ANA Meeting 2021

28–30 September 2021 l Salzburg, Austria

Content

Welcome from the ANA President

Dear ANA members, dear Colleagues,

It is with great pleasure that I welcome you all to Salzburg on behalf of the Executive Board of the Austrian Neuroscience Association.

Thanks to the superb work of the Local Organizers (LO), I can confidently say that the 17th ANA Meeting will be a memorable event for Austrian Neuroscience. The LO have put together an outstanding program addressing a number of topical Neuroscience issues and striking an optimal balance between disciplines. The organization of this meeting has not been an easy task because of the recent extraordinary circumstances, but the LO have been able to secure a great venue for an in person meeting and in compliance of the highest safety requirements. I express here my gratitude together with that of the whole ANA community for their efforts and time.

Besides keynote lectures from distinguished national and international speakers, the meeting offers parallel sessions of selected short oral presentations from younger scientists and thematic mini-symposia alternated with poster sessions. A meeting of this quality allows us to demonstrate the power and vivacity of Austrian Neuroscience. I am sure we will all enjoy this exciting opportunity to meet again, interact and share ideas for promoting neuroscience.

I take this opportunity to thank also all the sponsors for making this meeting possible and you for your contribution!

I wish you a productive and highly successful 17th ANA Meeting and an enjoyable stay in Salzburg.

Francesco Ferraguti

President of the Austrian Neuroscience Association

Welcome from the President of the FWF

Dear Colleagues,

It is a pleasure to welcome you to the ANA Meeting 2021 also on behalf of the FWF, Austria's National Funding Agency in charge of financing fundamental research. Over the years the FWF has supported many projects in the field of neuroscience, ranging from individual grants, international projects, and post doc fellowships, all the way to a large doctoral training program headed by ANA's president Prof. Ferraguti and the Wittgenstein prize awarded to Prof. Jonas in 2016. The outstanding success of your community in securing funding from the FWF underlines the excellence of your work, as well as the important role of neuroscience in Austria.

Beyond the national scale, neuroscience is one of the focus areas of research worldwide, with aspects ranging from clinical and psychological applications, insight to the human mind and consciousness, all the way to new ideas in computer science. Several of these topics will also be addressed at the ANA Meeting 2021 and I wish you, the participants of this year's meeting, a productive exchange of ideas, as well as interesting and inspiring discussions.

Christof Gattringer

President of the FWF

Society Committees

ANA Board

Francesco Ferraguti – Medical University
of Innsbruck
Isabella Sarto-Jackson – Konrad Lorenz Institute for Evolution and Cognition Research

René Seiger – Karolinska Institutet, Stockholm
Ramon Tasan – Medical University
of Innsbruck

Petra Scholze – Medical University of Vienna
Rupert Lanzenberger – Medical University of Vienna
Sébastien Couillard-Després – Paracelsus Medical University, Salzburg
Simon Hippenmeyer – Institute for Science and Technology Austria, Klosterneuburg

ANA Extended Board

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Magdalena Daurer – QPS Austria GmbH, Grambach

Peter Jonas – Institute for Science and Technology Austria, Klosterneuburg
David Keays – Research Institute of Molecular Pathology, Vienna

Andreas Lieb – Medical University
of Innsbruck

Gerald Obermair – Karl Landsteiner University Krems
Kristin Tessmar-Raible – Max F. Perutz Laboratories, University of Vienna

Organizing Committee

Sébastien Couillard-Després – Paracelsus Medical University, Salzburg

General Information

Dates & Venue

Tuesday, September 28th to Thursday, September 30th, 2021
University of Salzburg, Unipark Nonntal
Erzabt-Klotz-Straße 1
5020 Salzburg

Registration

Please note that on-site registration is not available due to COVID-19 restrictions!
Online registration ends on September 19th, 2021.

Fees:

Early bird registration fee, ANA members	EUR	120,–
Early bird registration fee, ANA student members *(certificate required)*	EUR	80,–
Early bird registration fee, non-members	EUR	180,–
Early bird registration fee, student non-members *(certificate required)*	EUR	100,–
Late registration fee, ANA members	EUR	150,–
Late registration fee, ANA student members *(certificate required)*	EUR	100,–
Late registration fee, non-members	EUR	210,–
Late registration fee, student non-members *(certificate required)*	EUR	120,–
Speakers' Dinner, 29th September 2021	EUR	53,–

Fees include:

- Access to all sessions
- Welcome reception (risotto & beer) at the venue on Tuesday evening
- Delegates´ documents (printed program booklet, online abstract book)
- Certificate of attendance (sent electronically after the meeting)
- Free lunch on Wednesday and Thursday
- Free drinks sponsored by Red Bull & Trumer Privatbrauerei
- Coffee breaks
- Exhibition of ANA partner companies

Health & Safety

Please wear your personal badge at all times while at the venue. It is the official entrance pass to scientific sessions as well as evidence that your 3G status was checked upon admission (colored stickers on your badge indicate 3G checks on subsequent days).

IMPORTANT:

If you experience symptoms of COVID-19 while participating in the conference or if your PCR test comes back positive while at the conference or within 5 days after the conference, **please inform us immediately** via the **hotline +43 677 606 404 0380** or via email: office@austrian-neuroscience.at (Petra Scholze and Isabella Sarto-Jackson).

Further information concerning on-site health and safety measures can be found online (hygiene concept for the ANA Meeting 2021).

Photos & Filming

Please take note that we are obligated – following § 17 Abs. 1 and 2 of the 2nd COVID-19-Opening Regulations – to collect name, email address, and telephone numbers of participants for the purpose of contact tracing if necessary. Data will be stored for 28 days and deleted thereafter.

In order to trace possible chains of infection, recorded material (photos or films) from individual sessions will used by the Covid-appointees to assess putative contact persons should a COVID-19 case occur. For sessions with seat assignments, electronic registration records will be consulted. In case of a confirmed COVID-19 infection, we will contact all persons seated within a radius of 5 meters of the infected person.

Social Events

Welcome Reception: Risotto & Beer

The welcome reception takes place on Tuesday, September 28th, 20.00 at the venue. Foodtruck RisotTomas provides creamy and tasty risotto; a big thanks to Trumer Brauerei who has sponsored drinks and equipment for the gathering.

Young ANA Get-Together

Young ANA (Chair: Bruno Benedetti) organizes an informal get-together at the famous Stiegl Keller taking place on September 29th, 20.00. Food for registrants is covered by Young ANA, drinks at own expenses. Due to space limitation, advance booking is required.

Speakers' Dinner

Speakers' Dinner will take place on September 29th, 20.00 at the traditional restaurant "Blaue Gans." Dinner is not included in the conference fee and advance booking is required.

Technical Information

WIFI Access

All registered participants can use the Eduroam network of the University of Salzburg for the duration of the conference.

Oral Presentations

Speakers should transfer their presentation (powerpoint file) from a USB key, on to a PC in the lecture rooms before the start of their session. We discourage speakers to use their own laptop computer to avoid delays in the session due to technical glitches. Speakers are asked to check their presentation to ensure that their file is not corrupted.

We kindly ask chairs to be present at least 10 minutes before the start of the session that they will chair.

The maximum duration of keynote lectures is 60 minutes (50' + 10' for questions), of talks in minisymposia 20 minutes (15' + 5' for questions), of contributed talks (oral presentations) 15 minutes (10' + 5' for questions), and speaking time for an individual a poster flash is exactly 1 min. Time slots for special lectures and symposia can be found in the detailed program.
Due to the large number of talks, we ask speakers to strictly keep to the allotted time for their talk and session chairs to be very strict in not allowing speakers to go over the allotted time.

Poster Presentations

The posterboards are suitable for posters of A0 format (1.2m height x 0.9m width).
All posters can remain posted throughout the meeting. Presenters should put up their posters on Tuesday morning. Please be present at your poster during the time assigned to your poster.
Materials for fixing posters on the boards will be available on site. Posters should be removed before the end of the meeting. Poster sessions were sponsered by Science Services.

Poster Flash

Poster flashes take place immediately before the poster sessions and aim at drawing the audience's attention to subsequent poster presentations. Each poster flash is exacly one minute.

Awards

Competition: Poster Prize / Oral Presentation Prize / Poster Flash Prize

Speakers presenting at the ANA Meeting 2021 (oral contributions, poster presentations, poster flash) will be eligible to enter our competition to win the following prizes:

- Best Poster Presentations: 5 x Ski goggles from Zeiss or 5 x EUR 100,-
- Best Oral Presentations: 5 x EUR 100,- (sponsored by the Government of Lower Austria)
- Best Poster Flash: 3 x book vouchers by Springer (EUR 180,-)

We thank our sponsors for their generous support.

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Springer

ZEISS
Seeing beyond

For the competions we use Slido polls (Best Poster, Best Flash, Best Oral presentation). To make the voting objective, we have distributed QR codes of each poll randomly among participants, putting them in the meeting-bags (each QR code can vote for one poll only). Winners of the prizes will be announced in the last session at the final conference day.

In addition, Young ANA offers 3 Picture Prizes of EUR 100,-. Winners are drawn by October 15th, among those who send photos and videos taken at the ANA meeting to office@austrian-neuroscience.at.

Otto Loewi Prize

The awardee of the Otto Loewi Prize, selected by the Otto Loewi Prize jury, will be announced at the Otto Loewi Prize cermony on Wednesday, September 29th. The prize is generously sponsored by Peter and Traudl Engelhorn Stiftung.

PETER UND TRAUDL ENGELHORN STIFTUNG
zur Förderung der Lebenswissenschaften

Pioneer in Austrian Neuroscience Award

ANA members were invited to vote for the Pioneer in Austrian Neuroscience Award. The winner elected by ANA members from a short-list of 3 candidates will be announced at the ceremony of the Pioneer in Austrian Neuroscience Award. The ceremony is generously sponsored by Siemens Healthineers.

SIEMENS Healthineers

Travel Grants

ANA provides a total of 28 travel grants for young ANA members who participate in the ANA Meeting 2021. Travel grants were supported by Land Salzburg and the Government of Lower Austria.

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LAND SALZBURG

Lecture Hall 1		Lecture Hall 2		Foyer & Gallery	
09.15 - 10.15	**ANA Board Meeting**			09.00 - 12.00	Admission for registered participants
		10.00 - 11.15	**VIRTUAL Young ANA Symposium** Job Opportunities in Research and Beyond **life-science Karriere Service** Chair: Bruno BENEDETTI (Paracelsus Medical University) g.tec medical engineering, Austrian Intitute of Technology, SAN Group, Red Noses, HOOKIPA Pharma		
12.00 - 12.30	**Opening Ceremony** Welcome Address Landesrätin **Andrea KLAMBAUER** **Simone WEISS** (ITG) Rector **Hendrik LEHNERT** (University of Salzburg) Rector **Wolfgang SPERL** (Paracelsus Medical University) ANA President **Francesco FERRAGUTI** (Medical University of Innsbruck)				
12.45 - 13.45	**Keynote Lecture** **Manuel SCHABUS** (University of Salzburg) Cognition in Altered States of Consciousness Chair: Maximilian JÖSCH (IST Austria)				Exhibition
14.00 - 15.00	**Oral Presentations I** Neuronal Networks & Activity Chair: Peter JONAS (IST Austria)				Exhibition
15.15 - 15.45	**Poster Flash I** Chair: Ramon TASAN (Medical University of Innsbruck)	15.15 - 16.15	**Special Lecture – Theoretical Neuroscience** **Steven VERMEULEN** (CIIO, EBRAINS AISBL) Discover EBRAINS Chair: David VANDAEL (IST Austria)		Exhibition
				15.45 - 17.00	Exhibition & **Poster Session I** Coffee & Snacks
17.00 - 18.00	**Mini-Symposium** Epigenetic Processes in Brain Development & Aging Chair: Simon HIPPENMEYER (IST Austria) **Nicole AMBERG** (IST Klosterneuburg) **Jerome MERTENS** (University of Innsbruck) **Valentina CINQUINA** (Medical University of Vienna)	17.00 - 18.00	**Special Lecture – Advanced Technologies** Superresolution Microscopy Chair: Rupert LANZENBERGER (Medical University of Vienna) **Johann DANZL** (IST Austria) **Stephan SIGRIST** (Freie Universität Berlin)		Exhibition
18.15 - 18.45	**Poster Flash II** Chair: Marco TREVEN (Medical University of Vienna; KLI)				Exhibition
		18.45 - 19.30	**Young ANA Symposium** The Real Power of Brain Networks: Community Driven Innovation **The Brainstorms** Chair: Bruno BENEDETTI (Paracelsus Medical University)	18.45 - 21.00	Exhibition & **Poster Session II** Risotto & Beer

Lecture Hall 1		Lecture Hall 2		Foyer & Gallery	
08.30 - 09.30	**Keynote Lecture** **Luca BONFANTI** (University of Turin) The Search for Young Neurons in the Adult Mammalian Brain: History, Twists and Future Perspectives Chair: Sébastien COUILLARD-DESPRÉS (Paracelsus Medical University)			08.00 - 12.00	Admission for registered participants
09.40 - 10.40	**Oral Presentations II** Neurological Disorders & Regeneration Chair: Sigismund HUCK (Medical University of Vienna)	09.50 - 12.10	**Interdisciplinary Symposium** Explaining Consciousness? – Searching Common Ground Between Neuroscience and Philosophy Chair: Markus KUNZE (Medical University of Vienna) **Thomas BUGNYAR** (University of Vienna) **Rupert LANZENBERGER** (Medical University of Vienna) **Georg GASSER** (University of Augsburg) **Daniel WEHINGER** (University of Innsbruck) **Stephen MÜLLER** (University of Salzburg)		Exhibition
11.00 - 12.00	**Oral Presentations III** Cognition & Behavior Chair: Johannes PASSECKER (Medical University of Innsbruck)				Exhibition
					Exhibition
12.00 - 12.30	**Poster Flash III** Chair: Simone SARTORI (University of Innsbruck)				Exhibition
				12.30 - 14.00	Exhibition & **Poster Session III** Lunch
14.00 - 15.00	**Mini-Symposium** Ion Channels & Channelopathies Chair: Gerald OBERMAIR (Karl Landsteiner University Krems) **Xaver KÖNIG** (Medical University of Vienna) **Petronel TULUC** (University of Innsbruck) **Yousra EL GHALEB** (Medical University of Innsbruck)	14.00 - 15.00	**Mini-Symposium** EEG, MEG and fMRI: Hot New Science in Cognitive Neuroscience Chair: Manuel SCHABUS (University of Salzburg) **Christopher HÖHN** & **Kerstin HÖDLMOSER,** **Fabian SCHMIDT** (University of Salzburg) **Martin KRONBICHLER** (University of Salzburg)		Exhibition
15.15 - 16.15	**Oral Presentations IV** Ion Channels & Neurotransmission Chair: Bernhard FLUCHER (Medical University of Innsbruck)				Exhibition
16.40 - 17.00	**Pioneer in Austrian Neuroscience Award** Awardee will be announced at the ceremony Chair: Francesco FERRAGUTI (Medical University of Innsbruck) Sponsored by Siemens Healthineers				Exhibition
17.00 - 17.30	**Otto Loewi Prize Lecture** Awardee will be announced at the ceremony Chair: Roman ROMANOV (Medical University of Vienna) Sponsored by Peter & Traudl Engelhorn Stiftung				Exhibition
17.45 - 18.45	**Keynote Lecture** **Vanja NAGY** (Medical University of Vienna) Deciphering the 'Sulfation Code' of the Brain Extracellular Matrix Chair: Sofia GRADE (IMBA)				Exhibition
		19.00 - 20.00	**Public Lecture – Öffentlicher Vortrag** *(GERMAN)* **Wolfgang KLIMESCH** (University of Salzburg) Gedächtnis, Gehirnschwingungen und Bewusstsein Chair: Isabella SARTO-JACKSON (KLI)		

Lecture Hall 1		Lecture Hall 2		Foyer & Gallery	
		08.30 - 09.30	**ANA General Assembly**	08.00 - 12.00	Admission for registered participants
09.30 - 10.30	**Keynote Lecture** **Ludwig AIGNER** (Paracelsus Medical University) Will We Ever Cure Neurodegenerative Diseases? Chair: Sandra SIEGERT (IST Austria)				Exhibition
10.50 - 11.50	**Oral Presentations V** Stem Cells & Development Chair: Lora SWEENEY (IST Austria)	11.00 - 15.00	**Satelliten-Symposium** *(GERMAN)* Elementarpädagogik & Neurowissenschaften Chair: Natascha TASLIMI (NeBÖ) **Manfred SPITZER** (University of Ulm) **Karin LANDERL** (University of Graz) PODIUMSDISKUSSION **Martina KÜNSBERG SARRE** (NEOS) **Sibylle HAMANN** (Die Grünen) **Karin LANDERL** (University of Graz) **Natascha TASLIMI** (NeBÖ) **Monika UDE** (NeBÖ) **Isabella SARTO-JACKSON** (KLI) **Sigismund HUCK** (Medical University of Vienna) Moderation: **Marlene NOWOTNY** (Ö1)		Exhibition
12.00 - 12.30	**Poster Flash IV** Chair: Andreas LIEB (Medical University of Innsbruck)				Exhibition
				12.30 - 14.00	Exhibition **Poster Session IV** Lunch
14.00 - 15.00	**Mini-Symposium** Clinical Neuroscience: Epilepsy Chair: Eugen TRINKA (Uniklinikum Salzburg) **Martha FEUCHT** (Medical University of Vienna) **Eugen TRINKA** (Uniklinikum Salzburg) PANEL DISCUSSION				Exhibition
15.15 - 15.45	**Best Thesis Lecture** **Simon NIMPF** (IMP) Investigating the Neuronal Basis of Magnetoreception in the Pigeon Chair: Johannes PASSECKER (Medical University of Innsbruck)				
15.45 - 16.15	**Best Poster, Best Oral Presentation, Best Poster Flash Awards** Chair: Bruno BENEDETTI (Paracelsus Medical University)				
16.30	**FAREWELL**				

Detailed Program

Detailed Program

09.15 – 10.15 **ANA Board Meeting**
LECTURE HALL 1

10.00 – 11.15 **VIRTUAL Young ANA Symposium**
LECTURE HALL 2 **Job opportunities in research and beyond**
Organizer: life-science Karriere Service
Chair: Bruno Benedetti
Online Discussion: g.tec medical engineering; Austrian Institute of Technology (AIT); SAN Group; Red Noses; HOOKIPA Pharma

12.00 – 12.30 **Opening Ceremony**
LECTURE HALL 1
Landesrätin Andrea KLAMBAUER
Simone WEISS (ITG)
Rector Hendrik LEHNERT (University of Salzburg)
Rector Wolfgang SPERL (Paracelsus Medical University, Salzburg)
ANA President Francesco FERRAGUTI (Medical University of Innsbruck)

12.30 – 12.45 Break

12.45 – 13.45 **Keynote Lecture**
LECTURE HALL 1 **Cognition in altered states of consciousness**
K-01 Manuel SCHABUS (University of Salzburg)
Chair: Maximilian JÖSCH (Institute of Science and Technology Austria, Klosterneuburg)

13.45 – 14.00 Break

14.00 – 15.00 **Oral Presentations I**
LECTURE HALL 1 **Neural networks & activity**
Chair: Peter JONAS (Institute of Science and Technology Austria, Klosterneuburg)

O-01 **VIP-expressing interneurons in the anterior insular cortex detect salience to promote adaptive behavior**
Arnau RAMOS-PRATS (Medical University of Innsbruck)

O-02 **Periventricular A14 dopamine neurons entrain the lateral septum for the circadian control of locomotor activity**
Roman ROMANOV (Medical University of Vienna)

O-03 **Behaviour of medial entorhinal cortex cells during a radial eight arm maze task**
Yosman BAPATDHAR (Institute of Science and Technology Austria, Klosterneuburg)

O-04 **One appendage – two behaviors**
Ruth GUTJAHR (University of Graz)

15.00 – 15.15 Break

15.15 – 16.15 **Special Lecture | Theoretical Neuroscience**
LECTURE HALL 2 **Discover EBRAINS**
S-01 Steven VERMEULEN (CIIO, EBRAINS AISBL)
Chair: David VANDAEL (Institute of Science and Technology Austria, Klosterneuburg)

15.15 – 15.45 **Poster Flash I**
LECTURE HALL 1
Chair: Ramon TASAN (Medical University of Innsbruck)

P-01 **Loss of autism-associated $\alpha_2\delta$-3 affects synaptic protein expression, presynaptic function, and mouse behavior**
Cornelia ABLINGER (Medical University of Innsbruck)

P-05 **Alteration in the retinal morphology due to a truncation mutation in the *cacna1f* gene**
Matthias GANGLBERGER (University of Innsbruck)

P-09 **Pentameric ligand-gated ion channels: can you get a hole-in-one?**
Filip KONIUSZEWSKI (Medical University of Vienna)

P-13 **A novel loss-of-function variant in Cav2.1 channel in a patient with spinocerebellar ataxia**
Yuliia V. NIKONISHYNA (University of Innsbruck)

P-17 **Channel-independent membrane expression of individual $\alpha_2\delta$ isoforms**
Ruslan STANIKA (Karl Landsteiner University of Health Sciences, Krems)

P-21 **Decoding adaptive performance**
Mohamed S. AMEEN (University of Salzburg)

P-25 **Neural signatures of contextual learning strategies**
Heloisa S. C. CHIOSSI (Institute of Science and Technology Austria, Klosterneuburg)

P-29 **Co-treatment with aripiprazole and escitalopram reversed the schizophrenia-like behaviour and enhanced the BDNF mRNA expression in adult Sprague-Dawley rats induced by glutathione deficit during early postnatal brain development**
Marta LECH (Polish Academy of Sciences, Kraków)

P-33 **Behavioural characterization of the *Fmr1* knock-out mouse model of Autism Spectrum Disorder (ASD)**
Shirin SHARGHI (QPS Austria GmbH, Grambach; University of Graz)

P-37 **Modulation of microglia function via omega-3 polyunsaturated fatty acids in the context of Alzheimer's disease**
Barbara ALTENDORFER (Paracelsus Medical University, Salzburg)

