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Development of the Dopaminergic System: from Stem Cells to Circuits

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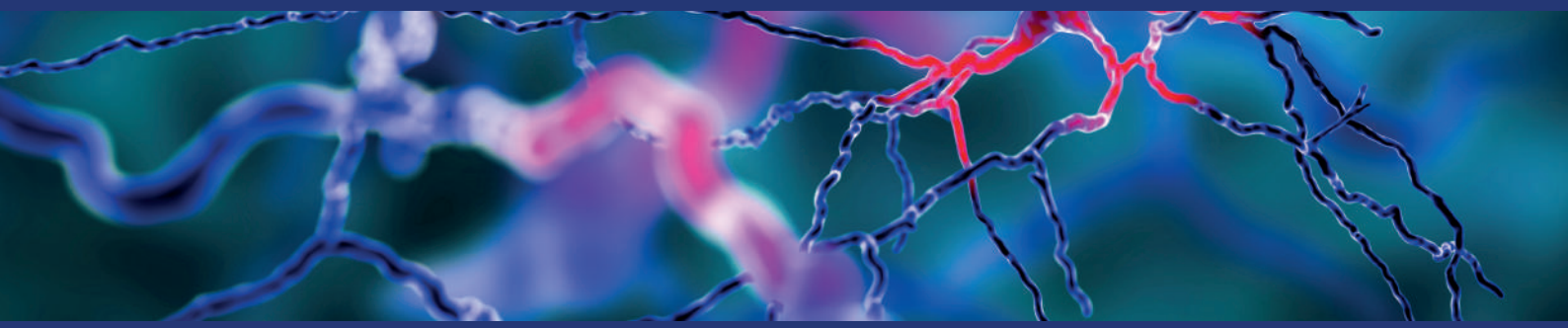

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Introduction

The mammalian brain is anatomically and functionally complex, and susceptible to diverse forms of neuropathology. A fundamental goal of developmental neuroscience is to understand the molecular, cellular and activity-based mechanisms that control the formation and maintenance of neural circuits. This knowledge is fundamental to better understand how these mechanisms become compromised in neurodevelopmental and neurodegenerative/psychiatric disorders.

In recent years, the development and function of dopamine neurons has come under intense focus, driven by the ambition of generating dopaminergic neurons for cell replacement strategies in Parkinson's disease (PD).To deepen our understanding of dopamine biology in the healthy brain and to develop strategies to ameliorate disease states, it is essential to bring together neurodevelopmental research, approaches to dissect complex neuronal networks, and advanced pluripotent stem cell technologies.

The 2019 conference "Development of the dopaminergic system-from stem cells to circuits" will feature an exciting and diverse scientific programme focused on recent advances and future directions in fundamental and applied developmental neuroscience centred on the midbrain dopaminergic system. We look forward to you joining us in this interesting and informative meeting and help us form a growing network of interactions and collaborations aiming at pushing the boundaries of research in the field of dopaminergic development.

Scientific Organising Committee

Prof. Rajeshwar Awatramani
ASSOCIATE PROFESSOR OF NEUROLOGY, NORTHWESTERN UNIVERSITY, USA

The focus of Prof. Rajeshwar Awatramani's research has been the development and diversity of dopamine neurons. His lab has described the floor plate origin of DA neurons, and the key role of Wnt signaling in DA neuron production. They are continuing to explore mechanisms of DA neuron development. Recently, the Awatramani Lab has developed a logical strategy to classify DA neurons. This involved a multilayered approach, initially involving single cell profiling which provided genetic entry points to dissect the DA system. The lab is now developing sophisticated genetic approaches to manipulate DA subtypes, to determine their transcriptome, projection and functions.

Prof. Sandra Blaess
HEISENBERG-PROFESSORSHIP IN NEURODEVELOPMENT, UNIVERSITY OF BONN, GERMANY

Prof. Sandra Blaess holds a diploma degree in Molecular Biology from the University of Basel, Switzerland. She finished her PhD under the supervision of Prof. Denis Monard and Dr. Ulrich Müller at the Friedrich Miescher Institute and the University of Basel in 2002. Subsequently, she joined the research group of Prof. Alexandra Joyner at the Skirball Institute, New York University and the Memorial Sloan Kettering Cancer Center, New York as a Postdoctoral Fellow. Since 2008 she is head of the Neurodevelopmental Genetics Group at the Institute of Reconstructive Neurobiology at the University. In 2017, she was awarded a Heisenberg-Professorship in Neurodevelopment. Her present research is focused on elucidating the mechanisms that underlie the generation of neuronal and functional diversity in the dopaminergic system.

Dr Emmanouil Metzakopian
TEAM LEADER, UK DEMENTIA RESEARCH INSTITUTE (UK DRI), UNIVERSITY OF CAMBRIDGE

Dr Emmanouil Metzakopian holds a BSc in Biochemistry and Biotechnology from the University of Thessaly, Greece. He received his PhD in midbrain development from University College London under the supervision of Dr. Siew-Lan Ang at the National Institute for Medical Research. In the last 5 years Emmanouil has been working on genome scale genetic screens using the CRISPR-Cas9 gene editing tool in the Lab of Dr. Allan Bradley at the Wellcome Trust Sanger Institute. Emmanouil now leads a team at the UK Dementia Research Institute (UK DRI) in Cambridge. The aim of his projects is to identify genes which confer resistance to stress (oxidative and ER) and synaptic maintenance in dopamine neurons.

Prof. Martin Lévesque
ASSOCIATE PROFESSOR AT LAVAL UNIVERSITY, CANADA & HEAD OF NEURODEVELOPMENTAL GROUP AT CERVO BRAIN RESEARCH CENTRE

Prof. Martin Lévesque holds a bachelor degree in Biology from Laval University (Canada). He then completed a PhD in Neurobiology under the supervision of Prof. André Parent at Laval University in 2006. Afterward, he joined the laboratory of Dr Frederic Charron at the Montreal Clinical Research Institute (Canada) as postdoctoral fellow working on developmental neurobiology. He then completed a second postdoctoral training on the development of dopamine neurons in the group of Dr Siew-Lan Ang at the National Institute for Medical Research (London, UK). Since 2012, he is head of Neurodevelopmental group at the CERVO Brain Research Centre and associate professor at Laval University (Canada). His current research focuses on understanding the mechanisms regulating dopaminergic circuit development and the mechanisms leading to neurodegeneration in Parkinson's disease.

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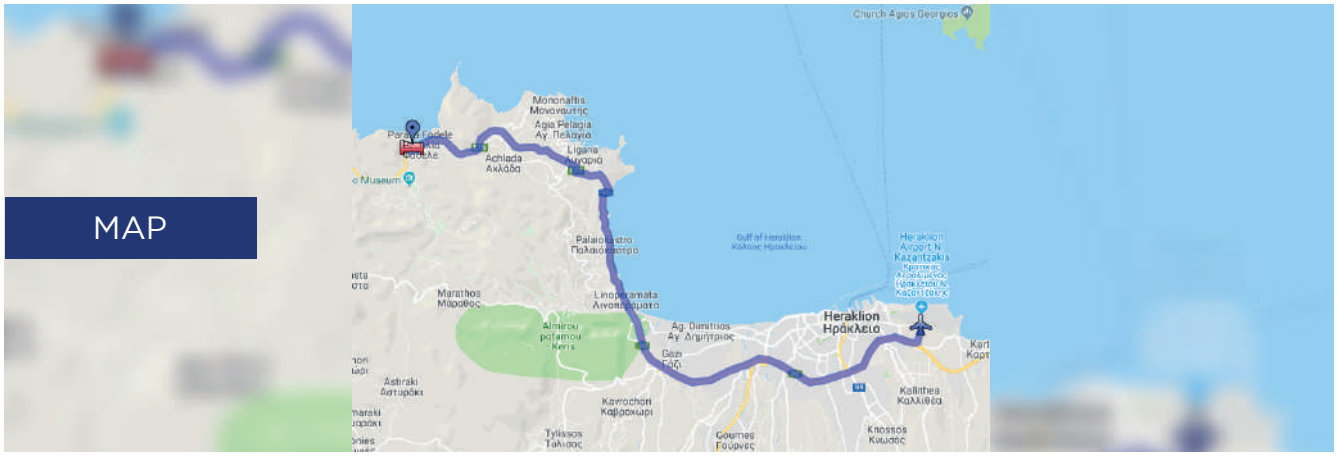
Venue Information

The Fodele Beach is situated in wonderful bay of Fodele. The hotel's terraced layout means that stunning views are to be had out over the sea and the surrounding beautiful landscape. The hotel offers a fun-filled waterpark, stylish accommodation and a range of dining venues. Airy rooms and suites offer flat-screens, plus features such as balconies, direct pool access, and/or terraces with garden access.
Some suites add living or dining rooms, bars, private pools and/or kitchens.



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SESSION 1: Early events in ventral midbrain fate specification | Sandra Blaess

9:30 – 10:00 Juha Partanen

Development of brainstem neurons associated with dopaminergic nuclei

Juha Partanen

University of Helsinki, Finland

The anterior brainstem contains nuclei important for regulation of the behavioral state, including mood, motivation, movement and memory. In addition to monoaminergic neurons, these nuclei include diverse inhibitory GABAergic and excitatory glutamatergic neurons important for the function of dopaminergic neurons and basal ganglia. In mice, the development of specific subtypes of these neurons is controlled by Gata and Tal family transcription factors, which select a GABAergic over a glutamatergic neuron identity in early neuronal precursors of the anterior brainstem. To get insights into the molecular diversity and development of the tegmental GABAergic and glutamatergic neurons, we combined gene modified mouse models, transcriptomics and single cell transcriptomics to profile Gata/Tal dependent gene products and neuronal precursor subtypes in the embryonic anterior brainstem. Our work suggests a framework for development of the GABAergic subtypes in the anterior brainstem.

