Description of the projects in alphabetical order:

Title: Resolving cell fate decisions in neural crest lineage during development, regeneration and cancer

Igor Adameyko

Project Description: Cells compute by processing external stimuli according to their internal state, abiding to rules that remain poorly understood (Rubens et al., 2016). Cell fate decisions are the key for constructing a multicellular organism. Errors, bias or aberrant delays in making such decisions can lead to neoplasia or in extreme cases, tumorigenesis. Our key question is how cells make decisions in a context of a multicellular organism. Here we propose an interdisciplinary study to uncover the detailed molecular logic driving cell fate decisions in a context of multipotent neural crest lineage. To achieve this, we will combine large-scale single-cell transcriptional measurements (Fan et al., 2016) with color-multiplexed lineage tracing (“Confetti”) performed in collaboration with Peter Kharchenko lab at Harvard Medical School. Applying advanced newly-proposed methods, we will analyze developmental trajectories and fate splits in the transcriptional space, lineage-specific modules and fate-biasing genes, and in addition will perform inference of early transcriptional regulatory events and signaling pathways associated with cell fate decisions in normal development and pathology. We expect the proposed computational and experimental pipelines will be widely applicable to other developmental and regenerative biology problems. We aim to predict and validate fate decisions in the entire neural crest lineage in mouse and human embryos, and to uncover the critical events leading from neural crest to different malignancies and congenital abnormalities. More precisely, we will focus on cancers arising from the neural crest-derived sympathoadrenal cells including pheochromocytoma (PCC), paraganglioma (PGL) and neuroblastoma (NB). In preliminary data we observed that these tumors are highly heterogeneous and resemble some stages of neural crest differentiation into chromaffin or glomus cells or sympathetic neuroblasts. Identifying the origins of different malignancy subtypes will help to reveal the causes of disease heterogeneity and to predict clinical behavior. Creating “comparative identity maps” using single cell analyses of tumors and healthy progenitor populations will allow us to identify the differentiation statuses for different subtypes of PCC, PGL and NB. This, in turn, we allow us to design new drugs that will drive the tumor in a more differentiated and less malignant state.

The Project entails the combination of developmental biology, comparative embryology, cancer research and advanced bioinformatics. Strong candidates are expected to have a good knowledge of developmental neurobiology or be strong in computer sciences or both.

More information on the research unit:
http://cbr.meduniwien.ac.at/organisation/dept-molecular-neurosciences/home

More information on the PI:
www.adameykolab.eu
Inflammation in X-linked Adrenoleukodystrophy

Johannes Berger

Project Description: X-linked adrenoleukodystrophy (X-ALD) is a rare, inherited disorder with a broad clinical variability. Whereas essentially all male X-ALD patients develop a slowly progressive dying-back axonopathy affecting both ascending and descending spinal cord tracts in young adults, in about 60%, a devastating, rapidly progressive form of cerebral inflammatory demyelination occur either in childhood or adulthood. Only if the cerebral inflammation is diagnosed and treated at an early stage, the exchange of the immune cells by hematopoietic stem cell transplantation can stop the inflammation and rescue the patients. We could demonstrate that, among the different immune cell types, predominantly the macrophages are metabolically affected in X-ALD. Moreover, we found, both in vitro and in vivo, that the intrinsic defect of X-ALD macrophages impairs their ability to convert from a pro-inflammatory to an anti-inflammatory status. A direct comparison between pro-inflammatory lesions of post-mortem brain tissue from patients with multiple sclerosis or cerebral X-ALD clearly revealed a lack of anti-inflammatory macrophages in X-ALD. This inability to establish an anti-inflammatory milieu would explain why spreading of the inflammatory, demyelinating lesion in X-ALD cannot halt spontaneously and why it is refractory to anti-inflammatory treatments. These findings may also explain the success of hematopoietic stem cell transplantation in X-ALD, whereby — among other cell types — also the patient’s macrophages are exchanged by those derived from the donor stem cells. However, often the inflammatory demyelination in X-ALD patients is diagnosed at an advanced stage, too late for hematopoietic stem cell transplantation to be beneficial, leaving many patients without any curative treatment. Our research results identified macrophages as a main therapeutic target for stopping the devastating, inflammatory demyelination in X-ALD. Moreover, we have identified compounds that are able to induce a homologous gene, which is intact in X-ALD, to an extent that may be sufficient for rescue of the inherited defect in X-ALD macrophages. The application of such compounds to cerebral X-ALD patients potentially represent a novel pharmacological therapeutic approach to halt the inflammation in X-ALD. By joining the X-ALD research team at the Berger Lab, you will become one of the driving forces moving forward international attempts to develop novel therapeutic strategies to treat the inflammation in X-ALD patients. Your research experiments will reach from cell biology/immunology to neuropathology/neurimmunology applying a broad range of modern techniques. On the clinical side, this project has strong cooperations with leading experts on X-ALD, Florian Eichler (Harvard Medical Schol) and Wolfgang Köhler (Leipzig, Germany).

