Age-related decline in functional connectivity of the human vestibular cortical network

Boegle R[2,3], Cyran CAM[1], Stephan T[1,2,3,6], Dieterich M[1,3,4], Glasauer S[3,5,6]
[1]LMU, Department of Neurology
[2]Graduate School of Systemic Neurosciences (GSN[LMU])
[3]Integrated Research and Treatment Center (IFB[LMU])
[4]Excellence Cluster Systems Neurology (SyNergy)
[5]LMU, Center for Sensorimotor Research
[6]LMU, Institute for Clinical Neurosciences

Question: In the elderly, major complaints concern dizziness and increasing number of falls [1], which may be related to altered central processing of vestibular sensory input [2]. Therefore, we studied the effects of healthy aging on central vestibular processing to aid in examining diseases of central vestibular processing across the ages.

Study design and hypothesis: Most fMRI studies examining the effects of healthy aging on brain activity focused either on the traditional analysis of the BOLD amplitude of task-related responses (e.g. [3]), or resting-state activity (e.g. [4]), involving no task and only minimal sensory input.
However, these designs have drawbacks which we like to avoid. For example task performance is often confounded with age and it can be difficult to disentangle task-related and age-related effects. Furthermore, resting-state paradigms might not reveal aging effects that are linked to the processing of sensory information and may thus only become evident with stimulation. Therefore, we chose a task-free paradigm using galvanic vestibular stimulation (GVS) during functional imaging (e.g., [5]) to activate brain networks specifically devoted to the processing of vestibular input. We hypothesized that healthy aging may affect brain function in multiple ways to maintain overall optimal function while brain areas change during aging. We therefore assessed signatures of brain function beyond BOLD-signal amplitude, such as functional connectivity or temporal BOLD-signal variability [6-8] and controlled for changes in brain volume and structural connectivity.

Methods: Functional and structural MRI were performed at a 3T scanner (GE Signa Hdx) in 39 healthy volunteers (right handed, age 20-70 years, 22 female). Data was preprocessed and analysed regarding the following characteristics: significant age-dependent changes in BOLD-signal amplitude, in voxel-based morphometry, in functional connectivity assessed by tensor independent component analysis, in structural connectivity expressed as fractional anisotropy, and in temporal BOLD-signal variability using partial-least-squares analysis.

Results: Age-correlated decreases of functional connectivity and increases of temporal BOLD-signal variability were associated with multisensory vestibular networks. In contrast, no age-related functional connectivity changes were detected in somatosensory networks or during a motor paradigm used as control. The functional connectivity decrease was not due to structural changes but to a decrease in response amplitude undetected by SPM analysis.

Conclusion: Our data suggest the possibility that both the age-dependent functional connectivity decrease and the variability increase are connected to deteriorating reciprocal cortico-cortical inhibition with age, related to vestibular multimodal integration of sensory inputs.

Acknowledgements: The authors are grateful for support by Bundesministerium für Bildung und Forschung (Grant Number BMBF - FKZ 01 E0 0901 and - 01GQ0440 (BCCN)) and Deutsche Stiftung Neurologie.

References:

[1] Barin K, Dodson EE; Otolaryngol Clin North Am 2011.
[2] Jahn K et al.; Brain 2003.
[3] Aizenstein et al.; J Cogn Neurosci 2004.
[4] Meier et al., Neuroimage 2012.
[5] Dieterich M, Brandt T; Brain 2008.
[6] Biswal BB et al.; Proc Natl Acad Sci USA 2010.
[7] Beckmann CF, Smith SM; Neuroimage 2005.
[8] Garrett DD et al.; J Neurosci 2010.

Reversibly light-controlled antimitotics: towards intelligently targeted chemotherapy

Borowiak M[1], Reynders M[1], Nahaboo W[2], Jalinot P[2], Hasserodt J[3], Zahler S[4], Vollmar A[4], Delattre M[2], Trauner D[1*], Thorn-Seshold O[1]
[1]LMU, Department of Chemistry
[2]Laboratoire de Biologie Moléculaire de la Cellule, Ecole Normale Supérieure de Lyon
[3]Laboratoire de Chimie, Ecole Normale Supérieure de Lyon
[4] LMU, Department of Pharmacy

Current chemotherapeutic drug design is in general highly unsatisfying, mainly due to a failure in controlling the specificity that results in severe side effects on the systemic level.
Here we introduce a new approach to chemotherapy, using light to allow precise spatial and temporal control of drug cytotoxicity. We synthesized and tested the biological activity of a series of azobenzene-based tubulin binding agents. We showed a light-controlled inhibition of tubulin polymerization *in vitro*, as well as *in cellulo* by immunofluorescence confocal microscopy. Moreover we demonstrated that light-dependent drug activation results in cell cycle arrest in the G_2/M phase with subsequent induction of apoptosis and cell death. *In vivo* live cell imaging in *C. elegans* embryos, showed a possibility of mitotic arrest induction involving precisely selected cell, not disturbing the division of the neighbouring ones. Those cytotoxic effects were observed only upon *trans->cis* isomerisation of the azobenzene following illumination with 400 nm light; while the more stable *trans* isomer was non-toxic at concentrations up to 50 times higher than the EC_{50} for the *cis* isomer.
This promises the possibility of side effect-free systemic administration of drugs, with spatio-temporally precise activation of cytotoxicity by local illumination of the zone of interest. Additionally, these azobenzenes offer the possibility of *cis*→*trans* isomerisation upon illumination with light of longer wavelength (~520 nm), which can be used to protect surrounding cells or healthy tissues from any toxic *cis* form diffusing away from the desired zone.
We hope that compounds presented herein can one day be used in clinics as novel selectively targeted chemotherapeutics, which toxicity may be exclusively restrained to a tumoural zone without systemic side effects. They may also be used as tools with applications in developmental and cellular biology, to answer numerous questions concerning the role of microtubule assembly and dynamics in intracellular transport, cell division and motility. Above all, we also hope that this research will inspire a new molecular approach to conceiving and proving photopharmaceuticals as “smart drugs” for a variety of important biomedical applications.

Modularity and predictability in naturalistic behavior

Sandeep Robert Datta, M.D., Ph.D.
Assistant Professor | Department of Neurobiology | Harvard Medical School

Specialized neural circuits devoted to motor planning and movement execution create complex patterns of motion that underlie all overt behaviors, ranging from the primal (e.g. hunting for prey) to the sublime (e.g. completing a pirouette). Although animal behavior appears nearly infinite in variety and form, neuroethologists have long hypothesized that the brain flexibly organizes a finite set of behavioral primitives — relatively invariant motifs of three-dimensional movement — into sequences to create behaviors that enable animals to interact with the world. However, to date no framework has been developed that is capable of segmenting the complex patterns of naturalistic behavior exhibited by vertebrates into likely behavioral primitives without the imposition of significant human supervision or bias.