10:00 – 10:30 Claude Brodski

BMP/SMAD Pathway Promotes Neurogenesis of Midbrain Dopaminergic Neurons In Vivo and in Human Induced Pluripotent and Neural Stem Cells

Claude Brodski

Ben Gurion University, Israel

Studying the molecular pathways controlling the development of midbrain dopaminergic (mDA) neurons is essential to better understand the maintenance, function and vulnerability of these cells in adulthood. Moreover, investigating the embryonic formation of mDA neurons *in vivo* provides critical guidelines for the *in vitro* differentiation of mDA neurons from stem cells, currently being developed for Parkinson's disease cell replacement therapy. BMP/SMAD inhibition is routinely used during early steps of stem cell differentiation protocols, including for the generation of mDA neurons. However, the function of the BMP/SMAD pathway for *in vivo* specification of mammalian mDA neurons is virtually unknown. We found that BMP5/7 deficient mice (*Bmp5*$^{-/-}$*; Bmp7*$^{-/-}$) lack mDA neurons, caused by reduced neurogenesis in the mDA progenitor domain, but not in the adjacent basal plate. As molecular mechanisms accounting for these alterations in *Bmp5*$^{-/-}$*; Bmp7*$^{-/-}$ mutants, we identified expression changes of the BMP/SMAD target genes MSX1/2 and SHH. Conditionally inactivating SMAD1 in neural stem cells of mice *in vivo* (*Smad1*^{Nes}) hampered the differentiation of progenitor cells into mDA neurons by preventing cell cycle exit, especially of TH^{+}SOX6^{+} and TH^{+}GIRK2^{+} substantia nigra neurons. In contrast, red nucleus neurons that develop in the basal plate formed normally. Notably, BMP5/7 robustly increased the *in vitro* differentiation of human induced pluripotent stem cells and induced neural stem cells to mDA neurons by up to 3-fold. In conclusion, we have identified BMP/SMAD signaling as a novel critical pathway orchestrating essential steps of mammalian mDA neurogenesis *in vivo* that balances progenitor proliferation and differentiation. Moreover, we demonstrate the potential of BMPs to improve the generation of stem cell-derived mDA neurons *in vitro*, highlighting the importance of sequential BMP/SMAD inhibition and activation in this process.

11:00 – 11:30 Andrea Wizenmann

Dopaminergic precursor marker PITX3 differs in spatiotemporal expression and epistatic gene regulation during chick and mouse development

Ruth Klafke[a], A. Alwin Prem Anand[b], Wolfgang Wurst[a,c,d,e§], Nilima Prakash[a,c,1§] and Andrea Wizenmann[b§]

[a] Institute of Developmental Genetics, Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
[b] Institute of Clinical Anatomy and Cell Analysis, University of Tuebingen, Oesterbergstrasse 3, 72074 Tuebingen, Germany
[c] Technische Universität München-Weihenstephan, Lehrstuhl für Entwicklungsgenetik c/o Helmholtz Zentrum München, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
[d] Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Standort München, Schillerstr. 44, 80336 München, Germany
[e] Munich Cluster for Systems Neurology (SyNergy), Adolf-Butenandt-Institut, Ludwig-Maximilians-Universität München, Schillerstrasse 44, 80336 München, Germany

The mesodiencephalic dopaminergic (mdDA) neurons are located in the ventral mesencephalon and caudal diencephalon of all tetrapod species studied so far, and these are the most prominent DA neuronal population. These neurons are implicated in the control and modulation of motor, cognitive and rewarding/affective behaviors, and their degeneration or dysfunction is intimately linked to several neurological and neuropsychiatric human diseases. To gain further inhsights into the generation of mdDa neurons, we studied the spatiotemporal expression patterns and epistatic interactions in developing chick embryos of selected marker genes and signaling pathways associated with mdDA neuron development in the mouse. We detected striking differences in the spatiotemporal expression patterns of the chick orthologs of mouse mdDA marker genes Pitx3 and Aldh1a1 suggesting important differences in the generation of these cells between these species. We also discovered that the Sonic hedgehog signaling pathway is both necessary and sufficient for the induction of ectopic Pitx3 expression in chick mesencephalon downstream of chick WNT9A-induced Lmx1a transcription. These aspects of early chicken development resemble more the ontogeny of zebrafish diencephalic DA neuronal populations, and suggest that they have diverged between birds and mammals during evolution.

11:30 – 11:45 Selected Abstract: Tae Wan Kim

Derivation of Enriched Engraftable Midbrain Dopamine Neurons from Human Pluripotent Stem Cells in a cGMP-qualified Condition for the Cell Replacement Therapy to Parkinson Patients

Tae Wan Kim[1,4], So Yeon Koo[1], Jinghua Piao[2], Eveline M Gutzwiller[1], Se Joon Choi[3], Eugene V Mosharov[3], Mark J Tomishima[1], Viviane Tabar[2], Lorenz Studer[1]

[1]Center for Stem Cell Biology, Memorial Sloan Kettering Cancer Center, New York [2]Department of neurosurgery, Memorial Sloan Kettering Cancer Center, New York [3]Department of Neurology, Columbia University Medical Center, New York, New York, USA [4]New York Stem Cell Foundation – Druckenmiller Fellow

hPSC-derived midbrain dopamine (mDA) neurons are considered a promising avenue for cell replacement therapy in Parkinson's disease (PD). Despite rapid developments for deriving mDA neuron from hPSC towards human translation, several questions remain as to define the most optimal cell product for treating PD patients. Here we developed our protocol for deriving clinical relevant mDA neurons by optimizing activation and timing of WNT signaling. The conditions yield mDA neurons giving rise to authentic functional TH positive neurons *in-vitro* and *in-vivo*, and rescuing amphetamine-induced rotation in rat Parkinson model. However, when applying those optimized mDA neuron induction conditions to *EN1-Knockout* hPSC, we observe expression of contaminating PAX6 and STN markers, suggesting a pivotal role for EN1 in mediating induction of mDA neuron and suppression of alternative markers. Furthermore, we established a NURR1-H2B-GFP reporter line that enables us to derive nearly pure population of engraftable mDA neurons, which was used for candidate surface markers. These studies take advantage of our ability to generate pure population of mDA neurons in a clinically relevant culture system. The work should be geared towards developing a "2.0" version of our GMP mDA neuron product to offer the best possible cell therapy to PD patients in the near future.

11:45 – 12:00 Selected Abstract: Gerard W. O'Keeffe

BMP/SMAD Pathway Promotes Axonal Growth Of Developing Dopaminergic Neurons

Gerard W. O'Keeffe

Department of Anatomy and Neuroscience, Cork Neuroscience Centre, University College Cork, Cork, Ireland.

The establishment of midbrain dopaminergic connectivity requires neuronal specification, differentiation, axon growth, and target innervation. While the specification and differentiation of midbrain dopaminergic neurons are being extensively studied, the molecular mechanisms that regulate axon growth and target innervation of midbrain dopaminergic neurons are less clear. One group of developmental signals that are expressed in the embryonic midbrain are the bone-morphogenetic proteins (BMP) which are a group of neurotrophic factors that are the largest subgroup of the transforming growth factor beta superfamily. These ligands and their receptors show a developmental expression profile that coincides with the period of development during which midbrain dopaminergic axons are growing towards their targets *in vivo*. These ligands signal through BMP receptors (BMPRs) to activate intracellular transcriptions factors called Smads. Exposure of developing midbrain dopaminergic neurons to specific BMPs promotes axon growth and branching. Moreover pharmacological and siRNA-based manipulation of the BMPR-Smad pathway shows that the axon growth promoting effects of BMPs require BMPR1B-Smad1/5 signalling. In agreement with this, overexpression of constitutively active BMPR1B in isolated midbrain dopaminergic neurons is sufficient to promote axon growth. Therefore, these findings show that as well as recently its described role in promoting neurogenesis of midbrain dopaminergic neurons, BMP-Smad signalling may also be important for promoting midbrain dopaminergic axon growth during development, and for the protection of midbrain dopaminergic axons in Parkinson's disease.

12:00 – 12:30 Sandra Blaess

Molecular mechanisms underlying the diversification and migration of midbrain dopaminergic neurons

Ankita Ravi Vaswani[1], Beatrice Weykopf[2], Cathleen Hagemann[1], Hans-Ulrich Fried[2], Oliver Brüstle[2] and Sandra Blaess[1]

[1]Neurodevelopmental Genetics, Institute of Reconstructive Neurobiology, University of Bonn School of Medicine & University Hospital Bonn, Bonn, Germany.
[2]Institute of Reconstructive Neurobiology, University of Bonn School of Medicine & University Hospital Bonn, Bonn, Germany.
[3]Light Microscope Facility, German Center for Neurodegenerative Diseases, Bonn, Germany.

Midbrain dopaminergic (mDA) neurons migrate from their progenitor domain in the ventral midbrain floor plate to form the laterally-located substantia nigra pars compacta (SN) and medially-located ventral tegmental area (VTA). SN and VTA-mDA neurons migrate radially away from the floor plate, followed by a tangential migration step of SN-mDA neurons that allows them to take up lateral positions. Still, little is known about the underlying cellular and molecular processes of these migratory processes. Using two-photon excitation time-lapse imaging to monitor SN-mDA tangential migration in organotypic slice cultures, we demonstrate that slow migration is the default mode in SN-mDA neurons, while fast, laterally-directed migration occurs infrequently and is strongly associated with bipolar cell morphology. We show that Reelin signaling directly regulates lateral, tangential migration of mDA neurons by promoting the lateral directionality of small, slow movements, by increasing the frequency of laterally-directed fast migration events that cover larger distances and by stabilizing the morphology of migrating SN-mDA neurons. We thus provide new mechanistic insight into how Reelin signaling regulates the formation of the SN and how Reelin signaling controls tangential neuronal migration.

SESSION 2: Dopamine circuits and axon guidance | Martin Lévesque

14:30 – 15:00 Louis-Eric Trudeau

Towards a better understanding of the development of the neurochemically complex axonal arborization of dopamine neurons

Louis-Eric Trudeau

Professor, Department of Physiology and Physiology, Faculty of Medicine, CNS Research Group (GRSNC), Université de Montréal

Dopamine neurons in the brain play key roles in a number of key physiological functions including motivation, learning and movement selection. A gradual loss of these neurons and of their axonal connections is tightly linked with the appearance of the cardinal motor symptoms of Parkinson's disease. A striking characteristic of these neurons is their highly branched nature, with the axonal arbor of single neurons covering a substantial portion of their target fields such as the striatum. We are presently exploring the development of the axonal arbor of mouse dopamine neurons, focussing on the links between axonal arborization size and bioenergetics, in an effort to explain the selective vulnerability of subsets of dopamine neurons in Parkinson's disease. We are also characterizing the development of the neurochemical identity and structure of release sites established by these neurons, highlighting the surprizing dichotomy between the minority of release sites that have a synaptic structure and that appear to be specialized for release of glutamate and GABA and the majority of release sites that have a non-synaptic structure and that appear to mediate dopamine volume transmission.

15:00 – 15:30 Cecilia Flores

Making dopamine connections in adolescence

Cecilia Flores

Department of Psychiatry, McGill University, Canada

Adolescence is a period of increased vulnerability to mental health disorders. Yet, there is a significant gap in our knowledge about basic mechanisms of adolescent brain development and about how they are influenced by experience, including drugs of abuse and stressors. This talk focuses on the emerging role of guidance cues in the adolescent development of dopamine systems and on its implications for susceptibility and resilience to psychiatric disorders. I discuss our recent findings from rodent and human studies on the role of the Netrin-1 receptor, DCC, on the development of the dopamine projections to the prefrontal cortex in adolescence and how this process is impacted by exposure to drugs of abuse and to social stressors. I show that DCC receptors on dopamine neurons in adolescence control the development of the prefrontal cortex itself, impacting cognitive abilities in adulthood. Variations in DCC expression are linked to psychiatric conditions of prefrontal cortex dysfunction and may be a key determinant of adolescent vulnerability or resilience. This new line of research may have significant implications for the development of data-driven prevention and intervention strategies for the youth.