More information on the research unit:
http://cbr.meduniwien.ac.at/organisation/dept-pathobiol-nerv-system/home

More information on the PI:
hhttps://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/johannes-berger/
Presynaptic Cl⁻ Channels

Stefan Böhm

**Project Description:** Ca²⁺-activated Cl⁻ channels (CaCCs) are involved in the fine-tuning of the vascular tone and thus constitute targets for novel antihypertensive drugs (Pedemonte and Galietta 2014). In a previous PhD project focusing on the signalling of muscarinic receptors in sympathetic neurons, blockers of CaCCs were found to enhance electrically evoked noradrenaline release (Salzer et al 2014). This suggests that postganglionic sympathetic neurons are equipped with CaCCs that may impinge on the cardiovascular system. In preliminary investigations aimed at characterising these channels, CaCC blockers with presumed preferential activities at two different CaCC proteins, TMEM16A (anoctamin-1) and bestrophin-1, were found to increase electrically evoked release patterns characteristic of TMEM16A. Experiments of the proposed PhD project will test the effects of subtype preferring CaCC blockers such as niflumic acid, DIDS, CaCC\textsubscript{inh}-A01 and T16A\textsubscript{inh}-A01 (Pedemonte and Galietta 2014) on transmitter release from sympathetic neurons triggered by stimulation paradigms that either rely on or bypass action potential propagation to reveal whether CaCCs operate at presynaptic sites. Immunocytochemistry will be employed to confirm expression and subcellular localization of TMEM16A and/or bestrophin-1 in these neurons. Pharmacological results will be supplemented by shRNA-mediated knockdown of either of the two proteins. The Ca²⁺- and voltage-dependence of currents through CaCCs will be determined in voltage clamp experiments using Ca²⁺ flurometry, Ca²⁺ chelators, and photolysis of caged Ca²⁺ in the absence and presence of CaCC blockers. In current clamp experiments, effects of CaCC blockers on membrane potential and action potential propagation will be analysed. The results will decipher the functions of CaCCs in postganglionic sympathetic neurons to provide a better understanding of their role in blood pressure regulation.

**More information on the research unit:**
https://www.meduniwien.ac.at/hp/1/zpp/departments/neurophysiology-and-neuropharmacology/

**More information on the PI:**
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/stefan-boehm/
Autoimmune Diseases of the CNS