P-41 **Development of adrenal gland hides the origin of neuroblastoma**
Polina KAMENEVA (Karolinska Institutet, Solna)

P-45 **The anti-asthmatic drug Montelukast improves motor coordination and balance in the Line 61 mouse model of Parkinson's disease**
Katharina STREMPFL (Paracelsus Medical University, Salzburg; QPS Austria GmbH, Grambach)

P-49 **Direct excitatory afferents onto hypothalamic tanycytes control metabolic states**
Marco BENEVENTO (Medical University of Vienna)

P-53 **Parvalbumin-positive GABAergic neurons in the basal forebrain – role for neuropathic pain**
Marie-Luise EDENHOFER (Medical University of Innsbruck)

P-57 **Alterations in basal forebrain-to-medial prefrontal cortex cholinergic signaling in a mouse model of neuropathic pain**
Kai KUMMER (Medical University of Innsbruck)

P-61 **Functional asymmetry of medial habenula output in mice**
Cihan ÖNAL (Institute of Science and Technology Austria, Klosterneuburg)

P-65 **The role of hippocampal cholecystokinin-expressing interneurons in spatial coding**
Damaris K. RANGEL-GUERRERO (Institute of Science and Technology Austria, Klosterneuburg)

P-69 **Acute anesthetic ketamine triggers sexual dimorphic microglia response in the mouse and human cortex**
Alessandro VENTURINO (Institute of Science and Technology Austria, Klosterneuburg)

P-73 **MorphOMICs: a new algorithm to unravel region- and sex-dependent microglia morphological plasticity in health and disease**
Gloria COLOMBO (Institute of Science and Technology Austria, Klosterneuburg)

P-77 **Gemcitabine Ionic Pump (GemIP) brain tumor treatment on the chick embryo chorioallantoic membrane**
Verena HANDL (Medical University of Graz)

P-81 **Spreading of P301S aggregated tau investigated in organotypic mouse brain slice cultures**
Dhwani S. KORDE (Medical University of Innsbruck)

P-85 **Dissecting the hormonally controlled loss of regenerative potential using a cell-based approach**
Alexander W. STOCKINGER (University of Vienna)

P-89 **Linking the moonlight interpreter L-Cry to the circalunar clock in the marine annelid *Platynereis dumerilli***
Aida CORIC (University of Vienna)

P-93 **Onecut transcription factors in the developing hypothalamus**
Maja ZUPANCIC (Medical University of Vienna)

15.45 – 17.00
GALLERY

Poster Session I & Coffee Break

Ion channels & neurotransmission
P-01 | P-05 | P-09 | P-13 | P-17

Cognition & behavior
P-21 | P-25 | P-29 | P-33

Neurological disorders & regeneration
P-37 | P-41 | P-45

Neural networks & activity
P-49 | P-53 | P-57 | P-61 | P-65 | P-69

New methods & disease models
P-73 | P-77 | P-81 | P-85

Stem cells & development
P-89 | P-93

17.00 – 18.00
LECTURE HALL 1

Minisymposium
Epigenetic processes in brain development and aging

Chair: Simon HIPPENMEYER (Institute of Science and Technology Austria, Klosterneuburg)

M-01 Distinct and sequential functions of PRC2 in radial glia lineage progression
Nicole AMBERG (Institute of Science and Technology, Klosterneuburg)

M-02 Direct neuronal reprogramming to study aging and disease
Jerome MERTENS (University of Innsbruck; Salk Institute for Biological Studies, La Jolla)

M-03 Life-long epigenetic programming of cortical architecture by maternal 'Western' diet during pregnancy
Valentina CINQUINA (Medical University of Vienna)

17.00 – 18.00 **Special Lecture | Advanced Technologies**
LECTURE HALL 2 **Superresolution microscopy**
Chair: Rupert Lanzenberger (Medical University of Vienna)

S-02 **Super-resolution optical microscopy for analysing nervous tissue across scales**
Johann DANZL (Institute of Science and Technology Austria, Klosterneuburg)

S-03 **Active zone long-term plasticity in behavioural control of *Drosophila***
Stephan SIGRIST (Freie Universität Berlin)

18.00 – 18.15 Break

18.15 – 18.45 **Poster Flash II**
LECTURE HALL 1
Chair: Marco TREVEN (Medical University of Vienna; Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg)

P-02 **Investigation of α4-containing $GABA_A$ receptors in the rat brain**
Maximilian AUGÉ-STOCK (Medical University of Vienna)

P-06 **Consequences of an autism-associated mutation of α2δ-1 on calcium channel trafficking and synapse composition**
Sabrin HADDAD (Medical University of Innsbruck)

P-10 **Western blot characterization of a human GABRA4 variant**
Georg KRAMER (Medical University of Vienna)

P-14 **Structural determinants for channel gating and excitation contraction coupling in CaV1.1**
Simone PELIZZARI (Medical University of Innsbruck)

P-18 **HIF-1α induces Cav3.2 and paves the way for post-ischemic epileptogenesis**
Anna TRÖSCHER (University of Bonn Medical Center; Kepler University Linz)

P-22 **Drifting memories: spontaneous long-term evolution of memory representations in the hippocampus**
Lars BOLLMANN (Institute of Science and Technology Austria, Klosterneuburg)

P-26 **Role of the VIP/VPAC receptor system in the regulation of stress and anxiety reactions in the rodent brain**
Federico FERRO (University of Innsbruck)

P-30 **Correlation between progesterone and word recall in a list-method forgetting paradigm in pregnant women**
Lisa Marie MAYER (University of Salzburg)

P-34 **Can predictive processing account for the spectrum of stereotyped repetitive behaviours in Parkinson's disease?**
Marco TREVEN (Medical University of Vienna; Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg)

P-38 **Understanding the role of platelets in Alzheimer's disease**
Diana M. BESSA DE SOUSA (Paracelsus Medical University, Salzburg)

P-42 **Activation of macrophages DRG (Dorsal Root Ganglion) in Fabry disease**
Jeiny LUNA-CHOCONTA (Medical University of Innsbruck)

P-46 **Transcriptomic characterization of brain CD8+ T-cells identifies gene signature of tissue-resident memory T-cells in Alzheimer's disease transgenic mice**
Michael S. UNGER (Paracelsus Medical University, Salzburg)

P-50 **Fast and slow – precision tuning of spinal locomotor networks**
Maximilan S. BOTHE (University of Graz)

P-54 **Retinal adaptation to natural luminance and contrast statistics**
Divyansh GUPTA (Institute of Science and Technology Austria, Klosterneuburg)

P-58 **Neuroinflammation in pain affective disturbances: role of the parabrachial nucleus**
Valeria MUSSETTO (Medical University of Vienna)

P-62 **Electrical synapses improve motion extraction in a natural visual environment**
Victoria POKUSAEVA (Institute of Science and Technology Austria, Klosterneuburg)

P-66 **Visualising priority maps: attentional modulation of neuronal population dynamics in the superior colliculus**
Florian H. SCHMIDT (Institute of Science and Technology Austria, Klosterneuburg)

P-70 **Cell-specific synaptic wiring within the hippocampal CA3 network**
Jake WATSON (Institute of Science and Technology Austria, Klosterneuburg)

P-74 **Plasma, brain and spinal cord pharmacokinetic profile of tetrahydrocannabinol from cannabis sativa extract or THC administration**
Cristiana DUMBRAVEANU (Medical University of Innsbruck; Bionorica research GmbH, Innsbruck)

P-78 **Generation of a human neuronal *in vitro* model for Rett Syndrome by non-viral expression of lineage factors**
Anna M. HUBER (Medical University of Vienna; University of Vienna)

P-82 **A fibrous nature of hydrogels causes directed migration in Schwann cells**
Flavia MILLESI (Medical University of Vienna; Austrian Cluster for Tissue Regeneration, Vienna)

P-86 **Modeling α-synuclein spreading in whole brain sagittal organotypic slices**
Buket UÇAR (Medical University of Innsbruck)

P-90 **Exploring a novel model of neurogenic plasticity as a function of endogenous timing mechanisms**
Nadja MILIVOJEV (University of Vienna)

P-94 **Long and short RNA transcriptomics giving insight into human iPSC-derived sensory neuron development**
Maximilian ZEIDLER (Medical University of Innsbruck)

18.45 – 21.00
GALLERY

Poster Session II &
Welcome Reception: Risotto & Beer

Ion channels & neurotransmission
P-02 | P-06 | P-10 | P-14 | P-18

Cognition & behavior
P-22 | P-26 | P-30 | P-34

Neurological disorders & regeneration
P-38 | P-42 | P-46

Neural networks & activity
P-50 | P-54 | P-58 | P-62 | P-66 | P-70

New methods & disease models
P-74 | P-78 | P-82 | P-86

Stem cells & development
P-90 | P-94

18.45 – 19.35
LECTURE HALL 2

Young ANA Symposium
The real power of brain networks: community driven innovation

S-04 The Brainstorms

Chair: Bruno Benedetti (Paracelsus Medical University, Salzburg)

08.30 – 09.30 **Keynote Lecture**
LECTURE HALL 1
The search for young neurons in the adult mammalian brain: history, twists and future perspectives
K-02 Luca BONFANTI (University of Turin)
Chair: Sébastien COUILLARD-DESPRÉS (Paracelsus Medical University, Salzburg)

09.40 – 10.40 **Oral Presentations II**
LECTURE HALL 1
Neurological disorders & regeneration
Chair: Sigismund HUCK (Medical University of Vienna)

O-05 **Spinal cord injury affects HCN channels activation and intrinsic excitability of cortical motor neurons**
Bruno BENEDETTI (Paracelsus Medical University, Salzburg)

O-06 **The role of enkephalin in hypoxic preconditioning**
Lisa BERGMEISTER (Medical University of Innsbruck)

O-07 **gp130 induces TRPA1 upregulation in uninjured neurons in a mouse model for neuropathic pain**
Theodora KALPACHIDOU (Medical University of Innsbruck)

O-08 **Intralesional administration of extracellular vesicles improves functional recovery in a rat model of traumatic spinal cord injury**
Lara BIELER (Paracelsus Medical University, Salzburg)

10.40 – 11.00 Coffee Break

09.50 – 12.10 **Interdisciplinary Symposium**
LECTURE HALL 2
Explaining consciousness? - Searching common ground between neuroscience and philosophy
S-05
Chair: Markus KUNZE (Medical University of Vienna); Isabella SARTO-JACKSON (Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg)
Georg GASSER (University of Augsburg)
Thomas BUGNYAR (University of Vienna)
Rupert LANZENBERGER (Medical University of Vienna)
Stephen MÜLLER (University of Salzburg)
Daniel WEHINGER (University of Innsbruck)

11.00 – 12.00
LECTURE HALL 1

Oral Presentations III
Cognition & behavior

Chair: Johannes PASSECKER (Medical University of Innsbruck)

O-09 **Histamine 3 receptor deletion reduces aggression and alters neuronal activation in zebrafish**
Florian REICHMANN (Medical University of Graz; University of Leicester)

O-10 **Exploring the role of Neurokinin B neurons of bed nucleus of the stria terminalis in emotional and metabolic processing**
Pradeepa MOHAN BETHURAJ (Medical University of Innsbruck)

O-11 **Serotonergic modulation of an associative relearning network**
Murray Bruce REED (Medical University of Vienna)

O-12 **Role of anterior insula cortex in context-induced relapse of nicotine seeking**
Hussein GHAREH (Medical University of Innsbruck)

12.00 – 12.30
LECTURE HALL 1

Poster Flash III

Chair: Simone SARTORI (University of Innsbruck)

P-03 **Selective targeting of α6-containing $GABA_A$ receptors**
Rebecca BAUER (Medical University of Vienna)

P-07 **Establishment of a gene therapeutic approach for Cav1.4 voltage-gated calcium channel knock-out and truncation mutant mice**
Thomas HEIGL (University of Innsbruck)

P-11 **Topological comparison of mRNA expression patterns with receptor distributions in the human cerebral cortex**
Matej MURGAS (Medical University of Vienna)

P-15 **A novel Ca channel β2 splice variant is predominant in the retina**
Hartwig SEITTER (University of Innsbruck)

P-19 **STAC proteins inhibit calcium and voltage dependent inactivation in L-type calcium channels**
Wietske TUINTE (Medical University of Innsbruck)

P-23 Innate sensorimotor processing deficits across mouse models of autism
Laura E. BURNETT (Institute of Science and Technology Austria, Klosterneuburg)

P-27 Secretagogin marks amygdaloid PKCδ interneurons and modulates NMDA receptor availability
Zsófia HEVESI (Medical University of Vienna; Hungarian Academy of Sciences, Budapest; Semmelweis University, Budapest)

P-31 Dopamine drives extinction-promoting effects in deficient fear extinction
Simone SARTORI (University of Innsbruck)

P-35 Silk fibroin-based conduits filled with native spider silk fibers successfully promoted nerve regeneration in a 10 mm sciatic nerve defect in rats
Lorenz SEMMLER (Medical University of Vienna; Austrian Cluster for Tissue Regeneration, Vienna)

P-39 Alterations of the leukotriene signaling pathway in aged and cognitively impaired rats
Jennifer FORSTER (Paracelsus Medical University, Salzburg; University of Salzburg)

P-43 The effect of low-energy extracorporeal shockwave treatment on the functional, morphological and molecular level in sub-acute and chronic phases of traumatic SCI
Alexander RÜHRNÖSSL (Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in AUVA Research Center, Vienna)

P-47 Insights into the development of a brain implant for local chemotherapy
Linda WALDHERR (Medical University of Graz)

P-51 Sex-dependent brain activity to anaesthetic ketamine exposure in mouse primary visual cortex
Ryan John A. CUBERO (Institute of Science and Technology Austria, Klosterneuburg)

P-55 Spinal locomotor circuits and their functionality in *Xenopus laevis*
Mara Jean JULSETH (Institute of Science and Technology Austria, Klosterneuburg)

P-59 **Medial entorhinal cortex integrates hippocampal offine inputs into independent, persistent memory representations**
Michele NARDIN (Institute of Science and Technology Austria, Klosterneuburg)

P-63 **The role of hippocampal VIP-expressing interneurons in the pathophysiology of temporal lobe epilepsy**
Sadegh RAHIMI (Medical University of Innsbruck)

P-67 **Spontaneous intrinsic spatiotemporal dynamics in superior colliculus**
Anton SUMSER (Institute of Science and Technology Austria, Klosterneuburg)

P-71 **Does the kappa-opioid receptor derived DREADD have a therapeutical potential in temporal lobe epilepsy?**
Melanie WIDMANN (Medical University of Innsbruck)

P-75 **Maternal high-fat diet during pregnancy and lactation provokes epigenetic modifications in the offspring brain**
Kinga GAWLINSKA (Maj Institute of Pharmacology Polish Academy of Sciences, Kraków)

P-79 **Long-term intranasal application of alarin is safe with no effects on food intake and body weight**
Sara HUBER (Paracelsus Medical University, Salzburg)

P-83 **The influence of Cox-1 on microglia reactivity after optic nerve crush**
Florianne SCHOOT UITERKAMP (Institute of Science and Technology Austria, Klosterneuburg)

P-87 **Improved grid-glued method of freeze-fracture replica labeling for molecular and structural identification of neuronal profiles**
Pradeep BHANDARI (Institute of Science and Technology Austria, Klosterneuburg)

P-91 **Molecular design of hypothalamus development**
Evgenii O. TRETIAKOV (Medical University of Vienna)

P-95 **Modeling rare neurodevelopmental disorders using human iPSC-derived neurons**
Venkat Swaroop ACHUTA (Medical University of Vienna; Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna; Austrian Academy of Sciences, Vienna)

12.30 – 14.00
GALLERY

Poster Session III & Lunch

Ion channels & neurotransmission
P-03 | P-07 | P-11 | P-15 | P-19

Cognition & behavior
P-23 | P-27 | P-31

Neurological disorders & regeneration
P-35 | P-39 | P-43 | P-47

Neural networks & activity
P-51 | P-55 | P-59 | P-63 | P-67 | P-71

New methods & disease models
P-75 | P-79 | P-83 | P-87

Stem cells & development
P-91 | P-95

14.00 – 15.00
LECTURE HALL 1

Minisymposium
Ion channels & channelopathies

Chair: Gerald OBERMAIR (Karl Landsteiner University Krems)

M-04 **Ion channel defects in the dystrophic heart**
Xaver KÖNIG (Medical University of Vienna)

M-05 **Deletion of α2δ-1 calcium channel subunit reduces Ca2+ influx and alters electrical excitability of catecholamine-secreting mouse chromaffin cells**
Petronel TULUC (University of Innsbruck)

M-06 ***CACNA1I* gain-of-function mutations differentially affect channel gating and cause neurodevelopmental disorders**
Yousra EL GHALEB (Medical University of Innsbruck)

14.00 – 15.00
LECTURE HALL 2

Minisymposium
EEG, MEG, and fMRI: hot new science in Cognitive Neuroscience

Chair: Manuel SCHABUS (University of Salzburg)

M-07 **The impact of smartphone use and short-wavelength light during the evening on circadian rhythm, sleep and alertness**
Christopher HÖHN (University of Salzburg)

M-08 **Neural speech tracking shifts from the syllabic to the modulation rate of speech as intelligibility decreases**
Fabian SCHMIDT (University of Salzburg)

M-09 **The role of the occipitotemporal cortex in normal and impaired readings**
Martin KRONBICHLER (University of Salzburg; Paracelsus Medical University, Salzburg)

15.00 – 15.15 Break

15.15 – 16.15
LECTURE HALL 1

Oral Presentations IV
Ion channels & neurotransmission

Chair: Bernhard FLUCHER (Medical University of Innsbruck)

O-13 **Presynaptic $GABA_B$ receptors induce phasic neurotransmitter release from medial habenula terminals**
Peter KOPPENSTEINER (Institute of Science and Technology Austria, Klosterneuburg)

O-14 **Interactions of tricyclic antipsychotic and antidepressant medications with a novel binding site in $GABA_A$ receptors**
Konstantina BAMPALI (Medical University of Vienna)

O-15 **Collaborative roles of calcium channel CaV1.1 and β-catenin in neuromuscular synaptogenesis**
Mehmet M. KAPLAN (Medical University of Innsbruck)

O-16 **Biophysical and pharmacological characterisation of de novo *CACNA1D* mutations associated with a severe neurodevelopment disorder**
Ferenc TÖRÖK (University of Innsbruck)

16.15 – 16.40 Coffee Break

16.40 – 17.00 **Pioneer in Austrian Neuroscience Award**
LECTURE HALL 1 **Awardee will be announced at the ceremony**
Chair: Francesco FERRAGUTI (Medical University of Innsbruck)
Sponsored by Siemens Healthineers

17.00 – 17.30 **Otto Loewi Prize Lecture**
LECTURE HALL 1 **Awardee will be announced at the ceremony**
Chair: Roman ROMANOV (Medical University of Vienna)
Sponsored by Peter und Traudl Engelhorn Stiftung

17.30 – 17.45 Break

17.45 – 18.45 **Keynote Lecture**
LECTURE HALL 1 **Deciphering the 'sulfation code' of the brain extracellular matrix**
K-03 Vanja NAGY (Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases (LBI-RUD); Research Centre for Molecular Medicine (CeMM) of the Austrian Academy of Sciences, Vienna; Medical University of Vienna)
Chair: Sofia GRADE (Institute of Molecular Biotechnology (IMBA) of the Austrian Academy of Science, Vienna)

19.00 – 20.00 **Public Lecture | Öffentlicher Vortrag**
LECTURE HALL 2 *GERMAN*
Kognitive Neurowissenschaften: Gedächtnis, Gehirnschwingungen und Bewusstsein
K-04 Wolfgang KLIMESCH (University of Salzburg)
Chair: Isabella SARTO-JACKSON (Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg)

08.30 – 09.30 **ANA General Assembly**
LECTURE HALL 2

09.30 – 10.30 **Keynote Lecture**
LECTURE HALL 1 **Will we ever cure neurodegenerative diseases?**

K-05 Ludwig AIGNER (Paracelsus Medical University, Salzburg)
Chair: Sandra SIEGERT (Institute of Science and Technology Austria, Klosterneuburg)

10.30 – 10.50 Coffee Break

10.50 – 11.50 **Oral Presentations V**
LECTURE HALL 1 **Stem cells & development**
Chair: Lora SWEENEY (Institute of Science and Technology Austria, Klosterneuburg)

O-17 **Mapping the macroscopic evolution of the neocortex in primates, rodents and related species – implications in ecology and behavior**
Ernst SCHWARTZ (Medical University of Vienna)

O-18 **Molecular characterization of non-cephalic sensory cells reveals a function of rhabdomeric Opsin in light modulation of mechanosensory neurons**
Florian RAIBLE (University of Vienna)

O-19 **Wnt/beta-catenin signalling is dispensable for adult neural stem cell homeostasis and activation**
Noelia URBÁN (Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna)

O-20 **Mechanisms of neuronal maturation in the adult and aging brain**
Maximilian REISINGER (Paracelsus Medical University, Salzburg; Austrian Cluster for Tissue Regeneration, Vienna)

11.00 – 15.00
LECTURE HALL 2

Satelliten-Symposium
Elementarpädagogik & Neurowissenschaften

GERMAN

Chair: Natascha TASLIMI (Netzwerk elementare Bildung Österreich (NeBÖ)); Isabella SARTO-JACKSON (Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg)

S-06 **Entwicklungsneurobiologie und Elementarpädagogik: Was wir wissen und was daraus folgt**
Manfred SPITZER (University of Ulm)

S-07 **Der Übergang vom Kindergarten in die Volksschule aus Perspektive der neurokognitiven Entwicklungspsychologie**
Karin LANDERL (University of Graz)

PODIUMSDISKUSSION
Martina KÜNSBERG SARRE (NEOS)
Sibylle HAMANN (Die Grünen)
Karin LANDERL (University of Graz)
Natascha TASLIMI (Netzwerk elementare Bildung Österreich (NeBÖ))
Monika UDE (Netzwerk elementare Bildung Österreich (NeBÖ))
Isabella SARTO-JACKSON (Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg)
Sigismund HUCK (Medical University of Vienna)