15:30 – 16:00 Mary Hynes

Netrin-1-dependent guidance of dopaminergic axons; RNA expression in early neurogenesis

Ze Yang[1], Shaoyi Yi[1], Leonardi Gozali[1], Arif Kocabas[2], Jie Li, Ana Marija Sola[2], Caitlin Gilbert[1], Eliza Adams[1], Marc Tessier-Lavigne[1], and Mary Hynes[1]

[1]Stanford University, Biology, Stanford, CA [2]Rockefeller University, Biology, New York , NY

Axons from the two main groups of midbrain dopaminergic (DA) neurons, the ventral tegmental area (VTA), and the more laterally located substantia nigra (SN) neurons show spatially segregated innervation of the striatum. VTA axons project ventro–laterally (VL) and SN axons doro–medially (DM). Unlike axons that project to layered structures, DA axons in the striatum do not innervate discrete regions and instead arborize widely within either the VL or DM zones- however they do not stray from one zone to the other. Here we show that Netrin-1 acts in a novel fashion to topographically pattern midbrain DA axons into these two striatal zones by a gradient of Netrin-1 in the striatum and differential attraction of axons to overlapping but distinct concentrations of Netrin-1. In mice lacking Netrin-1, DA axons that reach the striatum fail to segregate into their two terminal zones. Netrin-1 signaling via the Netrin-1 receptor DCC has also been implicated in patterning of DA innervation of the mPFC. To assess how changes in Netrin-1 signaling impact DA circuit function, we performed unbiased whole-brain c-Fos mapping in DCC heterozygous mice. We show, using iDISCO tissue clearing and automated ClearMap voxel mapping, that DCC heterozygous mice show a blunted functional response to stimulants, and region-specific reductions in neural activity as compared to WT and saline controls. We have also been examining the role of the widespread expression of isolated 3'UTR sequences, first noticed in RNAseq samples from embryonic DA neurons. Early studies examining the role of such isolated 3'UTRs in early cell fate decisions and neurogenesis will be presented.

16:30 – 17:00 Jeroen Pasterkamp

Using mouse genetics approaches for dissecting dopamine neuron subset-specific axon guidance and cell migration events

Brignani S[1], Raj D[1], Schmidt ERE[1], Adolfs Y[1], Moreno-Bravo JA[2], Van Battum EY[1], Chedotal A[2], Pasterkamp RJ[1]

[1]Department of Translational Neuroscience, Brain Center Rudolf Magnus UMC Utrecht, Utrecht University, The Netherlands [2]Sorbonne Universités, UPMC Université Paris 06, INSERM, CNRS, Institut de la Vision, Paris, France

Most regions of our central nervous system (CNS) have classically been considered as homogeneous structures on basis of biochemical markers or neuronal morphology. However, techniques such as single cell RNA sequencing and advanced neuronal tracing challenge this view and support the idea that most CNS regions are composed of many different neuron types with their own molecular make-up, connectivity patterns and functional roles. We exploit the midbrain dopamine system to understand how such neuronal subsets develop and function. The midbrain dopamine (mDA) system is involved in the control of various cognitive and motor behaviours. mDA neurons are grossly divided into two anatomically and functionally distinct subpopulations: substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) neurons. SNc neurons make precise connections with dorsal striatum (nigrostriatal projections), while VTA neurons target ventral striatum and cortex (mesocorticolimbic projections). Both pathways collectively run in the medial forebrain bundle (MFB) towards the forebrain. Recent data support the idea that the SNc and VTA can be subdivided into smaller and functionally more relevant neuronal subsets on basis of gene expression and connectivity patterns. However, tools to study such subsets, especially during embryonic and postnatal development, are lacking. Therefore, we recently developed novel mouse genetics tools to distinguish between different subsets of dopaminergic projections *in vivo* (called *Pitx3-ITC* mice). The subtractive genetic strategy we have developed relies on the expression of different fluorescent proteins in different subsets of mDA neurons in a single mouse. *Pitx3-ITC* mice display labelling of SNc neurons and selective visualization of nigrostriatal projections in the MFB and striatum, from early embryonic development onwards. Combination of *Pitx3-ITC* mice with 3D-imaging of solvent cleared organs (3DISCO) technology and light sheet microscopy allows for 3D analysis of neuronal subset-specific migration and axonal/dendritic development of mDA neurons. Using this approach, we have identified a previously unexplored role for Netrin-1 in the positioning of SNc mDA neurons. Intriguingly, we find that the lateral-ventral positioning of SNc mDA neurons and the ventrally directed outgrowth of their dendrites in the midbrain relies of the Netrin-1-dependent migration of GABAergic interneurons. This work identifies a novel mechanism in which the positioning of one brain nucleus relies on the migration of molecularly distinct neurons that will occupy an adjacent brain structure.

17:00 – 17:15 Selected Abstract: Tiago Cardoso

hESC-derived dopaminergic transplants integrate into basal ganglia circuitry in a preclinical model of Parkinson´s disease

Tiago Cardoso[1,2], Andrew F. Adler[1,2], Sara Nolbrant[1,2], Bengt Mattsson[1], Deirdre B. Hoban[1,2], Ulla Jarl[1], Jenny Nelander Wahlestedt[1,2], Shane Grealish[1], Anders Björklund[1], Malin Parmar[1,2*]

[1]Developmental and Regenerative Neurobiology, Department of Experimental Medical Science, Wallenberg Neuroscience Center, Lund University, 22184 Lund, Sweden [2]Lund Stem Cell Center, Lund University, 22184 Lund, Sweden

Dopamine (DA) neurons derived from human embryonic stem cells (hESCs) are a promising cell source for cell replacement therapy in Parkinson´s disease (PD). When transplanted into preclinical models of PD, these neurons survive long-term, release dopamine, extensively innervate target host structures and provide functional recovery. Using rabies-based monosynaptic tracing, we have recently shown that grafted hESC-derived neurons appropriately integrate into the host circuitry.

In this study, ventral midbrain (VM) and forebrain (FB) patterned hESCs-derived neural progenitors were transplanted into the striatum or into the substantia nigra of 6-OHDA-lesioned rats, to elucidate factors controlling target-appropriate innervation and synaptic integration 6 months post-transplantation. We show that cell intrinsic factors determine the pattern of graft-derived axonal innervation, while synaptic inputs from the host primarily reflect the location of the graft. Furthermore, we provide evidence that hESC-derived DAergic grafts transplanted to the striatum (reflecting clinical graft placement) receive synaptic input from the same subtypes of host cortical, striatal, and pallidal neurons which are known to regulate the function of endogenous nigral DA neurons.

This refined understanding of factors controlling graft-derived axonal outgrowth and circuitry integration will be important for the optimization of the design of clinical cell replacement therapies for PD.

17:00 – 17:15 Selected Abstract: Åsa Mackenzie

Disentangeling subtypes of midbrain dopamine in neurocircuitry and reward-related behavior

Åsa Wallén-Mackenzie, Zisis Bimpisidis, Niclas König, Stefanos Stagkourakis, Vivien Zell, Bianca Vlcek, Bruno Giros, Christian Broberger, Thomas S. Hnasko, Sylvie Dumas

Reward-related behavior is complex and its dysfunction correlated with neuropsychiatric illness and non-motor symptoms of Parkinson´s disease. Some of this complexity is likely correlated with the striking heterogeneity of some of the brain areas that regulate the behavior. Dopamine neurons of the ventral tegmental area (VTA) have long been associated with different aspects of reward function, but it remains to be disentangled how distinct VTA dopamine neurons contribute to the full range of behaviors ascribed to the VTA. In our studies, we implement conditional mouse genetics and optogenetics to achieve selective manipulation of molecularly defined subtypes of VTA dopamine neurons for the study of their role in neurocircuitry and behavior. The findings that will be presented show that upon comparison, molecularly defined subtypes of VTA dopamine neurons show different patterns of contribution to the regulation of reward behavior. By disentangling the role of subtypes that together form the heterogeneous habitat of the VTA, the current understanding of VTA neurocircuitry in health and in disorders of the midbrain dopamine system can be improved.

17:30 – 18:00 Martin Lévesque

Axon Guidance of midbrain dopamine neurons

The central pathology of Parkinson' disease (PD) is characterized by the selective loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). There is a large unmet need for a treatment that addresses the cause of the disease and modifies its clinical course. Graft of 19 dopaminergic neurons newly generated from stem cells represents a promising therapeutic avenue. However, a major factor limiting success in transplantation studies is the inappropriate re-innervation of the grafted neurons. It is thus primordial to identify factors regulating axon projection and connectivity of mDA neurons. The midbrain dopaminergic neurons are organized in two major populations: the neurons of the SNpc, which project their axons to the dorsal striatum and are highly vulnerable in PD; and the neurons of the ventral tegmental area (VTA), which innervate parts of the ventral striatum, the nucleus accumbens and the prefrontal cortex and are relatively spared in PD. Here, I will present our recent data revealing the role of Semaphorin7A-PlexinC1 pathway controlling axon innervation of DA neurons. PlexinC1 is a transmembrane receptor that binds Semaphorin7A (Sema7a). Upon binding to PlexinC1, Sema7a act as a chemo-repulsive cue that repels DA axons. Sema7a is expressed in the dorsal region of the striatum. Our results show that the Sema7a-PlexinC1 interaction mediates the segregation of dopaminergic axon projections of VTA and SNpc. Our in vitro and in vivo experiments also show that Sema7a-PlexinC1 guidance effect are mediated by Src family kinases. At the light of our data, we propose a novel model to explain the segregation of the nigrostriatal and mesolimbic pathways. Data generated here will shed a new light on mechanisms that regulate dopamine neuron connectivity and will certainly help in the effort to understand the molecular factors contributing to the efficiency of cell replacement therapies in PD.

SESSION 3: Dopamine neuron diversity | Rajeshwar Awatramani

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8:30-9:00 Siew-Lan Ang

LIM-only domain proteins define and regulate the survival of specific subsets of midbrain dopaminergic neurons

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Midbrain dopaminergic (mDA) neurons are a heterogeneous group of neurons important for control of voluntary movement, reward processing and a range of cognitive and emotional behaviours. Underpinning these diverse processes are distinct subgroups of cells with diverse axon projections, cellular properties and molecular signatures which are only beginning to be uncovered. In this study, we demonstrate that LMO3, a LIM-domain only transcriptional co-factor, is expressed heterogeneously within mDA neurons throughout development and marks a specific population of mature mDA neurons in the ventral medial Substantia Nigra pars compacta (SNc). Through retrograde labelling we demonstrate that this LMO3+ population preferentially projects to the dorsal medial striatum, but not to the more lateral dorsal striatum, corresponding to specific functional connections that have previously been described. Furthermore, we demonstrate that LMO3 acts in a redundant manner with another LIM-domain only factor, LMO4, to promote survival of specific subgroups of mDA neurons. Using *Lmo3*-deficient *LacZ* knock-in mice and mice with conditional deletion of *Lmo4*, we show that loss of *Lmo3* or *Lmo4* alone does not affect mDA neuron development but that when both *Lmo3* and *Lmo4* are removed from developing mDA neurons, approximately 40% of mDA neurons die at late embryonic stages. Double mutant mice show a preferential loss of ventral tier SNc neurons and of the corresponding axon projections to the dorsal striatum. Together these results demonstrate that LIM-only domain factors are important for developmental survival of SNc neurons, in particular ventral tier SNc neurons that are vulnerable in neurodegenerative Parkinson's disease.