Monika Bradl

Project Description: Neuromyelitis optica (NMO) is a severe autoimmune disease of the CNS characterized by the presence of pathogenic aquaporin 4 (AQP4)-specific antibodies, which bind to AQP4 on astrocytes and initiate the formation of large astrocyte-destructive lesions in spinal cords and optic nerves (Zeka et al., 2015). Peripheral organs also express AQP4, but are normally protected from AQP4-directed autoimmune responses. Yet, a second hallmark of NMO is co-existing autoimmunity, which may culminate in antibody-mediated destruction of additional organs and tissues. The causes for this condition are essentially unclear. We have recently identified AQP4 expressing muscles as additional targets in NMO (Pohl et al., 2011), and demonstrated that extraocular muscles are particularly prone to inflammation in experimental NMO. AQP4 expressing muscles also express nicotinic acetylcholine receptors, the antibody-targets of myasthenia gravis (MG), a severe autoimmune disease of the neuromuscular junction with involvement of extraocular muscles, which may co-exist with NMO. We propose to use our animal models of experimental NMO and MG to identify and characterize the mechanisms leading to a loss of tissue protection from antibody-mediated damage, and to decipher the key players involved in recruitment, activation and expansion of novel autoantigen-specific T and B cells over time, using an array of immunohistochemical, immunological, and molecular tools. We also suggest intravital two-photon imaging of antigen-specific T cells carrying a genetically encoded calcium sensor to visualize activation events in situ (cooperation with N. Kawakami in Munich). Our work may identify therapeutic targets to prevent the development of co-existing autoimmunity, which would significantly improve the quality of life of many NMO patients.

More information on the research unit:
http://cbr.meduniwien.ac.at/organisation/dept-neuroimmunology/staff-scientists/monika-bradl/home

More information on the PI:
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/monika-bradl/
Glial cells in spinal nociception and pain
Ruth Drdla-Schutting

**Project Description:** The amplification of nociceptive information at synapses in the spinal cord dorsal horn contributes to the generation of chronic pain, representing a major clinical problem worldwide. A growing body of evidence now suggests that astrocytes powerfully control the processing of nociceptive information at the spinal level. In the proposed project we aim to test whether the activation of astrocytes is a common denominator for various forms of chronic pain. If true, selective activation of astrocytes should be sufficient for the amplification of nociceptive information at spinal synapses. We will combine new powerful chemogenetic tools (DREADDs, Designer Receptors Exclusively Activated by Designer Drugs, in cooperation with C. Agulhon in Paris) with state-of-the art electrophysiological *in vivo* and *in vitro* techniques (Drdla et al., 2009; Drdla-Schutting et al., 2012) to assess the impact of selective activation of spinal astrocytes on basic transmission and plasticity at spinal synapses. Cell-specific depletion models and pharmacological blockade of mediators involved in intercellular communication will help to identify key players in the cross-talks between astrocytes and microglia in the pathogenesis of chronic pain. Furthermore, we aim to substantiate the effect of DREADD-mediated astrocyte activation on nociception using behavioural tests. We expect to gain new information about major open questions in the field regarding astrocyte-to-neuron/microglia communication in the spinal cord dorsal horn as well as the role of astrocytes in pain amplification, which will provide new background for the development of new analgesic drugs.

**More information on the research unit:**
http://cbr.meduniwien.ac.at/organisation/dept-neurophysiology/home

**More information on the PI:**
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/ruth-drdla-schutting/
GABA<sub>A</sub> receptors in homeostasis

Margot Ernst

**Project Description:** This project aims to study GABA<sub>A</sub> receptor isoforms that occur in the hypothalamus and may act as a mechanistic basis linking stress and addiction risks. GABA<sub>A</sub> receptors are pentameric anion channels assembled of 5 subunits, drawn from a pool of 19 in mammalian species. The major receptor isoforms for benzodiazepine sensitivity of most CNS neurons are well studied, yet very little is known about more minor isoforms that contain rare subunits and are spatially restricted. Among these, the gene product of the GABRG3 (γ3) gene was found in a specific cell population of hypothalamic GABA neuron subtypes by single-cell RNA-sequencing (Romanov et al. 2017). GABRG3 has been associated with alcohol dependence (Dick et al. 2004). However, the molecular composition of receptor assemblies containing this subunit remains unknown. Predictive RNA-seq shows that GABRB1 (β1), implicated in eating and affective disorders, is chiefly co-expressed with GABRG3 in hypothalamic neurons. These observations infer highly specific receptor isoforms involved in the regulation of food or drug craving, which are impacted by stress. Here, the hypothalamic GABA<sub>A</sub> receptor subtypes that contain these subunits will be probed with immunoprecipitation combined with LC-MS/MS, radioligand binding assays and Western blot analysis. Once their subunit composition is elucidated, their pharmacology will be determined by recombinant subunit co-expression in frog oocytes. Alterations of their expression patterns, synaptic sites and subcellular trafficking will be studied in rodents utilizing acute and chronic stress models. The long-term impact is a detailed understanding of GABAergic plasticity in the control of hypothalamic homeostasis.