To address this key issue, we have developed an analytical system that first captures continuous three-dimensional posture data from freely behaving mice, and then identifies repeatedly-used patterns of motion (that likely correspond to behavioral primitives) through the use of recently-developed methods in computational inference. By analogy to natural language we refer to each behavioral primitive identified by our methods as a behavioral “syllable.” Inspection of the 3D video reveals that each behavioral syllable corresponds to a brief motif of 3D motion — a head bob, a turn to the right, a curl of the body to the left and so on. Complex observed behaviors — like locomotion — can be described as specific sequences of these syllables; by quantitating how often any given syllable follows any other given syllable in sequence, we can characterize the “grammar” used by the brain to build complex behaviors out of component syllables. We find that this grammar is probabilistic but not random: the brain imposes context-specific rules on the sequencing of behavioral syllables that imparts order and regularity to patterns of action. To reveal behavioral vulnerabilities to alterations in internal or external state, we have used our methods to characterize the structure of behavior in a variety of experimental contexts. These experiments demonstrate that behavioral grammar is adaptively modified in response to defined sensory cues, enabling mice to generate new patterns of behavior by re-writing the statistics of interconnection between various behavioral syllables. Behavioral syllables and their associated grammar therefore are constituents of a stereotyped body language that we can now — for the first time — decipher and use to objectively organize and quantitate information about mouse behavior. We are currently leveraging our access to this rich description of mouse behavior to directly implicate individual genes and neural circuits within the olfactory system in the regulation of ethologically-relevant behaviors; we are also using neural recording and manipulation techniques to ask more general questions about how brain circuitry encodes the statistics that govern patterned behavior.

TYPE II CADHERINS GUIDE ASSEMBLY OF A DIRECTION-SELECTIVE RETINAL CIRCUIT

Xin Duan, Arjun Krishnaswamy, Irina De la Huerta, and Joshua R. Sanes
Ctr for Brain Sci., Dept. of Mol. & Cell. Bio, Harvard Univ., Cambridge, MA, 02138

Elucidating the mechanisms that instruct circuit assembly is a main item in the agenda of developmental neurobiology. An increasingly feasible program for accomplishing this goal involves (a) categorizing the cell types that comprise the neuronal ensemble; (b) gaining genetic access to them, so they can be marked and manipulated; (c) mapping their connectivity; (d) identifying candidate mediators of specific connectivity among them; and (e) assessing their roles. We are using the well-organized and accessible mouse retina to implement this plan.

One of the best-studied retinal circuits computes object trajectory from object position. Starburst amacrine cells (SACs) provide directional inhibition that tunes ON-OFF direction-selective retinal ganglion cells (ooDSGCs); the ooDSGCs send signals to the brain. It was not known, however, which of ~12 bipolar cell (BC) subtypes provide visual information to the SACs and ooDSGCs. We therefore began by identifying molecular markers for Type 2 OFF BCs (BC2s) and Type 5 ON BCs (BC5s), which laminate with ooDSGCs and SACs, and generated "cre-knock-in" lines for each. Cre-dependent channel-rhodopsin (ChR2) was delivered virally in either of the two BC types for optogenetic stimuli; we recorded from ooDSGCs and SACs in lines that marked them with YFP. We demonstrated monosynaptic excitatory connections from BC2s and BC5s to ooDSGCs and SACs.

We then inventoried recognition molecules and found that the Type II cadherins, *cdh8* and *cdh9*, are expressed by BC2s and BC5s, selectively. Using BC axonal lamination as a readout, alterations of laminar patterns between ON and OFF sublaminae were observed in (a) loss-of-function experiments analyzing BC2s and BC5s in *cdh8* and *cdh9* null mice; and (b) gain-of-function experiments ectopically overexpressing *cdh9* in BC2s and *cdh8* in BC5s. Additionally synaptic connectivity from BC2s and BC5s to ooDSGCs decreased in *cdh8* and *cdh9* mutants respectively, while overexpression of *cdh9* in BC2s led to mis-wiring between OFF BCs to the ON SACs. . Furthermore, we also examined visually-evoked responses from the ooDSGCs: ON responses were eliminated in the cdh9 mutants, while OFF responses were compromised in the cdh8 mutants. Together, these structural and functional findings suggest that cdh8 and cdh9 play both instructive and permissive roles during the retinal circuit assembly.

Reference

Duan, X.*, Krishnaswamy, A.*, De la Huerta, I., and Sanes, J.R. (2014) Type II cadherins guide assembly of a direction-selective retinal circuit. **Cell** (*in press*).

Fundings

NIH (NS029169 & EY022073) to J.R.S.; NSERC (Canada) & Banting Fellowships to A.K; HHMI-LSRF Award to X.D.

Mouse models for human brain evolution

Enard W
LMU, Department Biology II, Anthropology and Human Genetics

Identifying the genetic changes responsible for the phenotypic differences between humans and their close primate relatives is important from an evolutionary, medical and cultural perspective. The primary challenge facing researchers today, after analyzing the genomic data, is experimentally testing hypotheses concerning the genetic basis for human-specific traits. Similar to studying mutations in a biomedical context, mouse models are indispensable for such an endeavour, especially if the traits of interest are embedded in a complex developmental context as is the case for brain functions. I will present recent progress made on a mouse modelling human amino acid changes in the speech and language associated transcription factor FOXP2 and I will also touch upon mouse models for human-specific properties of ASPM, a gene associated with primary microcephaly.

The Evolution Machine: Engineering Optogenetic Biosensors

Fabritius A, Ng D, Litzbauer J, Griesbeck O
Max Planck Institute (MPI) of Neurobiology, Cellular Dynamics

Genetically encoded fluorescent biosensors have become an important tool to answer questions not only in Neurobiology but in Cell biology as a whole. Each of these questions requires biosensors tailored to the specific needs of these applications. To date the engineering of these molecular probes is a long and cumbersome process. Owing to the fact that the relation between the sequence of a given protein on the one side and its structure and function on the other is still poorly understood, the capabilities of the rational design approach to protein engineering is very limited. Directed evolution - the generation and successive selection of randomized libraries - is a good method to circumvent potential biases of theoretical models, but poses its own challenges. Here we show a streamlined process for the generation of fluorescent biosensors, from the early prototyping to high throughput screening, with a focus on reduction of manual labor, costs and time requirements. This allows for faster development of novel and advanced tools for the molecular toolbox available to neurobiologists and cell biologists alike.