9:00-9:30 Wolfgang Driever

Functional and molecular subtype diversity in the zebrafish diencephalospinal dopaminergic system

Wolfgang Driever

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Dopaminergic neurons constitute a major neuromodulatory system in vertebrates, affecting a wide range of circuits and behaviors. The diencephalospinal dopaminergic system appears highly conserved from fish to mammals, and provides the sole dopaminergic input into hindbrain and spinal cord. Dopaminergic neurons of this system (called A11 in mammals) have been linked to several diseases, including Restless Legs Syndrome / Willis-Ekbom Disease and chronic pain. In zebrafish, these dopaminergic neurons are located in the anatomical region of the posterior tuberculum in the ventral forebrain. Interestingly, they do not only send descending projections, but also ascending projections into subpallial / striatal regions. In addition, the same dopaminergic somata may also send peripheral projections to the ear, peripheral ganglia, and all lateral line sensory organs. Therefore, the diencephalospinal dopaminergic system has the potential to be a major modulator of both sensory and motor circuits. Given the absence of dopaminergic neurons from the zebrafish ventral midbrain, potential similarities between zebrafish posterior tubercular and mammalian VTA / midbrain A8-A10 dopaminergic neurons have been discussed extensively in the literature.

We will report on two aspects of the diencephalospinal dopaminergic system: Molecular and functional subtype diversity.

Correlation of calcium activity with specific sensory stimuli types or motor behavior revealed that these ventral diencephalic dopaminergic neurons may constitute a "sensory" dopamine system: anatomically distinct dopaminergic subgroups selectively respond to mechanosensory or visual stimulation. Mechanosensory-related dopaminergic activity is tuned to stimulus intensity. The activation of posterior tubercular dopaminergic neurons by sensory stimuli, and their projections onto peripheral mechanosensory systems, suggests a participation of these A11-type neurons in the feedback regulation of sensory systems. Together with the adjacent hypothalamic dopaminergic neurons, they may serve to set basic behavioral states. We have previously shown that diencephalospinal dopaminergic neurons have a dual neurotransmitter phenotype, dopaminergic and glutamatergic, while other dopaminergic groups are also gabaergic. To begin to dissect dopaminergic from second neurotransmitter-based activities of the diencephalospinal system, we have genetically engineered catecholamine-free zebrafish. These zebrafish develop morphologically normal "non-dopaminergic" diencephalospinal A11-type neurons.

Given the complexity of the projection patterns of the posterior tubercular, A11-type dopaminergic neurons, we aim at understanding the molecular basis of their anatomical and functional diversity. Therefore, we performed single cell RNAseq experiments on neurons FACS sorted based on coexpression of the *tyrosine hydroxylase* catecholaminergic marker and *orthopedia*, which encodes the transcription factor Otp required for specification of these dopaminergic neurons. Cluster analysis of the single cell data reveals subtype diversity both with respect to neurotransmitter content as well as receptor expression. We will discuss potential mechanisms how this subtype diversity may be generated.

9:30-10:00 Marten Smidt

Genetic and epigenetic programming of dopaminergic subsets

Marten P. Smidt

University of Amsterdam, The Netherlands

Over the last decade several components have been identified to be differentially expressed in subsets of mesodiencephalic dopaminergic (mdDA) neurons. These differences in molecular profile have been implied to be involved in the selective degeneration of the SNc neurons in Parkinson's disease. The emergence and maintenance of individual subsets is dependent on different transcriptional programs already present during development. In addition to the influence of transcription factors, recent studies have led to the hypothesis that modifications of histones might also influence the developmental program of neurons. Here we focus on the histone methyltransferase EZH2 and its role in the specification of the mid-hindbrain region and its direct influence on the development and maintenance of mdDA neurons. We generated two different conditional knock out (cKO) mice; an *En1Cre* driven cKO, for deletion of *Ezh2* in regional progenitors and a *Pitx3Cre* driven cKO, to study the effect of post-mitotic deletion of *Ezh2* on mdDA neurons maturation and neuronal survival. Loss of *Ezh2* changed the molecular coding of the anterior ventral hindbrain leading to a fate switch and the appearance of ectopic dopaminergic neurons. The correct specification of the isthmic region is dependent on the signaling factors produced by the Isthmic organizer (IsO), located at the border of the mid- and hindbrain. We propose that the change of cellular fate is a result of the presence of *Otx2* in the hindbrain of *Ezh2* conditional knock-outs and a dysfunctional IsO, as represented by the loss of *Fgf8* and *Wnt1*. During dopaminergic development *Ezh2* was found to be important for the generation of the proper amount of TH+ neurons. The loss of neurons primarily affected a rostrolateral population, which is also reflected in the analysis of the subset marks, *Ahd2* and *Cck*. In contrast to early genetic ablation, post-mitotic deletion of *Ezh2* did not lead to major developmental defects at E14.5. Finally, *Pitx3Cre* driven deletion of *Ezh2* led to a progressive loss of TH+ cells in the VTA and these animals display reduced climbing behavior. Together, our data demonstrates that *Ezh2* is important for the early regionalization and decision between dopaminergic or serotonergic programming. Moreover, Ehz2 is essential for the correct generation of mdDA neurons during development and that during adult stages *Ezh2* is important for the preservation of proper neuronal subset identity and survival.

10:00-10:30 Thomas Perlmann

Interrogating dopamine neurogenesis, diversity and clinical utility using single cell approaches

Thomas Perlmann

Department of Cell and Molecular Biology, Karolinska Institutet, Sweden

Stem cell engineering and grafting of midbrain dopamine (mDA) neurons provides a promising strategy for brain repair in Parkinson's disease. However, essential improvement of differentiation protocols will require deeper interrogation of mDA neuron lineage development. Here we present findings from studies using single-cell RNA sequencing (scRNA-seq) of mDA neurons. We have analyzed early specification steps and revealed a remarkably close relationship between developing mDA and suthalamic nucleus neurons. Combined with additional analysis, these results were essential in efforts to improve human embryonic stem (hESC) engineering protocols for cell replacement therapy. In additional studies we analyzed the maturation of Pitx3-expressing cells during late embryogenesis up into adult stages. The studies revealed several sub-lineages of mDA neurons that are subdivided very early in development, and also identified Pitx3-expressing ventral midbrain non-dopaminergic neuronal lineages. Finally, we have used scRNA-seq to analyze the graft composition of both ventral midbrain human fetal and hESC-derived cells after transplantation to rat striatum. The analysis revealed interesting differences between grafts from either hESCs or human fetal cells. Accordingly, while both cell preparations gave rise to neurons and astrocytes, oligodendrocytes were only detected in grafts of fetal cells. On the other hand, a cell type closely resembling a class of newly identified barrier forming fibroblasts was identified as a unique component of hESC-derived grafts. Thus, these experiments have addressed an important question in the field of cell replacement in neurological disease by revealing graft composition and differences between hESC- and fetal cell-derived grafts.

11:00-11:30 Alain Prochiantz

Protection of midbrain dopaminergic neurons by ENGRAILED transcription factors

Alain Prochiantz

Collège de France, Paris, France

ENGRAILED transcription factor is expressed in different adult central nervous system (CNS) regions, including the Substantia Nigra Pars Compacta (SNpc) and Ventral Tegmental Area (VTA). Studies from different groups have established that ENGRAILED-1 (EN1) and ENGRAILED-2 (EN2) are involved in the survival of mesencephalic dopaminergic (mDA) neurons in the SNpc and, at a lesser degree, in the VTA. Protection is through the regulation of Complex I mitochondrial mRNA translation, gene transcription and heterochromatin maintenance.

As most homeoprotein transcription factors, EN1 and EN2 pass between cells and are thus secreted and internalized. The latter property has allowed us to infuse or inject EN1 and EN2 at the level of the SNpc and to verify that the resulting cytoplasmic and nuclear gain of function has curative effects in rodents and non-human primate models of Parkinson Disease. Curiously, a single injection of the EN1 protein protects the cells for several weeks or months, suggesting an epigenetic mechanism. This was verified leading to the observation that EN1 internalization is followed by changes in chromatin marks disrupted after an acute oxidative stress.

To be more specific, heterochromatin disruption that accompanies ageing or follows oxidative stress is paralleled by an upregulation of mobile elements of the LINE-1 family. As a consequence, the expression of the endonuclease encoded by LINE-1 Open Reading Frame 2 (ORF2) is also increased, leading to the formation of an abnormally high number of DNA-brakes and to cell death. Conversely, the repression of LINE-1 expression and activity, using reverse transcriptase inhibitors, anti-ORF1 siRNAs or the LINE repressor Piwil1 rescues mDA neurons from oxidative stress deleterious effects.

The link between mobile elements and ENGRAILED protective activity is through the ability of the transcription factor to repress LINE-1 expression through chromatin refolding, as already mentioned, but also through direct transcriptional repression following its high affinity binding to LINE-1 promoters.

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11:30-12:00 Rajeshwar Awatramani

Molecular genetic approaches to uncover DA neuron diversity

Rajeshwar Awatramani

Northwestern University, USA

Midbrain dopamine (DA) neurons have diverse functions that can in part be explained by their heterogeneity. Although molecularly distinct subtypes of DA neurons have been identified by single-cell gene expression profiling, fundamental features such as their projection patterns, and developmental basis of diversification has not been fully elucidated. Progress in this regard has been hindered by the lack of genetic tools to study DA neuron subtypes. Here, we develop intersectional genetic labeling strategies, based on combinatorial gene expression, to map the projections of molecularly defined DA neuron subtypes. We reveal distinct genetically-defined DAergic pathways arising from the substantia nigra *pars compacta* and from the ventral tegmental area that innervate specific regions of the caudate putamen, nucleus accumbens and amygdala. We also use these approaches to begin to study the diversification of DA neurons. Together our work, in conjunction with other recent studies, paints a richly heterogeneous picture of midbrain DA neurons, and will provide a foundation for functional interrogation of DA neuron subtypes, in normal and diseased states.

