specialized niche that regulate adult neurogenesis, and how these change and impact stem cell dynamics in different states such as aging. Insights into these processes in the adult mouse brain may be translatable to other species, including humans, where V-SVZ stem cells are largely dormant.

Olivier Raineteau

Stem Cell and Brain Research Institute,
Université de Lyon, France
E-mail: olivier.raineteau@inserm.fr

Origin and potency of postnatal forebrain glutamatergic progenitors

Our laboratory investigates lineage specification in the postnatal SVZ, the main germinal zone of the postnatal forebrain. We have participated to the demonstration that the SVZ is not a homogeneous germinal region, but is composed of microdomains that have different embryonic origins, distinct transcriptional signatures and are biased in generating specific neural cell types. Over the last few years, we have progressively focused on characterizing the dorsal-most domain of the postnatal SVZ (dorsal SVZ or dVSZ), where a population of glutamatergic (Glu) progenitors persists and continue producing a discrete population of olfactory bulb glutamatergic neurons early after birth, as well as in early adulthood. We have shown that Glu progenitors are not only observed in mice but also in primates, early after birth. Finally, we have used pharmacological and genetic approaches to demonstrate a role of the Wnt canonical pathway in promoting Glu progenitors proliferation and specification in the postnatal and adult SVZ, as well as following premature brain injury.

Investigating the 3D distributions of proliferative progenitors and committed neuroblasts in the marmoset forebrain highlights a persistent germinal activity in the dentate gyrus and rostral most regions of the lateral ventricles. Expression of lineage-specific transcription factors (e.g. *dlx2*, *tbr2*) reveal that glutamatergic (Glu) progenitors are not only observed in the dentate gyrus, but also in the most dorsal regions of the neonatal marmoset subventricular zone (SVZ).

This regional heterogeneity of the postnatal SVZ as well as the presence of postnatal Glu progenitors is also observed in rodents, where it can be studied more extensively. We used

a transgenic mouse line that allows the conditional labeling of birth-dated cohorts of glutamatergic progenitors and their progeny. Our results show that recombination at embryonic stages allows the targeting of progenitors of specific cortical layers. Recombination at postnatal stages reveals a surprisingly large population of Glu progenitors that persist in the dorsal most region of the SVZ for several weeks after birth. Fate mapping of these postnatal progenitors reveal that whereas most undergo delayed cell death, some give rise to cortical neurons that express markers of late born neurons, but ectopically migrate to deep cortical layers where they survive for at least several weeks. Birth dating and RNA-sequencing experiments suggest that postnatal Glu progenitors represent a heterogeneous population in term of embryonic origin and reveal their transcriptional specificities when compared to their embryonic counterparts. In line with the presence of postnatal Glu progenitors, premature brain injuries result in their reactivation and in the regeneration of some glutamatergic neurons in both upper and deeper cortical layers. Finally, a pharmacogenomics approach identified Gsk3b inhibitors to stimulate Glu progenitors and promote this spontaneous regenerative attempt.

Altogether, these results highlight the regional diversity of neural progenitors at early postnatal stages, both in rodents and primates. Further characterization of Glu progenitors in mice reveals their heterogeneity and amenability to pharmacological manipulations following premature brain injuries.

Anton Tonchev

Medical University – Varna, Bulgaria

E-mail: anton.tonchev@mu-varna.bg **Transcriptional profiles of adult monkey anterior subventricular zone progenitors**

Neurogenesis persists in the brain of adult mammals, including primates, in restricted brain regions. There are differences between the primates and non-primate species regarding the extent of neurogenesis and possibly regarding the localization of neurogenic sites. We are interested in finding the specific features of the neurogenic niche in primates using adult monkey model and postmortem human specimens.

Monika Chongtham¹, Haifang Wang², Christina Thaller¹, Nai-Hua Hsiao¹, Ivan Vachkov¹, Stoyan Pavlov³, Lorenz Williamson³, Tetsumori Yamashima⁴, Anastassia Stoykova¹, Jun Yan³, Anton B. Tonchev^{1,3}, Gregor Eichele¹.