**More information on the research unit:**
[http://cbr.meduniwien.ac.at/organisation/dept-molecular-neurosciences/staff-scientists/margot-ernst/home](http://cbr.meduniwien.ac.at/organisation/dept-molecular-neurosciences/staff-scientists/margot-ernst/home)

**More information on the PI:**
[https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/margot-ernst/](https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/margot-ernst/)
Receptor/Transporter Trafficking

Michael Freissmuth

**Project Description:** Our earlier work showed that the exocyst was required for the surface expression of the GABA transporter-1 (GAT1/SLC6A1) into the plasma membrane; this required the presence of the last three amino acids of GAT1, which conform to a type II-PDZ-binding motif. The dopamine transporter (DAT/SLC6A3) also belongs to the solute carrier 6 family. Axonal targeting and surface expression of DAT is contingent on an intact C-terminal type II PDZ-binding motif (Rickhag et al., 2013). The working hypothesis of this project posits that (i) DAT requires the exocyst to reach the cell surface and that (ii) the first component(s) is/are already recruited at the level of the ER. The aim of the project is to understand how and when DAT recruits which exocyst component. The ultimate goal is to understand how DAT traffics through the secretory pathway to reach the presynaptic specialization and why some misfolded mutants fail to do so and thus give rise to infantile dystonia/Parkinson's disease. The experimental approach relies on measuring protein interactions in vitro (GST pull-downs), ex vivo (immunoprecipitation) and in living cells (FRET microscopy), on time lapse microscopy of tagged versions of DAT and on cross-linking studies with DAT versions, where artificial amino acids (e.g. benzoyl-phenylalanine) are incorporated into the protein using amber suppressor codons and modified tRNAs. At a later stage, the phenotypic consequences of interfering with exocyst recruitment to DAT will be studied in Drosophila melanogaster (Kasture et al., 2016) and in dopaminergic neurons derived from iPS cells of patients (with Manju Kurian).

**More information on the research unit:**
https://www.meduniwien.ac.at/hp/zpp/institute-abteilungen/zentrum/

**More information on the PI:**
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/michael-freissmuth/
Cellular diversity in the developing hypothalamus

Tibor Harkany

Project Description: The mammalian hypothalamus exhibits the undoubtedly largest heterogeneity of neurons in the brain. The diversity of hypothalamic neurons is tailored to their multimodal functions, interfacing the periphery and the nervous system. Therefore, some hundred-to-thousand neurons (Romanov et al., 2017) and their interplay with neighboring glia can drive fundamental neuroendocrine output along the life-span of an individual. Nevertheless, the molecular mechanisms that shape neuronal identity, determine and synchronize hormonal (endocrine motor) and synaptic connectivity and output during fetal and postnatal development remain largely unknown. This graduate program will rely on single-cell RNA-seq (also correlated with electrophysiology; “Patch-seq”; Fuzik et al., 2016), mouse genetics, developmental biology and imaging in optically-cleared intact tissues in mouse and human (Romanov et al., 2017) to describe organizational principles of the developing hypothalamus including: i) the temporal dynamics of neuronal diversification encompassing the spatial segregation of progenitors and their migratory routes, ii) the emergence of neuropeptide and hormonal heterogeneity and redundancy, their transitions and final lay-out across neuronal cohorts, and their role in neuronal function determination, and iii) guidance mechanisms of axonal patterning to specify neuroendocrine vs. synaptic sites along axon collaterals of identified neurons. iv) the selective ablation of neuronal subtypes that exhibit gender bias (e.g. galanin/AVP neuroendocrine cells) will be causally linked to one of the predicted behavioral outputs: sexual preference, offspring nursing, bodily metabolism or endocrine illnesses. Thus, a unifying concept on the molecular and cellular enrichment of the developing hypothalamus will emerge and serve as a blueprint for functional analysis in conjunction with projects by Margot Ernst and Daniela Pollak. International collaborators are Tomas Hökfelt (Karolinska Institutet) and Tamas L. Horvath (Yale) on the organization and function of the hypothalamic circuitry.