Pre- and postsynaptic refinements in the medial superior olive (MSO) during late postnatal development

Franzen D[1,2]*, Berner S[1,2]*, Blank S[1], Kellner C[1,2], Felmy F[1,2,3]
[1]LMU, Department Biology II
[2]Graduate School of Systemic Neurosciences (GSN[LMU])
[3]LMU, BioImaging Center
*These authors contributed equally to this work

Binaural coincidence detector neurons of the MSO process sound localization cues with microsecond precision by integrating excitatory and inhibitory inputs. The inhibitory input to these neurons has been shown to refine around hearing onset in an activity-dependent manner, and is accompanied by severe developmental changes in the cell's biophysical properties. To further understand the refinement mechanism, we investigated the developmental regulation of calcium entry in MSO neurons. Calcium is an important second messenger, potentially associated with developmental alterations. Whole-cell recordings were carried out in acute slices from Mongolian gerbils around hearing onset at postnatal day (P) 10-15 and at mature stages (P60) to image calcium transients evoked by synaptic and action potential (AP) stimulations. Even after hearing onset, a strong calcium influx could be evoked by both APs and EPSCs. However, from P13 onwards the AP-evoked calcium influx decreased rapidly to undetectable levels at P60, as a consequence of reduced AP amplitude and a downregulation predominantly of a T-type calcium current. Moreover, the NMDAR component of the EPSC declined drastically from P13 onwards. Additionally, the quantal content of the EPSC increased in agreement with morphological changes indicated by calretinin stained input fibres. Nevertheless, at mature stages a significant dendritic calcium transient was still driven locally by the AMPAR component. Our data suggests that calcium entry shifts from its pre- and postsynaptic activity dependence to predominantly presynaptic activity dependence. However, the presence of AP- and EPSC-evoked calcium signals just after hearing onset suggests the persistence of refinements after hearing onset.

Identification and successful negotiation of a metabolic checkpoint in direct neuronal reprogramming

Gascon S[1,2], Murenu E[1], Ortega F[1#], Masserdotti G[1,2], Berninger B[1,3,4] Götz M[1,2,5]
[1]LMU, Department of Physiological Genomics
[2]Helmholtz Center Munich (HMGU)
[3]Johannes Gutenberg University, Institute of Physiological Chemistry
[4]Johannes Gutenberg University, Focus Program Translational Neuroscience,
[5]Excellence Cluster Systems Neurology (SyNergy)
[#]present address: Johannes Gutenberg University, Institute of Physiological Chemistry

Direct neuronal reprogramming provides a straightforward strategy to generate induced neurons of various identities. However, both the cellular and molecular mechanisms of direct neuronal reprogramming are still ill-understood. Using continuous single cell live imaging we identify here a critical point in fate conversion after which cells either successfully convert to a neuronal phenotype or succumb to cell death. This checkpoint is more efficiently negotiated after treatment with forskolin, resulting in improvements in neuronal reprogramming independent of species, cell type of origin or the transcription factor used. We further identify Bcl-2 as the key mediator of this effect with Bcl-2 mutants deficient in Bax-interaction retaining the capability to sponsor fate conversion, therefore identifying a novel function of Bcl-2 with wide-spread applicability in direct reprogramming. We next observed that Bcl-2 overexpression also highly improves *in vivo* neuronal conversion of glial cells mediated by neurogenic transcription factors in a murine model of brain injury. Genome-wide expression analysis revealed a core signature of metabolic and pro-inflammatory pathways regulated by either forskolin or Bcl-2, indicating that these molecular checkpoints are crucial in cell fate conversion. Thus our work identified critical molecular pathways in direct neuronal reprogramming with a potent widespread effect *in vitro* and *in vivo*.

Interaction of the circadian clock and neurodegenerative processes in *C. elegans*

Geibel M, Koritala BSC, Olmedo M, Merrow M
LMU, Institute of Medical Psychology

The circadian clock is a temporal process that generates circa 24---hour oscillations in protein and metabolite levels as well as in behavior. These endogenous oscillations are a key feature of the process of circadian entrainment, whereby organisms anticipate and synchronize to zeitgeber cycles such as light, temperature or food availability. Components of several pathways involved in proteostasis (protein homeostasis) such as chaperones, the ubiquitin/proteasome degradation machinery and the UPR system are regulated by the circadian clock. Impaired proteostasis results in an increase in misfolded proteins and in aggregate formation as seen in aging organisms and in neurodegenerative diseases. Entrainment To circadian temperature or light cycles might therefore be beneficial for maintenance of proteostasis and be used to control protein aggregate formation. We are testing a possible interaction between circadian clock entrainment and protein aggregate formation in the nematode *C. elegans* in neurodegenerative disease models.

How learning of visual associations affects sensory processing in neocortical networks

Goltstein PM[1,2], Bonhoeffer T[1], Hübener M[1], Pennartz CMA[2]
[1]Max Planck Institute (MPI) of Neurobiology, Department Synapses, Circuits, Plasticity
[2]University of Amsterdam, Department Cognitive and Systems Neuroscience

The response properties of neurons in the primary visual cortex change following perceptual learning and the acquisition of stimulus-reward associations. This form of plasticity has been proposed to improve encoding of stimulus features, serve enhanced detection and discrimination of relevant stimuli and be part of the memory trace that represents the behaviorally acquired association. Here, I will present work in which we investigated how learning of a stimulus-reward association affects sensory representations in the visual cortex of the mouse.

To induce visual stimulus-reward associations, we either trained freely moving animals in an operant conditioning chamber, or employed a head-restrained stimulus presentation paradigm. Mice acquired the association between an oriented moving grating and a food reward over the course of multiple training sessions (40-65 trials/day, 4-7 weeks for freely moving; 40-60 trials/day, 2-3 weeks for head restrained). After mice had mastered the behavioral paradigm, cells in the primary visual cortex were bulk-loaded with the fluorescent calcium indicator OGB1-AM. Activity patterns of populations of neurons in response to moving gratings were recorded with a two-photon microscope under anesthesia.

After successful learning, orientation-tuned cells that preferred the rewarded (or a similar) orientation, responded with larger amplitudes to the trained orientation. While this effect broadened tuning curves having a preferred orientation slightly off from the conditioned stimulus, it led to a sharpening of direction selectivity. Moreover, population coding for the discriminating feature was improved. These experiments leave open, however, whether the memory of the association was mediated by synaptic changes on those very cells' dendrites, or if the observed effects in the primary visual cortex resulted from changes in higher cortical areas. To shed light on this question, we are now studying how learning of visual associations affects synaptic inputs and somatic responses of neurons in higher cortical areas using repeated imaging of neurons expressing genetically encoded calcium indicators.

Evolution of spatial hearing

Grothe B[1,2]
[1]LMU, Department Biology II
[2]Graduate School of Systemic Neurosciences (GSN[LMU])

TBD

Two-pore channel 2 – a regulator of endo-lysosomal trafficking

Hassan S
LMU, Faculty for Chemistry and Pharmacy (CUP)

An intact endo-lysosomal system is elementary for eukaryotes' cellular physiology. It is involved in uptake, trafficking and processing of nutrients or macromolecules from the extracellular space, as well as the recycling and degradation of intracellular structures like signaling molecules or damaged organelles. A key structure in these processes is the lysosome because it is the responsible organelle for the final degradation of macromolecules along the endo-lysosomal route. Defects and mutations of lysosomal proteins can therefore lead to accumulation of undegraded material and are the reason for a group of pathologies in man, the so called lysosomal storage diseases. So far, mainly enzymatic defects has been described as a cause, but recently also lysosomal ion channels are appreciated as the reason for lysosomal malfunction. One of these channels is the Two-pore channel 2 (TPC2), a member of the superfamily of voltage gated cation channels. It is widely expressed in mammalian tissue and in the past we and other groups reported the fundamental functional properties of these channels. The channel's physiological role and involvement in pathophysiologies however, remained elusive so far. Here, I present data about TPC2's involvement in intracellular trafficking of lysosomes and endosomes and also evidence, that a loss of functional TPC2 can lead to diverse effects like cholesterol accumulation in the liver as well as changed neurotransmission in the hippocampus.