¹ Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

² CAS-MPG Partner Institute for Computational Biology, Shanghai, China

³ Medical University - Varna, Bulgaria

⁴ Kanazawa University, Japan

Understanding the specificity of regenerative neurogenesis in primate brain would have a great impact for understanding the mechanisms of neuronal replacement in human brain, and thus is with a great therapeutic significance. Given the high diversity of cortical progenitors with uniquely proliferative potential in primate/human brain, one direction of investigations is to develop batteries of reliable endogenous markers for characterization of neural precursors and neurogenesis.

We have previously shown that cerebral ischemia in adult monkey brain increases progenitor cell proliferation in the anterior subventricular zone (SVZa) during the second

postischemic week (Tonchev *et al.*, 2005). In order to deepen the knowledge related to this progenitor response, we investigated the gene expression changes on a transcriptome level in the adult monkey SVZa at the end of the first postischemic week. We performed transient global brain ischemia or sham operation to adult macaque monkeys, snap froze the brains on day 7 after surgery and dissected SVZa to subsequently perform Deep RNA Sequencing. Bioinformatics analysis revealed 539 up-regulated and 476 down-regulated genes after ischemia. Gene ontology and gene set enrichment analyzes disclosed distinct molecular features in up- and down-regulated genes. The up-regulated genes were enriched in those involved in cell adhesion, nervous system development, cell communication, transcriptional regulation, and stimulus response. The down-regulated genes were enriched in oxidation reduction, mitochondrial membrane, cation transport, and ribosome.

In order to investigate the expression at cellular resolution and identify the cell- subtypes showing differential expression pattern upon ischemia, we used chromogenic or fluorescent *in situ* hybridization (CISH/FISH). We selected 150 up-regulated genes, generated monkey-specific ISH probes and performed high-throughput ISH analysis. We noticed differential pattern of expression of those genes in the ependymal zone (EZ), subependymal zone (SEZ) or in both EZ and SEZ. Aiming at identification of new markers for distinct zones of the macaque SVZa, for some of the upregulated genes we applied dual FISH analysis combined with immunofluorescence for known markers of SVZ cell subtypes. Based on these results, we generated for our knowledge the first data base illustrating expression pattern in SVZa of adult macaques brain upon ischemia.

Paul Lucassen

Swammerdam Institute for Life Science,
University of Amsterdam, The Netherlands
E-mail: p.j.lucassen@uva.nl

Hippocampal neurogenesis in relation to (early) stress and major depression

Our main interest is structural plasticity in relation to stress and disease. In addition to apoptosis, we focus on the regulation of adult neurogenesis and its relevance for cognition, depression and dementia. Neurogenesis is prominent particularly during early development, and our primary focus is effects of early stress on (development of) the hippocampus. This brain structure is very sensitive to stress hormone action, implicated in learning and memory and affected in both these disease conditions.

PJ Lucassen¹, L. Reneman², A.G.M. Schranter^{1,2}, A. Koroš¹, MM Valenzuela³, M Maletić-Savatić⁴.

¹ Swammerdam Institute for Life Science, University of Amsterdam, The Netherlands

² Dept Radiology, Academic Medical Centre, University of Amsterdam, The Netherlands

³ Regenerative Neuroscience Group, Brain & Mind Centre, University of Sydney, Australia

⁴ Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA

Hippocampal neurogenesis in animals is well regulated by exercise or antidepressants, a.o. and has been implicated in cognition, mood and antidepressant efficacy. Interestingly, exposure to stress, particularly during the early-life period, increases the vulnerability to develop later psychopathologies like depression, and accelerates cognitive decline. Here, we

histological and imaging approaches to study neurogenesis changes in relation to (early) stress in animals and in human depression.

In mice, exposure to stress in the first week of life, reduced adult neurogenesis, and lastingly affected cognition and the immune system. Notably, also early nutrition modified later neurogenesis and cognition and dietary supplementation could even rescue lasting effects of early stress. Early stress and nutrition may thus be key players in the organization of adult brain structure, function and disease vulnerability.

In a model for adult social stress in marmoset monkeys, no changes were found in hippocampal neurogenesis after a short recovery period, indicating a rapid normalization at adult age. Surprisingly, extensive neurogenesis was present in the amygdala of this primate species.

To further address neurogenesis in human brain, we studied postmortem tissues of established, well-characterized, major depressed patients, and further optimized automated procedures to detect the 1.28 ppm NMR spectroscopic signal associated with neurogenesis in the human brain in vivo. We studied changes in neurogenic markers in relation to age, depression and in association with treatment with selective serotonin reuptake inhibitors (SSRIs), that are known to stimulate hippocampal neurogenesis in rodents, in an age-dependent manner. We conclude that, consistent with recent literature, a significant reduction with age is found in neurogenic signal and in older depressed patients, also in the human hippocampus in vivo.