More information on the research unit:
http://cbr.meduniwien.ac.at/organisation/dept-molecular-neurosciences/home

More information on the PI:
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/tibor-harkany/
Molecular Mechanisms of Neuromuscular Development and Diseases

Ruth Herbst

Project Description: The receptor tyrosine kinase MuSK is the central regulator of neuromuscular junction (NMJ) formation and maintenance. Mutations in MuSK cause congenital myasthenic syndromes and autoantibodies against MuSK instigate myasthenia gravis, both of which are severe neuromuscular disorders hallmarked by fatigable skeletal muscle weakness (Burden et al., 2013). The underlying pathomechanisms are poorly understood. In order to unravel the causal molecular mechanisms we intend to develop in vitro NMJs starting from induced pluripotent stem (iPS) cell-derived motor neurons and skeletal muscle myoblasts (Demestre et al., 2015). We will establish co-cultures of iPS cells differentiated into motor neurons and myotubes derived from skeletal myoblasts to build NMJs in vitro. The validity of formed NMJs will be functionally analyzed by gene expression profiling, morphological analysis and electro-physiological recordings (in collaboration with Prof. Hashemolhosseini). We will use this in vitro NMJ model system to dissect the role of MuSK mutations on NMJ development and function by integrating muscle cells carrying mutant MuSK. Subsequent functional and molecular analysis of signaling events will be performed. Further, we will use in vitro NMJ models to analyze the role of MuSK autoantibodies in so far unsolved pathological alterations such as reduced quantal content and impaired presynaptic active zones. In vitro NMJs will be exposed to MuSK autoantibodies and presynaptic differentiation will be studied by quantifying active zone areas, studying the localization of marker proteins followed by morphometric imaging and quantitative 3D analysis. We expect that using an in vitro NMJ model system will allow the molecular characterization of pathomechanisms of neuromuscular disorders for better treatment of patients and the development of future therapeutic strategies.

More information on the research unit:
http://immunologie.meduniwien.ac.at/science-research/research-groups/ruth-herbst/?L=1

More information on the PI:
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/ruth-herbst/
Antibody-associated autoimmunity in the CAN and PNS

Romana Höftberger

**Project Description:** Anti-neuronal autoimmune encephalitis (AIE) is an inflammatory disease of the central nervous system that mainly affects children and young adults and is caused by antibodies targeting neuronal surface antigens. Despite intensive investigation, there are certain AIE subgroups, in which anti-neuronal surface antibodies can only rarely be identified, although there is substantial evidence that they could be antibody-mediated. Of particular clinical importance are syndromes with severe and potentially life-threatening disease courses that show an impressive response to immunotherapy. We have recently shown that anti-neuronal surface antibodies recognize a conformation dependent epitope and their detection relies on the use of specific assays that preserve the immunological target (Höftberger et al. 2015). We propose to identify novel autoantibodies against neuronal and glial surface antigens in sera and CSF of suspected AIE patients, using novel in house tissue based assays on post-fixed rat brain, cell-based assays, primary neuronal and glial cell cultures, immunoprecipitation, and mass spectrometry (cooperation with J. Dalmau in Barcelona). We will assess the neurological symptoms, response to treatment, neuroradiological features, and laboratory biomarkers. In previous studies we could demonstrate that some anti-neuronal surface antibodies may be associated with neuropathological alterations that would not suggest an autoimmune origin at first glance (Gelpi et al. 2016). Here we propose to decipher the functional effects of these antibodies on the targeted antigen in live cell cultures and human biopsy and autopsy specimens derived from affected patients. The identification of novel anti-neuronal surface antibodies will allow the development of useful diagnostic kits designed to the commercial exploration and the creation of new patents, and the functional analysis will improve our understanding about the mechanism of humoral immunity that operate in patients with AIE.