Imaging and quantification of chemistry in the brain, from synthesis methods to studies in man.

Jacob Hooker, Assistant Professor of Radiology, Harvard Medical School

Abstract: Chemistry when combined with molecular imaging is extraordinary at answering questions about human brain function. Positron emission tomography (PET) is a translational imaging tool that is enabled by chemical innovation and small molecule radiotracer design. The HookerLab at MGH and Harvard Medical School develops and uses PET radiotracers to study the human brain. This lecture will highlight the breadth of research in the lab and then focus on our progress toward the development of a PET radiotracer for histone deacetylases (HDACs). HDACs, as drug targets, have shown broad potential in treatments against cancer and emerging data support HDAC-targeting in the context of cardiovascular disease and CNS dysfunction.

Subclass-specific capture of maleimide-antibiotics with customized nucleophilic probes

Koch M
Technical University Munich (TUM), Excellence Cluster for Integrated Protein Science Munich (CIPSM)

Nature offers a vast diversity of molecular motifs that exhibit promising pharmacological activities. They constitute up to 60% of all currently applied drugs in medicine and therefore serve as a great source for privileged structures in biomimetic compound screening programs. Hereby, small electrophilic molecules are of special interest as they selectively and covalently bind specific and essential target proteins. Possible sources of these reactive compounds are i.e. microorganisms that over time developed their own potential capacity to protect against their constantly changing environmental influences.
One interesting and well-studied microorganism are *Streptomyces*, the largest genus of Actinobacteria covering over 500 different species. The different strains are known to produce various substances showing promising antibiotic activity against other microorganisms like *Staphylococcus aureus* and multiresistant species. Thereby the maleimide showdomycine which is produced by *S. showdoensis* revealed to play an important role in disarming bacteria by addressing two proteins related to cell wall biosynthesis. Intrigued by this mode of action we aimed to precisely explore maleimide antibiotics by establishing a selectively directed isolation procedure. We were able to synthesize an aromatic nucleophile that showed promising selectivity for the maleimide scaffold in an artificial mixture containing different electrophiles. Transferring this as prove of concept for further secretome studies, we established a fermentation protocol and used an analytical platform based on LC-HRMS to verify the production of showdomycine in the Streptomyces strain. Furthermore we observed that the selectivity of our nucleophile was still preserved in the complex natural secretome. The addition product of showdomycine and the nucleophile had a different polarity compared to the initial compounds and additionally showed characteristic UV-absorption at high aromatic wavelength. Thus, it was possible to further reduce the complexity of the secretome, which is crucial for ongoing fishing experiments.
With this proof-of-principle we aim to perform further secretome studies for diverse *Streptomyces* strains in order to reveal and investigate possible new maleimide related antibiotic substances.

CD33 Targeted Immunotherapy in AML

Krupka C[1,2], Köhnke T[1,2], Lichtenegger FS[1,2], Vick B[3], Konstandin N[1,4], Metzeler K[1], Jeremias I[3], Spiekermann K[1,4], Riethmüller G[5], Hiddemann W[1,4], Subklewe M[1, 2]
[1]LMU, Department of Internal Medicine III
[2]Helmholtz Center Munich (HMGU), Clinical Cooperation Group Immunotherapy
[3]Helmholtz Center Munich (HMGU)
[4]Helmholtz Center Munich (HMGU), Clinical Cooperation Group Leukemia
[5]LMU, Institute for Immunology

Antibody-based immunotherapy represents a promising strategy to specifically target and eliminate chemoresistant leukemic cells in acute myeloid leukemia (AML). We evaluated a CD33/CD3 BiTE (bispecific T-cell engager) antibody (AMG 330) for its suitability as immunotherapy in AML. A prerequisite for successful immunotherapeutic approaches using this molecule is the expression of CD33 on AML blasts including leukemic stem cells (LSCs). Therefore, we quantified CD33 expression on AML blasts and LSCs by flow cytometry (specific fluorescence intensity, SFI) and correlated expression intensity with cytogenetic and molecular disease characteristics. CD33 expression was detected in >99% of patient samples (n=621, SFI ≥ 1.5) although highly variable. A strong correlation between high CD33 expression levels and *NPM1* mutations (*p<0.001*) was found. In contrast, low CD33 expression levels were significantly associated with complex karyotypes and t(8,21) translocations (*p<0.001*). Furthermore, LSCs within the $CD34^{+}/CD38^{-}$ compartment displayed CD33 at higher levels than healthy donor (HD) stem cells (*p=0.047*). As genetic variations in the CD33 gene could also impact CD33 expression levels, we genotyped 4 CD33 single nucleotide polymorphisms (SNPs) in 13 primary AML patient samples. Patients homozygous for the reference allele GG of rs35112940 and CC of rs12459419 showed a trend towards higher CD33 expression compared to the other genotypes. Functional relevance of CD33 expression level was shown by faster lysis kinetics of $CD33^{BRIGHT}$ vs. $CD33^{DIM}$ AML cell lines in an *in-vitro* cytotoxicity assay.
To simulate the natural setting of target and T-cells in AML patients for functional testing of AMG 330, we successfully developed a long-term culture system for AML blasts based on a mouse feeder cells in combination with a AML propagating cytokine cocktail. Thus, we were able to show effective elimination of AML blasts within primary samples by AMG 330-activated and expanded residual $CD3^{+}/CD45RA^{-}/CCR7^{+}$ memory T-lymphocytes. At low effector to target ratios (up to 1:79), the recruited T-cells lysed autologous blasts completely in the majority of samples. Limiting Dilution Transplantation (LDTA) assays were performed to test the potential of AMG 330 to effectively mediate lysis of LSCs. Patient-derived AML cells were lentivirally transduced with luciferase and co-cultured with HD T-cells and either AMG 330 or control BiTE for 7 days. Residual $CD3^{-}$ cells were injected into NSG mice and monitored for AML outgrowth by *in-vivo* imaging and peripheral blood analysis. Control mice developed leukemia within 21 days post injection (3/3). In contrast, cells from AMG 330 treated cultures did not initiate leukemia in NSG mice (0/6; median follow up 119 days), suggesting that *ex-vivo* treatment with AMG 330 successfully eliminated AML stem cells. Taken together, these results suggest high therapeutic potential for AMG 330 and strongly encourage clinical development for patients with AML.