Masanori Sakaguchi

University of Tsukuba, Japan

E-mail: sakaguchi.masa.fp@u.tsukuba.ac.jp

Function of adult-born neurons in memory consolidation during sleep

Our group utilizes optogenetics to manipulate adult-born neurons during specific stage of sleep. Our goal is to clarify the mechanisms by which adult-born neurons incorporate into memory circuits during sleep, which could contribute to the prevention or treatment of neurodegenerative diseases.

Robust human adult neurogenesis takes place in the dentate gyrus (DG) of the hippocampus. The endogenous neurogenic mechanism could be used to regenerate damaged adult brain. For this, it is essential to know the mechanism of how adult-born neurons integrate into existing neuronal circuits. We have shown that the DG adult-born neurons can

be integrated into memory circuit by post-training ablation method (Okano H, Sakaguchi, M et al. J. Neurochem.; 2007). Furthermore, we present the essential role of the adult-born neurons in memory consolidation during sleep by taking advantage of the excellent time resolution and reversibility of optogenetic intervention (Kumar et al., in prep.). Currently, in collaboration with Prof. Hideyuki Okano of Keio University, we are analyzing a sleep dependent behavioral disorder in transgenic non-human primate (marmoset). We will apply the knowledge from mouse study to preclinical research using primate in hope of utilizing the adult-neurogenesis to treat human neurodegenerative disorder in the future.

Dimitre Staykov

Eisenstadt, Austria

E-mail: staykov@gmx.at

Challenges in the design of clinical trials in ischemic stroke and intracerebral hemorrhage

Dr Staykov has graduated from the University of Vienna, Austria and received his training in neurology, neurocritical care and gerontology at the University Hospital Erlangen, Germany. Since April 2015 he chairs the Department of Neurology at the Hospital of the Brothers of St. John Eisenstadt, Austria. Dr Staykov's recent research is related to the treatment of intraventricular hemorrhage, the development of imaging analysis techniques and quantification of perihemorrhagic brain edema, and therapeutic hypothermia in ischemic and hemorrhagic stroke. Dr Staykov has published more than

50 peer-reviewed articles in international scientific journals and has co-authored guidelines of the Neurocritical Care Society, the European Stroke Organization and the German Neurological Society.

Ischemic and hemorrhagic stroke are responsible for more than 50% of disease burden in neurology and belong to the major causes of morbidity, mortality and disability in general. Although stroke has been in the focus of clinical research in the past decades, only few evidence based specific acute treatment strategies exist for ischemic stroke and

practically none for intracerebral hemorrhage. Therefore new treatments for those diseases are urgently needed and there are many promising emerging approaches including neural stem cell treatment. Many successful experimental treatment strategies, especially those aiming at neuroprotection after stroke, have failed in the clinical setting. The

reasons for this failure can at least partially be explained by issues concerning the design of clinical trials in stroke. Some important aspects are reviewed in the current talk including the sensitivity and reliability of endpoints typically employed in clinical trials and failures in the execution of phase II and III clinical trials.

Hagen B. Huttner

Department of Neurology,
University of Erlangen, Germany
E-mail: hagen.huttner@uk-erlangen.de

14C-based analysis of neurogenesis after stroke

The lab of Hagen B. Huttner covers research of neurovascular diseases and consists of a clinical and an experimental section. Clinical research mainly relates to acute and long-term management of patients with severe ischemic stroke and intracranial hemorrhages. Here, both retrospective studies and prospective clinical trials are performed aiming to explore experimental treatment approaches such as hypothermia, intraventricular thrombolysis or prognostic parameters. The experimental lab, which is linked to the labs of Jonas Frisén and Olaf Bergmann (Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden), performs studies on neuroprotection and neurogenesis after stroke. We are focusing on neuroprotective agents after focal ischemia, or perifocal edema management in hemorrhage mice models. Neurogenesis is studied using post-mortem human tissue samples. Further focus are cell culture experiments on DNA repair after hypoxia.