More information on the research unit: [https://www.meduniwien.ac.at/hp/kin/allgemeine-informationen/mitarbeiterinnen/assocprof-dr-romana-hoeftberger/](https://www.meduniwien.ac.at/hp/kin/allgemeine-informationen/mitarbeiterinnen/assocprof-dr-romana-hoeftberger/)

More information on the PI: [https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/romana-hoeftberger/](https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/romana-hoeftberger/)
Molecular and Cellular Machineries in Cortical Networks

Thomas Klausberger

Project Description: The Wisconsin card sorting test has been used for many decades (Grant and Berg, 1948) to examine patients with mental illness including schizophrenia. During this test a subject has to find, apply and change strategies for sorting cards based on the number, color or nature of symbols shown and on a trial and error basis. The Wisconsin card sorting test requires the subject to alter the response strategy and use previously irrelevant information to solve a new set of problems, which is impaired during pathological conditions with unknown neuronal mechanisms. Recently, we have succeeded to implement a modified version of this task for head-fixed mice navigating a “choice-ball” in a virtual environment. In the proposed PhD project we aim to determine neuronal circuit activity underlying the different aspects of task performance. For this, we will perform electrophysiological recordings from head-fixed mice performing the modified Wisconsin card sorting test. Silicon probes will be used to determine the activity of up to a hundred simultaneously recorded neurons in the prefrontal cortex and to detect gamma oscillations in the network, which are considered to contribute to cognitive operations. Glass electrodes will be used to record and juxtacellularly fill recorded cells to determine how distinct types of neuron contribute to task performance (Lagler et al., 2016). In addition, we will use optogenetic manipulations to up- or downregulate neuronal activity and elucidate associations between firing patterns and behavioral performance. This project will identify neuronal circuit activity in the prefrontal cortex supporting cognitive flexibility and attentional set shifting.

More information on the research unit:
http://cbr.meduniwien.ac.at/organisation/dept-cognitive-neurobiology/home

More information on the PI:
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/thomas-klausberger/
Protein-based biomarker research in neurodegenerative dementias

Gabor G. Kovacs

**Project Description:** Neurodegenerative diseases are characterized by progressive loss of neurons and deposition of physicochemically altered proteins in the brain. Recent investigations together with our observation in a longitudinal community-based study on ageing (Kovacs GG, et al., 2013) revealed that in neurodegenerative dementias a combination of different proteinopathies, with influence on the phenotype and prognosis, are more the rule than exception. We hypothesized that the evaluation of different proteins in body fluids, which we called protein coding of neurodegenerative disease (Kovacs GG et al., 2010), can lead to a better prediction of prognosis and stratification of patients for therapy trials. Therefore, we aim to evaluate modifications of the five most relevant proteins (amyloid-beta, prion protein, tau, alpha-synuclein, and TDP-43) associated with neurodegenerative dementias in the cerebrospinal fluid (CSF) in cases where we have the possibility to examine post mortem brain tissue. In the corresponding brain tissue, we will evaluate the spectrum of modifications and correlate with disease progression. In cooperation with Dr. Lachmann, Leipzig, Germany and Dr. Perret-Liaudet, Lyon, France we will use ELISA to examine proteins in the CSF; immunohistochemistry and Western blot to evaluate modified proteins in the diseased human brain and the novel Real-Time Quaking-Induced Conversion (RT-QuIC) method to detect small amount of disease-associated proteins in the CSF and brain. This project will contribute to the definition of clusters of patients based on the patterns of altered proteins. These patterns (“codes”), which will be evaluated gender-specifically as well, are expected to have higher prognostic predictive value and to be useful for monitoring therapy, and may open new avenues for research on pathogenesis.