12:00-12:30 Huaibin Cai

Distinct connectivity and functionality of nigrostriatal ALDH1A1-positive dopaminergic neurons in motor control

Junbing Wu[1], Justin Kung[1], Jie Dong[1,2], Lisa Chang[1], Chengsong Xie[1], Nannan Yang[1,3], Vivian Chen[1], Zhenhua Liu[1,3], Rebekah Evans[4], Sarah Hawes[1], Bo Liang[5], Lixin Sun[1], Jinhui Ding[6], Jia Yu[7], Sara Saez-Atienzar[1], Ahsan Habib[1], Weidong Le[2], Beisha Tang[3], Zayd Khaliq[4], Da-Ting Lin[5], and Huaibin Cai[1]

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We and others demonstrated previously that Parkinson's disease preferentially affects the nigrostriatal aldehyde dehydrogenase 1A1-positive dopaminergic neuron (NalDAN) subpopulations located in the ventral tier of substantia nigra pars compacta (SNC). The connectivity and functionality of NalDANs, however, remain poorly understood. In this study, we constructed a comprehensive input and output map of NalDANs in the rodent brain and discovered an essential physiological function of NalDANs in motor control. We found that NalDANs receive diverse monosynaptic inputs from a variety of brain regions, but project predominantly to the dorsal portions of dorsal striatum, a striatal subregion particularly important in motor control. Our current work represents an initial attempt to understand specific physiological functions of a molecularly defined midbrain dopaminergic neuron subpopulation in regulating specific behavioral phenotypes.

SESSION 4: In vitro models of dopamine neurons | Lia Panman

14:30-15:00 Lia Panman

Directed differentiation of substantia nigra dopaminergic neurons from mouse and human pluripotent stem cells

[1,2]Tony Oosterveen, [1,2]Pedro Garcao, [2]Emma Garcia Moles, [1,2]Clement Soleilhavoup, [1]Marco Travaglio, and [1,2]Lia Panman

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Midbrain dopaminergic neurons (mDA) constitute a highly diverse neuronal population controlling important brain functions such as voluntary movements, cognition and reward. These neurons can be broadly subdivided into two major groups, which form the substantia nigra (SN) and ventral tegmental area (VTA). SN DA neurons selectively degenerate in Parkinson's disease, while the neighbouring VTA neurons remain relatively unaffected despite their commonalities in developmental origin and gene expression profile. How these subpopulations are specified and innervate their target areas during embryonic development and the reason for the difference in vulnerability are not fully understood.

Based on our gained insight into the specification of SN and VTA neurons we have developed an ES/iPS cell-based model system that allows us to investigate the differences in vulnerability between distinct dopaminergic subpopulations. DA cultures containing mainly VTA neurons lack the sensitivity to mitochondrial toxicity and hampers PD disease modelling in vitro. Therefore, we identified culture conditions that can direct the differentiation of ES/iPS cells into enriched dopaminergic cultures displaying SN specific characteristics. Our platform is highly amendable for compound screening and led to the identification of pathways that can protect SN from degeneration.

15:00-15:30 Jens Schwarmborn

Modeling Parkinson's disease in vitro with 3D cultures and organoids

Jens Schwamborn

University of Luxembourg, Luxembourg

Modeling Parkinson's disease (PD) using advanced experimental *in vitro* models is a powerful tool to study disease mechanisms and to elucidate unexplored aspects of this neurodegenerative disorder. Here, we demonstrate that 3D differentiation of expandable midbrain floorplate neural progenitor cells (mfNPCs) leads to organoids that resemble key features of the human midbrain. These organoids are composed of midbrain dopaminergic neurons (mDANs), which produce and secrete dopamine. Midbrain-specific organoids derived from PD patients carrying the *LRRK2*-G2019S mutation recapitulate disease-relevant phenotypes. Automated high-content image analysis shows a decrease in the number and complexity of mDANs in *LRRK2*-G2019S compared to control organoids. The floor plate marker FOXA2, required for mDAN generation, increases in PD patient-derived midbrain organoids, suggesting a neurodevelopmental defect in mDANs expressing *LRRK2*-G2019S. Thus, we provide a robust method to reproducibly generate 3D human midbrain organoids containing mDANs to investigate PD-relevant patho-mechanisms.

15:30-16:00 Vania Broccoli

Novel in vitro human models of functional and diseased neuronal circuits

Vania Broccoli

San Raffaele Scientific Institute/CNR Institute of Neuroscience, Italy

Neuronal cultures obtained by in vitro differentiation of pluripotent stem cells are an ideal model to assess physiological and disease-relevant processes occurring in human biology. However, stem cell-derived neurons are obtained in mass cultures that lack spatial organization and without any meaningful connectivity. We implemented a novel system for long-term culture of human neurons with patterned organization of projections and synaptic terminals. Co-culture of human midbrain dopaminergic and striatal medium spiny neurons on a chip established an orchestrated nigro-striatal circuitry with functional dopaminergic synapses. We employed this platform to evaluate the impact of mitochondrial dysfunctions associated with a genetic form of Parkinson's disease (PD) with OPA1 mutations. Remarkably, we found that axons of OPA1 mutant dopaminergic neurons exhibited a significantly reduction of mitochondria mass, functionality and dynamics.

16:30-17:00 Nilima Prakash

WNT/b-catenin dosage-dependent differentiation of midbrain dopaminergic neuron subsets

Nilima Prakash

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Dopamine (DA)-synthesizing nerve cells located in the human midbrain are involved in the control and modulation of voluntary movements, rewarding/aversive behaviors and other cognitive functions of the brain. The age-dependent and progressive degeneration of these neurons leads to the motor symptoms of Parkinson's Disease (PD), whereas their dysfunction is associated with neuropsychiatric disorders such as addiction, schizophrenia, attention deficit hyperactivity disorder and depression. The etiology of these diseases, in particular of the neuropsychiatric disorders, is thought to have a neurodevelopmental component. Therefore, a precise knowledge of the developmental pathways directing the generation of the midbrain dopaminergic (mDA) neurons is needed for a better understanding of these still incurable neurodegenerative and psychiatric disorders, and for the design of new therapeutic approaches to these diseases.
The mammalian mDA neurons arise from the ventral midline (floor plate) of the mesencephalon (midbrain) and caudal diencephalon (forebrain) between embryonic days 9.5 to 14.5 (E9.5-14.5) of mouse gestation. Several intercellular signaling pathways control the early steps of mDA neuron generation, including the WNT1/b-catenin pathway. Initially (around E10.5), WNT1/b-catenin signaling is implicated in the establishment of the mDA domain in the ventral midbrain and promotes the proliferation of floor plate progenitors, particularly in the medial and caudal midbrain. From E11.5 onwards, this signaling pathway directs the specification of the mDA cell fate in these progenitors and their correct differentiation into the two main mDA neuron subsets in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). Moreover, WNT1/b-catenin signaling promotes the survival of mature mDA neurons through a network of downstream transcription and neurotrophic factors. My talk will focus on the identification, quantification and transcriptome profiling of the WNT-responsive cells in the developing ventral midbrain of WNT/b-catenin signaling reporter (*BAT-gal*) mouse embryos, which revealed several novel and/or additional pathway components and putative WNT/b-catenin target genes in these cells. I will also present data showing that an attenuation of WNT/b-catenin signaling in the mDA progenitors appears to be essential for their correct differentiation into specific mDA neuron subsets, thus providing new insights into the stem cell-based in vitro modeling of mDA neuron-associated neurological and psychiatric diseases.

17:00-17:30 Su-Chun Zhang

Reconstruction of the Nigra-Striatal Circuit by Human Dopamine Neurons

Man XIONG, Yezheng TAO, Yuejun CHEN, Su-Chun ZHANG

University of Wisconsin-Madison, Duke-NUS Medical School

Degeneration of the midbrain dopamine (mDA) neurons results in disruption of the nigra-striatal circuit, which underlies Parkinson's disease. Transplantation of neural cells to repair the damaged circuitry is a potential treatment, but whether and to what extent by which the function of the repaired circuit is restored in the adult brain is not known. By transplanting mDA neurons, derived from human pluripotent stem cells (hPSCs), into the substantia nigra (SN) of adult Parkinson's disease model mice, we found that mDA neurons projected axons predominantly to the dorsal striatum via the nigra-striatal pathway. The grafted mDA neurons also received area-specific synaptic inputs and these inputs became functional 3-6 months after transplantation. The transplanted animals showed motor functional recovery, which was abrogated or enhanced by regulating the activity of the grafted mDA neurons via DREADDs. These results highlight the capacity of hPSC-derived mDA neurons for functionally reconstructing the nigra-striatal circuit in the adult brain, contributing to therapeutic outcomes.

17:30-18:00 Lorenz Studer

Dopaminergic differentiation of human pluripotent stem cells – from Development to Cell Therapy

SESSION 5: Cell replacement strategies for Parkinson's disease | Emmanouil Metzakopian

8:30-9:00 Anders Björklund

Use of embryonic stem cells for dopamine replacement in Parkinson´s disease

Anders Björklund,

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Cell replacement therapy for Parkinson´s disease is based on the idea that implanted dopamine neurons can substitute for the lost nigrostriatal neurons, restore dopaminergic neurotransmission and reverse the Parkinson-like motor impairments induced by damage to the nigrostriatal system. Open-label clinical trials in patients with PD have shown that dopamine neuroblasts obtained from fetal human midbrain tissue can survive and function over many years in the brain of PD patients, restore striatal dopamine release, and provide sustained and long-lasting improvements in motor behavior. The ethical and practical problems associated with the use of fetal tissue is a serious obstacle to further developments of this approach. Further progress, therefore, is critically dependent on the development of transplantable dopamine neurons from stem cells. The most promising results so far have been obtained using pluripotent stem cells, ESCs or iPSCs, as starting material. Recently developed and optimized protocols allow efficient generation of midbrain dopamine neurons from human ES cells that survive well following transplantation to the striatum, in the absence of any contaminating tumor-forming cells, and differentiate into genuine midbrain dopamine neurons of both A9 and A10 subtypes. In recent experiments performed in immunosuppressed and immunodeficient rats we have shown that the hESC-derived neurons grow to form extensive axonal terminal networks in appropriate striatal, limbic and and cortical targets and reverse PD-like motor impairments. The results indicate that transplantable and fully functional midbrain dopamine neurons can be generated from human ES cells. Clinical trials using these cells are now under way.

9:00-9:30 Wolfgang Wurst

Prodromal Models of Parkinson Disease

Wolfgang Wurst

Helmholtz Centre Munich, Institute of Developmental Genetics, Munich/Neuherberg, Germany

Parkinson´s Disease (PD) is clinically characterized by the progressive loss of dopaminergic neurons in the ventral midbrain but increasingly recognized also by the loss and dysfunction of other neuronal populations. Based on human genome-wide association studies we have previously generated mouse mutants reproducing mutations in human PD patients: DJ-1, PINK1, and LRRK2. All these mouse models exhibit several symptoms of PD indicative for the prodromal phase of the disease, including gait deficits resembling the human phenotype and represent ideal models to interrogate the biological basis of gait disturbances in PD. We further characterized these mutants and identified alterations in behavior and metabolic, and bioenergetics parameters. Furthermore, we established idiopathic PD patient-derived iPSC, NPCs and DA neurons, which are currently molecularly, and cellular characterized as well. The comparative analysis of human cellular in vitro and mouse in vivo models of PD will be presented and discussed.