It has long been thought that neurons that were lost because of trauma or degenerative disease cannot be replaced in the human adult brain. More than two decades ago this dogma started to prove untrue as immunohistochemical studies revealed evidence of neurogenesis within the subgranular

zone of the dentate gyrus of the human hippocampus. However, given the lack of a sound methodological approach to study cell turnover in humans – similar to BrdU incorporation into DNA in newly generated cells in animals – over a long period the dynamics of neurogenesis in the human brain remained unstudied.

Since the establishment of the radiocarbon technique in 2005, which allows a retrospective birth dating of cells by taking advantage of the above-ground nuclear bomb test-derived carbon isotope ^{14}C , this radiocarbon-approach represents the most valid approach to explore dynamics of human cell turnover in humans. Using this technique – next to further studies not mentioned here – our investigations have demonstrated that (i) there is no evidence of human neurogenesis within the healthy adult cerebral cortex, (ii) there is continuous lifelong adult neurogenesis within the human hippocampus, and (iii) there is no induction of neurogenesis within the cerebral cortex after cortical ischemic stroke.

The talk will cover the presentation of the technique and will summarize the main results of above studies. Further, unpublished data on striatal (and hippocampal) neurogenesis following basal ganglia stroke in humans will be presented.

Thorsten R. Döppner

Department of Neurology, University Medical
Center, Göttingen, Germany
E-mail: Thorsten.Doeppner@med.uni-goettingen.de

Post-conditioning - a tool to modify post-stroke neurogenesis?

Being both a neurologist and a neuroscientist with special interest in cerebrovascular diseases, our group focuses on studying mechanisms of post-stroke neuroregeneration. In that respect, we analyze and manipulate post-stroke angiogenesis in mice, striving to better understand the cellular and molecular mechanisms that are involved in the process. Besides, we also focus on analyzing signaling pathways that are critical for induction of acute neuroprotection, both *in vitro* and *in vivo*, in order to help establish novel adjuvant therapeutic tools next to thrombolysis and endovascular therapy.

Causal therapeutic approaches against cerebral ischemia (i.e., stroke), are limited to systemic thrombolysis and endovascular recanalization, albeit the majority of patients is not eligible to either form of therapy. Hence, novel therapeutic concepts are in order, among which the manipulation of the endogenous restorative capacities of the brain has gained special interest. Although cerebral ischemia itself stimulates endogenous neurogenesis, the latter's therapeutic potential is critically impaired as new-born neural progenitor cells (NPCs) do not replace lost tissue due to low differentiation rates and high cell death rates. In view of the failure of pharmacologi-

cal therapies manipulating neurogenesis, alternative strategies promoting post-stroke brain repair are therefore needed. Post-conditioning, i.e., the induction of a short-lasting ischemic episode prior to an injurious one, is a potentially promising therapeutic strategy, which is known to induce acute neuroprotection against ischemic injury. However, the therapeutic potential of post-conditioning in terms of long-lasting effects and especially towards post-stroke neuroregeneration are not fully understood, yet. Using a mouse model of transient focal cerebral ischemia, our group has thus studied effects of both cerebral post-conditioning and remote post-conditioning (i.e., hind leg ischemia) in order to modify post-stroke neuroregeneration. Indeed, induction of post-conditioning stimulates post-stroke neuroregeneration such as increased neurogenesis, although these effects are in part transient only. Yet, post-conditioning also modifies the post-stroke extracellular milieu, which facilitates the transplantation of ectopic NPCs, thus helping boost endogenous neuroregeneration as well. Nevertheless, additional pre-clinical studies are in order before the concept of post-conditioning may be a feasible tool for stroke treatment in man.

Marin Guentchev

Trinity Medical Center, Sofia, Bulgaria
E-mail: guentchev@gmail.com

Neural stem cells and glioma

Gliomas account for more than half of adult primary intracranial tumors, with glioblastoma multiforme being

the most common. As a practicing neurosurgeon, my research is mainly focused on identifying the cell population

determining the prognosis in malignant glioma patients.

Plethora of evidence supports the notion that malignant glioma arise from cells with stem cell/progenitor cell properties. These cells form a small subset of all cancer cells and share some features with CNS stem cells, e.g. a capacity for self-renewal, multipotency and relative quies-

cence. Interestingly specific glioma cell population were shown to be responsible for invasiveness, tumorigenicity, chemo- and radioresistance. I would review our and other results on the cellular complexity of malignant glioma and discuss it in the light of possible new therapeutic strategies.