Proteome-wide analysis for identification of functional SNPs

Lehm M[1,2], Prestel M[1], Viturawong T[2], Dichgans M[1], Mann M[2]
[1]LMU, Institute for Stroke and Dementia Research (ISD)
[2]Max Planck Institute (MPI) of Biochemistry, Department of Proteomics and Signal Transduction

Genome-wide association studies (GWAS) have identified more than 50 chromosomal loci associated with large artery atherosclerotic stroke (LAS) or coronary artery disease (CAD). Both cardiovascular phenotypes, LAS and CAD, share several risk factors and many aspects of their underlying pathophysiology. However, most disease-associated single nucleotide polymorphisms (SNPs) are located in non-coding regions of the genome, indicating that these SNPs might alter transcriptional regulation. Our objective is to characterize the genome-wide molecular interaction landscape for all SNPs that are highly associated with both LAS and CAD.
Here, to systematically identify functional SNPs from non-coding regions and to delineate their molecular mechanisms, we employ “Proteome-Wide Analysis of Disease-Associated SNPs” (PWAS). By combining a DNA-protein pull-down approach followed by mass-spectrometric analysis, we detect differential binding of transcription factors in an allele-specific manner. Based on this resource, allele-specific interactors can be subjected to transcriptional reporter assays and additional functional follow-up; this will allow to establish transcription factor-mediated regulation of target genes and to identify key molecular regulators and pathways of LAS and CAD.
Taken together, we demonstrate the feasibility of a DNA-centric, genome-wide screen for functional SNPs in cardiovascular disease – a novel approach that can be applied to virtually any complex phenotype.

Neuroproteomics and neurodegeneration: substrates and functions of Alzheimer secretases

Lichtenthaler S
Technical University Munich (TUM), German Center for Neurodegenerative Diseases (DZNE), Neuroproteomics

Alpha-, beta- and gamma-secretases are membrane-bound proteases and play a central role in Alzheimer¹s disease, but also in basic physiological processes such as embryonic development, cell adhesion, axon guidance and the immune system. This talk demonstrates how novel proteomic methods can be used for the identification of substrates and functions of membrane-bound secretases *in vitro* and *in vivo*, for the evaluation of the therapeutic potential of secretases and for the analysis of basic mechanisms in the vertebrate brain and CSF.

High throughput methods for characterization of lethargus in *C. elegans.*

Olmedo M, Geibel M, Koritala BSC, Merrow M
LMU, Institute of Medical Psychology

Biological clocks introduce temporal structure into biological processes, synchronizing events between each other and to the external environment. They can time repeated processes during the life of an organism. One of these recurrent events is the molting of the nematode *C. elegans* [1], which occurs at the end of each larval stage. *C. elegans* progresses through 4 larval stages from egg to adult. The transitions between larval stages are characterized by a lethargic period that precedes the molt, during which the animals show behavioural quiescence and cessation of feeding. Furthermore, lethargus is a sleep-like state [2].

We have established a high throughput method to monitor the timing of this event, following individual worms from the first larval stage to the adult. The method is based in luminometry and relies on the cessation of pumping during lethargus. The assay is performed in 96-wells, allowing acquisition of large amounts of data that provide quantitative information about the process.

[1] Monsalve, G. C. & Frand, A. R. Toward a unified model of developmental timing: A ‘molting’ approach. *Worm* 1, 221–230 (2012).
[2] Raizen, D. M. *et al.* Lethargus is a Caenorhabditis elegans sleep-like state. *Nature* 451, 569–572 (2008).

Effects of cell type-specific manipulations on cognitive and emotional behaviour in mice

Reichel JM[1], Bedenk B[1], Rogel G[1], Härtig W[2], Kao C-Y[1], Gassen NC[1], Rein T[2], Turck C[1], Deussing J[1], Czisch M[1], Wotjak CT[1]
[1]Max Planck Institute (MPI) of Psychiatry, Neuronal Plasticity
[2]Leipzig University, Paul Flechsig Institute for Brain Research

Molecular and/ or functional lesions are part of the pathophysiology of most neurological disorders ranging from Schizophrenia to Alzheimer's disease. These neurological diseases are defined by distinct CNS lesions often leading to the failure of specific behavioral aspects, e.g. cognitive or emotional abilities.
There are however many ways to "acquire" such regional dysfunctions in the CNS, ranging from neurotoxins and physical injuries to neurodegenerative diseases as well as "normal" aging.
Here we analyzed the (i) spatio-temporal effects of GABAergic lesions via Saporin-conjugated immunotoxins for selective brain regions and time courses and (ii) the consequences of lacZ expression – as used in many transgenic mouse models – in distinct neuronal subpopulations both for constitutive and inducible expression with and without progressed age.
We found that (i) GABAergic lesions in the dorsal HPC impair the acquisition of a spatial learning paradigm (WCM) whereas GABAergic lesions in the Prelimbic Cortex hinder the re-learning of this task which requires a shift in the learning strategy. Furthermore we found that GABAergic neurons in the Hippocampus are necessary for the acquisition of a place learning strategy, but not for the recall of a learned strategy.
Regarding the (ii) consequences of LacZ expression, we found that constitutive expression causes distinct and severe phenotypic, morphological and molecular changes (on the protein level) depending on the driving promoter. LacZ expression induced in adulthood causes less severe but nonetheless significant behavioral and morphological alterations (measured by Manganese enhanced MRI scan). These effects also interact with progressed aging.
Taken together these results demonstrate the variety of mechanisms underlying neurological lesions and their consequences and may therefor help to further identify common principles as well as disease defining key-components for each pathophysiology.

Pale, small eyes, big belly - the genetic basis of unattractiveness - or why cavefish prefer the dark.

Nicolas Rohner, *Ariel Aspiras, Richard Borowsky, Cliff Tabin*

Cave animals have equally fascinated laymen and scientist for decades. Trapped in total darkness and left in isolation for thousands of years, cave animals evolved as some of nature's most bizarre and best adapted creatures. The cavefish *Astyanax mexicanus* is an example of an organism with a well characterized set of cave (troglomorphic) traits. This model is particularly useful because surface forms still exist and are interfertile with the cave fish, allowing for genetic analysis of the derived traits. Additionally, there are numerous independently evolved cave populations that have converged on troglomorphy, resulting in replicated natural experiments.

These advantages have led to the discovery of numerous Quantitative Trait Loci (QTL) - regions in the genome linked to these cave traits. The analysis of candidate genes in QTL has been hampered so far by the absence of genomic information for *Astyanax mexicanus*. This deficiency has recently been overcome and a fully annotated genome is now available on Ensembl (www.ensembl.org).