9:30-9:45 Partner Talk: Giovanna De Filippi – Axion BioSystems

Modeling neurodegenerative diseases in-a-dish: Exploring life's circuitry with next generation MEA

9:45-10:00 Selected Abstract: Alessandra Zanon

Establishment of a 3D culture system for the generation of dopaminergic neurons for disease modeling in PD

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Human induced pluripotent stem cells (hiPSCs) represent an unlimited cell source for the generation of 3D *in vitro* models for neurological diseases. Neurons cultured in 3D aggregates might not only serve to facilitate the development of more precise human brain models for basic mechanistic studies but also represent better predictors of drug responses *in vivo*. To evaluate the capacity of alginate to support the differentiation of hiPSCs to midbrain-specific dopaminergic neurons for Parkinson's disease (PD) modeling, we encapsulated iPSCs in alginate microcapsules (1% or 2%) with or without fibronectin (Fn). Cells grown in 1% alginate-Fn and 2% alginate-Fn present increased differentiation capacity towards neural lineages with respect to both alginate (1%-2%) alone and 2D conditions. Gene expression analysis suggests an increase in TH^+ neurons and a higher maturity compared to the neurons differentiated in 2D. Immunofluorescence analysis further supports these results, showing expression of synaptic markers as well as specific DA neuronal markers. The 3D neurons can be maintained in culture for more than 200 days. We envision that this differentiation protocol might enable the generation of midbrain-specific dopaminergic neurons in a shorter time-frame as compared to 2D cultures and will allow successful phenotype assessment and advanced therapy development.

10:00-10:30 Janelle Drouin-Ouellet

Direct reprogramming of patient skin fibroblasts to induced dopaminergic neurons to model idiopathic Parkinson's disease

Understanding the pathophysiology of Parkinson's disease (PD) has been hampered by the lack of models that recapitulate all the critical factors underlying its development. We have developed a novel and highly efficient approach to generate functional induced dopaminergic neurons (iDANs) that are directly reprogrammed from dermal fibroblasts of patients with idiopathic PD. We further investigate whether such cells have deficits in autophagy and show that iDANs derived from PD patients exhibit lower basal chaperone-mediated autophagy as compared to iDANs of healthy donors. Furthermore, stress-induced autophagy resulted in an accumulation of macroautophagic structures in induced neurons derived from PD patients, independently of the specific neuronal subtype but dependent on the age of the donor. Our results show that iDANs provides a patient-specific model to study neuronal features relevant to idiopathic PD.

11:00-11:30 Ernest Arenas

From dopaminergic neuron development to cell replacement strategies for Parkinson's disease

Ernest Arenas

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of midbrain dopaminergic neurons (mDA) neurons in the substantia nigra. Current therapeutic interventions focus on restoring the levels of the neurotransmitter dopamine, by pharmacological means, or on balancing neurotransmission, by stimulating the subthalamic nucleus with deep brain stimulation. Three factors in recent years have contributed to the development of cell replacement therapies for PD: 1) Proof of concept that clinical fetal ventral midbrain tissue grafting can change the course of disease in PD patients. 2) The advent of human pluripotent stem cells (hPSCs) and reprogramming technologies. 3) Progress in understanding mDA neuron development.

However, our knowledge of the developing human midbrain is still limited. In order to address this challenge, we recently examined of the human embryonic ventral midbrain by single-cell RNA-sequencing (scRNA-seq) and identified 25 distinct cell-types as well as their transcriptional signature. This data together with newly available single-cell data covering the entire adult mouse brain, new genome wide association studies of PD as well as bioinformatics analysis and functional studies are currently being used to address critical questions in the field, such as:

1) What are the transcriptional networks controlling mDA neurogenesis?

2) What are the cell types affected in PD that need replacement?

3) What is the quality of current hPSC-derived midbrain cell preparations aimed for PD cell replacement therapy?

4) Can single cell technologies contribute to develop next generation hPSC- or reprogramming-based cell replacement therapies for PD?

11:30-12:00 Emmanouil Metzakopian

Defining human iPSC derived dopamine neurons via single cell RNA-Seq

UK Dementia Research Institute (UK DRI), University of Cambridge

In vitro human induced pluripotent stem cell derived neurons consist a versatile model of studying diseases such as Parkinson's and Alzheimer's. However, the differentiation course is susceptible to experimental variability which can skew experimental outcomes from the resulting culture. The cellular development and heterogeneity of these in vitro models require proper characterization before use for downstream experiments such as CRSIPR-Cas9 genetic screens. Single cell transcriptomics has proven to be a powerful tool to achieve in depth understanding of cellular states and tissue culture complexities. Here we are capturing the tissue culture states using single-cell transcriptomics by studying the developmental time course and assessing clonal heterogeneity. Results highlight the different culture outcomes and the intermediate state transitions through the course of differentiation.

Poster Abstracts

Abstract number	Name	Title
1	Milagros Pereira Luppi	Development and application of a Nestin-anchored intersectional fate mapping strategy to reveal Dopaminergic neuron cell fate
2	Mateja Rybiczka-Tešulov	Circular RNAs in developing midbrain dopamine neurons
3	Parivash Nouri	LEF1-mediated WNT1/b-Catenin signaling in subtype-specific midbrain dopaminergic neuron differentiation
4	Tae Wan Kim	Derivation of Enriched Engraftable Midbrain Dopamine Neurons from Human Pluripotent Stem Cells in a cGMP-qualified Condition for the Cell Replacement Therapy to Parkinson Patients
5	Alessandro Petese	Investigating Lmo3 (LIM-domain Only Protein 3) as a Marker for a Subset of midbrain Dopaminergic Neurons
6	So Yeon Koo	Molecular Characterization of Human Stem Cell-Derived Dopaminergic Neuron Subtypes
7	Marco Travaglio	Altered striatal innervation in Nolz1 deficient embryos provides novel insight into dopaminergic circuitry formation
8	Marianna Tolve	The Role of the Transcription Factor Bcl11a in Midbrain Dopaminergic Neurons
9	Yojet Sharma	Role of ion-channels in development of mouse mDA neurons
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11	Alessandra Zanon	Establishment of a 3D culture system for the generation of dopaminergic neurons for disease modeling in PD
12	Laura Lahti	Single-cell RNA sequencing reveals midbrain dopamine neuron diversity emerging during mouse brain development
13	Zachary Gaertner	Molecular Classification of Dopamine Neurons: Towards a consensus
14	Nikolaos Patikas and Stefanie Foskolou	Single-cell characterization of mid-brain Dopamine & Cortical Neurons from in vitro models
15	Divya Raj	Role of long-range Netrin-1 signaling in dopaminergic migration and cellular organization in the ventral midbrain

Abstract 1: Milagros Pereira Luppi

Development and application of a Nestin-anchored intersectional fate mapping strategy to reveal Dopaminergic neuron cell fate

Milagros Pereira Luppi, Jean-Francois Poulin, Pei-Ken Hsu, Raj Awatramani

Department of Neurology, Northwestern University

Lineage analysis has led to a better understanding of how the embryonic neural tube progenitors give rise to the enormous complexity of cell types in the adult CNS. Lineage tracing methods indelibly label a progenitor or subset of progenitors. In particular, site-specific recombinases driven by gene-specific promoters activate a reporter in a permanent manner, allowing tracking of the descendants of the progenitor of interest. The accuracy of these methods, however, is limited by how specific to such progenitors is the promoter driving the recombinase. Intersectional approaches increase the specificity of the analysis, rendering more refined fate maps. Nonetheless, driver genes are often expressed in progenitors and in related or unrelated postmitotic neurons simultaneously. Thus, in such cases, progenitor–progeny relationships cannot be correctly ascertained. Inducible-recombinase approaches restrict labeling to a specific time window avoiding reporter induction in postmitotic cells expressing the driver gene, yet, this method is hampered by mosaicism and toxicity. To circumvent these limitations, we propose a new platform for lineage analysis anchored in the Nestin locus. This method is compatible with most Cre drivers; thus, it will be broadly applicable to study important lineage related questions. Here, we demonstrate the validity and efficiency of this approach and preliminary studies towards understanding DA neuron fates.

Abstract 2: Mateja Rybiczka-Tešulov

Circular RNAs in developing midbrain dopamine neurons

Mateja Rybiczka-Tešulov 1 , Roland E. van Dijk 1, Morten T. Venø 2, Lieke L. van de Haar 1, Jørgen Kjems 2, R. Jeroen Pasterkamp 1

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Non-coding (nc) RNAs play a crucial role during neuronal development, but their precise function in midbrain dopamine (mDA) neurons remains incompletely understood. Circular (circ) RNAs are a novel class of ncRNAs that form as the result of covalent linkage of 5' and 3' pre-mRNA splice sites. CircRNAs can originate from many different genes, while expression levels are tissue-, cell type- and developmental stage-dependent. The mammalian brain is particularly rich in this novel class of ncRNAs, where circRNAs have been proposed to regulate processes such as synaptogenesis and synaptic activity. Here, we study the spatio-temporal expression and function of circRNAs during the early development of the mDA system. Using novel mouse reporter lines and immunohistochemical approaches, we generated a detailed map of mouse mDA neuron development. On basis of this analysis, we selected a number of developmental timepoints at which mDA neurons were isolated by FACS and subjected to RNAseq and circRNA prediction. We aim to dissect the functional role of specific circRNAs during mDA neuron development by knockdown or overexpression of circRNAs in primary cultures or in vivo by *in utero* electroporation. Overall, these experiments will provide insight into the role of a novel class of ncRNAs in mDA neuron development.