Ara Kaprelyan

Department of Neurology,
Medical University – Varna, Bulgaria
E-mail: ara.kaprelyan@mu-varna.bg

Therapeutic windows in epilepsy and neurodegeneration

Multiple sclerosis and neurodegenerative diseases such as Alzheimer's and Parkinson's disease are debilitating neurological disorders. Our group is interested in clinical imaging such as positron emission tomography (PET) as an early biomarker in multiple sclerosis and neurodegenerative diseases. We aim at finding novel biomarkers with clinical usefulness in these diseases.

Nowadays, despite the advances in molecular and cellular neuroscience, modern knowledge in neurogenetics, and introduction of novel anatomical and functional neuroimaging techniques, the human brain still presents an enigma for neuroscientists and physicians. Therefore, nuclear medicine plays a crucial role in diagnosis and differentiation of dementia, movement disorders, cerebral neoplasms, epilepsy, cerebrovascular diseases, neuroinfections, and headache. The detection of hypo- or hypermetabolic zones in brain is

of great importance both for the accurate diagnosis and adequate conservative or surgical treatment. Obviously, the use of brain scintigraphy enables the understanding of disease underlying mechanisms and the development of novel drugs and therapeutic strategies. In addition, based on the principles of personalized and translational medicine, recent application of nanotechnology and stem cell transplantation shows promising results, especially in patients with poor drug response and worse prognosis. Accordingly, several clinical trials in patients with progressive neurodegenerative disorders (AD, PD, ALS, and MS) and traumatic cerebral or spinal cord injuries are in progress and goal to improve the efficacy and safety of disease control.

We will present our own experience with ^{18}F -FDG PET use and discuss the perspectives of stem cell transplantation in different neurological disorders.

Aggressive behavior in aged male mouse potentiate the expression of endogenous brain NGF and BDNF and their main receptors, TrkA and TrkB, respectively: possible implication for brain progenitor cells activation

Marco Fiore¹, Luigi Aloe¹, Valentina Carito¹,
George Chaldakov², Mauro Ceccanti³
E-mail: marco.fiore@cnr.it

¹ Institute of Cell Biology and Neurobiology, IBCN-CNR,
Rome, Italy

² Laboratory of Cell Biology, Department of Anatomy and
Histology, Medical University, Varna, Bulgaria

³ Centro Riferimento Alcolologico Regione Lazio, Sapienza
University of Rome, Italy

The condition of dominance or submission following agonistic encounters in the adult male mouse is known to differentially affect brain nerve growth factor, a neurotrophin playing a role in brain remodeling, in the fine tuning of behaviour and in the regulation of the basal forebrain cholinergic neurons. During development and adult life nerve growth factor regulates brain expression of neurotransmitters and the stimulation of progenitor cells which, under different external stimuli, may differentiate into neuronal and/or glial cells promoting the recovery of the injured brain. However, little information is available for the aged brain. Thus in the present study we investigated the effect of the social status ('dominance' vs. 'submission') in the aged mouse on the presence of nerve growth factor, brain-derived neurotrophic factor, choline acetyltransferase, neuropeptide Y and progenitor cells of selected brain regions.

We found that aged dominant mice showed increased brain-derived neurotrophic factor in the subventricular zone

and hippocampus and increased choline acetyltransferase in the septum and basal nuclei, which were associated with increased presence of progenitor cells in the subventricular zone Ki67 stained. Conversely, in aged subordinate mice the data showed a marked brain increase in nerve growth factor in the subventricular zone and hippocampus, choline acetyltransferase in the septum and basal nuclei and neuropeptide Y in the hippocampus and parietal cortex. It was also found that mRNA-TrkA is over-expressed in the subventricular zone and hippocampus of dominant and subordinate mice, whereas mRNA-TrkB is increased in the subventricular zone of both dominants and subordinates and in the hippocampus of dominant mice. Fighting was also associated with increased presence of proliferating cells in the hippocampus stained with the nuclear marker bromodeoxyuridine. Moreover, the brain of subordinate mice displayed tyrosine hydroxylase immunoreactivity in the wall of the periventricular region of the third ventricles and a marked neuropeptide Y presence in the hippocampus co-expressed with bromodeoxyuridine.