12.00 – 12.30
LECTURE HALL 1

Poster Flash IV

Chair: Andreas LIEB (Medical University of Innsbruck)

P-04 **Visualization of nanoscale phosphoinositide distribution on neuronal cell membranes**
Kohgaku EGUCHI (Institute of Science and Technology Austria, Klosterneuburg)

P-08 **Biochemical characterization of voltage-gated calcium channel α1-α2δ subunit interactions**
Manuel HESSENBERGER (Karl Landsteiner University of Health Sciences, Krems)

P-12 **Enantioselective drug-binding kinetics shape the psychostimulant effect of dopamine transporter inhibitors**
Marco NIELLO (Medical University of Vienna)

P-16 **Impact of partially duplicated α7 subunits on α7 nicotinic acetylcholine receptor function in human iPSC-derived neurons**
Gökce Ilayda SÖZTEKIN (Medical University of Vienna)

P-20 **Acetaldehyde blocks the activation of BK channels by the ethanol metabolite acetate in GH3 cells**
Thomas M. WEIGER (University of Salzburg)

P-24 **A predictive processing framework for single arm use in octopuses**
Sidney CARLS-DIAMANTE (University of Konstanz)

P-28 **Gender-affirming hormone therapy for transgender people makes sexual arousal in the ventral striatum more gender-congruent for lesbian scenes**
Manfred KLÖBL (Medical University of Vienna)

P-32 **Dynamic and state-dependent switching of behaviour in response to competing visual stimuli in *Drosophila***
Roshan Kumar SATAPATHY (Institute of Science and Technology Austria, Klosterneuburg)

P-36 **A rodent lumbosacral spinal cord injury model reflecting neurological and urological deficits of humans**
Behnaz AFRASHTEH (Paracelsus Medical University, Salzburg)

P-40 **Galanin receptors 2 and 3 modulate the inflammatory response following experimental traumatic brain injury**
Stefanie GAISBAUER (Paracelsus Medical University, Salzburg)

P-44 **MRI findings in atypical presentation of fibrous meningioma**
Antonio Gomes LIMA JUNIOR (Mount Sinai Hospital. New York)

P-48 **Unravelling the role of spinal astrocytes in nociception and pain**
Sibel ADA (Medical University of Vienna)

P-52 **Ca^{2+}-mediated adaptation of neuronal metabolism to neuronal electrical activity**
Ankit DHOUNDIYAL (Medical University of Vienna)

P-56 NMDA receptor subunit GluN2B c-terminus orchestrates hippocampal left-right asymmetry
David KLEINDIENST (Institute of Science and Technology Austria, Klosterneuburg)

P-60 Presynaptic modulation by cAMP-PKA pathway at hippocampal mossy fiber synapses
Yuji OKAMOTO (Institute of Science and Technology Austria, Klosterneuburg)

P-64 Ponto-genicular waves dynamics in the sleeping rat brain
Juan F. RAMIREZ-VILLEGAS (Institute of Science and Technology Austria, Klosterneuburg)

P-68 Anti-aversive drugs modulate inputs to the lateral parabrachial nucleus
Hannah Luise TEUCHMANN (Medical University of Vienna)

P-72 Using Xenopus to define tetrapod motor circuit cell types across evolution
Lora B. SWEENEY (Institute of Science and Technology Austria, Klosterneuburg)

P-76 Effect of modified maternal diets on expression of autism spectrum disorder-related genes in the offspring limbic areas
Dawid GAWLINSKI (Maj Institute of Pharmacology Polish Academy of Sciences, Kraków)

P-80 Dipeptide repeat protein toxicity and its contribution to DNA damage, nucleolar stress and heterochromatin dysregulation in ALS patients with underlying C9orf72 repeat expansions
Sophie IMHOF (Medical University of Vienna)

P-84 Using synthetic biology to dissect G protein-coupled receptor signaling in microglia
Rouven SCHULZ (Institute of Science and Technology Austria, Klosterneuburg)

P-88 Developmental regulation of coupling between presynaptic Ca^{2+} channels and release sensors at basket cell–Purkinje cell synapses in cerebellum
Jingjing CHEN (Institute of Science and Technology Austria, Klosterneuburg)

P-92 Two light sensors decode moonlight versus sunlight to adjust a plastic circadian clock to moon phase
Martin ZURL (University of Vienna)

12.30 – 14.00
GALLERY

Poster Session IV & Lunch

Ion channels & neurotransmission
P-04 | P-08 | P-12 | P-16 | P-20

Cognition & behavior
P-24 | P-28 | P-32

Neurological disorders & regeneration
P-36 | P-40 | P-44

Neural networks & activity
P-48 | P-52 | P-56 | P-60 | P-64 | P-68 | P-72

New methods & disease models
P-76 | P-80 | P-84

Stem cells & development
P-88 | P-92

14.00 – 15.00
LECTURE HALL 1

Minisymposium
Clinical neuroscience: epilepsy

Chair: Eugen TRINKA (Uniklinikum Salzburg)

M-10 **Tuberous sclerosis complex (TSC) as disease model for investigating mTOR-related epileptogenesis – identification of potential new targets of therapy**
Martha FEUCHT (Medical University of Vienna)

M-11 **Clinical antiepileptogenesis trials – time for translation?**
Eugen TRINKA (Uniklinikum Salzburg)

PANEL DISCUSSION

15.00 – 15.15 Break

15.15 – 15.45 **Best Thesis Lecture**
LECTURE HALL 1 **Investigating the neuronal basis of magnetoreception in the pigeon**
S-08 Simon NIMPF (Research Institute of Molecular Pathology (IMP), Vienna)
Chair: Johannes PASSECKER (Medical University of Innsbruck)

15.45 – 16.15 **Award Ceremony**
LECTURE HALL 1 Chair: Bruno BENEDETTI (Paracelsus Medical University, Salzburg)
Best Poster Awards
Best Oral Presentation Prizes
Best Poster Flash Prizes

16.15 – 16.30 **Acknowledgments & Farewell**
LECTURE HALL 1 Sébastien COUILLARD-DESPRÉS (Paracelsus Medical University, Salzburg)
Isabella SARTO-JACKSON (Konrad Lorenz Institute for Evolution and Cognition Research, Klosterneuburg)

Abstracts of Keynote Lectures

Keynote Lectures

K-01 Cognition in altered states of consciousness

Manuel Schabus
University of Salzburg, Department of Psychology & Centre for Cognitive Neuroscience Salzburg (CCNS)

In our research we use electrophysiological methods (hdEEG and MEG) in order to better understand how the brain works in „altered states of awareness". In particular, we address the question of what still can be processed by the human mind in the full absence of awareness. I am therefore particularly interested in information processing during (i) sleep, during (ii) prenatal learning, (iii) in the newborn and also in pathological states like post-comatose "Disorders of Consciousness". One of the most exciting findings is that the brain can perform astonishingly complex tasks even in apparently fully "unconscious" states, that is for example auditory processing of complex stimuli even in deep NREM and dreaming REM sleep. Last but not least we are interested in translating our knowledge into practice and work on eHealth solutions for more healthy sleep in disturbed sleepers or on learning during sleep in healthy individuals. In my talk I will try to give an overview of that research and allow insights into the fascinating world of cognitive neuroscience on a "systems" level.

K-02 The search for young neurons in the adult mammalian brain: history, twists and furture perspectives

Luca Bonfanti
Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Italy

Plasticity, and in particular, neurogenesis, is a smart tool used by the nervous system to build up and sculpt its neural circuits throughout life on the basis of experience. It is also a promising target to prevent and treat a wide variety of diseases (e.g., dementia). The discovery of stem cell-driven adult neurogenesis in mammals has opened the possibility to have "new neurons" in the rather "static" brain. Nevertheless, adult neurogenesis is a sort of exception, being restricted to very small neurogenic sites, dramatically decreasing with age and likely to be reduced in humans. In fact, there are different types of plasticity, which vary with age, brain region, and species, representing theme variations not easy to identify in the dynamic complexity of brain structural plasticity. One of these consists of "immature" neurons that are generated prenatally, then persisting in an immature state for long time, with the possibility to mature and integrate in the pre-existing neural circuits. This process, provisionally called "neurogenesis without division", remarkably vary among mammals and

might represent an evolutionary "smart" trick to place new neurons in brain regions not endowed with stem/progenitor cells (e.g., the cerebral cortex), especially in large-brained species. These "young" neurons are emerging in the heterogeneous landscape of brain plasticity as a fully new field of research, which has been slowed down by the subtle similarities existing between these cells and the newly generated neurons.

K-03 Deciphering the 'sulfation code' of the brain extracellular matrix

Vanja Nagy

Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases (LBI-RUD), Vienna; Research Centre for Molecular Medicine (CeMM) of the Austrian Academy of Sciences, Vienna; Department of Neurology, Medical University of Vienna (MUW), Vienna

Background:

It has long been established that the extracellular matrix (ECM) intimately participates in embryonic and postnatal development and function of the brain. It is largely composed of chondroitin sulfate proteoglycans (CSPGs), glycoproteins with covalently attached sugar polymers called glycosaminoglycans (GAGs). GAGs are postranslationally modified by attachment of a sulfate group at different positions of the disaccharide subunits in a template-free manner, orchestrated by sulfonyl transferases. It is believed that this sulfation pattern, also known as the 'sulfation code', is an instructive molecular signature critical during discrete developmental periods (critical periods) of the brain. It is not clear, however, how neurons detect the sulfation code. Indeed, while receptors with high affinities for differentially sulfated GAGs have been reported, how (and whether) their known neuronal function is related to their GAG recognition has not yet been elucidated. . Here we report a relatively uncharacterized receptor, FIBCD1, to recognize a specifically sulfated GAG on glycosaminoglycans in mouse and human brain.

Methods:

We generated Fibcd1 knock-out mouse and knock-down fly models and validated our findings in CRISPR-Cas9 edited H9 human ES-derived cortical neurons.

Results:

Using behavioral, electrophysiological, cellular and molecular techniques in knock-out mouse and knock-down fly models we demonstrate that Fibcd1 is a conserved component of cellular sulfation code recognition and is crucial for neuronal function. Furthermore, we find that FIBCD1 is also highly expressed in the human foetal and adult brain, and seems to regulate human CNS development and function.

Discussion:

Our studies shed light on the fundamental neuronal ability to sense the changing landscape of the biochemical properties of the brain ECM, and elucidate the critically important biological function of an evolutionary conserved lectin receptor, previously unsuspected to have any function in the brain.

K-04 Kognitive Neurowissenschaften: Gedächtnis, Gehirnschwingungen und Bewusstsein

Wolfgang Klimesch
University of Salzburg, Centre for Cognitive Neuroscience Salzburg (CCNS)

Die kognitiven Neurowissenschaften wurden in den 70er und 80er Jahren des vergangenen Jahrhunderts von mehreren Forschergruppen gegründet. Im Vordergrund stand die funktionelle Anatomie ('was passiert wo im Gehirn?'), die sich durch die damals neuen Methoden der regionalen zerebralen Durchblutung und der funktionellen Kernspintomographie gut untersuchen ließen. Bald wurde aber klar, dass eine rein funktionell-anatomische Beschreibung des Gehirns unbefriedigend ist: Es fehlte der systemische Ansatz. Die – geschichtlich gesehen – parallele Erforschung der funktionellen Bedeutung von Gehirnwellen (welche elektromagnetischen Frequenzen - untersucht mit dem EEG und MEG – haben welche Bedeutung?) führte zu völlig neuen Einsichten in die zeitliche Verarbeitungsstruktur des Gehirns, die einem systemischen Ansatz sehr nahe kommen und der es erlaubt, die zeitliche und die örtliche Verarbeitung im Gehirn gemeinsam zu verstehen. Die Bedeutung dieser Forschungsergebnisse für höhere kognitive Funktionen und des menschlichen Bewußtseins werden diskutiert.

K-05 Will we ever cure neurodegenerative diseases?

Ludwig Aigner
Institute of Molecular Regenerative Medicine, Paracelsus Medical University Salzburg; Spinal Cord Injury and Tissue Regeneration Center Salzburg, Paracelsus Medical University, Salzburg

Most neurodegenerative disease are complex diseases. That means that genetic as well as non-genetic factors are at their origin. A minor percentage of neurodegenerative diseases are purely genetic, and for these, gene therapy approaches are currently under development and might provide a cure, especially in the context of personalized medicine. For the vast majority of neurodegenerative diseases, the cause is still unclear, but many factors and systems contribute to the disease. These are, among others, neuronal cell death, neuroinflammation, blood brain barrier leakage, gliosis and scarring, and systemic and peripheral factors such as adaptive immunity and the sytemic mileu, and even the microbiome. Considering this, I hypothesize that by addressing only one of these contributing factors, which is what the community has done over the past decades, will not provide a cure for patients with neurodegenerative diseases. In consequence, we need to target neurodegenerative diseases by multimodal approaches, which address the various mechanisms that are involved. Future research will hopefully identify the cause of neurodegenerative diseases at the personalized level, which might be the base for individual cures.

Abstracts of Special Lectures & Satellite-Symposium

Special Lectures

S-01 Discover EBRAINS

Steven Vermeulen
CIIO, EBRAINS AISBL

EBRAINS is a new digital research infrastructure (RI), created by the EU-funded Human Brain Project (HBP), that gathers an extensive range of data and tools for brain-related research.

It draws on cutting-edge neuroscience, big data, computing, robotics and related technologies to help translate the latest scientific discoveries into innovation in medicine and industry, for the benefit of patients and society.

EBRAINS' ambition is to provide the scientific community at large with an open state-of-the-art capability that fosters collaborative brain science, opens the way to ground-breaking discovery and aims to secure Europe's leading position in the dynamically growing field of multidisciplinary brain research and its exploitation.

EBRAINS aims at accelerating collaborative brain research with a comprehensive set of services for the research community. In this talk, EBRAINS will give a general overview of its service categories and address the importance of combining these services as a game-changer, enabling brain researchers to tackle their questions effectively and allowing other disciplines to benefit from brain research.

S-02 Super-resolution optical microscopy for analysing nervous tissue across scales

Johann Danzl
Institute of Science and Technology Austria, Klosterneuburg

The incredibly complex arrangement of neurons and their synaptic connections in brain tissue forms the basis for the brain's information processing capabilities. Light microscopy excels at following the time evolution of living systems, visualizing signalling activity, and highlighting specific molecules. However, its spatial resolution is classically limited by diffraction of light waves to about half the wavelength of light (~200 nm), which is too coarse to decode the details of brain tissue structure or the molecular architecture of synapses. Super-resolution optical imaging approaches provide resolution much better than the optical diffraction limit and have enabled unexpected insights into key aspects of neuronal organization. I will introduce some of the tools that modern light microscopy offers to neuroscientists, including stimulated emission depletion (STED) microscopy, increasing resolution by optically controlling fluorophore states, and expansion microscopy, reaching effective nanoscale resolution by embedding the sample in a swellable hydrogel and isotropically

expanding the tissue-hydrogel hybrid. I will discuss how we strive to improve these technologies, inspired by specific unmet needs in biological measurement technology for neuroscience, unravelling synaptic to cellular and tissue architecture in a molecularly informed way.

S-03 Active zone long-term plasticity in behavioural control of *Drosophila*

Stephan Sigrist
Freie Universität Berlin

Homeostatic synaptic plasticity is an adaptive form of plasticity, which serves to maintain transmission strength in response to altered pre- or postsynaptic function. It is conserved from invertebrates through humans, but perhaps best illustrated in Drosophila NMJ synapses. Homeostatic presynaptic plasticity at the active zones (AZs) of NMJs can be triggered by the application of a glutamate receptor blocker Philanthotoxin (PhTx), resulting in a compensatory enhancement of presynaptic neurotransmitter release. This homeostatic plasticity increases both the release probability for docked SVs at existing release sites as well as the number of new functional release sites. Using STED super-resolution light microscopy, we revealed a molecular sequence promoting release site addition. Within 10-20 minutes after PhTx treatment, numbers of BRP scaffold protein nanoclusters increased, likely representing the addition of discrete SV release sites to pre-existing AZs. This AZ remodeling we found is likely especially critical for sustained homeostatic plasticity.

We recently also established intravital single molecule imaging of endogenously-tagged Ca2+ channels together with the lab of Martin Heine (Mainz) using single particle tracking photoactivation localization microscopy ("sptPALM") imaging. Notably, PhTx application here triggered a "freezing" of individual Ca2+ channel and a compaction of Ca2+ channels on population level, potentially mediated via the extensive remodeling of the AZ BRP-matrix.

In result, we seek to arrive at a detailed nanoscopically resolved scenario of how presynaptic AZs, which in their molecular organization are fairly conserved, remodel in order to mediate behavioral adaptations.

S-04 The real power of brain networks: community driven innovation

The Brainstorms Scientific GmbH
Vienna

Good scientific research has a meticulous process in which results are pixels of an intricated image, gathered bit by bit. However, the more data is gathered, the harder it gets to see the big picture. Nevertheless, the journey is not in vain: experimental work generates valuable knowledge, methods and insights. An intellectual property that can be efficiently applied if you look at your work with someone's "new eyes", from a different perspective. It's not a

question that tackling the incredible complexity of neuroscience requires various expertise of many fields. While collaboration and transdisciplinarity are encouraged in academia, working overtime, often isolated from each other leaves us with little space to have an open mind and follow other disciplines' breakthrough, let alone reach out for help to them.

In this session, we will show you how ideas around the world are turned into exciting and surprising products at the service of patients and technological progress through collaboration. From brain-inspired AI to COVID sniffing robots, from human optogenetics to soft gel brain electrodes and wearables, the possibilities are endless. We will also talk about how we built the Brainstorms, our global neuroscience innovation network with thousands of scientists from 50+ countries and how this network is used to speed up breakthroughs. Finally, we'll give you tips how you can use it to make your own research actionable and join the neurotech revolution with the Brainstorms.

S-05 Explaining consciousness? - Searching common ground between neuroscience and philosophy

Markus Kunze 1, Georg Gasser 2, Thomas Bugnyar 3, Stephen Müller 4, Rupert Lanzenberger 5, Daniel Wehinger 6, Isabella Sarto-Jackson 7

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Neuroscientists and philosophers share interests in human experience, cognition and thinking, but their approaches differ drastically in the methods, the argumentation, but most importantly in what the respective field considers a valid or justified argument. Consequently, interdisciplinary exchange as a joint approach to foster better understanding of a common research topic is inherently different, but can be exceptionally fruitful and rewarding when put into praxis. Thus, this symposium is devoted to practice such interdisciplinary discussion using the phenomenon of consciousness as the general theme. On the one hand, consciousness has been amply investigated by neuroscientific studies and is implicitly presumed when performing behavioral studies in humans and complex model organisms, but on the other hand, the phenomenon of consciousness has attracted philosophical curiosity across many centuries and thus conceptual frameworks complementary to the empirical approaches have been developed. In this symposium, we will explore concepts of consciousness for their ability to integrate very different modes of consciousness ranging from those of non-mammalian animals or very young children to those of adults, but also to cope with different ways of accessing consciousness either by introspection, but also by quantifying experimental approaches.

In the first session, we will search for a proper description of the phenomenon of consciousness, which starts from personal experience, but takes the

whole width of manifestations into consideration and relates them to other forms of consciousness-like phenomena. Introducing a basic terminology and a conceptual framework the philosopher Georg Gasser will discuss the close link between consciousness and bodily existence, in which consciousness and body are not two separate concepts but are complementarily related to each other. This does not lead to a reduction of consciousness to the bodily processes but to the insight that we are both, embodied and conscious individuals. This implicates that human consciousness can only be adequately understood by considering the human body and the human body can only be adequately understood by considering human consciousness. The cognitive biologist Thomas Bugnyar argues from an evolutionary perspective starting from the observation that consciousness is considered characteristic for humans, but is typically questioned for non-human animals, which tend to be guided by instinctive responses to environmental stimuli. However, sophisticated cognitive skills like forms of reasoning and mental time travel such as episodic memory and future planning can be found in a variety of non-human animals where they probably serve to achieve high behavioral flexibility. It is still unclear, whether such skills are mediated by consciousness, partially due to difficulty of studying consciousness in animals. Characteristic approaches are the independent investigation of building blocks like emotions, self-recognition, reasoning, mental time travel with a set of tailored behavioral experiments and to measure neural correlates while animals are engaged in these tasks. Comparing the results of such tests between species of different taxonomic groups aims at identifying the elements of consciousness in these animals and to relate different levels of consciousness with the environmental conditions fostering their selection. Finally, the cognitive scientist Stephen Müller will reflect on the question, what evidence for consciousness obtained in animal experiments (e.g. in crows) actually means and under which presumptions such experiments can be related to human consciousness. This will allow our construal of full-blown everyday human consciousness to be contrasted with evidence for it in animals.

In the second session, we will discuss the relation between higher order cognitive abilities in humans and measurable activities observed in the material foundation, namely neuronal activity in the brain. Usually, neuroscientists conceptualize consciousness as biological phenomenon as many others and thus presuming that the todays observation of neural correlates of consciousness will (on the long run) be converted into a mechanistic explanation how consciousness emanates from its material substrate. The clinical neuroscientist Rupert Lanzenberger will present recent results from studies in psychiatry, neurology, neurosurgery and psychopharmacology. Using cutting-edge technologies as functional MRI and molecular neuroimaging with PET, electrical brain/cortex stimulation in awake patients during neurosurgery, and challenges with consciousness altering drugs (e.g., LSD, psilocybin, ketamine) he will question classical views regarding the first/third order perspectives and limitations of conscious experience. With regards to such results, some

philosophers have objected that consciousness escapes pure observation from a third person perspective, because qualitative experience from the first order perspective is a necessary prerequisite of conscious experience. Thus, an explanatory gap exists. In this context, the philosopher Daniel Wehinger asks, what specifically an explanation of consciousness needs to provide to satisfy the demands of neuroscientists and philosophers, respectively. For that purpose, he tries to uncover implicit presuppositions on both sides by first asking what specifically is asked for when the term "consciousness" is to be explained. Furthermore, he will address the question of what qualifies an explanation as good and which property of the concept of consciousness can be addressed.