Taking advantage of this new resource, we have analyzed multiple candidate genes for the loss of pigmentation in cave forms of *Astyanax mexicanus* and identified coding mutations in Ednrb1, Edn3 and Mitfa. Furthermore we have focused on some of the impressive physiological changes that accompany the transition to the dark and food deprived environment. Because there are no primary producers in the cave, cavefish rely on external food input such as seasonal floods. To survive, cavefish need to binge when food is becoming available in order to gain weight rapidly. Here, we show the genetic basis of the insatiable appetite found in some populations of cavefish. We provide evidence that this binging behavior is derived from standing genetic variation present in surface populations. Intriguingly, the same mutated residue has been shown to be linked to obesity in humans. Our results suggest that drastic metabolic and behavioral changes can occur by a single point mutation in natural populations.

Dendritic cell vaccination in cancer immunotherapy: from biology to translational medicine

Lichtenegger FS[1,2], Schnorfeil FM[1,2], Altmann T[1], Koehnke T[1], Bücklein V[1], Krupka C[1,2]., Geiger C[3], Wagner B[4], Henschler R[4], Kvalheim G[5], Hiddemann W[1], Schendel DJ[3], **Subklewe M**[1,2]

[1]LMU, Department of Internal Medicine III
[2]Helmholtz Center Munich (HMGU), Clinical Cooperation Group Immunotherapy
[3]Helmholtz Center Munich (HMGU), Institute of Molecular Immunology
[4]LMU, Department of Transfusion Medicine, Cellular Therapeutics and Hemostaseology
[5]Oslo University Hospital, Department of Cellular Therapy

First identified and isolated in 1973 by Ralph Steinman 1973, dendritic cells (DCs) have since evolved in our understanding from mere “accessory” cells to essential initiators and modulators of innate and adaptive immune responses. Acting as professional antigen-presenting cells, they effectively stimulate naïve and memory T cells. Due to their high potency to induce tumor-specific T cells, DCs have been used in cancer immunotherapy for 17 years (*first clinical trial at Stanford by Ronald Levy in lymphoma pts*). Although antigen-specific immune responses were elicited in the majority of patients, clinical effects have been limited. However, with new knowledge of DC biology rapidly increasing, several impediments are now better understood and can be overcome in the design of future studies. We have developed a GMP-compliant 3-day protocol for the generation of *next-generation* DCs from monocytes using a defined cytokine cocktail containing a TLR7/8 agonist. The resulting DCs are characterized by a positive costimulatory profile and high IL-12p70 production. Both *in vitro* and *in vivo*, they have been shown to polarize $CD4^+$ T cells into Th1, to induce antigen-specific $CD8^+$ T cells and to activate NK cells. The appropriate disease state to administer immunotherapeutics is most likely in the setting of minimal residual disease (MRD) in which tumor-mediated immunosuppression is minimal. In the setting of acute myeloid leukemia (AML) the potency of immunotherapy has already been demonstrated by the successful eradication of residual leukemic cells through allogeneic stem cell transplantation. We are currently conducting a proof-of-concept phase I/II clinical trial evaluating *next-generation* DCs as postremission therapy for AML patients with a non-favorable risk profile (NCT01734304). DCs are loaded with ivt-RNA encoding WT1 and PRAME as well as CMVpp65 as an adjuvant and surrogate antigen. The primary endpoint of the trial is feasibility and safety. Secondary endpoints are immune responses and disease control, with particular focus on MRD conversion. Adaptive immune responses are hampered by upregulation of inhibitory molecules (“*immune check points*”) on tumor cells, which initiate inhibitory pathways in responding immune cells. Many of the immune checkpoints are initiated by ligand-receptor interactions that can be blocked by antibodies or immunmodulatory agents. The relevance of these immune checkpoints has been shown in several trials demonstrating clinical efficacy using blocking antibodies of immune inhibitory signals in cancer therapy. Cytotoxic T-lymphocyte-associated antigen-4 (CLTA-4) antibodies were the first of this class of immunotherapeutics to achieve FDA approval in advanced malignant melanoma. The results of the phase III clinical trials with immune check point inhibiting antibodies have put immunotherapy in the spotlight. The journal “Science” has chosen cancer immunotherapy as the most significant milestone reached in 2013 (“*scientific breakthrough of the year 2013*”). The future will need to integrate vaccine approaches with checkpoint-blockade agents to enhance immune responses and vaccine efficacy. Our growing understanding of how tolerance, immunity and immunosuppression regulate antitumor immune responses together with the uprising novel treatment options, represent a promising path to successful immunotherapy in cancer patients.

Zebrafish to study cholesterol involvement in axon degeneration

Trovó L, Misgeld T
Technical University Munich (TUM), Institute for Neuronal Cell Biology

Many neurodegenerative diseases are linked to defects in lipids metabolism. Cholesterol in particular, is a fundamental plasma membrane lipid involved in neuronal function and neurodegeneration. Nonetheless, most of the mechanisms that link cholesterol to neurodegeneration are not well understood. My aim is to use zebrafish, which is optically accessible and genetically malleable, to further improve our understanding of the cellular, subcellular and molecular mechanisms that link cholesterol membrane content and axon stability.

Initially, I chose a pharmacological approach to lower cholesterol using statins (inhibitors of cholesterol synthesis). Zebrafish larvae treated with statins show two-fold accelerated Wallerian degeneration after axotomy. Exogenous cholesterol substitution after blocking synthesis can rescue this phenotype. In subsequent experiments I am currently exploring subcellular mechanisms that might mediate this effect. First, as the lag phase of Wallerian degeneration is believed to depend on the diminishing transport of axon protective factors, I investigated axonal transport of mitochondria. Time-lapse recordings revealed that base-line axonal transport seems undisturbed, suggesting that alternate subcellular mechanisms are involved – on-going analysis is focused on assaying cytoskeletal stability and calcium influx.

Parallel to these global pharmacological experiments, I am pursuing genetic approaches in zebrafish, as well as in mice, to modulate cholesterol availability in a more controlled way, to interrogate the contribution of neurons versus glial cells, and to explore the role of cholesterol provision in models of chronic axon degeneration.

Support for this project comes from an EMBO long-term fellowship

Investigating the role of CNS histamine in mediating sleep-wake states using DREADDs
Anne Venner, Research Fellow in Neurology, Beth Israel Deaconess Medical Center

The histamine-containing neurons of the tuberomamillary nucleus (TMN) of the hypothalamus have long been thought to play an important role in regulating arousal. Antagonists of the histaminergic system are well known to induce drowsiness whilst juxtacellular recordings of histamine neurons *in vivo* indicate that they are predominantly active during wake. Intriguingly however, chronic global disruption of histaminergic transmission appears to have little effect on total sleep-wake amounts, thereby questioning the necessity of histamine to promote or maintain arousal.