One possible implication of this observation is that mild stress and/or physical activity may improve brain cells functions also throughout the endogenous synthesis and release of neurotrophic factors in the aged brain. Because a consistent number of neurotrophins-responsive cells die during brain aging, we do strongly believe that increasing the endogenous brain level of neurotrophins as a result of behavioral or environmental stimuli (i.e. fighting, physical exercise, enriched environment, etc.) natural occurring brain cell death could be reduced in mammals.

Microglial activation is attenuated by allogeneic intracerebral grafts

Nikola Tomov¹, Lachezar Surchev^{1,2}, Clemens
Wiedenmann³, Máté Döbrössy³, Guido Nikkhah⁴
E-mail: tomovmd@gmail.com

¹ Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

² Department of Anatomy, Histology and Embryology, Medi-

cal University of Sofia, Sofia, Bulgaria

³ Neurocenter, University Medical Center Freiburg, Freiburg, Germany

⁴ Neurosurgical Clinic, Klinikum Stuttgart, Stuttgart, Germany

Any influence to the central nervous system is associated with microglial activation, cell transplantation is no excep-

tion. However, it is not completely clear what the relative role of the introduced cells themselves and of the mechanical influence is. Comparing the two could shed light on some of the mechanisms of graft-host interaction.

In the present study we compare the microglial activation caused by intracerebral transplantation of allogeneic cell suspension to the one caused by introduction of the transplantation instrument only to the brain.

Single-cell suspension of E14 ventral mesencephalic tissue was transplanted to the striatum of unilaterally 6-OHDA lesioned rats, using a standard steel cannula. Control animals were subjected to an introduction of the same cannula into the striatum, without injecting anything. Animals were sacrificed 7 and 28 days after surgery, brains were processed for immunohistochemistry. Microglial activation was assessed

using Iba1+ cell density and immunoreactive area fraction in different zones, surrounding the grafts or areas of mechanical influence.

Results show the presence of activated microglia around grafts, as well as around areas of mechanical influence. Interestingly, the parameters of microglial activation were significantly lower in zones, intimately adjacent to the grafts, compared to the respective zones adjacent to the area of mechanical influence.

The present findings suggest attenuation of microglial reaction by grafts. We attribute this mostly to the functional integration of transplanted cells with the host striatum. Further investigation of mechanisms of microglial activation would contribute to better understanding of neurodegeneration and to the development of neuroprotective strategies.

Antidepressant effect of chronic agomelatine treatment in kainate model of epilepsy-associated depression involves a suppression of inflammatory signaling

Jana Tchekalarova¹, Dimitrinka Atanasova^{1,2},
Milena Atanasova³, Nikolai Lazarov^{1,4}
E-mail: janetchekalarova@gmail.com

¹ Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

² Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

³ Department of Biology, Medical University-Pleven, Pleven, Bulgaria

⁴ Department of Anatomy and Histology, Medical University-Sofia, Sofia, Bulgaria

Inflammatory signal molecules are suggested to be involved in mechanism underlying comorbid depression in epilepsy. In the present study, we tested the hypothesis that the novel antidepressant agomelatine, which is a potent MT₁ and MT₂ melatonin receptor agonist and 5HT_{2C} serotonin receptor antagonist, could prevent depressive symptoms developed during the chronic epileptic phase through suppression

of markers of inflammation. Long-term agomelatine treatment (40 mg/kg) was carried out after onset of kainate acid (KA)-induced status epilepticus (SE) in Wistar rats for ten weeks. Development of chronic epileptic condition was verified by a detection of spontaneous recurrent seizures through continuous video-monitoring (24-h) and EEG recording. Comorbid depression was confirmed during the chronic epileptic phase by a battery of behavioral tests. Epileptic rats exhibited depressive symptoms with decreased grooming, anhedonia and despair-like pattern. A positive correlation between these behavioral deficit and markers of inflammation (increased plasma levels of interleukin (IL)-1 β and activated neuroglia) was demonstrated. Chronic agomelatine treatment prevented depression accompanying chronic epileptic condition, decreased plasma IL-1 β to control levels and alleviated activated astrocytes and microglia. Taken together, the present results suggest that agomelatine treatment starting during SE can provide effective therapy of comorbid depression in chronic epileptic state through suppression of inflammatory signaling in plasma and brain.