S-06 Entwicklungsneurobiologie und Elementarpädagogik: Was wir wissen und was daraus folgt

Manfred Spitzer
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Das Gehirn kann sich nur durch Auseinandersetzung mit der Umwelt, der physikalischen und vor allem sozialen Umwelt, entwickeln. Es ist im Laufe der Evolution des Menschen entstanden, um Kultur aufzunehmen und weiterzugeben - vielleicht DIE wichtigste Besonderheit des Menschen im Vergleich zur Tierwelt. Anhand von Beispielen aus den Bereichen Sprachentwicklung, kognitive Entwicklung und soziale Entwicklung wird gezeigt, wie das Gehirn von Kindern auf kulturelle Inhalte reagiert und welche Muster verarbeitet werden müssen, um die jeweiligen Entwicklungsschritte zu vollziehen. Anhand der hierzu vorhandenen medizinisch-wissenschaftlichen Literatur wird dies aus entwicklungsneurobiologischer Sicht anhand der Beispiele diskutiert.

S-07 Der Übergang vom Kindergarten in die Volksschule aus Perspektive der neurokognitiven Entwicklungspsychologie

Karin Landerl
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Lesen, Schreiben und Rechnen sind zentrale Lerninhalte in den ersten Schuljahren und stellen wesentliche Basiskompetenzen für weitere Bildungsverläufe dar. Schriftsprache und mathematische Kognition basieren auf spezifischen neurokognitiven Netzwerken. Die Ausbildung und Spezifizierung dieser neurokognitiven Systeme dauert viele Jahre und hängt wesentlich von Anregung und Unterricht ab. Bestimmte neurokognitive Kernmechanismen für diese Netzwerke haben eine neurobiologische Grundlage und ihre Entwicklung kann bereits im Kindergarten beobachtet werden. Phonologische Bewusstheit, also das explizite Verständnis, dass Sprache aus Lauten besteht, und die Fähigkeit Abfolgen von Bildern oder Ziffern schnell zu benennen sind signifikante Prädiktoren der späteren Schreib- und Leseleistung. Die Entwicklung der Rechenleistungen wird durch das frühe Zahlenverständnis

prädiziert. Auf diesen Forschungsbefunden basiert ein neu entwickeltes förderorientiertes Screening für die Schuleingangsphase. Dieses Screening soll Elementarpädagog*innen bei der Einschätzung des individuellen Entwicklungsstandes bildungsrelevanter kognitiver Fähigkeiten unterstützen, damit Kinder frühestmöglich gezielte und individualisierte Anregung und Förderung erhalten können. Die spielerisch gestalteten Aufgaben des Screenings können digital am Tablet oder in Buchform durchgeführt werden. Das Screening wird derzeit im Rahmen einer umfangreichen Längsschnittstudie, in der wir Kinder vom letzten Kindergartenjahr bis zum Ende der 2. Klasse in ihrer Lernentwicklung begleiten, empirisch validiert und für den Einsatz zum Zeitpunkt der Schuleinschreibung und am Beginn der 1. Klasse normiert. Der Einsatz derartiger Screenings setzt umfassendes Wissen über die Variabilität und Komplexität frühkindlicher Entwicklungsverläufe voraus. Regelmäßiges Monitoring der individuellen Lernverlaufsentwicklung ist in dieser Entwicklungsphase besonders wichtig und kann helfen, systemische Schwierigkeiten an der Schnittstelle Kindergarten – Schule gut zu überbrücken.

S-08 Investigating the neuronal basis of magnetoreception in the pigeon

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The remarkable ability of animals to navigate over long distances is mediated by the sensory perception of the Earth's magnetic field. Behavioral experiments on a large number of taxonomically diverse species support the existence and utilization of magnetoreceptive systems, however the underlying sensorineural structure mediating this unusual sense remains elusive. In this thesis I set out to investigate where and how a magnetic stimulus might be transduced into a neuronal impulse and how this information is integrated in the central nervous system of pigeons.

Employing neuronal activity mapping I report that exposing pigeons to rotating magnetic fields leads to increased activity in the brainstem vestibular nuclei and the hippocampus of pigeons. Physical calculations and modeling further support the hypothesis that magnetic fields might be detected by voltage sensitive ion channels in the semicircular canals of the vestibular system through a process called electromagnetic induction (Nimpf, Nordmann et al., Current Biology, 2019). Using a newly established in vivo 2-photon calcium-imaging set-up, I provide additional preliminary evidence for magnetosensitive neuronal populations in the pigeon hippocampus. Finally, I investigated the molecular machinery associated with the formation, development and function of an iron-rich organelle in pigeon sensory hair cells and its potential involvement in magnetoreception (Nimpf et al., eLife, 2017). Taken together, these data support the hypothesis that magnetic field information might be detected in the pigeon inner ear and relayed to higher order brain structures for central integration.

Abstracts of Minisymposia

Minisymposia

Epigenetic Processes in Brain Development and Aging

M-01 Distinct and sequential functions of PRC2 in radial glia lineage progression

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Background:
Radial glial progenitor cells (RGPs) generate different populations of neocortical projection neurons, glia and adult neural stem cells. We recently uncovered sequential temporal transcriptional fingerprints in RGPs correlating with their lineage progression during cortical neurogenesis, and RGP proliferation behavior appears to be controlled by PRC2-mediated H3K27me3. Yet, the mechanism how PRC2 instructs RGP lineage progression in vivo remains elusive.
Methods:
Here we utilized Mosaic Analysis with Double Markers (MADM) to genetically dissect the cell-autonomous and non-cell-autonomous function of the PRC2 core component Eed.
Results:
Our genetic loss-of-function approaches show that global tissue loss of Eed results in precocious depletion of RGPs and strong microcephaly. However, we reveal that Eed does not regulate RGP behavior and neuron output in a cell-autonomous manner at single cell level. Furthermore, we discover a novel cell-autonomous Eed function which is essential for cortical astrogliogenesis. On the transcriptional level, absence of PRC2 activity from astrocytes correlates with downregulation of genes implicated in proliferation and synapse formation. Accordingly, cell-autonomous loss of PRC2 function results in reduced proliferation rates, impaired surface expansion and reduced complexity of mutant astrocytes.
Discussion:
Altogether, our data reveal distinct sequential requirements of Eed and thus PRC2-mediated H3K27me3 in RGP lineage progression during cortical development and an essential role in cortical astrocyte production and maturation.

M-02 Direct neuronal reprogramming to study aging and disease

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Background:
Old age is a major risk factor for many human diseases. Alzheimer's Disease (AD) represents a prime example, as it exclusively affects people at old age. Sporadic AD represents the overwhelming majority of all cases, and familial genetically defined early-onset cases are rare. Still, most research on AD has been performed on genetic causes and their directly related pathways, also because we were in lack of models that can reflect complex human genetics, physiology, and age in an appropriate human neuronal context.
Methods:
While patient-specific iPSC-based models represent an attractive solution, iPSC reprogramming results in cellular rejuvenation and thus yields phenotypically young neurons. By contrast, direct conversion of old patient fibroblasts into induced neurons (iNs) preserves endogenous signatures of aging. To control for the involvement of aging in human neuronal models for AD, we combined both technologies and generated age-equivalent fibroblast-derived iNs, as well as rejuvenated iPSC-derived neurons from a large cohort of AD patients and controls.
Results:
In addition to their rejuvenated state, we found that iPSC neurons transcriptionally resemble prenatal developmental stages, while iNs reflect adult-like neuronal stages and show little correlation with the prenatal brain. Thus not surprisingly, only age-equivalent adult-like iNs, but not rejuvenated prenatal-like iPSC neurons, revealed strong AD patient-specific signatures. Our iN model further revealed high concordance with previous human post-mortem AD studies.
Discussion:
Sporadic AD patient-specific iNs highlight a pathological de-differentiated hypo-maturity state of AD neurons as a major phenotype that might underlie many hallmark pathologies typically observed in the human AD brain.

M-03 Life-long epigenetic programming of cortical architecture by maternal 'Western' diet during pregnancy

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Background:
The evolution of human diets led to preferences toward polyunsaturated fatty acid (PUFA) content with 'Western' diets enriched in ω-6 PUFAs. Mounting evidence points to ω-6 PUFA excess limiting metabolic and cognitive processes that define longevity in humans. When chosen during pregnancy, ω-6 PUFA-enriched 'Western' diets can reprogram maternal bodily metabolism with maternal nutrient supply precipitating the body-wide imprinting of molecular and cellular adaptations at the level of long-range intercellular signaling networks in the unborn fetus. Even though unfavorable neurological outcomes are amongst the most common complications of intrauterine ω-6 PUFA excess, cellular underpinnings of life-long modifications to brain architecture remain unknown.
Methods:
Female C57Bl6/J, cholecystokinin (CCK)$^{BAC/DsRed}$ and CCK$^{BAC/DsRed}$::GAD67$^{gfp/+}$ transgenic mice were randomly assigned to either a hypercaloric diet enriched in ω-6 PUFAs or to a standard diet. The effect of maternal diets on fetal brain development was evaluated at E18.5 after both the 'priming protocol' (consuming a high ω-6 PUFA diet starting 2 weeks prior to conception) or 'programming protocol' (consuming a high ω-6 PUFA diet 8 weeks prior to conception). Global proteomic analysis, DNA methylation and open chromatin mapping were performed to search for molecular determinants underpinning neuronal deficits.
Results:
We show that nutritional ω-6 PUFA-derived endocannabinoids desensitize CB1 cannabinoid receptors, thus inducing epigenetic repression of transcriptional regulatory networks controlling neuronal differentiation. We found that cortical neurons lose their positional identity and axonal selectivity when mouse fetuses are exposed to excess ω-6 PUFAs in utero. Conversion of ω-6 PUFAs into endocannabinoids disrupted the temporal precision of signaling at neuronal CB1 cannabinoid receptors, chiefly deregulating Stat3-dependent transcriptional cascades otherwise required to execute neuronal differenti-

ation programs. Global proteomics identified the immunoglobulin family of cell adhesion molecules (IgCAMs) as direct substrates, with DNA methylation and chromatin accessibility profiling uncovering epigenetic reprogramming at >1400 sites in neurons after prolonged cannabinoid exposure. We found anxiety and depression-like behavioral traits to manifest in adult offspring, which is consistent with genetic models of reduced IgCAM expression, to suggest causality for cortical wiring defects.

Discussion:

In the present report, we show transgenerational consequences of increasing the ω-6 PUFA nutritional content during pregnancy in mice on the development of the offsprings' nervous system. We outline that a critical mechanism involved is the engagement of the endocannabinoid system. As such, nutritional ω-6 PUFA-derived endocannabinoids desensitize CB1Rs thereby altering neurogenesis, neuroblast commitment to the cerebral cortex and the formation of axonal connectivity. We suggest a link between long-lasting changes of cortical architecture and epigenetic repression of regulatory transcription factor networks downstream from CB1Rs that control neuronal differentiation. The finding that many of the transcription factor networks associated with axonal growth are epigenetically controlled by endocannabinoids might be relevant to public health in view of the ever-increasing world-wide pandemic of excess 'Western' diet and ensuing metabolic and behavioral dysfunctions.

Ion Channels and Channelopathies

M-04 Ion channel defects in the dystrophic heart

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Duchenne muscular dystrophy (DMD) is a devastating disease characterized by progressive muscle weakness and degeneration. Besides the relatively well characterized skeletal muscle pathology, DMD is associated with cardiomyopathy development and the occurrence of cardiac arrhythmias. Respective pathomechanisms are only poorly understood but emerging evidence suggests that impaired ion channel function plays an important role.

DMD is caused by mutations in the gene encoding for the large cytoplasmic protein dystrophin (DYS). DYS is a pivotal part of a structural protein complex that links the actin cytoskeleton to the extracellular matrix. At the sarcolemma DYS is known to interact with various ion channels either directly or indirectly. Consequently, loss of DYS is associated with altered ion channel expression and function. Abnormalities in sodium, calcium, and potassium channels, including the isoforms Nav1.5, Cav1.2, and Kir2.x, have been reported amongst others, which translated to altered action potential waveforms and electrocardiogram parameters. Some ion channel defects manifest already prior to cardiomyopathy development and may thus be considered a potential disease trigger. Ion channel defects are observed in the working myocardium

but also penetrate into the cardiac conduction system where they represent a potential source of lethal cardiac arrhythmias.

In conclusion, functional abnormalities of key cardiac ion channels caused by the absence of DYS are likely involved in disease development and the generation of cardiac arrhythmias in DMD patients.

M-05 Deletion of α2δ-1 calcium channel subunit reduces Ca2+ influx and alters electrical excitability of catecholamine-secreting mouse chromaffin cells

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Background:

High voltage gated calcium channels (HVCC) shape the electrical activity and controls hormone release in most endocrine cells. HVCCs are multi-subunit protein complexes formed by the pore forming β1 and the auxiliary α2δ and γ subunits. Previously, we and others have shown that α2δ subunits controls α1 membrane incorporation and biophysical properties in many cell types. Four genes encode for α2δ subunits with α2δ-1 being the isoform with the highest expression level in endocrine cells.

Methods:

Here we report the functional characterization of the role of α2δ-1 HVCC subunit in mouse chromaffin cell electrical activity and hormone release.

Results:

α2δ-1 genetic deletion did not affect adrenal gland medulla size or morphology however it led to ~60% smaller HVCC Ca2+ currents with slower inactivation kinetics. Pharmacological dissection demonstrated that HVCC composition remained similar in $\alpha2\delta\text{-}1^{-/-}$ MCCs compared to WT demonstrating that the altered kinetics are caused by changes in HVCC biophysical properties and not an isoform switch. The reduced HVCC Ca2+ influx altered the action potential (AP) properties with $\alpha2\delta\text{-}1^{-/-}$ MCCs showing a lower AP peak amplitude, and faster rising and decay slopes resulting in shorter half-maximal AP duration. Interestingly, while the spontaneous activity was reduced the induced electrical activity showed a higher frequency and plateau potential in $\alpha2\delta\text{-}1^{-/-}$ MCCs compared to WT despite a similar rheobase. Additionally, capacitance measurements demonstrated that following a 500ms Ca2+ preloading step the readily releasable pool and total vesicle exocytosis and endocytosis were unaltered in $\alpha2\delta\text{-}1^{-/-}$ compared to WT MCCs.

Discussion:

Our study suggests that α2δ-1 deletion leads to increased catecholamine secretion due to increased excitability and preserved vesicle exocytosis despite a lower Ca2+ influx.

M-06 *CACNA1I* gain-of-function mutations differentially affect channel gating and cause neurodevelopmental disorders

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Background:

T-type calcium channels (Cav3.1 to Cav3.3) regulate low-threshold calcium spikes, burst firing and rhythmic oscillations of neurons and are involved in sensory processing, sleep, and hormone and neurotransmitter release. In this study, we examined four heterozygous missense variants in the CACNA1I gene, encoding the Cav3.3 channel, in patients with variable severity of epilepsy and/or intellectual disability (ID).

The p.(Ile860Met) variant, affecting a residue in the putative channel gate at the cytoplasmic end of the IIS6 segment, was identified in three family members with variable cognitive impairment. The de novo p.(Ile860Asn) variant, changing the same amino acid residue, was found in a patient with severe developmental delay and seizures. The two additional variants examined in this study were found in individuals with global developmental delay and epilepsy, p.(Ile1306Thr) and p.(Met1425Ile), substituting residues at the cytoplasmic ends of IIIS5 and IIIS6, respectively.

Methods:

Structure modelling indicated that the amino acid substitutions differentially affect the flexibility of the channel gate, we analysed possible effects on Cav3.3 channel function by using patch-clamp analysis in HEK293T cells and mouse chromaffin cells. Additionally we examined the effect of the variants on excitability by using a computer model of thalamic reticular nuclei (TRN) neurons.

Results:
The mutations resulted in slower kinetics of current activation, inactivation, and deactivation, as well as in hyperpolarizing shifts of the voltage-dependence of activation and inactivation. Cav3.3-I860N consistently showed the strongest and Cav3.3-I860M the weakest effect. Structure modelling predicts that the mutations introduce stabilizing hydrogen bonds that slow the kinetics of the channel gate, and cause the gain-of-function effect in Cav3.3 channels. The gating defects resulted in left-shifted and increased window currents, causing an increased calcium influx during repetitive action potentials and even at resting membrane potentials. This increased calcium influx could lead to calcium toxicity in developing neurons that express the Cav3.3 variants, which is a plausible cause of the neurodevelopmental phenotype. Computer modelling of TRN neurons indicated that the altered gating properties of the Cav3.3 disease variants result in a lower firing threshold and an increased burst duration and frequency of action potential firing. Expressing the Cav3.3-I860N/M mutants in excitable mouse chromaffin cells resulted in a shift in the firing mode from low-threshold spikes and rebound burst firing observed in wildtype Cav3.3 to slow oscillations for Cav3.3-I860N and an intermediate firing mode for Cav3.3-I860M.
Discussion:
Such neuronal hyper-excitability could explain seizures in the epilepsy patients. Thus, our study links CACNA1I gain-of-function mutations to neurodevelopmental disorders, with a phenotypic spectrum ranging from mild ID to a severe neurodevelopmental disorder with epilepsy.

EEG, MEG, and fMRI: Hot New Science in Cognitive Science

M-07 The impact of smartphone use and short-wavelength light during the evening on circadian rhythm, sleep and alertness

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Background:
Smartphone ownership and exposure to smartphones has strongly increased over the last decade, especially during the late evening. There is already growing evidence that short-wavelength light in general can affect circadian timing, alertness and sleep. However, research on the impact of artificial light during the evening, which is emitted by smartphones is still limited and even less is known about the protective functions and effects of blue light filtering software as a countermeasure to short-wavelength light exposure.
Methods:
Data were recorded in a within-subjects crossover study from 33 healthy men

(21.70 ± 1.91 years), who spent one adaptation night and three experimental nights in the sleep laboratory. On each experimental night, participants had to read for 90 min either on a smartphone (1) without or (2) with a blue light filter, or (3) a printed book during the late evening before bedtime. Before and after reading, as well as in the next morning, sleepiness, alertness and melatonin concentration were measured. Additionally, EEG data was recorded during all tasks as well as during reading and sleep was monitored with polysomnography.

Results:

No consistent effects of the light conditions were observable on subjective sleepiness ratings in the evening. Objective alertness (assessed with an auditory GO/NOGO task) was slightly reduced in the next morning but only when the subjects had to read on the smartphone without a blue light filter on the preceding evening. As expected, melatonin secretion was suppressed during reading and before bedtime in both smartphone conditions with the strongest effect without a blue light filter. During the whole reading session, an increase in centroparietal alpha power was observable for both smartphone conditions. Additionally, during the first 30 min of reading fast beta frequencies were enhanced but again only in the smartphone condition without a blue light filter. Regarding sleep, we were able to detect an increased amount of sleep fragmentation and a reduction in total sleep time in both smartphone conditions. Finally, the amount of slow wave sleep and slow wave activity during the first night quarter was diminished, especially when no blue light filter was used on the preceding evening.

Discussion:

Our analyses revealed that just the exposure to the emitted light from smartphones during the evening might be sufficient to trigger an implicit alerting response and negatively affect subsequent sleep. However, these effects might remain unnoticeable for the subjects as no behavioural changes in sleepiness and alertness could be detected and effect sizes were limited. Using a blue light filter helped to reduce some of the effects but was not sufficient to suppress all effects of the artificial light exposure. It has to be noted that our effects might be stronger under unrestricted conditions when the smart-phones are used until the last minutes before bedtime and over longer periods than in our study.

M-08 Neural speech tracking shifts from the syllabic to the modulation rate of speech as intelligibility decreases

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Background:
The most prominent acoustic features in speech are intensity modulations, represented by the amplitude envelope of speech. Synchronization of neural activity with these modulations is vital for speech comprehension. As the acoustic modulation of speech is related to the production of syllables, investigations of neural speech tracking commonly do not distinguish between lower-level acoustic (envelope modulation) and higher-level linguistic (syllable rate) information. Here we manipulated speech intelligibility using noise-vocoded speech and investigated the spectral dynamics of neural speech processing, across two studies at cortical and subcortical levels of the auditory hierarchy, using magnetoencephalography.
Methods:
Subjects (N=52; split across 2 studies) listened to an audiobook narrated by a female speaker whilst magnetoencephalography was recorded. Parts of the audiobook were noise vocoded (using either 7- or 3-Channels). We measured the coherence between the speech envelope and the recorded sub-/cortical activity.
Results:
Overall, we find that cortical regions mostly track the syllable rate of speech, whereas subcortical regions rather track the acoustic envelope. Furthermore, with less intelligible speech, tracking of the modulation rate becomes more dominant (in both cortical and subcortical regions).
Discussion:
Our study highlights the importance of distinguishing between envelope modulation and syllable rate and provides novel possibilities to better understand differences between auditory processing and speech/language processing disorders.

M-09 The role of the occipitotemporal cortex in normal and impaired reading

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Background:
The critical importance of the left ventral occipitotemporal cortex (vOTC) for reading has been shown in numerous studies. However, there are different accounts on the precise function of this region for visual word recognition.
Methods:
Data from several functional MRI studies on visual word recognition in normal and impaired readers will be presented.
Results:
The presented results wil show that the left vOTC is sensitive to orthographic familiarity. Furthermore, the left vOTC is involved in accessing these orthographic whole-word representations when performing spelling tasks for auditory

presented words. Results also highlight a surprising sensitivity of this orthographic lexicon to the visual format of words and argue for the position that orthographic whole-word representations in the left vOTC are not completely visually abstract. Results will also highlight a consistent dysfunction of this region in impaired readers.

Discussion:

These results underscore a critical function of the left vOTC for orthographic whole-word processing and provide evidence for the existence of an orthographic whole-word lexicon in this brain region.