In order to further investigate the precise role of histaminergic transmission in modulating sleep-wake, we sought to acutely, reversibly and selectively manipulate histaminergic neurons *in vivo*. To this end, we placed stereotaxic brain injections of various adeno-associated viral (AAV) vectors into the TMN of histidine decarboxylase (HDC)-cre driver mice and monitored sleep and wake using surgically implanted EEG/EMG headstages. The AAVs contained cre-dependent cassettes that expressed DREADDs (Designer Receptors Exclusively Activated by Designer Drugs (AAV-FLEX-mCherry-hM3Dq or AAV-FLEX-mCherry-hM4Di)) for acutely activating or inhibiting HDC neurons respectively. DREADDs have very little sensitivity for endogenous ligands but are selectively activated by the synthetic compound, clozapine-N-oxide (CNO), a drug which is otherwise pharmacologically inert. We examined a comprehensive array of sleep-wake parameters in HDC-cre mice transfected with either excitatory or inhibitory DREADDs, following injection of saline or CNO. Consistent with previous reports of global histamine disruption, changes in sleep-wake architecture following excitation or inhibition of HDC neurons were unremarkable. We posit that, although histaminergic transmission may not be necessary or sufficient to induce or maintain arousal under baseline recording conditions, it remains possible that histamine-containing neurons may influence more qualitative aspects of wake.

New insights into the heterogeneity of adult NG2 glia

Viganò F. and Dimou L.
LMU, Department of Physiological Genomics

Glial cells in the adult brain are very heterogeneous and some of them represent the stem and progenitor cells of the CNS. The only proliferating cells in the healthy, adult brain outside the neurogenic niches are the NG2-glia, also named oligodendrocyte progenitor cells (OPCs). Adult NG2-glia in the mouse cerebral cortex regulate their proliferation, self-renewal and differentiation in a rather dynamic manner.

Recently we have shown that the differentiation properties of adult NG2-glia depend on their location in the brain (Dimou et al., 2008; Viganò et al., 2013). These data revealed not only important environmental differences in NG2-glia maturation into oligodendrocytes, but also supported the idea of intrinsic heterogeneity among adult NG2-glia across different cortical regions. This concept became more complex when we observed heterogeneity amongst these cells also in the same cortical area. Indeed, we could show that the membrane G-protein coupled receptor GPR17 is transiently expressed by a subpopulation of NG2-glia in the adult brain (Boda et al., 2011). Genetic fate mapping indicated that this subpopulation of NG2-glia differentiates into mature oligodendrocytes with a slower rate. Interestingly, in the grey matter of the cortex after acute injury, NG2-glia show a fast and very heterogeneous reaction. In vivo 2-photon imaging of NG2-glia reacting to stab wound injury provided new insights into the role of these cells upon lesion: the fast process orientation towards the injury site and their substantial proliferation imply a contribution to wound closure and scar formation.

Taken together our data indicate that adult NG2-glia comprise a very heterogeneous cell population with probably different functions and roles in the brain.

Pharmacological and genetic modulation of the endocannabinoid system in a chronic epilepsy model

von Rüden EL[1, 2], Bogdanovic RM[1, 2], Jafari M[1, 2], Wotjak CT[3], Potschka H[1]
[1]LMU, Institute for Pharmacology
[2]Graduate School of Systemic Neurosciences (GSN[LMU])
[3]Max Planck Institute (MPI) of Psychiatry, Neuronal Plasticity

The endocannabinoid system serves as a negative retrograde feedback mechanism in the central nervous system. The endocannabinoid system essentially consists of endogenous ligands (endocannabinoids), cannabinoid receptors (CB) and enzymes, which coordinate endocannabinoid synthesis, release and degradation. The most well characterized endocannabinoids are 2-arachidonoylglycerol (2-AG) and anandamide (AEA). The synthesis of the endogenous ligands is located in post-synaptic terminals and initiated by presynaptic axonal depolarization. Once the endocannabinoids are released into the synaptic cleft, they travel backwards to activate CB1 receptors on pre-synaptic terminals. When activated, CB1 receptors regulate ion channels to decrease neurotransmitter release of excitatory as well as of inhibitory neurons. To complete the feedback-loop, specific uptake transporters and degrading enzymes terminate the physiological effects of the ligands.
Since the endocannabinoid system balances excitatory and inhibitory neurotransmission it is discussed as a potential target for treatment and prevention of epilepsies.

Here, we modulated the endocannabinoid system pharmacologically and genetically *in vivo* and analyzed the impact on the generation of a hyperexcitable neuronal network in a chronic model of temporal lobe epilepsy.
In the course of our work we demonstrated that endocannabinoid signaling affects seizure susceptibility and endogenous termination of seizure activity, dependent on the neuronal subpopulation being modulated. In addition, CB1 receptor agonism proved to interfere with the development of a hyperexcitable network.
Further investigations in chronic epilepsy models with spontaneous seizures are necessary to confirm whether the endocannabinoid system has a preventive potential against epilepsy generation.

Regulated intramembrane proteolysis and its therapeutic potential in Alzheimer Disease

Wanngren J

Technical University Munich (TUM), German Center for Neurodegenerative Diseases (DZNE), Neuroproteomics

Regulated intramembrane proteolysis (RIP) is a highly conserved process that enables the signaling between cells and the extracellular environment. During the RIP processing, membrane proteins are sequentially cleaved with various sheddases of the ADAM-family or by BACE1 followed by the γ-secretase complex. Importantly, a dysregulation of the RIP process can lead to diseases such as Alzheimer's disease (AD). One of the most extensive studied RIP substrate is the amyloid precursor protein (APP). During the sequential cleavage of APP by BACE1 and γ-secretase, the amyloid β-peptide (Aβ) is generated, which aggregates and form plaques in brains of Alzheimer's disease patients. Thus, both γ-secretase and BACE1 are considered key drug targets in AD.
However, since γ-secretase mediate the cleavage of many substrates involved in cell signaling, it is crucial to sustain theses pathways while altering the Aβ secretion. Unfortunately, it has been extremely challenging to develop γ-secretase inhibitors with a sufficient therapeutic window between APP and the processing of other substrates. This has led to the interruption of many large clinical trials with γ-secretase inhibitors during the last years. An alternative strategy is to use compounds that modulate the enzyme instead of inhibiting γ-secretase. Recently, several pharmaceutical companies have entered clinical trials with BACE1 inhibitor drug candidates. However, recent studies have shown that BACE1 deficient mice display several neurological phenotypes. This raises the concern that possible mechanism-based side effects of BACE1 inhibition in patients also may result from reduced cleavage of other BACE1 substrates. Therefore, it is crucial to understand how a loss-of-BACE1 cleavage affects BACE1 substrates in order to better evaluate the therapeutic potential of BACE1 in AD and the function of BACE1 in basic neurobiological processes.