Abstract 3: Parivash Nouri

LEF1-mediated WNT1/b-Catenin signaling in subtype-specific midbrain dopaminergic neuron differentiation

Parivash Nouri[1*], Sebastian Götz[2*], Benedict Rauser[2*], Martin Irmler[3], Changgeng Peng[2], Dietrich Trümbach[2], Yojet Sharma[1], Andrea Dlugos[1], Wolfgang Wurst[2,4,5,6], Johannes Beckers[3,7,8] and Nilima Prakash[1]

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*These authors contributed equally to this work

The dopamine producing neurons located in substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) neurons, which are subgroups of Mesodiencephalic dopaminergic (mdDA) neurons, play significant roles in the neural network responsible for movement, cognition and emotion. The development of mdDA neurons has attracted particular attention, due to their critical functions in neuropsychiatric and neurodegenerative disorders, such as Parkinson disease (PD). Previous research on the importance of the WNT1/b-catenin signaling pathway in the differentiation and survival of mdDA neurons suggests that a disrupted activation or inhibition of the WNT1/b-catenin signaling pathway leads to the incorrect specification or differentiation of mdDA neurons and/or their subsets?. This study set out to investigate the identity of the WNT/b-catenin-responsive cells in the murine ventral midbrain (VM) and the precise mechanism of WNT/b-catenin action in the mdDA cells. We found that only a fraction of all mdDA progenitors, precursors, and neurons in the murine VM respond to this pathway. The WNT/b-catenin-responsive cells are located mostly in the *Wnt1*+, *Rspo2*+ and *Lef1*+ lateral floor plate of the medial and caudal midbrain, and give preferentially rise to caudomedial (VTA) mdDA neurons. We also show that the strong activation of the WNT/b-catenin signaling pathway by RSPO2, a WNT/b-catenin agonist, and LEF1, a nuclear effector of this pathway, inhibits the differentiation of WNT/b-catenin-responsive mdDA progenitors into mature mdDA neurons. This is in part due to the direct repression of the murine *Pitx3* gene promoter by LEF1-mediated WNT/b-catenin signaling. Our results indicate that the correct differentiation of mdDA progenitors into specific mdDA neuron subtypes, particularly SNc DA neurons, requires the attenuation of WNT/b-catenin signaling in these cells, thus providing new insights to the directed differentiation of these cells means for stem cell-based regenerative therapies of PD and other *in vitro* models of neuropsychiatric diseases.

Abstract 4: Tae Wan Kim

Derivation of Enriched Engraftable Midbrain Dopamine Neurons from Human Pluripotent Stem Cells in a cGMP-qualified Condition for the Cell Replacement Therapy to Parkinson Patients.

Tae Wan Kim[1,4], So Yeon Koo[1], Jinghua Piao[2], Eveline M Gutzwiller[1], Se Joon Choi[3], Eugene V Mosharov[3], Mark J Tomishima[1], Viviane Tabar[2], Lorenz Studer[1]

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hPSC-derived midbrain dopamine(mDA) neurons are considered a promising avenue for cell replacement therapy in Parkinson's disease (PD). Despite rapid developments for deriving mDA neuron from hPSC towards human translation, several questions remain as to define the most optimal cell product for treating PD patients. Here we developed our protocol for deriving clinical relevant mDA neurons by optimizing activation and timing of WNT signaling. The conditions yield mDA neurons giving rise to authentic functional TH positive neurons *in-vitro* and *in-vivo*, and rescuing amphetamine-induced rotation in rat Parkinson model. However, when applying those optimized mDA neuron induction conditions to *EN1-Knockout* hPSC, we observe expression of contaminating PAX6 and STN markers, suggesting a pivotal role for EN1 in mediating induction of mDA neuron and suppression of alternative markers. Furthermore, we established a NURR1-H2B-GFP reporter line that enables us to derive nearly pure population of engraftable mDA neurons, which was used for candidate surface markers. These studies take advantage of our ability to generate pure population of mDA neurons in a clinically relevant culture system. The work should be geared towards developing a "2.0" version of our GMP mDA neuron product to offer the best possible cell therapy to PD patients in the near future.

Abstract 5: Alessandro Petese

Investigating Lmo3 (LIM-domain Only Protein 3) as a Marker for a Subset of midbrain Dopaminergic Neurons

Alessandro Petese1, Cathleen Hagemann1, Pietro Pilo Boyl2, Philipp Hatzfeld3, Joachim Degen4, Walter Witke2, Irmgard Förster3, Sandra Blaess1

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Midbrain dopaminergic (mDA) neurons are located in in three distinct anatomical regions, the substantia nigra pars compacta (SNc), the ventral tegmental area and the retrorubal field. mDA neurons are diverse in their projection targets, physiological properties and impact on behavior. On a molecular level, single-cell RNA sequencing studies of developing and adult mDA neurons have identified mDA subpopulations defined by specific gene expression profiles. To gain insight into how the functional heterogeneity of mDA neurons is linked to these molecular profiles, it is crucial to understand whether genetically-defined subpopulations contribute to specific circuits and functions. *Lmo3*, a transcriptional co-regulator, is expressed in ventral SNc neurons (E15.5 and E18.5) during embryonic development. This expression pattern is largely maintained in adulthood. Thus, *Lmo3* is a suitable candidate to define a subset of mDA neurons. Our goal is to characterize *Lmo3*-expressing mDA neurons within the context of neurodevelopment, circuits and functions using cell-specific tracing and optogenetic approaches. To this end we are generating a knock-in line in which the Cre recombinase gene is expressed under the control of the *Lmo3* promoter. To establish this *Lmo3*-Cre knock-in line, we are using CRISPR/Cas-mediated targeted integration in zygotes.

Abstract 6: So Yeon Koo

Molecular Characterization of Human Stem Cell-Derived Dopaminergic Neuron Subtypes

So Yeon Koo[1, 2], Tae Wan Kim[1], Lorenz Studer[1,2]

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Parkinson's disease (PD) is due by the progressive loss of dopaminergic neurons in the narrow region of the brain called substantia nigra (SN). The Studer lab has recently developed a clinically-relevant protocol to derive functionally authentic midbrain dopaminergic neurons (mDA) from human pluripotent stem cells (hPSCs) under defined cGMP conditions. However, the differentiation of hPSC *in vitro* does not yield pure population of A9 substantia nigra neurons but likely contains other mDA subtypes. Thus, we set out to define molecular signatures for each mDA neuron subtype (A9 vs. A10) and investigate the developmental origin of such subtype identity in hPSC-derived neurons.

SOX6 and OTX2 have been reported to exhibit exclusive expression in dopamine neurons of the SN and VTA respectively in both the developing and adult murine brain (Panman et al., 2014; Di Salvio et al., 2010; Vernay et al., 2005). However, their role during human mDA neuron development remains unknown. I developed an endogenous SOX6-tdTomato reporter in our well-characterized NURR1-GFP hPSC reporter line. This double reporter may enable us to denote a potential SN-type mDA neuron subtype. Furthermore, I established inducible SOX6 and OTX2 knockout approaches to manipulate expression of either gene at defined temporal windows. These tools will enable us to examine the SN(A9)/VTA(A10) mDA neuron subtype ratio in our culture system, facilitate efforts to further optimize the protocol for maximum SN yield and clarify the role of SOX6 and OTX2 during mDA neuron subtype specification and maintenance in vitro and in vivo.

Abstract 7: Marco Travaglio

Altered striatal innervation in Nolz1 deficient embryos provides novel insight into dopaminergic circuitry formation

Marco Travaglio[1,2], Clement Soleilhavoup[1,2], Kieran Patrick[1,2], Brian Brooks[3], Elangovan Boobalan[3], Jeroen Pasterkamp[4], Fokkoliena Panman[1,2]

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Dopaminergic (DA) neurons in the midbrain give rise to ascending axonal projections to the striatum. Despite their fundamental role in locomotion, reward and memory, relative little is known about how DA neurons innervate their target areas. Although multiple axon guidance molecules have been suggested to direct DA axon growth toward their final targets, the identity of secreted cues expressed by the striatum to promote DA innervation remains unknown. Here, we found that that the transcriptional regulator Nolz1 expressed in both dopaminergic neurons and the striatum has a key role in establishing dopaminergic circuitry. Using a conditional gene targeting approach, we show that genetic ablation of Nolz1 leads to reduced nigrostriatal axonal projections, indicating its involvement in DA axon development. Transcriptomic analysis of striatal cells from Nolz1 deficient mice further indicates that a repertoire of striatum-enriched genes is dysregulated in mutant embryos. This finding was confirmed by immunohistochemistry and the chemotrophic effect of these genes is currently being investigated through integrated microfluidic platforms. Our results indicate a fundamental role of Nolz1 in dopaminergic axon guidance and shed light on novel mechanisms involved in DA circuitry formation.

Keywords: axon guidance, dopaminergic neurons, striatum, transcriptional regulator

Abstract 8: Marianna Tolve

The Role of the Transcription Factor Bcl11a in Midbrain Dopaminergic Neurons

Marianna Tolve[1], Ayse Ulusoy[2], Alesja Dernst[1], Gabriela O. Bodea[1], Pentao Liu[3], Walid Khaled[4], Neal Copeland[5], Heinz Beck[6], Stephan Baader[7], Donato Di Monte[2], Sandra Blaess[1]

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Midbrain dopaminergic (mDA) neurons of the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) project to multiple forebrain target regions and modulate various behaviors. Transcription factor codes established during development may define the identity of specific mDA neuronal subsets and could ultimately contribute to the determination of their projection targets, their functional properties and their susceptibility to neurodegeneration. Bcl11a is a Krüppel-like zinc finger transcription factor expressed in a subset of mDA neurons in the lateral VTA and the SNc. Using viral tracing experiments, we show that the olfactory tubercles are a major projection target of Bcl11a-expressing VTA-mDA neurons. To examine Bcl11a function in development and maintenance of mDA neurons, we inactivated *Bcl11a* specifically in mDA neurons during development (*Bcl11a* cko mice). We find that differentiation and maintenance of Bcl11a-positive mDA neurons is not obviously affected in *Bcl11a* cko mice. However, *Bcl11a* cko mice show behavioral impairments suggesting that the loss of Bcl11a leads to functional deficits in mDA neurons. Moreover, Bcl11a could play a neuroprotective role within SN-mDA neurons, since overexpression of alpha-synuclein in the SNc leads to a significantly greater reduction in the number of SNc-mDA neurons in *Bcl11a* cko animals as compared to controls.

Abstract 9: Yojet Sharma

Role of ion-channels in development of mouse mDA neurons

Yojet Sharma[1]*, Benedict Rauser[2]*, Sebastian Götz[2]*, Martin Irmler[3], Parivash Nouri[1]*, Johannes Beckers[3,4,5] Birgit Liss[6] and Nilima Prakash[1]

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The dopamine (DA)-synthesizing neurons are crucial for facilitation of voluntary movements, regulation of emotional behavior and reward pathways. These neurons are localized in the adult ventral midbrain. To precisely outline the developmental profile of mDA neurons, could prove beneficial in modeling neurological disorders such as Parkinson's disease (PD). The degeneration of DA neurons in the substantia nigra pars compacta (SNpc) leads to anomalies in the motor functions of the PD patients. One of the most important signaling pathways in the generation of midbrain-DA (mdDA) neurons is the canonical WNT/β-catenin signaling pathway. We have found out that a WNT1 signal directs the specification and differentiation of progenitor cells into mdDA neurons and their subsets. To understand which genes are expressed in the developing WNT-responsive mdDA domain, we carried out a microarray-based comparative transcriptome analyses. To our surprise, around 51% of the overrepresented transcripts in the WNT-responsive mdDA domain encode proteins belonging to the ion channel and neurotransmitter receptor/transporter gene ontology categories. Further, *in situ* validation of the voltage-gated potassium channel *Kcnd3* (*Kv4.3*), the inwardly-rectifying potassium channel *Kcnj6* (*Girk2*) and the voltage-dependent calcium channel subunit *Cacna1d* (*Cav1.3α1*), revealed that they are selectively transcribed in the ventral midbrain of the mouse embryo, exhibiting in some cases a very localized expression pattern in what appears to be mdDA subdomains. Because the neural connectivity of the mdDA neurons has not yet been established at these early developmental stages, our data points out alternative roles of these ion channels during early mdDA neuron development.