Clinical Neuroscience: Epilepsy

M-10 Tuberous sclerosis complex (TSC) as disease model for investigating mTOR-related epileptogenesis - identification of potential new targets of therapy

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Tuberous sclerosis complex (TSC, MIM#191100) is a rare genetic autosomal dominant multisystem disorder, characterized by the occurrence of hamartomas in different organs. (i.e. heart, brain, skin, kidneys, and lungs). The brain is affected in more than 90% of individuals with TSC, and neurological symptoms are the major source of morbidity and mortality.

Epilepsy is the most frequent neurological manifestation (occurring in up to 93% of patients, and starting before the age of 2 years in 75%). Despite the availability of an increasing number of anti-seizure medications (ASMs), TSC-associated epilepsy usually remains difficult to treat and often pharmacoresistant and – to a high degree – associated with neuropsychiatric disorders (TAND).

However, increasing knowledge of both genetic background and pathophysiology underlying TSC has also revolutionized the understanding of TSC-related epileptogenesis and by this opened perspectives for new treatment concepts:

TSC is caused by loss-of-function mutations in the tumor suppressor genes TSC1 (located on chromosome 9q34, encoding hamartin) or TSC2 (located on chromosome 16p13.3, encoding tuberin). Hamartin and tuberin form a heterodimeric complex, acting as an upstream regulator of the mammalian target of rapamycin (mTOR) complex signaling pathway. Most of TSC-related manifestations are a consequence of loss of inhibition/over-activation of the (mTOR) complex caused by the mutation.

The discovery of this underlying mechanism paved the way for the use of mTOR inhibitors specifically targeting this pathway. Management of TSC-associated epilepsy is now moving from traditional "reactive" treatment with conventional ASMs initiated after the onset of clinical seizures, to a proactive

'P4-medicine' (predictive, preventive, personalized, and participatory) approach, aimed at predicting epilepsy via repeated EEG recording in patients at risk and preventive disease modifying treatment with mTOR inhibitors (initiated at a "point of no return" defined by specific inter-ictal EEG abnormalities prior to the onset of overt seizures).

This preventive individualized approach (i.e, early suppression of abnormal mTOR signalling before seizure onset using mTOR inhibitors) seems to be an effective anti-epileptogenic and disease-modifying strategy in patients with TSC and also a model for other "mTORpathies, e.g. focal cortical dysplasia (FCD) 2b.

M-11 Clinical antiepileptogenesis trials – time for translation?

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tba

Abstracts of Oral Presentations

Oral Presentations

Neural Networks & Activity

O-01 VIP-expressing interneurons in the anterior insular cortex detect salience to promote adaptive behavior

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Background:
Adaptive behavior critically depends on the detection and attribution of salience. The anterior insular cortex (aIC) has long been proposed as a key player in the detection of behaviorally relevant stimuli, as part of the brain system known as the "salience network". However, to date, little is known about the contribution of aIC interneurons to the processing of salient stimuli.
Methods:
To adress this, we used a combination of whole-brain connectivity tracing, imaging of neural calcium dynamics and optogenetic modulation in freely moving mice across different experimental paradigms and during the presentation of a wide variety of salient events.
Results:
We describe here a role for vasoactive intestinal polypeptide-expressing (VIP+) interneurons in the aIC in the detection and processing of salient stimuli and in mediating adaptive behaviors such as social interactions and associative learning in response to salient events of aversive and positive nature.
Discussion:
Our findings enlighten novel cellular mechanisms underlying salience processing in the aIC and show that VIP+ interneurons are centrally positioned within the salience network to participate to the broadcasting of salience.

O-02 Periventricular A14 dopamine neurons entrain the lateral septum for the circadian control of locomotor activity

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Background:
Locomotion is an essential animal behavior, allowing for exploration, foraging, social interactions and the initiation of escape upon threat. Locomotion predominates during the active diurnal phase that is at night in nocturnal rodents. Recently, feed-forward command from the lateral septum has been implicated in initiating locomotion, yet without specifying either the cell contingents or their possible wiring into the brain's clock network to allow for locomotor epochs be timed and executed during the active diurnal phase. The suprachiasmatic nucleus of the hypothalamus produces the primary neural command of the clock network ('Zeitgeber').
Methods:
We used a wide assortment of methods and techniques: single-cell transcriptomics anlaysis, anterograde and retrograde viral tracing, light-sheet imaging, immunostaining, fluorescent in situ hybridization, optogenetics, the chemogenetic approach combined with behavioral recordings.
Results:
Here, we show that dopamine neurons of the anterior subdivision of the periventricular nucleus of the hypothalamus (aPeVN) receive neuropeptidergic innervation from the suprachiasmatic nucleus, which modulates their activity in a diurnal fashion. By combining anterograde viral tracing and light-sheet imaging in intact tissues, we find that the aPeVN preferentially innervates the lateral septum. Reciprocal viral tracing identified somatostatin-containing septal neurons expressing both D1 and D2 dopamine receptors as preferred synaptic targets of extrahypothalamic afferents originating in the aPeVN. Optogenetic stimulation of hypothalamic dopaminergic terminals in brain slices recapitulated diurnal rhythms in septal networks. In vivo chemogenetic activation of dopamine neurons of the aPeVN stimulated locomotion. Conversely, chemogenetic silencing of dopamine neurotransmission led to the cessation of locomotion. Notably, the power of dopamine output was sufficient to occlude amphetamine-induced hyperlocomotion, which substantiates the diurnal control of spontaneous activity and pinpoints a novel midbrain-independent cellular target mediating the stimulatory action of common psychostimulants.
Discussion:
Altogether, our data reveal distinct sequential requirements of Eed and thus PRC2-mediated H3K27me3 in RGP lineage progression during cortical development and an essential role in cortical astrocyte production and maturation.

O-03 Behaviour of Medial entorhinal cortex cells during a radial eight arm maze task

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Background:
The medial temporal lobe (across species) is known to be an important brain region involved in spatial navigation. The entorhinal cortex, hippocampus

and parahippocampal regions are all a part of the medial temporal lobe. Historically the grid cells, discovered in 2005, of the medial entorhinal cortex (mEC) have been of interest. Their (relatively) rigid, grid-like firing pattern in space has played a significant role in furthering our understanding of how space is represented in the brain. The mEC, however, has a myriad of other very interesting cells that exhibit spatial properties but are not grid cells per say. The role of these non-grid spatial cells in spatial navigation, learning and memory is still not well understood. In addition, the mEC has been shown to replay independently of the hippocampus. The role of this independent replay during a complex spatial navigation task is as yet unknown. The aim of the project is to elucidate the role of this independent replay while trying to understand how different types of cells in the mEC behave during a complex navigation task.

Methods:

We performed multichannel, extracellular electrophysiology recordings from 4 adult, male Long Evans rats while they performed a radial eight arm maze task. Of the 4 rats 2 were bilaterally implanted while 2 were unilaterally implanted with microdrives targeting the dorsal CA1 and layers II/III of the medial entorhinal cortex. All 4 rats performed an eight arm maze task where, everyday, they were expected to learn which 3 (out of the 8) arms were rewarded. At the start of each trial the animal was enclosed in the centre of the maze for a delay period of 1min. At the end of the delay period the central doors were opened and the animal was allowed to look for the rewards. Animals performed 30 trials per day. This learning block of 30 trials was flanked by sleep and open field recordings (30mins in a familiar, square environment). For each animal we tracked it's position while it performed the task. The recorded data was preprocessed and putative single units were isolated using a semi-automated clustering algorithm. All further analysis was then performed on these isolated units.

Results:

We establish how the different kinds of spatial cells in the entorhinal cortex behave during a complex navigation task. We show how grid cells and non-grid spatial cells behave differently at different phases during the task - during the 1min delay period, during the first half of the learning block (when the animal is still learning the rewarded locations and makes mistakes) and during the second half of the learning block (once the animal has learnt the rewarded locations).

We show how at key moments of immobility (when the animal is at the centre - a decision point or when the animal is at the rewarded location) the trajectory replayed by entorhinal cortex cells isn't always the same as that replayed by hippocampal CA1 cells.

Discussion:

The complex behaviours of spatial navigation and learning are a consequence of a wide number of cells, networks and brain regions working together. Here

we show what the role of the independent replay and the non-grid spatial cells of the rodent medial entorhinal cortex is during complex spatial navigation tasks. These results are a demonstration of the influence of non-geometric factors on the firing properties of the MEC space coding cells. The results also demonstrate how the independent replay in the MEC could be part of a parallel memory formation circuit in the brain.

O-04 One appendage – two behaviors

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Background:
Behavioral flexibility of pectoral appendages is a common feature throughout the animal kingdom. One fish group that shows specialized pectoral behaviors are hatchet fish. Their hypertrophied abductor muscles enable them to become airborne in response to a potential threat. In contrast to this explosive behavior, we found a second behavior, which is characterized by its smaller abduction amplitude. This behavior we refer to as fin flickering. Here we investigated how these two opposing behaviors are controlled at the neuronal level.
Methods:
Using high-speed cinematography, we filmed hatchet fish fin moving behavior. Flickering occurred during resting conditions, while jumping behavior was performed as a response to a startle stimulus. We analyzed both behaviors in terms of amplitude and latency to the fin abduction peak. To investigate the underlying neuronal control, we performed retrograde tracing experiments on nerves innervating the pectoral fin. We used a combination of gap junction passable and impassable tracers. In cleared whole-brain experiments neurons were reconstructed using the software Neurolucida.
Results:
During behavior observations, we found that hatchet fish leave the water in a jump-like motion, as previously described. We found an additional previously undocumented behavior, which we termed fin flickering that likely serves a station holding function. In contrast to the escape behavior, fin flickering is characterized by a smaller amplitude and more variable duration of pectoral fin motion.
Backfill experiments revealed a set of motoneurons characterized by a broad range of different sizes and a widespread spatial distribution within the caudal hindbrain and rostral spinal cord. However, neuronal tracings did not label any pre-motoneurons.
Discussion:
Gap junction passable tracers did not reveal trans-synaptic labelling, even though gap junctions are known to occur between moto- and pre-motoneurons in the hatchet fish pectoral system. While the pre-motoneuronal network of escape responses is associated with the Mauthner escape system,

and has been detailed previously, this system is, however, not able to generate fin flickering behavior. This suggests that additional neuronal populations are involved in pectoral control. Our data thus indicates that the observed pectoral fin abductive behavior dualism is likely associated to differences in motoneuron size and pre-motoneuronal control. We propose that large motoneurons likely only contribute to escape behavior. In future studies involving electrophysiological experiments, we expect to find prominent differences in the action potential firing pattern and membrane properties of differently sized motoneurons.

Neurological Disorders & Regeneration

O-05 Spinal cord injury affects HCN channels activation and intrinsic excitability of cortical motor neurons

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Background:
Altered cortical excitability is a well-known consequence of spinal cord injury (SCI) and controlling cortical pathophysiology after SCI may help to promote recovery. Regrettably, attempts in this direction are hindered by poor resolution over cellular and molecular mechanisms that control the process of cortical dysfunction. Focusing on the primary motor cortex layer V (M1LV), we hypothesized that injured principal neurons, axotomized upon SCI, would become hyperexcitable and we questioned the role of hyperpolarization cyclic nucleotide gated channels (HCN channels) in such context.
Methods:
To model SCI, adult female rats underwent transection of the corticospinal tract at vertebral level C4. One week after injury, acute pharmacological manipulation of HCN channels ex vivo allowed to analyse the mechanisms of derangement affecting M1LV. To this end, patch clamp experiments were carried out on acute brain slices, targeting axotomized M1LV neurons that were identified by retrograde tracer applied at the time and point of injury.
Results:
After SCI, some M1LV principal neurons were hyperexcitable and excessively depolarized. The latter issue caused the resting membrane potential to escape the permissive range for HCN channel activation. As consequence, the homogenous response to the pharmacological blockade of HCN channels observed in healthy neurons was lost and heterogeneous responses were detected in injured neurons.

Discussion:
These data imply that loss of HCN channel activation aggravates the conditions of some M1LV neurons after SCI. However, such loss is not the root-cause of M1LV neuron depolarization and hyperexcitability, which rather appears initiated by other factors. Under physiological conditions, modulating HCN channels pharmacologically allows to control cortical excitability and output. However, targeting HCN channels after SCI is expected to lead to convoluted effects due to the wide spectrum of altered functional states prevailing in injured M1LV neurons.

O-06 The role of enkephalin in hypoxic preconditioning

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Background:
Hypoxic preconditioning (HPC) is the application of mild transient hypoxia which protects the brain against a following more severe hypoxic insult as it occurs during epileptic seizures. HPC decreases seizure susceptibility and severity as well as neuronal damage in the hippocampus. The delta opioid receptor (DOR) and its primary endogenous ligands, the neuropeptides met- and leu-enkephalin (Enk), are thought to be involved in the neuroprotective actions of HPC. Recently, we showed that Enk/DOR influences mitochondrial respiration that may contribute to the neuroprotective effects of the Enk/DOR system. The present study aims at investigating the effects of the Enk/DOR system on structural and functional alterations of mitochondria in HPC.
Methods:
Wild type (WT) and preproenkephalin knockout (ppEnk KO) mice were exposed to hypoxia (9 % O_2) for 7 h. Seventeen hours later, we determined the seizure threshold (infusion of GABAA receptor antagonist pentylenetetrazol), analyzed mitochondrial function (high-resolution respirometry) and, dynamics (real-time qPCR of key genes). Mitochondrial respiration was normalized to the protein amount (mass-specific flux) and the activity of a mitochondrial specific enzyme, the citrate synthase (mitochondrial-specific flux).
Results:
HPC increased the seizure threshold of WT, but not ppEnk KO mice. However, naive ppEnk KO mice already displayed an elevated seizure threshold. HPC improved mitochondrial reserve capacity in WT mice. Further, HPC decreased mitochondrial-specific flux yet increased mass-specific flux in WT mice, suggesting the generation of new mitochondria upon HPC in WT mice. This is supported by increased activity of the citrate synthase in those mice, which is a marker for mitochondrial quantity. However, real-time qPCR revealed no changes in PGC1α expression, the master regulator of mitochondrial biogene-

sis, in the same mice. In addition, we observed elevated mitochondrial fusion in ppEnk KO mice and after HPC in WT mice.

Discussion:

Our results indicate that HPC in WT mice elevates the seizure threshold, improves mitochondrial reserve capacity, increases mitochondrial fusion, and induces mitochondrial biogenesis. A rather long time window between treatment and analysis could explain the missing changes in PGC1α expression. ppEnk KO mice had an increased seizure threshold and increased mitochondrial fusion but no changes upon HPC. The observed mitochondrial alterations after HPC in WT mice could explain improved neuronal survival and increased seizure threshold. Enhanced mitochondrial reserve capacity improves energy supply in situations with increased energy demand, like epileptic seizures. Increased mitochondrial fusion is associated with neuronal survival, elevated Ca2+ storage capacity, reduced production of reactive oxygen species, and increased mitochondrial membrane potential improving ATP production. Which together support increased stress resistance and, thereby, neuronal survival. So far, the precise role of ppEnk in HPC is unclear but we observed adaptive mechanisms in WT mice which are absent in ppEnk KO mice.

O-07 gp130 induces TRPA1 upregulation in uninjured neurons in a mouse model for neuropathic pain

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Background:

Peripheral nerve injuries induce pronounced alterations in primary sensory neurons residing in dorsal root ganglia (DRG) that can result in the development of neuropathic pain. The transient receptor potential ankyrin 1 ion channel (TRPA1) is an emerging target for pain treatment, however, the mechanisms by which TRPA1 contributes in the pathogenesis of neuropathic pain are not fully understood.

Methods:

To explore the role of TRPA1 in neuropathic pain, we subjected a transgenic mouse model with a conditional depletion of the interleukin-6 signal transducer gp130 specifically in Nav1.8 expressing sensory neurons (SNS-gp130$^{-/-}$) to the spared nerve injury (SNI) model. Sensory testing in SNS-gp130$^{-/-}$ mice and gp130$^{fl/fl}$ controls was performed using the von Frey test, in vivo, and an ex vivo skin-nerve model. TRPA1 mRNA levels were assessed using RT-qPCR. Adenoviral re-expression of gp130 in primary cultures was used to evaluate regulation of TRPA1 expression by gp130. Finally, microfluorimetric calcium recordings were employed to identify functional differences between sensory neurons derived from SNS-gp130$^{-/-}$ and littermate controls, combined with retrograde labeling of injured and uninjured neurons.

Results:
SNI induced a severe mechanical hypersensitivity in control mice, evident by a profound reduction in mechanical thresholds in the von Frey behavioral test, as well as an increase in the mechanosensitivity of unmyelinated primary afferents. Importantly, microfluorimetric calcium measurements revealed that after SNI uninjured but not injured neurons derived from control mice increased responsiveness to the TRPA1 agonist cinnamon aldehyde (CA). In contrast, SNS-gp130$^{-/-}$ mice exhibited a complete absence of mechanical hypersensitivity at the injured paw, and low levels of Trpa1 mRNA in DRG sensory neurons. Neurons derived from SNI treated SNS-gp130$^{-/-}$ mice became increasingly insensitive to CA. Furthermore, Trpa1 downregulation could be partially rescued by adenoviral re-expression of gp130 in vitro.
Discussion:
Our study demonstrates that the differential increase in TRPA1 responsiveness after peripheral nerve injury exclusively in uninjured but not injured neurons depended on the IL-6 signal transducer gp130. We provide novel mechanistic insight into the role of TRPA1 in neuropathic pain pathogenesis and highlight the importance of TRPA1 as a relevant target in treating neuropathic pain disorders.

O-08 Intralesional administration of extracellular vesicles improves functional recovery in a rat model of traumatic spinal cord injury

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10 Austrian Cluster for Tissue Regeneration, Vienna; 11 Transfer Centre for Extracellular Vesicle Theralytic Technologies (EV-TT), Salzburg

Background:
Local inflammation plays a pivotal role in the process of secondary damage after spinal cord injury (SCI). Acute intravenous application of extracellular vesicles (EVs) secreted by human umbilical cord mesenchymal stromal cells have been shown to dampen the induction of inflammatory processes following spinal cord injury. However, systemic administration of EVs is associated with delayed delivery to the site of injury and the necessity for high doses to reach therapeutic levels. In this project, we compared the impact of a treatment based on EVs injected directly into the lesion site, to a treatment in which EVs were injected intravenously, for the functional regeneration and structural integrity of the tissue following spinal cord injury.
Methods:
A 200 kdyn contusion of the spinal cord (Th8) was performed in female Fisher 344 rats of 10 weeks of age which resulted in a moderate to severe incomplete spinal cord injury. The rats were randomly divided in treatment groups receiving acutely an intra-parenchymal administration of either vehicle, EVs or an intravenous injection of EVs. Sham rats only received a laminectomy. Gene expression of pro and anti-inflammatory cytokines in the spinal cord were determined at 24 hours post injury. Histological analyses were performed 2 and 8 weeks after contusion. Locomotor recovery was assessed until 8 weeks after contusion. MRI, DTI and µCT analysis were performed ex vivo 8 weeks after injury.
Results:
Local EVs application was found to be the most efficient to improve locomotor function recovery during the first 8 weeks post-injury based on the BBB and BBB-Subscore. The acute local application of EVs resulted in a marked reduction of pro-inflammatory cytokines expression measured at 24h post-tSCI. Even at 14 days post-injury, the accumulation of inflammatory cells around the lesion was significantly lower in rats treated with EVs. Furthermore, the deposition of collagen and CSPGs, as well as astrogliosis, were attenuated by the application of EVs. Finally, eight weeks post-injury, we also observed that an acute EVs i.pa. treatment could preserve more neuronal fiber bundles in the proximity of the lesion site, as compared to the vehicle administration measured with diffusion tensor imaging.
Discussion:
Our observation demonstrates that EVs are particularly potent to address the processes of inflammation and scarring when applied very early and close to the site of injury, which additionally resulted in a better motor function. Moreover, an improvement of the lesion microenvironment and the additional structural sparing obtained with EV treatment constitute valuable assets for follow-up interventions aiming for endogenous axonal regeneration or involving cell therapies.

Cognition & Behavior

O-09 Histamine 3 receptor deletion reduces aggression and alters neuronal activation in zebrafish

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Background:
Aggression is an adaptive behaviour expressed by animals and humans. Neurotransmitters such as serotonin, dopamine and noradrenaline play an important role in the expression of this behaviour, however the contribution of the histaminergic system to aggression is less well known. In this project, we have studied a novel zebrafish histamine 3 receptor (hrh3) mutant line. HRH3 is a heteroreceptor with both pre- and post-synaptic functions that modulates histaminergic signalling.
Methods:
Using CRISPR-Cas9 engineering we generated a null allele of hrh3 and studied larval and adult homozygous mutants in various behavioural tasks. In addition, we investigated neuronal activation in mutants using whole brain imaging in larvae and RpS6 immunohistochemistry in adults.
Results:
Larval fish displayed a behavioural phenotype consistent with enhanced anxiety in the open field and light/dark tests. Whole brain calcium imaging revealed that hrh3-/- larvae expressing GCaMP6s under the pan-neuronal promoter elavl3 have higher peak intensity values especially in the fore-brain, lower peak intensity values in various hindbrain areas and a number of changes in functional connectivity between the brain areas compared to WT. Adult hrh3-/- fish were less aggressive in a mirror-induced aggression task, a phenotype that could be reproduced by pharmacological means using the HRH3 inverse agonist pitolisant. Neuronal activation analysis after aggression showed that hrh3-/- fish display a higher number of RpS6+ cells in the medial area of the dorsal telencephalon (Dm), the homologue of the mammalian amygdala, and lower RpS6+ cell counts in the ventral part of the ventral telencephalon (Vv), the homologue of the mammalian septum.
Discussion:
In summary, this work provides evidence for a role of Hrh3 during aggression and anxiety. Genetic deletion and pharmacological blockade reduces aggression suggesting that Hrh3 might be an interesting novel target to develop treatments for human disorders characterized by heightened aggression.