More information on the research unit:  
[https://www.meduniwien.ac.at/hp/kin/forschung/neurodegenerationsgruppe/](https://www.meduniwien.ac.at/hp/kin/forschung/neurodegenerationsgruppe/)

More information on the PI:  
[https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/gabor-g-kovacs/](https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/gabor-g-kovacs/)
Neuroimaging with PET/fMRI in psychiatry

Rupert Lanzenberger

**Project Description:** Hybrid systems with combined positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) are promising approaches to link molecular (e.g., concentration or drug-occupancy of proteins) and functional data (e.g., brain activation or connectivity patterns) in humans in vivo. Using these systems, drug effects can be investigated in vivo in patients and healthy controls in longitudinal or challenge studies. We have an established programme to investigate the effects of frequently prescribed antidepressants (SSRI, ketamine) and hallucinogens (Carhat-Harris 2017). E.g., selective serotonin reuptake inhibitors (SSRIs), which are the current gold standard in the therapy of mood and anxiety disorders, raise 5-HT in the synaptic cleft. In vivo molecular neuroimaging of key players of the serotonergic system with PET has unveiled alterations associated with mood disorders. This method has been successful in predicting treatment response in patients suffering from major depressive disorder (MDD) (Lanzenberger et al., 2012). Furthermore, functional brain connectivity measured with fMRI has proven to be useful as a prediction biomarker of treatment response. However, current protocols are mainly based on successive measurements in different PET and MRI scanners with the disadvantages of oral SSRI treatment and time consuming measurement protocols. We have implemented a promising protocol to perform simultaneous molecular and functional pharmaco-neuroimaging using a PET/MR hybrid system combined with an acute SSRI challenge approach (Gryglewski et al., 2017), and EEG. Using this multimodal data it was possible to relate occupancy changes at the serotonin transporter by SSRIs with changes in functional brain connectivity (in a single measurement session of 90 minutes). The next important step will be the combination of these results with different brain protein distributions models of key molecules of the serotonergic system (5-HT1A receptor, 5-HT2A receptor, 5-HT1B receptor, monoamine oxidase A) based on our PET data collection from different studies. High-resolution voxel/vertex-wise maps (http://www.meduniwien.ac.at/neuroimaging/mRNA.html) of the human whole-brain transcriptome (as surrogate for proteonomic distribution data) based on the ALLEN Brain ATLAS (http://www.brain-map.org/) or/and MALDI imaging data will be integrated with the multimodal in vivo neuroimaging data (Gryglewski et al., 2018). Maschine / deep learning, clustering (James et al., 2018) and cutting-edge pattern recognition approaches in neuroimaging (www.prni.org) will be applied. These methods will significantly increase sensitivity and specificity of molecular/functional imaging results as treatment response biomarker & may foster implementation in clinical routine for treatment stratification and precision medicine of psychiatric patients.

More information on the research unit:
http://www.meduniwien.ac.at/neuroimaging/

More information on the PI:
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/rupert-lanzenberger/
Animal Model-Based Depression Research

Daniela Pollak

**Project Description:** Paternal exposure to positive and negative environmental influences has life-long consequences for offspring brain and behaviour (Braun K et al. 2014). We have demonstrated a significant impact of the paternal lineage on the transgenerational transmission of the consequences of maternal immune activation (MIA) on depression-like behaviour of female offspring in the first (F1) and second generation (F2) (Ronovsky et al. 2016). The observed modulation of maternal care in F1 female MIA and control offspring after mating with a F1 male MIA offspring suggests a possible impact of the father’s MIA heritage on the reproductive investment of the mother (Ronovsky et al. 2016). Here we aim to dissect germ-line-dependent from germ-line-independent mechanisms in paternally transmitted MIA history on offspring behaviour and to analyse underlying molecular mechanisms. In-vitro fertilization studies and sperm miRNA profiling will be used to investigate a potential direct impact of the father and its epigenetic correlates. Aggression and mating behaviour will be evaluated in male MIA and correlated to maternal care in order to reveal a possible indirect influence of the father on offspring depression-like behaviour through modulation of the mother-pup-relationship. Optogenetic activation/silencing of specific neuronal populations in selected target brain regions i.e. the ventromedial hypothalamus and the medial amygdala will be employed in order to revert potentially aberrant male aggression and mating behaviour in MIA offspring. The consequences of the manipulation of the male behaviour for maternal care of the dam and male and female offspring emotional behaviour will be evaluated. This study is expected to delineate the mediating role of maternal care in the paternal transmission of transgenerational effects of environmental adversities on offspring emotionality.