Abstract 10: Tiago Cardoso

hESC-derived dopaminergic transplants integrate into basal ganglia circuitry in a preclinical model of Parkinson´s disease

Tiago Cardoso[1,2], Andrew F. Adler[1,2], Sara Nolbrant[1,2], Bengt Mattsson[1], Deirdre B. Hoban[1,2], Ulla Jarl[1], Jenny Nelander Wahlestedt[1,2], Shane Grealish[1], Anders Björklund[1,] Malin Parmar[1,2*]

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Dopamine (DA) neurons derived from human embryonic stem cells (hESCs) are a promising cell source for cell replacement therapy in Parkinson´s disease (PD). When transplanted into preclinical models of PD, these neurons survive long-term, release dopamine, extensively innervate target host structures and provide functional recovery. Using rabies-based monosynaptic tracing, we have recently shown that grafted hESC-derived neurons appropriately integrate into the host circuitry.

In this study, ventral midbrain (VM) and forebrain (FB) patterned hESCs-derived neural progenitors were transplanted into the striatum or into the substantia nigra of 6-OHDA-lesioned rats, to elucidate factors controlling target-appropriate innervation and synaptic integration 6 months post-transplantation. We show that cell intrinsic factors determine the pattern of graft-derived axonal innervation, while synaptic inputs from the host primarily reflect the location of the graft. Furthermore, we provide evidence that hESC-derived DAergic grafts transplanted to the striatum (reflecting clinical graft placement) receive synaptic input from the same subtypes of host cortical, striatal, and pallidal neurons which are known to regulate the function of endogenous nigral DA neurons.

This refined understanding of factors controlling graft-derived axonal outgrowth and circuitry integration will be important for the optimization of the design of clinical cell replacement therapies for PD.

Abstract 11: Alessandra Zanon

Establishment of a 3D culture system for the generation of dopaminergic neurons for disease modeling in PD

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Human induced pluripotent stem cells (hiPSCs) represent an unlimited cell source for the generation of 3D *in vitro* models for neurological diseases. Neurons cultured in 3D aggregates might not only serve to facilitate the development of more precise human brain models for basic mechanistic studies but also represent better predictors of drug responses *in vivo*. To evaluate the capacity of alginate to support the differentiation of hiPSCs to midbrain-specific dopaminergic neurons for Parkinson's disease (PD) modeling, we encapsulated iPSCs in alginate microcapsules (1% or 2%) with or without fibronectin (Fn). Cells grown in 1% alginate-Fn and 2% alginate-Fn present increased differentiation capacity towards neural lineages with respect to both alginate (1%-2%) alone and 2D conditions. Gene expression analysis suggests an increase in TH^+ neurons and a higher maturity compared to the neurons differentiated in 2D. Immunofluorescence analysis further supports these results, showing expression of synaptic markers as well as specific DA neuronal markers. The 3D neurons can be maintained in culture for more than 200 days. We envision that this differentiation protocol might enable the generation of midbrain-specific dopaminergic neurons in a shorter time-frame as compared to 2D cultures and will allow successful phenotype assessment and advanced therapy development.

Abstract 12: Laura Lahti

Single-cell RNA sequencing reveals midbrain dopamine neuron diversity emerging during mouse brain development

Katarína Tiklová[1], Åsa K. Björklund[2], Laura Lahti[1], Alessandro Fiorenzano[3], Sara Nolbrant[3], Linda Gillberg[1], Nikolaos Volakakis1, Chika Yokota[4], Markus M. Hilscher[4], Thomas Hauling[4], Fredrik Holmström[1], Eliza Joodmardi[1], Mats Nilsson[4], Malin Parmar[3] & Thomas Perlmann[1,5]

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Midbrain dopamine (mDA) neurons constitute a heterogenous group of cells that have been intensely studied, not least because their degeneration causes major symptoms in Parkinson's disease. Understanding the diversity of mDA neurons – previously well characterized anatomically – requires a systematic molecular classification at the genome-wide gene expression level. Here, we use single cell RNA sequencing of isolated mouse neurons expressing the transcription factor *Pitx3*, a marker for mDA neurons. Analyses include cells isolated during development up until adulthood and the results are validated by histological characterization of newly identified markers. This identifies seven neuron subgroups divided in two major branches of developing *Pitx3*-expressing neurons. Five of them express dopaminergic markers, while two express glutamatergic and GABAergic markers, respectively. Analysis also indicate evolutionary conservation of diversity in humans. This comprehensive molecular characterization will provide a valuable resource for elucidating mDA neuron subgroup development and function in the mammalian brain.

Abstract 13: Zachary Gaertner

Molecular Classification of Dopamine Neurons: Towards a consensus

Zachary Gaertner[1], Jean-Francois Poulin[2], Oscar Moreno Ramos[1], Rajeshwar Awatramani[1].

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Dopamine (DA) influences a spectrum of behaviors including locomotion, learning, reward, motivation, and cognition. DA dysfunction is prominently implicated in a wide range of disorders, including Parkinson's disease (PD), schizophrenia, ADHD, addiction, chronic pain and depression. How a small group of neurons underpins a multitude of unrelated behaviors remains enigmatic. We have hypothesized that the midbrain DAergic system is composed of distinct DA neuron subtypes, each serving a precise purpose. Thus, characterizing DA neuron subtypes could be essential to understanding DA-related diseases. Towards this end, our lab and others have categorized DA neurons based on their molecular signatures obtained by single-cell gene expression profiling. While not in full agreement, these studies have shown overlapping results. Here we aim to synthesize points of congruence as well as highlight key differences between classification schemes to derive a consensus set of DA subtypes and their presumed anatomic localizations. Doing so will provide a theoretical framework for investigating the diversity within the dopaminergic system.

Abstract 14: Nikolaos Patikas and Stefanie Foskolou

Single-cell characterization of mid-brain Dopamine & Cortical Neurons from in vitro models

Nikolaos Patikas, Hugo Fernandes, Muhammad Kaiser Bin Abdul Karim, Stefanie Foskolou,Mark Cotter ,Andrew Bassett , Emmanouil Metzakopian

UK Dementia Research Institute, University of Cambridge, Wellcome Sanger Institute

In vitro differentiations of neurons consist a versatile model of studying diseases such as Parkinson's and Alzheimer's. However, the reprogramming course is susceptible to experimental variability that can skew the resulting culture. Here, we focus on the development of cortical neurons using the induced NGN2 protocol and the heterogeneity of midbrain dopaminergic neurons using the Kriks protocol. We are capturing the culture states using single-cell transcriptomics by studying the developmental time course and assessing the clonal heterogeneity. Results highlight the different culture outcomes and the intermediate state transitions through the course of reprogramming and differentiation.

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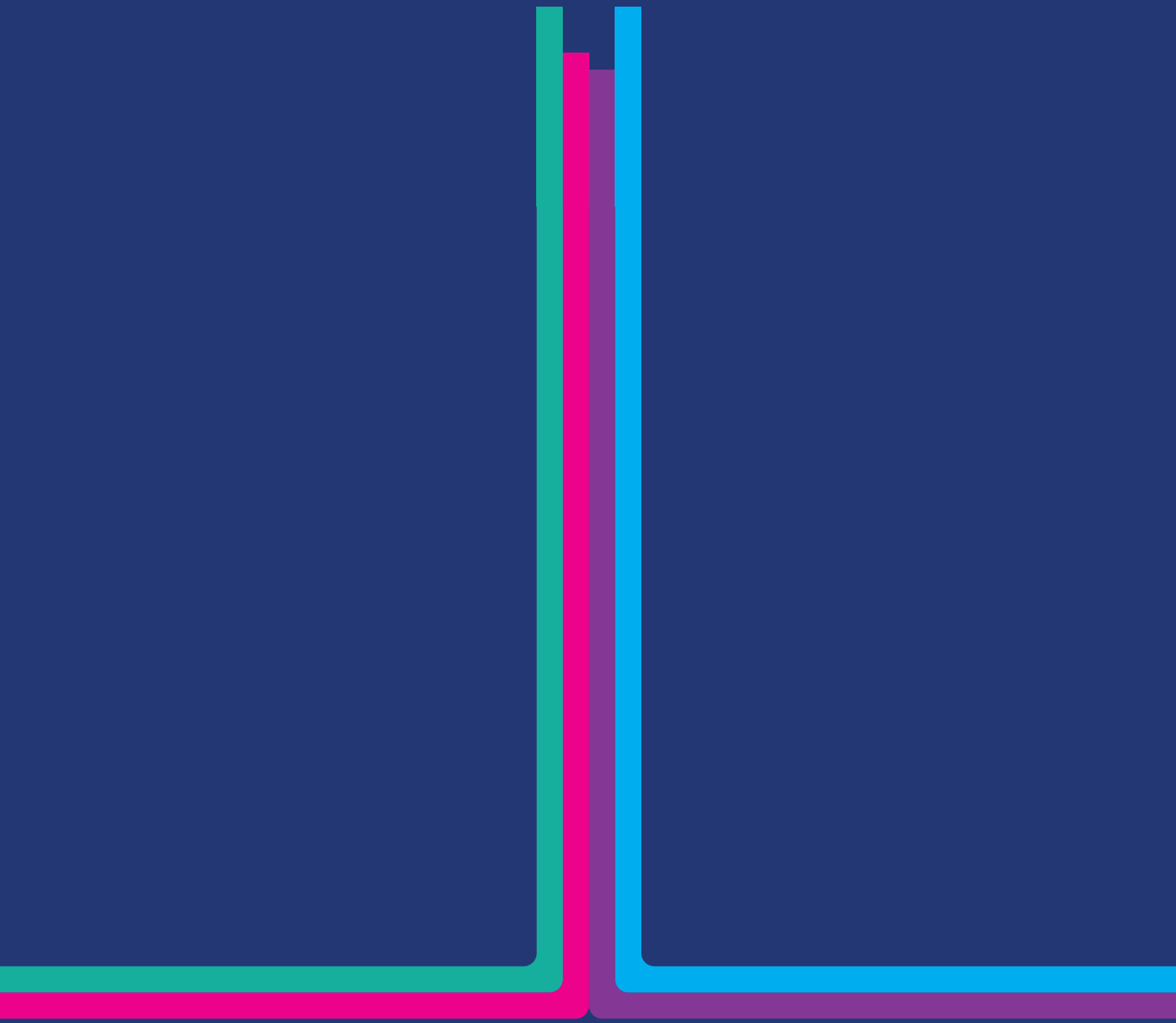

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