O-10 Exploring the role of Neurokinin B neurons of bed nucleus of the stria terminalis in emotional and metabolic processing

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Background:
The bed nucleus of the stria terminalis (BNST) is a basal forebrain structure that acts as a critical node for the integration of emotional behavior. In particular, sustained fear responses seem to be crucially dependent on BNST functioning. The heterogeneous neuronal populations with multiple neuropeptide transmitters and the complex intrinsic and extrinsic connectivity accentuates the BNST as coordinative hub of competing needs. The abundantly expressed tachykinin neurokinin B (NKB) may support such an integrative function of BNST neurons.
Methods:
Histochemistry and viral vector mediated neuronal tract tracing were used to identify, characterize and map the elaborated network of BNST NKB neurons. Using chemogenetic (DREADD) activation in transgenic Tac2cre mice, we studied the modulatory role of NKB neurons on anxiety and fear processing. Furthermore, BNST NKB expression was suppressed with targeted gene silencing using RNA interference technique followed by behavioral testing.
Results:
NKB was abundantly expressed in calretinin-expressing neurons of the anterior and posterior BNST, predominantly projecting to the amygdala, hypothalamus and periaqueductal grey. Chemogenetic stimulation of BNST NKB neurons increased anxiety-related behavior and sustained fear expression. Deletion of NKB form the BNST abolished anxiogenic behavior and reduced sustained fear expression to control levels. Furthermore, activation of NKB neurons in the BNST reduced normal food intake and fasting-induced refeeding, but did not affect water intake or home cage activity. In particular, the fasting increased re-feeding was dependent on the co-release of NKB.
Discussion:
Thus, our study demonstrates that NKB neurons of the BNST play a substantial role in coordinating emotional and metabolic behaviors, probably by interacting with distinct amygdala and brainstem nuclei.

O-11 Serotonergic modulation of an associative relearning network

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Background:
A vital core function of one's cognitive flexibility is the use of acquired knowledge and skills to adapt to ongoing situational and environmental changes.

Magnetic resonance imaging (MRI) represents a useful tool to explore the neuronal underpinnings of these processes. Animal studies combining selective serotonin reuptake inhibitors (SSRIs) and learning paradigms have demonstrated that serotonin is an important factor for learning flexibility. SSRIs may exert their clinical antidepressant effects via mechanisms of neuroplasticity but translation of these mechanisms to humans are missing. To this aim, we assessed the effective connectivity at rest and during associative learning as a proxy of neuroplastic changes in healthy volunteers.

Methods:

Thus, 76 subjects (age: 25.3 ± 4.7, 45 female) underwent 3 MRI sessions: at baseline, after three weeks of daily associative learning and after three weeks of relearning under escitalopram (10mg/day, randomized double-blind placebo-controlled design). During each learning session, subjects performed a (re)learning task and were shown a pseudorandomized selection of 52 out of 200 image pairs to memorize (face-matching or Chinese character–German noun matching). Psychophysiological interactions (PPI) was estimated to reveal a network involved in serotonergic associative (re)learning and dynamical causal modelling (DCM) used to elucidate the effective connectivity between all regions.

Results:

Escitalopram intake modulated relearning changes in a network encompassing the right insula, anterior cingulate cortex and right angular gyrus. Here, the process of relearning during SSRI intake showed a greater decrease in effective connectivity from the right insula to both the dorsal anterior cingulate cortex and right angular gyrus, with increases in the opposite direction when compared to placebo. In contrast, intrinsic connections and those at resting-state were only marginally affected by escitalopram.

Discussion:

Each of the regions, have been associated with learning, memory and error detection during task performance and have been further modulated by SSRIs. Furthermore, these findings show that the right insula plays a central role in the process of relearning and SSRIs additionally potentiate this effect. Overall, we have demonstrated that SSRIs strengthen learning-induced effective connections rather than affecting the intrinsic task connectivity or that of resting-state.

O-12 Role of anterior insula cortex in context-induced relapse of nicotine seeking

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Behavioral flexibility of pectoral appendages is a common feature throughout Smoking tobacco is one of the leading causes of preventable death worldwide. During abstinence, environmental contexts associated with nicotine use can induce craving and contribute to relapse. This phenomenon has previously been modeled in rodents using the classical ABA renewal procedure. However, the use of extinction to model abstinence has been argued to not appropriately capture the motivation for abstinence in humans. We have previously addressed this potential limitation in a rat model of context-induced relapse after punishment-imposed abstinence in rats trained for alcohol self-administration, however this has not been investigated in nicotine trained rats. In this study we report a novel rodent model of context-induced relapse to nicotine seeking after punishment-imposed abstinence. The insular cortex is thought to be a critical substrate of nicotine addiction, however its specific role in context-induced relapse of nicotine seeking is not known, therefore we used chemogenetic inhibition of anterior insular cortex (AI) to investigate its role in context-induced reinstatement of nicotine seeking after punishment-imposed abstinence. We trained male and female rats to self-administer nicotine in one context (context A), and subsequently punished nicotine self-administration in an alternative context (Context B). After punishment, rats tested in context A showed increased nicotine seeking, replicating past findings in rats trained to self-administer alcohol or cocaine. Chemogenetic inhibition of AI decreased context-induced nicotine seeking after punishment-imposed abstinence in female rats, but not male rats. In another group of rats, we found no effect of chemogenetic inhibition of AI on context-induced reinstatement of extinguished nicotine seeking. These findings highlight a critical role of AI in context-induced relapse after punishment-imposed abstinence. They demonstrate differences in the neural substrates of context-induced relapse of nicotine seeking depending on the method used to impose abstinence. Finally, our findings show that the neural substrates of context-induced relapse of nicotine seeking may be different for male and female rats.

Ion Channels & Neurotransmission

O-13 Presynaptic $GABA_B$ receptors induce phasic neurotransmitter release from medial habenula terminals

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Background:
Presynaptic $GABA_B$ receptors attenuate neurotransmitter release in most synapses. The only currently known exception is the synaptic connection from the

medial habenula to the interpeduncular nucleus which is crucial for emotion-related behaviors. In this pathway, presynaptic $GABA_B$ receptor activation strongly enhances neurotransmitter release but the underlying mechanism of this unique function of $GABA_B$ receptors is unknown.

Methods:

We performed electrophysiological recordings and "Flash and Freeze" experiments in acutely cut brain slices to identify the physiological mechanism and structural correlate of $GABA_B$ receptor-mediated enhancement of neurotransmitter release from medial habenula terminals.

Results:

Unexpectedly, the potentiation of neurotransmitter release by baclofen, an agonist of $GABA_B$ receptors, was associated with only a minor increase in neurotransmitter release probability, but with a six-fold increase in size of the readily releasable synaptic vesicle pool. Basal neurotransmission showed low Ca2+ sensitivity and short-term synaptic augmentation in response to repeated stimulation, characteristic properties of tonic neurotransmission. In contrast, the additionally recruited synaptic vesicles following $GABA_B$ receptor activation exhibited a high sensitivity to Ca2+ and rapidly depleted during repeated synaptic activity, indicating a transition to phasic neurotransmitter release. Using timed high-pressure freezing after optogenetic stimulation ("Flash and Freeze"), we identified presynaptic nano-anatomical changes associated with the rapid depletion and subsequent replenishment of synaptic vesicles during the activation of $GABA_B$ receptors.

Discussion:

We discovered that $GABA_B$ receptors on medial habenula terminals act as a molecular switch between tonic and phasic neurotransmitter release modes. This switch was mediated by the recruitment of additional, physiologically distinct synaptic vesicles to the readily releasable pool. The molecular identity of these vesicles remains to be determined.

O-14 Interactions of tricyclic antipsychotic and antidepressant medications with a novel binding site in $GABA_A$ receptors

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Background:

Many psychotherapeutic drugs including clozapine have a polypharmacological profile and act on $GABA_A$ receptors, where subtype-specific information is often lacking. Patients with schizophrenia show alterations in function,

structure and molecular composition of the hippocampus, and a recent study demonstrated aberrant levels of hippocampal α5 subunit containing $GABA_A$ receptors.

Methods:

Functional studies of GABA modulatory effects by antipsychotic and antidepressant medications were performed in several $GABA_A$ receptor subtypes by two-electrode voltage-clamp electrophysiology using Xenopus laevis oocytes. Computational structural analysis was employed to design mutated constructs of the α5 subunit probing a novel binding site. Computational ligand analysis complemented the functional and mutational data.

Results:

We show that the antipsychotic drugs clozapine and chlorpromazine have negative modulatory effects on multiple $GABA_A$ receptor subtypes, including α5-containing. On the latter, we show negative modulatory effects for five additional antipsychotic and antidepressant drugs. Based on a chlorpromazine binding site observed in a GABA-gated bacterial homologue, we identified a novel site in α5 $GABA_A$ receptor subunits.

Discussion:

Our findings support previous studies suggesting a link between some of the therapeutic effects of clozapine and its negative modulatory action on certain $GABA_A$ receptor subtypes. The novel site we describe in this study is a new potential target for optimizing antipsychotic medications with beneficial polypharmacology.

O-15 Collaborative roles of calcium channel CaV1.1 and β-catenin in neuromuscular synaptogenesis

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During neuromuscular synaptogenesis motor nerves project their axons towards the central muscle region where postsynaptic acetylcholine receptors (AChRs) are clustered, establishing the stereotypical central innervation pattern. This precise spatio-temporal organization of neuromuscular junctions (NMJ) requires a delicate interplay between anterograde and retrograde mechanisms between nerve and muscle. We have recently shown that activity-induced calcium signaling initiated by skeletal muscle L-type calcium channel CaV1.1 is a key controller of neuromuscular patterning, correct guidance of the axons to their target territory and of the differentiation of nerve terminals [1], [2]. However, how muscle calcium signaling interacts with downstream effectors to govern these processes remained unknown.

Muscle β-catenin was previously identified as a retrograde regulator of motor axon fasciculation and nerve terminal development [3]. Here we show that coordinated functions of CaV1.1 and β-catenin are required for proper neuromuscular synaptogenesis. By analyzing NMJ formation in the diaphragm of mice lacking CaV1.1, muscle β-catenin, or both, we found that the role of

CaV1.1 is to determine the innervation territory, while β-catenin determines the degree of nerve branching so that their opposite but complementary roles induce sufficient nerve branching specifically in the muscle center. On the other hand, in the double knockouts AChR clustering and synapse formation are severely perturbed, indicating a cooperativity of CaV1.1 and β-catenin in these processes. Furthermore, by using a β-catenin reporter mouse line, Western blot, and qRT-PCR analysis, we show that CaV1.1 does not directly regulate β-catenin expression but has opposite effects on the activity of transcriptional partners of β-catenin, TCF/Lef and YAP. While CaV1.1 promotes TCF/Lef-dependent transcription, it suppresses YAP expression, phosphorylation and transcriptional activity.

All together, these data show that CaV1.1 and β-catenin cooperate in various ways to regulate distinct aspects of the NMJ formation. And we identified possible signaling mechanisms by which CaV1.1 controls gene expression in the regulation of neuromuscular synapse formation.

References:

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[3] X.-M. Li et al., "Retrograde regulation of motoneuron differentiation by muscle beta-catenin.," Nat. Neurosci., vol. 11, no. 3, pp. 262–268, 2008, doi: 10.1038/nn2053.

O-16 Biophysical and pharmacological characterisation of de novo *CACNA1D* mutations associated with a severe neurodevelopment disorder

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Background:

Cav1.3 voltage-gated Ca2+ channels give rise to low voltage-activated dihydropyridine (DHP)-sensitive L-type currents in electrically excitable cells. They control important physiological processes including hearing, sinoatrial node pacemaking, aldosterone and insulin secretion as well as brain functions such as learning, memory and emotional behaviours. Germline gain-of-function missense variants in the pore forming Cav1.3 α1-subunit (CACNA1D gene) cause a severe neurodevelopmental disorder with or without endocri-

ne symptoms. Somatic CACNA1D gain-of-function variants drive enhanced aldosterone secretion in aldosterone-producing adenomas (APAs). Our goal was to investigate the potential pathogenicity of two novel germline Cav1.3 missense variants from patients with a severe neurodevelopmental disorder with (L271H) and without (A749T) congenital hyperaldosteronism and hyperinsulinemic hypoglycemia. In addition, the missense variant F747S, recently reported in an APA, was also tested. Moreover, we investigated the sensitivity of variants for the brain-permeable DHP Ca2+ channel blocker isradipine because DHPs could be used for the symptomatic treatment of patients with gain-of-function CACNA1D germline variants.

Methods:

F747S, A749T, and L271H variants were cloned into the human Cav1.3 α1-subunit (long C-terminal isoform) and heterologously expressed in HEK-293T cells in parallel with wild-type channels (plus accessory β2a- or β3-, and $\alpha 2\delta$1-subunits). Constructs were tested for pathogenic gating changes using the patch-clamp technique in whole-cell configuration and with 15 mM Ca2+ as the permeant ion.

Results:

With co-expressed β2a- and $\alpha 2\delta$1-subunits all three variants significantly shifted the voltage dependence of activation ($V_{0.5,act}$ [mV]: -17.2 (A749T), -19.4 (L271H) and -24.7 (F747S), 1.6 (wild-type)) and channel availability ($V_{0.5,inact}$ [mV]: -35.3 (A749T), -41.7 (L271H), -38.9 (F747S), -16.5 (wild-type)) to more negative voltages as compared to wild-type. This causes an increase of steady-state inward current („window current") and enhanced channel activity at negative (subthreshold) membrane potentials. In addition, the F747S variant significantly slowed the time course of current inactivation during 5-s depolarizations to the voltage of the maximum inward current. The A749T variant also induced a significant three-fold increase of the maximal current density ([pA/pF]: -16.8 (wild-type), -56.5 (A749T)).

Since we have previously found altered DHP sensitivity for other CACNA1D variants, we also measured the inhibition of A749T Ca2+ currents by isradipine. When co-expressed with β2a and $\alpha 2\delta$1, our pharmacological experiments revealed no major changes in drug sensitivity as compared to wild-type (n=6-11). In terms of gating changes, similar alterations were observed when the pore-forming subunits were co-expressed with β3 and $\alpha 2\delta$1 auxiliary subunits.

Discussion:

Our data confirm the pathogenicity of all three variants by demonstrating typical gating changes allowing a channel gain-of-function, including higher subthreshold "window currents." This extends the number of biophysically confirmed germline CACNA1D variants to 8 in a total of 11 different patients. For variant A749T we already provide preliminary evidence for no decrease in DHP-sensitivity, suggesting that symptomatic off-label treatment of patients carrying this variant is justified and could mitigate symptoms such as muscle hypotonia, self-aggressive and autistic behaviour in the affected patient.

Stem Cells & Development

O-17 Mapping the macroscopic evolution of the neocortex in primates, rodents and related species – implications in ecology and behavior

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Background:

Elucidating the nature of the relationships between the concerted or independent evolution of brain regions, ecological constraints and behavioral correlates lies at the heart of evolutionary neuroscience. As the region showing the largest evolutionary expansion in mammals and notably in primates, the neocortex has been at the center of research in both anatomical and functional approaches to studying these processes. However, the complex interplay of evolutionary changes in number, extent and functional homologies of cortical fields underlying both primary sensory and to an even larger extent inte-grative association capabilities renders this research challenging.

Methods:

To enable such studies, we performed surface-based alignment of the surface meshes of the two cerebral hemispheres of 90 species of Primata (58), Rodentia (28), Lagomorpha (2), Dermoptera (1) and Scandentia (1), proceeding via pairwise alignment steps between sister species in a genetically determined phylogeny. This procedure results in dense correspondences between the cortical surfaces, allowing for the application of phylogenetic comparative methods in the light of unclear notions of structural homology between neocortical areas.

Results:

Analysis of the morphospace of neocortical surface geometry showed a strong relationship between overall brain surface area, globularity and folding pattern, despite normalization to unit surface area of all surfaces. Furthermore, individual modes of shape variability showed significant correlation with ecological categories in terms of preferred habitat as well as the more behavioral aspect of average group size in the studied species. We approximated habitats as either fossorial, terrestrial or arboreal and found significant relationships between these categories and the relative expansion or contraction of visual and limbic areas. Mapping imaging-based measures of cortical myelin into the common space of aligned cortical surfaces revealed a positive correlation between the phylogenetic signal of myelin content and

its ancestral state estimate, indicating selective regional myelination of the cortical sheet as a possible vector of evolutionary adaptation. Analysis of the evolutionary changes of the relative extent between and geodesic distance of modality-specific neocortical areas indicates both an evolutionary segregation of sensory regions, as well as - in primates - stronger integration of these areas in diurnal species and species living in larger groups, possibly reflective of increased cognitive demands posed by these environments. Finally, we performed meta-analytic decoding of the estimated evolutionary expansion maps of the neocortex leading from the last common ancestor of rodents and primates some 77 Mya to present day humans by correlating them with activation maps associated with over 3200 terms derived from 14371 neuroscientific studies. Grouping of the time-courses of the correlation values of strongly correlated terms revealed 7 clusters relating to distinct sensorial, functional and cognitive capabilities that reveal a potential timeline of neocortical evolution, starting with the expansion of the visual areas, followed in sequence by temporal, primary auditory, motor, language-related and frontoparietal regions and lastly areas relating to retrieval and episodic memory.
Discussion:
We demonstrate that the phylogenetic analysis of the macroscopic shape of the neocortex yields neuroscientifically relevant results concerning the evolutionary history of this important anatomical structure. Most importantly, the multidimensional nature of the divergences between rodent and primate cerebra should leave a cautionary note on the translatability of research results from model animals to humans.

O-18 Molecular characterization of non-cephalic sensory cells reveals a function of rhabdomeric Opsin in light modulation of mechanosensory neurons

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Background:
Rhabdomeric Opsins (r-Opsins) serve as light-sensory molecules in cephalic eye photoreceptors of many invertebrates, but also play roles in additional sensory organs. This has prompted questions on the evolutionary relationship of the cell types, and specificall,y if ancient r-Opsins cells possessed non-photosensory functions.

Methods:
We have FAC-sorted and profiled cephalic and non-cephalic r-opsin1-expressing cell types of the marine bristleworm Platynereis dumerilii, taking advantage of a transgenic strain expressing EGFP under control of the r-opsin1 locus. Moreover, we have employed cell-culture-based second messenger assays for characterizing the action profile of Platynereis r-Opsin1, and established an r-opsin1 knock-out strain to assess the requirement of r-opsin1 for cellular characteristics and functionality of the non-cephalic cell type. Finally, we also established a supervised, deep learning-based quantitative behavioral analysis pipeline to assess animal trunk movements as a read-out for mechanosensory functionality
Results:
Molecular profiling revealed shared and distinct identities between cephalic and non-cephalic r-opsin1-positive cell types. We establish that non-cephalic cells possess a mechanosensory signature, but also a full set of phototransduction components. Cellular assays revealed r-Opsin1 to be a Gαq-coupled blue-light receptor. Profiling of cells in r-opsin1 mutant animals, and comparing cells under dim and bright light conditions reveals that in the non-cephalic cell type, light – mediated by r-Opsin1 – adjusts the expression level of a calcium transporter relevant for auditory mechanosensation in vertebrates. Consistently, we identify light-dependent differences in the fine-tuning of the animals' undulatory movements in mutant vs. wildtype headless trunks, which are known to require mechanosensory feedback.
Discussion:
Our results support an evolutionary concept in which r-Opsins act as ancient, light-dependent modulators of mechanosensation. Our findings thereby refine the reconstruction of sensory cell type evolution, and suggest that light-independent mechanosensory roles of r-Opsins likely result from secondary evolutionary processes.

O-19 Wnt/beta-catenin signalling is dispensable for adult neural stem cell homeostasis and activation

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Background:
Adult mouse hippocampal neural stem cells (NSCs) generate new neurons that integrate into existing hippocampal networks and modulate mood and memory. These NSCs are largely quiescent and are stimulated by niche signals to activate and produce neurons. Wnt/beta-catenin signalling acts at different steps along the hippocampal neurogenic lineage and has been shown to promote the proliferation of intermediate progenitor cells. However, whether it has a direct role in the regulation of NSCs still remains unclear.

Methods:
Here we used Wnt/beta-catenin reporters and transcriptomic data from in vivo and in vitro models to investigate how active and quiescent adult NSCs respond to Wnt/beta-catenin signalling.
Results:
Wnt/beta-catenin stimulation instructed neuronal differentiation of active NSCs and promoted the activation or differentiation of quiescent NSCs in a dose-dependent manner. However, we found that inhibiting NSCs response to Wnt by conditionally deleting beta-catenin did not affect their activation or maintenance of their stem cell characteristics.
Discussion:
Together, our results indicate that whilst NSCs do respond to Wnt/beta-catenin stimulation in a dose-dependent and state-specific manner, Wnt/beta-catenin signalling is not cell-autonomously required to maintain NSC homeostasis, which could reconcile some of the contradictions in the literature as to the role of Wnt/betae-catenin signalling in adult hippocampal NSCs.

O-20 Mechanisms of neuronal maturation in the adult and aging brain

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Background:
Maturation of neuronal precursors in the adult brain occurs spontaneously in the neurogenic niches and, as we recently documented, in some cortical areas. Such maturation requires necessarily mechanisms that are different from those regulating the embryonic and neonatal development, as precursors must interface with and wire into fully developed brain networks. In this project, we questioned brain aging effects on neuronal precursor maturation. Furthermore, we searched for molecular mechanisms that may trigger the maturation of latent precursors in the adult brain.
Methods:
A transgenic mouse model (DCX-CreRT2::fl-eGFP) allowed tracing the maturation of neuronal precursors in the adult (9 months) or aged (15 months) murine brain. In these genetically engineered animals, tamoxifen administration induces permanent green labelling of neuronal precursors, which were thereafter analysed six months after induction. Morphological and immunohistochemical traits of aged precursors were analysed ex vivo. Additionally, transcriptome analysis allowed to detect candidate proteins selectively enriched in the neuronal precursors that may control maturation.