**More information on the research unit:**
https://www.meduniwien.ac.at/hp/zpp/institute-abteilungen/neurophysiologie-und-pharmakologie/

**More information on the PI:**
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/daniela-pollak-monje-quiroga/
Neuroinflammation controls Synaptic Plasticity and Pain

Jürgen Sandkühler

Project Description: In the CNS nociceptive neurons comprise a heterogeneous group of neurons that are defined by their input from high threshold sensory nerve fibres. Despite being subject to intense investigation it is presently still not known which subgroups of nociceptive neurons mediate the various aspects of pain. Of particular clinical importance are the aversive components of pain which involve limbic structures. We have recently proposed that neuroinflammation is a common driver for pain amplification, widespread pain and various co-morbidities of pain (Kronschläger et al., 2016). In the proposed project we will optogenetically target rat spinal neurons with a projection to the parabrachial nucleus which in turn has projections to numerous limbic structures. Our previous studies revealed that spino-parabrachial neurons are nociceptive and express a special form of activity-dependent long-term potentiation at synapses from high threshold sensory fibres (Ikeda et al., 2006). Here we propose to selectively activate the spino-parabrachial pathway by light to assess the fundamental properties and potential plasticity of spino-parabrachial synapses both in the presence and in the absence of neuroinflammation. We suggest assessing the contribution of this pathway for aversive components of nociception by using a battery of behavioural tests in rodents during optogenetic stimulation of spino-parabrachial neurons. If time allows we plan to assess the functional impact of clinically used and experimental analgesics on this pathway.

More information on the research unit:
http://cbr.meduniwien.ac.at/organisation/dept-neurophysiology/home

More information on the PI:
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/juergen-sandkuehler/
Interplay of psychostimulants with monoamine transporters

Harald Sitte

**Project Description:** New psychoactive substances (NPS) are psychoactive compounds developed by rogue chemists to escape legislation (Sitte and Freissmuth, 2015). They come in different structures and target the clinically important monoamine neurotransmitter transporters such as the transporters for dopamine and serotonin, and, eventually, the cognate neurotransmitter receptors. NPS flood the illicit drug market and can be roughly divided in compounds that are transported substrates or non-transported inhibitors. The current project aims at understanding (i) how the transporters handle the respective NPS compound and (ii) how the transporter-embedding plasma membrane impacts on the translocation process. The PhD candidate will therefore characterize the novel compound pharmacologically in a number of standard assays to elucidate its properties, i.e. in biochemical uptake inhibition assays, superfusion assays, in electrophysiological approaches using the patch-clamp method in the whole-cell configuration. These approaches allow to clearly distinguish the substrates from inhibitors (Sandtner et al 2016). Next, the PhD candidate will examine the conformational cycling of the transporter by employing fluorescence resonance energy transfer (FRET) microscopy and determine how the compound moves or locks the transporter in certain conformations. This will also be the starting point to assess the impact of the plasma membrane on conformational cycling of the transporter: FRET microscopy will be used in a variety of different settings, either in living cells with exchanged plasma membrane constituents (e.g., phosphoinositides, cholesterol) or in reconstituted systems with well-defined membrane composition (Buchmayer et al 2013). The results will improve our understanding of the regulation of transport movement by the plasma membrane, will explore formerly unexplored compounds that flood the street market and finally help us to better understand the process of how neurotransmitter transporters work.

More information on the research unit:
https://www.meduniwien.ac.at/hp/zpp/institute-abteilungen/zentrum/

More information on the PI:
https://www.meduniwien.ac.at/hp/phd-neuroscience/research-laboratories/harald-sitte/