A molecular basis for specificity in the mammalian circadian clock feedback loop

Charles J. Weitz, M.D., Ph.D.
Robert Henry Pfeiffer Professor of Neurobiology
Department of Neurobiology
Harvard Medical School

Mammalian circadian rhythms are generated by a negative feedback loop in which PERIOD (PER) proteins accumulate, form a large nuclear complex (PER complex), and bind the transcription factor CLOCK-BMAL1, repressing their own expression. We found that mouse PER complexes include the NuRD (Mi-2/NUcleosome Remodelling and Deacetylase) transcriptional co-repressor. Unexpectedly, two NuRD subunits, CHD4 and MTA2, constitutively associate with CLOCK-BMAL1, with CHD4 functioning to promote CLOCK-BMAL1 transcriptional activity. At the onset of negative feedback, the PER complex delivers the remaining NuRD subunits to DNA-bound CLOCK-BMAL1, thereby reconstituting a NuRD co-repressor important for circadian transcriptional feedback and clock function. Full repressor activity of the PER complex thus requires successful targeting of CLOCK-BMAL1. Our results show how fidelity is generated in the clock despite its dependence on generic transcriptional regulators and reveal the existence of active communication between the positive and negative limbs of the circadian feedback loop.

A novel synthetic Toll-like-receptor 7 agonist for tumor therapy

Wiedemann G[1], Chaloupka M[1], Hamm S[2], Strobl S[2], Endres S[1], Kobold S[1]
[1]LMU, Department of Medicine IV, Clinical Pharmacology
[2]4SC Discovery GmbH

Introduction: Toll-like-receptors (TLR) are an evolutionarily ancient family of pattern recognition receptors belonging to the innate immune system. TLR7 is located in the endosome and is activated upon binding of viral ssRNA, which results in the release of proinflammatory cytokines such as IL-6, IL-12 and type 1 interferon and culminates in the activation of cells of the innate and adaptive immune system. This rapid initiation of an innate and adaptive immune response may be used for systemic anti-tumoral immunotherapy.
However, the clinical application of synthetic TLR7-agonists is currently restricted to the topical treatment of skin tumors and new compounds are needed for safe and efficient systemic application.

Results and methods: All experiments were performed using the novel synthetic small molecule TLR7-agonist SC1. We found SC1 to be specific for TLR7, as evidenced by a TLR7-HEK-reporter cell line and TLR7-/-splenocytes. SC1 induced a pro-inflammatory cytokine profile and lead to immune cell activation both *in vitro* and *in vivo*. SC1 induced interleukin-6, interleukin-12p70, MCP-1, MIP-1β, Interferon-γ and IP-10 in splenocytes and enhanced the expression of the early activation marker CD69 on T cells, B cells and natural killer cells (NK cells). Mechanistically, cytokine up-regulation and immune cell activation are TLR7-mediated, as they could not be observed in TLR7-/- mice. *In-vivo,* treatment of mice with SC1 lead to specific killing of β2-mikroglobulin-deficient target cells by NK cells as opposed to β2-mikroglobulin-competent target cells (92% specific lysis vs 0% specific lysis). Finally, treatment with SC1 of RMA-S tumor-bearing mice (an NK cell sensitive lymphoma model) significantly reduced tumor growth as compared to vehicle-treated mice (mean tumor size at day 32: 218 mm2 (vehicle); 38,5 mm2 (low dose SC1); 0 mm2 (high dose SC1); n = 6) and resulted in long-term tumor-free survival in SC1-treated mice (> 40 days after last treatment).

Conclusion: Our study demonstrates that the novel TLR7-agonist SC1 is a potent TLR7-specific immune activator and is effective in the treatment of a NK cell-sensitive tumor model. SC1 may thus be a promising new candidate for systemic use in anti-tumoral immunotherapy.

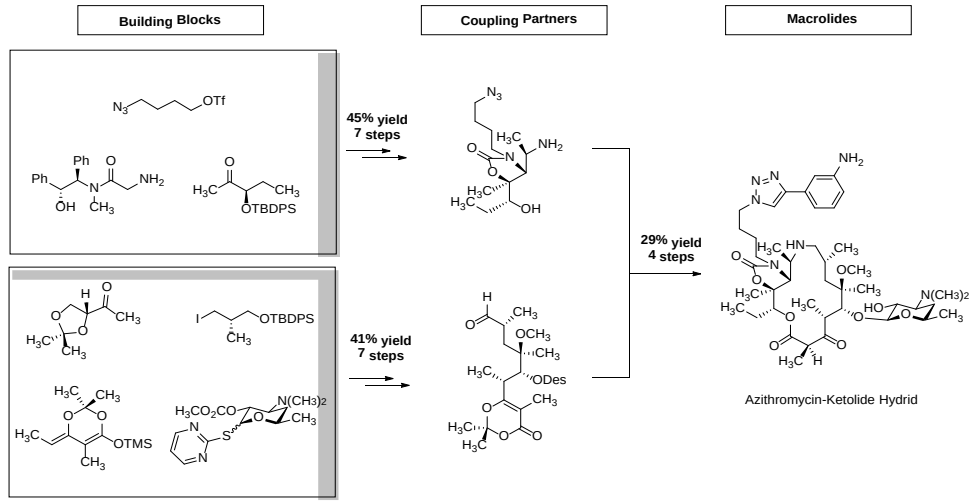
A Practical Platform for the Synthesis of Macrolide Antibiotics

Ziyang Zhang, Ian B. Seiple, Audrey Langlois, Peter M. Wright, Kazuo Yabu and Andrew G. Myers*

Department of Chemistry and Chemical Biology, Harvard University

This poster presents the development of the first robust platform for the synthesis of macrolides, a safe and effective class of anti-infective agents currently derived from complex fermentation products such as erythromycin. This platform holds extraordinary potential for the discovery of new antibiotics with heretofore inaccessible structural variety.

Our route is based upon the highly convergent assembly of modular building blocks. The final macrolides are constructed by a late-stage coupling of two halves of the molecule, which are prepared from 7 building blocks, each in 7 steps. Such a strategy has enabled efficient syntheses of a vast array of novel macrolides, covering multiple structural classes. As an example, an "azithromycin-ketolide hybrid" is prepared in 11 linear steps and 12% overall yield (Scheme 1).



Scheme 1. Modular assembly of building blocks allows for rapid construction of macrolides. Pym = 2-pyrimidyl. Des = b-1-(2-O-methoxycarbonyl)desosaminyl.

There are manifold benefits to such an approach: by its nature it reduces a complex structural problem to scalable components, it allows for multiplicative expansion of end products through variation of each component, and it permits independent evolution of the synthetic chemistry of each assembly component, allowing the overall route to evolve in efficiency. Several modifications that are not chemically feasible using a semi-synthetic approach will be accessible with this fully synthetic platform, enabling the discovery and development of a new generation of antibiotics and novel chemotypes.

To date, this platform has produced over 100 novel fully synthetic macrolide antibiotic candidates. Preliminary antibacterial testing has revealed highly potent compounds with greater activity than azithromycin. Improved activity was also observed in several multi-drug resistant strains (e.g. *S. aureus* USA300). Structure-activity relationships established from the preliminary screening will be used to refine structural components of each building block, and evolution of the route will enable the synthesis of an extraordinary number of new macrolide antibiotic candidates of unprecedented diversity.