Results:
We found that aging affects precursor maturation; i.e. neurons derived from latent immature precursors in the aged brain are morphologically different from neurons derived from precursors which maturation occurs in early-adulthood. For instance, precursors that matured in the aged brain become bigger, have more dendrites and have a shorter axon initial segment, implying different functional properties in comparison to early-matured precursors. Focusing on precursor maturation in cortical areas, we could identify the likely triggers of precursor maturation in the adult brain impinge on two specific neurotransmitter receptors, which expression is selectively enriched in these cells.
Discussion:
Our preliminary results indicate that although neuronal precursor maturation can take place regardless of the age of the brain, the results of such process vary according to the time of onset. Thus, immature precursors may mature in a different type of neurons after the brain has become old. In this context, a deeper understanding of molecular mechanisms controlling precursor maturation will allow to modulate precursor maturation pharmacologically and evaluate the effects of early VS late precursor integration in the brain in vivo.

Abstracts of Posters

Ion Channels & Neurotransmission

P-01 Loss of autism-associated α2δ-3 affects synaptic protein expression, presynaptic function, and mouse behavior

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Background:

In the central nervous system voltage-gated calcium channels (VGCC) exert critical roles by regulating, for example, neurotransmitter release, gene expression, and synaptic plasticity. α2δ subunits are part of the multi-subunit protein complex and were traditionally envisioned as modulators of VGCC. However, recent studies proposed specific synaptic and channel-independent functions. Moreover, human genes encoding for α2δ subunits have been linked to neurological disorders including epilepsy, autism, schizophrenia, and bipolar disorder. Particularly the gene CACNA2D3, encoding for α2δ-3, has been strongly associated with autism spectrum disorders and a recent study described α2δ-3 knockout mice as a model to study cross-modal activation and possibly synaesthesia. This led us to propose that α2δ-3 knockout mice are a potential model for studying autism-like disorders. We further hypothesize that in the knockout mice defects in specific synapses or synaptic connections can explain the autism-like phenotype.

Methods:

To test this, we examined brain structure in Nissl-stained serial sections and analysed synaptic protein expression in α2δ-3 knockout mice. Moreover, we tested synaptic functions in α2δ-3 knockout cultured hippocampal neurons using presynaptic calcium imaging. Behavioural phenotypes are characterized by using a series of tests including, for example, open field and forced swim testing.

Results:

Brain structure analysis in Nissl-stained sections revealed no gross morphological changes, but illustrated a slightly reduced brain volume in α2δ-3 knockout mice. Biochemical analysis of whole brain and synaptosomal lysates demonstrated a strong reduction of neuroligin 1 to 3 and NMDAR 2B expression in α2δ-3 knockout. α2δ-3 over-expression in cultured hippocampal neurons significantly reduced the size of presynaptic boutons, suggesting a specific presynaptic role. Hence, we next tested synaptic function by imaging presynaptic calcium signals using the genetically encoded calcium sensor GCaMP6F. Knockout of α2δ-3 resulted in a reduction of presynaptic calcium transients at 1AP, 3AP, and 10 AP stimulations, while over-expression

produced the opposite effect. Finally, preliminary behavioural characterization of the knockout mice suggests a mild anxiogenic phenotype together with reduced passive coping compared to wild type littermate controls.

Discussion:

Taken together, loss-of α2δ-3 causes changes of synaptic protein expression and interferes with proper presynaptic function. Moreover, α2δ-3 deficiency results in a slight reduction of brain volume. Furthermore, knockout mice display a mild anxiogenic phenotype and a reduced passive coping. Both behaviours are in line with previous reports of autism-like behaviour in mice. In order to ultimately test the suitability of α2δ-3 knockout mice as a model for autism spectrum disorders, we will next test for signs of autism-like behaviour, and examine synaptic structure and wiring.

P-02 Investigation of α4-containing $GABA_A$ receptors in the rat brain

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Background:

γ-Aminobutyric acid (GABA) is one of the most important inhibitory neurotransmitters in the central nervous system and acts mainly by binding GABAA receptors. These are chloride ion channels assembled of 5 (out of 19 known) subunits. The majority of receptors is composed of α1β2/3γ2 and will form receptors which contain the α1(+)γ2(-)-Interface necessary for the binding of classical benzodiazepines. In the brain we can however find multiple additional receptor-subtypes which have so far only been poorly investigated. We know from past studies, that some of those receptor subtypes contain two different α-subunits. In rat cerebellum, for example, we can find α1α6-and γ2 containing GABAA-receptors, which can occur in two different subunit arrangements: one population containing a diazepam insensitive α6(+)γ2(-)-Interface, and another population with a diazepam sensitive α1(+)γ2(-)-interface (Scholze et al, 2020).

During the current project we plan to closer investigate α4-containing GABAA receptors in the rat brain trying to learn which subunits co-assemble in different brain regions, and how those identified subunits arrange within the pentameric receptor. We are especially interested in receptors, which are assembled of two different α-subunits (one of them being α4) in combination with γ2, hypothesizing that those receptors can come in two different arrangements, and will form two receptor populations (one with a diazepam insensitive α4γ2- and one with a diazepam sensitive αxγ2-interface).

Methods:

We performed radioligand binding assays with 3H-Flunitrazepam and 3H-Ro 15-4513. 3H-Flunitrazepam binds to the classical diazepam-binding site at the interface between γ and α1/2/3/5, while 3H-Ro 15-4513 binds to all αγ-contact sites (including α4γ2).

Results:
First we determined radioligand binding affinities to GABAA receptors in the brain regions Hippocampus, Striatum, Cortex and Thalamus of rats, where α4 is known to be expressed at above-average levels. With this information we were able to choose a single saturating ligand concentration in order to be sure to fully label all different receptor subtypes present in the selected brain regions. We then compared the binding sites detected by our two radioligands and determined the number of receptors containing an α4γ2-interface, since those are labelled by 3H-Ro 15-4513 but not by 3H-Flunitrazepam.
Discussion:
Our data indicate, that the number of GABAA-receptors containing an α4γ2-diazepam insensitive binding site differs in the brain regions studied. Future experiments will aim to further characterize α4-assembling partners in different rat brain regions by immunoprecipitating α4-containing receptors using subunit specific antibodies followed by western blot detection and/or additional radioligand binding experiments.
Reference:
Scholze P. et al, (2020) Front Synaptic Neurosci. Oct 6;12:591129

P-03 Selective targeting of α6-containing $GABA_A$ receptors

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Background:
Cerebella granule cell function depends on a delicate balance of tonic and phasic inhibition, largely mediate by α6 subunit expressing $GABA_A$ receptors. These are pentameric ligand gated chloride ion channels assembled of 5 out of a set of 19 known subunits. Depending on the subunits, which assemble, receptors with unique pharmacological and physiological properties are being formed. The different subunits also show unique anatomical distribution.

Animal models of several neuropsychiatric conditions suggest that compounds selectively activating or positively modulating cerebellar α6-containing $GABA_A$ receptors can alleviate essential tremor and motor disturbances in Angelman and Down´s syndrome, as well as sensorimotor gating deficits which occurs in several neuropsychiatric disorders. Moreover, they have been suggested to reduce migraine and trigeminal-related pain by acting on α6-containing $GABA_A$ receptors on trigeminal ganglia. Moreover, human data from genetic studies strongly suggests that the GABRA6 gene is a major node in the stress-responding networks. We thus are interested in developing novel α6-selective ligands as therapeutic prospects in neuropsychiatric conditions involving central and peripheral α6-containing $GABA_A$ receptors. In a collaboration with chemists from the University of Vienna, a mini-library yielded several compounds which selectively modulate α6 containing $GABA_{A-}$

receptors. Their in-depth characterization and structure activity relationship is currently ongoing.

Methods:

α1 and α6 containing $GABA_A$ receptors are expressed heterologously in Xenopus laevis oocytes and chloride anion currents are measured with two electrode voltage clamp recordings. Mutational analysis and structural modelling complements the functional data to verify the targeting of the "etomidate site."

Results:

We have so far identified several novel positive allosteric modulators which enhance the response to the endogenous ligand GABA in α6 containing $GABA_A$ receptors with higher potency and or efficacy than in α1 containing $GABA_A$ receptors.

Discussion:

With this work we pave the path towards developing novel ligands that might be useful in treating neuropsychiatric conditions involving central and peripheral α6-containing $GABA_A$ receptors and to generate tool compounds with which the stress sensitivity of cerebellar circuits can be investigated.

P-04 Visualization of nanoscale phosphoinositide distribution on neuronal cell membranes

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Background:

Phosphoinositides (PIs) are minor phospholipid components on the cytoplasmic leaflet of eukaryotic cell membranes, but they play essential roles in many aspects of cellular functions, including synaptic transmission in neurons. Although it is important to know how PIs distribute on neuronal cell membranes to understand their roles in synaptic transmission, it is not unveiled yet. Recently, biofluorescent probes for PIs based on a PI-binding domain (PBD) of proteins tagged with a fluorescent protein have been developed to visualize PIs in living cells. However, this method does not have sufficient spatial resolution to observe the nanoscale distribution of PIs in small areas in synapses in brain tissues. Furthermore, the overexpressed PBD-based probes mask PIs and competitively interfere with the interactions between proteins and PIs in living cells. This study aimed to solve these issues to observe nanoscale distribution of PIs on neuronal cell membranes of mouse cerebellum at the electron microscopic level and address their potential roles in neuronal activities including synaptic transmission.

Methods:

To investigate the nanoscale distribution of phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) on neuronal cell membranes in brain tissues, we established a labeling method using the SDS-digested freeze-fracture replica labeling (SDS-FRL) with a recombinant GST-tagged pleckstrin homology (PH) domain of phospholipase Cδ1 (PLCδ1) as a specific probe of PI(4,5)P2. The PI(4,5)P2-

labeled replicas were observed under transmission electron microscopy (TEM) to analyze nano-scale distributions.

Results:

First of all, we verified the specificity of the PI(4,5)P2 labeling between the stereoisomers of PIs using liposomes containing each PI under TEM. Using SDS-FRL on replicas of mouse cerebellar tissues, we found that PI(4,5)P2 clusters broadly distributed on the cytoplasmic side of plasma membranes of Purkinje cells (PCs) and parallel fiber (PF) boutons in the cerebellum. At PF-PC synapses, PI(4,5)P2 was concentrated at active zones (AZs) and co-localized with P/Q-type Ca2+ channels (CaV2.1) in presynaptic boutons. At post-synaptic sides, PI(4,5)P2 was co-localized with a metabotropic glutamate receptor mGluR1α and a G protein-gated inward rectifing potassium channel subunit GIRK3 outside of the postsynaptic density area.

Discussion:

In this study, we successfully observed the nano-scale distribution of PI(4,5) P2 on neuronal cell membranes in mouse cerebellum. Especially, it was concentrated at AZs of presynaptic PF boutons, indicating that PI(4,5)P2 contributes to the regulation of synaptic transmission. Furthermore, our works showed the co-localization of PI(4,5)P2 with CaV2.1, GIRK3 and mGluR1α at pre- and postsynaptic membranes. These observations also suggest potential roles of PI(4,5)P2 in regulation of neurotransmitter release, excitability and synaptic plasticity through interaction with ion channels and receptors.

P-05 Alteration in the retinal morphology due to a truncation mutation in the *cacna1f* gene

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Background:

Voltage-gated L-type calcium channels exert different cellular events, including excitation-contraction coupling, transmitter release, enzyme regulation and hormone secretion. In the retina, Cav1.4 channels are predominantly expressed at the synaptic terminals of photoreceptors in the outer plexiform layer (OPL) supporting tonic glutamate release, required for normal visual function. Mutations in the CACNA1F gene encoding Cav1.4 channels are linked to congenital stationary night blindness type 2 (CSNB2). CSNB2 is a X-linked non-progressive retinal disorder with symptoms of abnormal visual acuity, myopia, nystagmus, strabismus and impaired night vision. In this study we investigated a C-terminal truncation mutation in the Cav1.4 channel, which is caused by exchanging the base pair cytosine to thymine at position 5446 in the CACNA1F gene and encodes a stop codon (TAA) instead of the amino acid arginine (CAA). The truncation of the Cav1.4 channel leads to CDI, which is normally not present in this isoform of calcium channels.

Methods:
The study examined morphological changes in photoreceptor terminals and second order neurons in mice carrying the truncation mutation Cav1.4 R1827X (Cav1.4 RX) compared to inbreed wild type (WT) controls by using immunohistochemical analyses. Vertical retina slices (14 µm) were examined by Zeiss Axio Observer.Z1/7 fluorescence imaging microscopy. Image analysis is being performed by image analyze software ZEN 3.2 (ZEN light) and ImageJ.
Results:
In the Cav1.4-RX retina, PKCα immunostainings delineated the aberrant morphology of rod bipolar cells with dendrites extending deep into the outer nuclear layer (ONL). Also, horizontal cells exhibited a similar pattern. Sprouting rod bipolar and horizontal cells might also try to form new connections with displaced photoreceptor terminals, because they approached synaptic ribbons, which were found also in the ONL. Synaptic ribbons in Cav1.4-RX mice showed a punctate shape compared to WT retinas, which expressed typical horse shoe shape structures in the OPL only. Cones seemed to be largely unaffected, concerning the sprouting pattern and the altering in the synaptic terminals. Along these lines, immunostainings with anti-secretagogin demonstrated no difference between WT and Cav1.4-RX cone bipolar cells.
Discussion:
Initial immunohistochemical studies showed significant changes in the retinal morphology caused by a truncation in the C-terminus of Cav1.4 channels. The retinal alteration in horizontal and rod bipolar cells of this novel mouse line were similar to mice carrying a Cav1.4 gain-of-function mutation or Cav1.4 deficient mice([1]; for review see [2]). In contrast to the previously reported mouse lines, the morphological changes in the Cav1.4-RX retina seemed to affect primarily the rod and not the cone pathway. This observation might be explained by differences in the protein composition in rod and cone photoreceptor terminals. Based on this assumption further investigations will also include Cav1.4 interactome analysis in WT and mutant retinas.
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P-06 Consequences of an autism-associated mutation of α2δ-1 on calcium channel trafficking and synapse composition

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Background:
The roles of auxiliary α2δ subunits of voltage-gated calcium channels in modulating functional membrane expression and calcium current properties

are widely recognized. In addition, recent literature suggests an important role of α2δ proteins in synapse formation and differentiation. Therefore, it is not surprising that α2δ genes have been linked to various neurological and neuropsychiatric disorders, emphasizing their importance in brain connectivity. Here we aimed to establish new state-of-the art procedures to characterize human mutations in α2δ proteins by addressing their synaptic function besides their role as channel subunit. Because "synaptopathy" is an important component of autism spectrum disorders, we chose to start establishing these techniques by using a de novo missense mutation in α2δ-1 (p.Arg351Thr) that was identified in a patient with autism. Investigating potential autism causing mutations in α2δ proteins may shed light on their physiological and pathophysiological synaptic functions.

Methods:

First, we used site directed mutagenesis to introduce the p.Arg351Thr mutation into the mouse coding sequence of α2δ-1. To test plasma membrane trafficking and subcellular targeting we introduced an extracellular double HA tag and used cultured hippocampal neurons as homologous expression system. Moreover, to address potential consequences on synapse composition, we immunolabeled presynaptic and postsynaptic markers of excitatory and inhibitory synapsis in neurons expressing wildtype or p.Arg351Thr α2δ-1. Analysis was performed using high resolution immunofluorescence microscopy.

Results:

Live-cell labelling of cultured hippocampal neurons co-transfected with 2HA-tagged α2δ-1 and soluble eGFP revealed somatodendritic and synaptic targeting of the p.Arg351Thr mutant. However, compared to wildtype α2δ-1 overall plasma membrane trafficking was significantly reduced. We have previously shown that expression of α2δ-1 and α2δ-2 subunits without the alternatively spliced exon 23 induces a mismatched localization of postsynaptic GABAARs opposite glutamatergic nerve terminals. This function was preserved in the p.Arg351Thr mutant suggesting that the mutation does not strongly affect trans-synaptic signalling.

Discussion:

Our experiments indicate that α2δ-1 harbouring the autism-related mutation p.Arg351Thr shows normal subcellular and synaptic targeting, however, strongly reduced plasma membrane targeting. Preliminary analysis further indicates that mismatched synapse formation of an α2δ-1 splice lacking exon 23 was not compromised by the mutation. Structure-homology modelling identified a critical position of arginine 351 within the von Willebrand factor type A domain (VWA) of α2δ-1. Mutation of this amino acid may therefore destabilize the interaction of the VWA with the α1 subunit as well as with the δ peptide.

P-07 Establishment of a gene therapeutic approach for Cav1.4 voltage-gated calcium channel knock-out and truncation mutant mice

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Background:

Cav1.4 L-type voltage-gated calcium channels are predominantly expressed in the retina. They are localized at ribbon synapses where they couple membrane potential changes to glutamate release; thus, enabling signal transmission. Mutations in the CACNA1F gene, encoding for Cav1.4, can cause congenital stationary night blindness type 2 (CSNB2). CSNB2 patients exhibit variable levels of night blindness together with nystagmus, strabismus and low visual acuity. Due to the phenotypic variability seen in CSNB2 patients, the only diagnostic tool is the electroretinography. A majority of the disease-causing mutations, however, are predicted to cause severe structural changes and are unlikely to form fully functional channels. Amongst these channels also C-terminal truncation variants are of interest as they lack important regulatory domains. Consequently, we proof the concept of a Cav1.4 gene augmentation therapy in mice by either delivering the full-length coding gene or a fragment encoding the distal Cav1.4 C-terminus.

Methods:

To transport the coding sequences (based on JF701915.1) into cells, we employ recombined adeno-associated virus vectors (rAAVs). Due to the limited AAV packaging capacity we split the genetic information of the full-length Cav1.4 and packaged the halves into separate vectors. Split-inteins, which enable protein trans-splicing, have been added to ensure reconstitution of Cav1.4 proteins in cells. HA and myc tags have been included in the first and second half, respectively, allowing for detection in immunofluorescence studies and Western blot analyses. Whole-cell patch-clamp analyses have been employed to verify the reconstitution to functioning full-length channels. The C-terminal peptide was V5-tagged and upstream a fluorescent protein was added which was linked with a self-cleaving 2A-element. rAAV2/2 vectors carrying a CMV promoter were tested in retinal explant cultures, which have been cultured for up to two weeks on self-made gel islets, for their transduction efficacy. Moreover, these vectors were administered to adult, wild type C57BL/6 mice at a dose above 1e+08 vector genomes per eye by subretinal injections.

Results:
Western blot and whole-cell patch-clamp analyses indicated the reconstitution of split channel halves to functioning full-length channels after transfection of HEK cells, which co-expressed auxiliary subunits β3 and α2-β1. In rAAV-transduced cells, split Cav1.4 halves were co-expressed as shown by our immunofluorescence analyses. Additionally, the C-terminal peptide has been successfully expressed in vitro. Successful cultivation of explanted retinas allowed us to show rAAV-mediated fluorescent protein expression also in the retinal environment. Of note, explanted retinas preserved the overall morphology and synaptic ribbons after being kept in culture for up to two weeks. We further evaluated the delivery of rAAV vectors following in-vivo subretinal or intravitreal injections.
Discussion:
In this project, we used a split-intein–mediated protein trans-splicing strategy to reconstitute Cav1.4 L-type calcium channels from two separate fragments. This approach will help to advance our concept for Cav1.4 gene augmentation therapy using rAAVs. Moreover, successful delivery of a C-terminal peptide will rescue the effect of C-terminal truncations and extend our knowledge on the role of the C-terminal regulatory mechanism in Cav1.4 channels.

P-08 Biochemical characterization of voltage-gated calcium channel α1-α2δ subunit interactions

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Background:
Neuronal signaling depends on the precise timing of synaptic transmission within the central nervous system. This is enabled by voltage-gated calcium channels (CaV), which regulate neurotransmitter release and postsynaptic signal integration. Three different families of CaV (CaV1-3) were identified that differ in their biophysical properties and subcellular localization. Overall, CaV complexes consist of a pore-forming α1 subunit, an intracellular β, and an extracellular membrane-anchored α2δ subunit. To date, four α2δ isoforms (α2δ-1 to -4) are known, with α2δ-1 to -3 being abundantly expressed in brain. α2δ subunits have crucial roles in regulating CaV channel trafficking and current kinetics as well as synapse formation. Consequently, mutations in α2δ subunits are linked to neurological disorders such as epilepsy, schizophrenia, and autism spectrum disorders. While α2δ proteins show a promiscuous function in both channel-dependent and synaptic functions, as seen by rescue experiments of α2δ knockout neurons, it is still unknown, which α2δ subunits preferably associate with which α1, and how the specificity of these interactions is regulated. Within this project, we are testing the hypothesis that preferential α2δ and α1 subunit interactions are regulated by distinct binding affinities.

Methods:
The previously published cryo-EM structures of CaV1.1 and CaV2.2 channel complexes show a specific interaction between the von Willebrand factor type A (VWA) domain of α2δ-1 with the extracellular loops of the α1 subunits. Hence, to compare surface binding interfaces, we use homology modeling of individual complexes based on these existing structures. To biochemically characterize the interaction and to map the binding affinities of distinct subunit combinations, we express and purify the VWA domains of the three neuronal α2δ isoforms and perform pull-down assays using the extracellular loops of individual α1 subunits, fused to a GST-tag.
Results:
Homology models of individual complexes of α2δ with α1 subunits indicate differences in binding interfaces. Hence, we established soluble VWA-domain expression of rabbit α2δ 1 in E.coli and optimized the purification protocol. This protocol is now applied to purify the VWA domains of all neuronal α2δ proteins. Next, the corresponding binding affinities (KD) of the VWA domain to α1 subunits and to δ peptides will be measured using isothermal titration calorimetry. Specific point and chimeric mutations will aid in specifying the distinct interaction surfaces. Finally, hypotheses regarding CaV complex formation and function derived from the in vitro measurements will be tested in in vivo in cultured hippocampal neurons.
Discussion:
Elucidating the molecular nature and function of specific CaV complexes is critical for understanding the pathophysiology and treatment options of associated disorders. Within the CaV complex, the VWA domain of α2δ is ideally positioned to regulate not only the interaction with the α1 subunit, but also the position of the δ peptide. Therefore, the VWA domain may also be important for stabilizing the entire α2δ protein. Together with the physiological readouts in cultured hippocampal neurons, mapped binding affinities will help us to understand the specificity and diversity of neuronal CaV signaling.

P-09 Pentameric ligand-gated ion channels: can you get a hole-in-one?

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Background:
Human pentameric ligand-gated ion channels (pLGICs), comprising the nicotinic acetylcholine receptors (nAChRs), 5-HT3 receptors, zinc-activated channels, GABAA receptors and glycine receptors, mediate and modulate neuronal communication. They are common therapeutic targets of some of the most prescribed drugs like general anaesthetics, anxiolytics, smoking

cessation aids, antiemetics and many more. Currently approximately 100 cryo-EM and crystal structures of pLGICs with ligands bound exist in the protein data bank (PDB). These atomic level 3D structures allow to generate a comprehensive binding site inventory for the superfamily. They also help to estimate the presence or absence of shared sites with common ligands by computational integration of structure data, sequence data and indirect evidence for binding sites (such as mutational data). The impact of conformational states on binding sites can also be examined.

Methods:

Structure data from the PDB is analyzed with MOE and python scripts are utilized for conformational analyses and clustering. Principal component analyses and K-means are used to examine conformational states. Human sequences from uniprot along with structural data are aligned with promals3D. Local sequence similarities and binding site properties are investigated with Z-Scale descriptors.

Results:

The structural data shows the existence of known and novel binding sites, some of which may be unique to individual receptors, while others are broadly conserved. Biochemical evidence suggests an etomidate site in nAChRs in a position homologous to the site observed in cryo-EM structural studies in $GABA_A$ receptors. This prompted a comprehensive analysis of the upper portion of the membrane spanning domain in search for conserved sites. In addition, we find that conformational motion impacts strongly on some binding pockets, and to a negligible degree on others.

Discussion:

With this study we explore the potential for polypharmacology among pLGICs as observed for picrotoxin, which is a channel blocker of multiple family members ($GABA_A$ receptors, 5-HT3 receptors, nicotinic acetylcholine receptors and glycine receptors). Our data suggests that additional binding sites may also contribute to polypharmacology. Conformational states need to be considered for in silico drug screening, as certain binding sites can exist in collapsed states. Therefore, the question still remains if there are ligands that can achieve a hole-in-one. The analysis of this study is bringing us a step closer to the answer, as well as to more informed in silico drug design.

P-10 Western Blot Characterization of a human GABRA4 Variant

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Background:

$GABA_A$ receptors are heteropentamers that are assembled by five protein subunits which themselves can be further differentiated into different subunit classes. As $GABA_A$ receptors play a major role in neuronal inhibition, the genes encoding the subunits that assemble these receptors, are often

considered as potential underlying causes of a wide spectrum of monogenic epilepsies. Subsequently, more than 60 de novo mutations in genes encoding subunits of GABA receptors have been associated with various types of epilepsy. Here, we examine a single de novo mutation in the GABRA4 gene, encoding the α4 $GABA_A$ receptor subunit, found in an individual with early-onset, treatment-refractory focal epilepsy and developmental delay. As of today, GABRA4 has not been associated with a monogenic condition, but evidence suggests it to play a role in autism-spectrum disorder and seizure susceptibility. As mutations in $GABA_A$ receptors have been shown to be able to hinder protein expression, receptor assembly and trafficking processes in the cell, these could well be pathoaetiological mechanisms underlying the case of epileptic disorder at hand. Through the usage of the western blotting technique, the possible alterations of receptor expression levels of $GABA_A$ receptor containing the mutated α4 subunit are explored in this study.

Methods:

HEK293 cells were transfected with plasmid cDNAs encoding a combination of $GABA_A$ receptor subunits containing the mutated or the wild-type α4 subunit. Cell membranes were prepared for western blotting and protein levels of wild-type and mutated α4 subunits detected using a subunit specific antibody. Protein levels were normalized by using actin as an internal loading control.

Results:

We found no statistically significant differences of the protein levels of the wild-type and mutated α4 subunit in transfected HEK cells.

Discussion:

$GABA_A$ receptors are heteropentamers assembled by five protein subunits. During their synthesis, subunits are co-translationally inserted into the membrane of the endoplasmatic reticulum, where folding and oligomerization processes are effectuated. Correctly folded and assembled pentamers are then trafficked to the Golgi apparatus which further processes the pentamers, before they are being transported to the cell surface. As mutations in $GABA_A$ receptors have been shown to be able to hinder these processes, we compared the protein levels of the wild-type and the mutated α4 subunit in transfected HEK cell membranes by performing Western Blots. We found no significant differences between the protein levels, suggesting that the mutation neither alters protein expression nor assembly and trafficking processes of receptors. Further characterization of the GABRA4 mutation is needed to conclusively understand its effects and potential causative nature of epileptic disorders.

P-11 Topological comparison of mRNA expression patterns with receptor distributions in the human cerebral cortex

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Background:

Unveiling the underlying links between molecular mechanisms and protein distributions associated with them could lead to measure and analysis of in

vivo changes in multi-receptor systems. Imaging transcriptomic could help to close the gap between basic science and clinical application to make treatment more effective and individualize medicine. The main aim of this work is to present a comparison of gene expression patterns with the distribution of the various receptors in the human cerebral cortex to shed light on the relations of mRNAs and proteins.

Methods:

To this end, we made use of four different data sets. In particular, autoradiography data published by Zilles et al. [1] and three transcriptomic data sets representing - interpolated mRNA expression patterns (int-mA) published by Gryglewski et al. [2], normalized microarray (mA) and RNA sequencing (RNA-seq) data, published by Allan Brain institute [3]. The spatial distribution of 15 receptors listed in [1] and 64 gene expression patterns, associated with at least one of the receptors, were represented by mean regional values of 38 cytoarchitectonic areas defined in the Julich-Brain atlas [4]. Spearman's rank correlation, complemented with spatial permutation test to assess the significance of the correlation, was used to compare proteomic and gene expression data sets.

Results:

Solid positive associations between autoradiography and transcriptomic data sets were found for e.g. serotonin 1A receptor (int-mA: r=0.71, mA: r=0.60, RNA-seq: r=0.54) or glutamate ionotropic receptor kainate type subunit 2 (int-mA: r=0.63, mA: r=0.60, RNA-seq: r=0.56). On the other hand, analysis revealed negative associations for e.g. $GABA_A$ receptor subunit alpha 3 (int-mA: r=-0.64, mA: r=-0.62, RNA-seq: r=-0.49) as well as for $GABA_A$ receptor subunit beta 1 (int-mA: r=-0.58, mA: r=-0.54) or $GABA_A$ receptor subunit theta (int-mA: r=-0.50, RNA-seq: r=-0.61). In general, most of the receptors showed low correlation values (int-mA: 60%, mA: 74%, RNA-seq: 76% of $r<0.35$).

Discussion:

Spatial distribution comparison of transcriptomic and proteomic data revealed few strong associations between receptor distributions and mRNA expression patterns. However, most of the evaluations revealed negative or no correlation, suggesting weak or no relation of a single gene expression pattern to protein distribution. Altogether, our results are highlighting the importance of further studies on splicing variants and potential mRNA markers indicating the role of post-transcriptional and post-translational modifications.

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P-12 Enantioselective drug-binding kinetics shape the psychostimulant effect of dopamine transporter inhibitors

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Background:
The pharmacology of different compounds is normally assessed under thermodynamic equilibrium, however, in the human body, the drug-target interaction is influenced by the constant flux of fluids and a variety of physiological processes. Hence, the in vivo properties of a drug differ from the in vitro thermodynamic equilibrium. α-Pyrrolidinovalerophenone (αPVP) is a psychostimulant and drug of abuse associated with severe intoxications in humans. αPVP exerts long-lasting psychostimulant effects, when compared to the classical dopamine transporter (DAT) inhibitor cocaine.
Methods:
We used radiotracer assays, computational approaches, transporter electrophysiology and behavioural assays in mice to investigate the role of binding kinetics in the psychostimulant effect elicited by αPVP enantiomers.
Results:
We found that αPVP enantiomers substantially differ from the DAT-inhibitor cocaine in their binding kinetics. The two enantiomers differ from each other in their association rates, but they show similar slow dissociation rates leading to pseudo-irreversible binding kinetics at DAT. The pseudo-irreversible binding kinetics of αPVP is responsible for the observed non-competitive pharmacology, correlates with persistent psychostimulant effects in mice and differ from the fast-acting DAT-inhibitor cocaine.
Discussion:
Our work shows that drug binding kinetics at DAT can have a significant impact on the psychostimulant effects of drugs in vivo. Given this informa-

tion, a more detailed assessment of drug-binding kinetics for DAT inhibitors might provide insights for the design of novel DAT inhibitors with improved clinical utility. Furthermore, it shows how psychopharmacological research on illicit drugs may help to reach a better understanding of physiological and toxicological processes.

P-13 A novel loss-of-function variant in Cav2.1 channel in a patient with spinocerebellar ataxia

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Background:

In the brain, voltage-gated Cav2.1 Ca2+ channels (encoded by the CACNA1A gene) mediate Ca2+ influx and fast neurotransmitter release at presynaptic active zones. They are strongly expressed in cerebellar Purkinje cells. It is well known that poly-glutamine expansions in the C-terminus of the Cav2.1 pore-forming α1-subunit cause spinocerebellar ataxia type 6 (SCA6), a progressive neurodegenerative disorder with predominantly cerebellar signs. Here we describe an atypical case of SCA6 which shows no poly-glutamine expansions but a heterozygous missense mutation, C256F, in the Cav2.1 α1-subunit. Our aim was to assess the pathogenicity of this novel mutation in a heterologous expression system.

Methods:

The effects of the C256F mutation on Cav2.1 channel gating was studied using the whole-cell patch-clamp technique in tsA-201 cells transiently transfected with Cav2.1 α1-subunit and auxiliary β4e and $\alpha 2\delta 2$ subunits. A Cav2.1 homology model of the α1-subunit in complex with the $\alpha 2\delta$-subunit was generated based on the known cryo-EM structure of the rabbit Cav1.1 channel.

Results:

The mutation C256F caused a significant reduction of the maximal Ca2+-current density compared to wild-type channels (pA/pF, mean ± SEM: WT: 79.67 ± 9.11, n = 39; C256F: 44.58 ± 5.12, n = 30; $p < 0.01$, Student's unpaired t-test). This could not be explained by a lower protein expression level of the mutant channel. No differences in the channel voltage dependence of

activation and steady-state inactivation between mutant and wild-type were observed. However, a small but statistically significant increase of current inactivation was found which was independent of current density. Our homology model predicted that this mutation disrupts one of two disulfide bonds formed by four cysteine residues conserved in all voltage-gated Ca2+ channel α1-subunits. This can cause increased flexibility of the extracellular pore loop of domain I which is known to bind auxiliary $\alpha 2\delta$ subunits.

Discussion:

Our results show that by reducing current density and promoting channel inactivation the SCA6-associated C256F mutation induces a loss of Cav2.1 channel function as observed for CACNA1A variants causing Episodic Ataxia Type 2. Thus, we provide functional evidence that episodic and spinocerebellar ataxia can both share a loss-of-function pathomechanism. Therefore, we recommend that polyglutamine repeat expansion-negative SCA cases should also be screened for CACNA1A missense variants. We also show that disruption of the disulfide bond between C256 and C281 is essential for normal Cav2.1 channel function. Our model predicts that its disruption leads to increased flexibility of the extracellular loop. If this impairs binding of auxiliary $\alpha 2\delta$ subunits required for Cav2.1 membrane targeting needs to be tested in further experiments.

P-14 Structural determinants for channel gating and excitation contraction coupling in CaV1.1

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Background:

CaV1.1 is a calcium channel exclusively expressed in skeletal muscle where it functions as voltage-sensor in excitation-contraction coupling (ECC). CaV1.1 is a polypeptide folded into four homologous repeats (I–IV), each with six trans-membrane segments (S1–S6). Helices S1–S4 of each repeat constitute the voltage-sensing domains (VSDs), while S5 and S6 form the channel pore. The S4 segment responses to voltage changes acting on its evenly distributed positive gating charges (R0-R5). Upon membrane depolarization S4 moves upwards, thus opening the channel gate and/or activating ECC. The voltage-dependence and kinetics of S4 movement are regulated by negative counter-charges located in the surrounding helices of each VSD. Here we hypothesize that mutations of counter-charges which alter specific interactions with gating charges will change the intrinsic properties of the VSD, and consequently reveal the role of that VSD in activating channel gating and/or ECC.

Methods:
To test this hypothesis, we applied site-directed mutagenesis of residues suggested by MD simulations of the CaV1.1 structure as well as disease-causing mutations of putative counter-charges in different VSDs. The channel constructs were expressed in CaV1.1-null myotubes; channel gating properties were analyzed with patch-clamp recordings and ECC using fluorescence calcium indicators.
Results:
Previous research from our lab showed the importance of outer counter-charges in VSD I on current kinetics (E90) and voltage sensitivity (E90/E87), demonstrating the role of VSD I in channel gating. Unpublished results indicate the involvement of VSD II in channel gating and ECC. Based on these results, and computational structure predictions, we identified and mutated analogous counter-charges in the VSD II (D465, N468) and in VSD III (D836) to test their involvement in the regulation of channel gating and ECC. Preliminary results on VSD II and VSD III show minor effects on voltage sensitivity and kinetics of calcium currents. Therefore, these VSDs appear secondary for determining gating properties, but might be important for controlling ECC. Furthermore, a disease causing mutation (E100K) in VSD I indicated the importance of E100 as counter-charge for the innermost gating charge R4. Probably, the charge reversal in E100K impedes voltage sensing and activation of L-type calcium current by prohibiting the formation of ion pairs and introducing repulsive forces. In order to discriminate between these two possible effects, we introduced a neutralizing mutation (E100A) and the charge-reversing disease mutation (E100K) into CaV1.1. Preliminary data support the role of E100 as counter-charge in VSDI and as regulator of the voltage-dependence of calcium current activation.
Discussion:
Together these results highlight both the similarities and differences of the voltage-sensing mechanisms of the four VSD of CaV1.1, and their respective roles in regulating channel gating properties. Their possible involvement in ECC is currently under investigation.

P-15 A novel Ca channel β2 splice variant is predominant in the retina

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Background:
The N-terminus of calcium channel β2 subunits is a hub of alternative splicing. These largely N-terminal splice variants can be grouped into non-membrane-anchored (β2b, -c, -d) and membrane-anchored (β2a and -e). The membrane-

anchoring feature has profound effects on the biophysical properties of the calcium channel complex they are part of, with β2a and β2e slowing down voltage-dependent inactivation. Here we report the discovery of a novel, as of yet unpublished, N-terminal splice variant of β2 we call β2i.

Methods:

Published RNA sequencing (RNAseq) datasets of mouse retina were screened for Cav β2 alternative splicing and expression of N-terminal variants was confirmed by 5'RACE PCR and quantitative real-time PCR (qPCR) in mouse retina and other tissues. The novel β2i variant was cloned and its basic properties were investigated in heterologous expression in HEK293T cells: I) We fused a fluorescent reporter to the β2i C-terminus and determined its membrane-targeting characteristics, independent of calcium channel complex association. II) We co-expressed β2i with Cav1.4 and β2δ-4 – the native retinal photoreceptor calcium channel complex – and measured the biophysical parameters, voltage-dependence and inactivation, of this channel composition.

Results:

RNAseq suggested the predominant expression of a novel N-terminal exon we called exon 2E upstream of β2 exon 3 in mouse retina, which our 5'RACE and qPCR experiments confirmed. The novel exon 2E-containing β2i variant was not found in mouse heart muscle, pancreatic islets, cochlea, cerebellum or hippocampus but was predominantly expressed in retina and pineal gland. The β2i coding sequence fused to a C-terminally attached mEmerald revealed a localization at the plasma membrane when expressed in HEK293T cells, but not comparable to β2a and β2e. β2i clustered and in addition accumulated in the Golgi apparatus. We identified residues critical for the targeting properties in the N-terminus by Alanine-substitution. The calcium currents in Cav1.4 channel complexes with β2i showed unchanged voltage-dependent inactivation in comparison to complexes with β2a or its derivative β2X13 but a slowed inactivation compared to β2d with comparable voltage-dependence of activation.

Discussion:

We identified a novel β2 splice variant that is predominant in the retina, thus expanding the repertoire of distinct N-terminal sequences of this isoform. As we did not observe substantially different biophysical properties in comparison to β2a N-terminus variants, we assume there is another aspect of β2i that makes it the variant of choice for retinal cells. The accumulation of β2i in the Golgi, together with the more clustered membrane localization might point towards an enrichment of β2i in specific target regions – possibly cholesterol-rich types of membranes – that might be relevant for their native photoreceptor terminal environment.

P-16 Impact of partially duplicated α7 subunits on α7 nicotinic acetylcholine receptor function in human iPSC-derived neurons

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Background:
Genome-wide association studies suggest a critical role of the CHRNA7 (coding for α7 nAChR) and/or CHRFAM7A genes in cognitive and psychiatric disorders including cognitive deficits, epilepsy, language impairment, acute stress disorder, attention deficit hyperactivity disorder, schizophrenia, hypotonia and many others. CHRFAM7A occurs only in humans and encodes a partially duplicated version of the α7 nAChR (dup α7) subunit lacking the agonist binding site. CHRNA7 and CHRFAM7A are located in an unstable region of the genome, thus resulting in copy number variations (CNVs) of either of the two genes. In our project, we aim to investigate the functional consequences of CHRFAM7A CNVs on α7 nAChR in human neurons using Fura-2 Ca2+-Imaging and Patch-Clamp electrophysiology.
Methods:
There is a lack of animal models for human specific genes and human neurons as a cell source are even more limited. Therefore, cortical neurons are differentiated from human induced pluripotent stem cells (hiPSCs) with either overexpressing or lacking CHRFAM7A. hiPSC derived neurons were stained by using fluorescently labelled neuronal markers for molecular characterization.
Results:
With patch clamp recordings in voltage-clamp mode, hiPSC-derived neurons responded to depolarizing voltage steps by inward-directed sodium currents and outward-directed potassium currents. After at least 4 weeks of differentiation, these currents formed the basis for trains of action potentials recorded in current-clamp mode. hiPSC-derived neurons carrying two copies of CHRNA7 and CHRFAM7A responded to a supra-threshold concentration of the α7-specific agonist PNU-282978 by firing series of action potentials. Moreover, we observed a large increase in the frequency of miniature excitatory post-synaptic currents upon combined applications of PNU-282987 and PNU-120596, a type II positive allosteric modulator (PAMII) of α7 nAChRs. Fura-2 Ca2+-Imaging revealed that in the presence of TTX, PNU-282987 or choline, when combined with PNU-120596, and 4BP-TQS, which has both PAMII and allosteric agonist properties, induced an increase of intracellular Ca2+ in a fraction of neurons.
Discussion:
We are able to differentiate hiPSC into functional cortical neurons and characterize them using Patch-Clamp electrophysiology and Fura-2 Ca2+-Imaging. In future experiments, we plan to repeat those experiments using hiPSCs with higher expression levels of dupα7 (after lentiviral infection), or with

reduced expression levels (through CRISPR/Cas9 technology). In conclusion, Fura-2 Ca2+-imaging and patch-clamp electrophysiology provide us with tools to study the impact of CHRFAM7A CNVs on the function of α7 nAChR in hiPSC-derived neurons and to understand the possible molecular mechanisms causing the phenotypes of this α7 nAChR polymorphism.

P-17 Channel-independent membrane expression of individual α2δ isoforms

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Background:
α2δ is an extracellular membrane-attached protein serving as an auxiliary subunit of voltage-gated calcium channels. It regulates membrane expression of the pore-forming α1 subunit and modulates current properties of the channel. Four isoforms of α2δ are known, three of which (α2δ-1, -2, and -3) are expressed in brain and α2δ-4 is exclusively expressed in the retina. Particularly α2δ-1 is the target of the antiepileptic and antiallodynic drugs gabapentin and pregabalin. Recent studies suggest a new, calcium channel-independent, critical role of α2δ subunits in the formation of glutamatergic synapses. Hence, the aim of this study is to investigate the ability of α2δ subunits to be expressed in the membrane of heterologous cells in a channel-independent matter.
Methods:
To this end, gene delivery of individual α2δ subunits into tsA201 cells was performed using calcium-phosphate transfection. To analyze surface expression of α2δ subunits, live cell immunostaining against an extracellularly introduce HA epitope was performed. Surface expression of α2δ subunits was calculated as a ratio of the summarized area of all α2δ clusters normalized to the total area of the plasma membrane.
Results:
Our data show that neuronal α2δ isoforms are strongly expressed in the membrane of tsA201 cells without forming a complex with α1 subunits. Expression levels of all three neuronal isoforms was at the comparable level. Importantly, membrane expression of the retinal isoform α2δ-4 was not detectable, although total protein was expressed at similar levels. To compare expression of single α2δ isoforms to those in calcium channel complexes, α2δ proteins were co-transfected with α1 and β subunits (CaV2.1+β4e+α2δ, CaV1.3+ β2a+α2δ, CaV1.4+ β2a+α2δ). Similarly to individual expression, all neuronal α2δ proteins within calcium channel complex are expressed in the plasma membrane, however, at a slightly reduced expression level. Interestingly, membrane expression of α2δ-4 was not detectable even in combination with its endogenous interaction partner CaV1.4.

Discussion:
All neuronal α2δ isoforms can be expressed in heterologous cells with or without forming a complex with α1 subunits. We assume that the observed reduction of surface expression of α2δ in complex with β1 subunits represents a higher local density of α2δ subunits in compact clusters formed by calcium channels. Surface expression of α2δ-4 most probably requires another interaction protein (along with CaV1.4), since α2δ-4 can be expressed in the surface of hippocampal neurons.
Because all three neuronal α2δ isoforms similarly increase surface expression and ionic currents through α1 subunits, our findings support a redundancy of α2δ subunit functions in heterologous cells. The mechanisms underlying surface expression of retinal α2δ-4 in neuronal and non-neuronal cells will be further investigated.

P-18 HIF-1α induces Cav3.2 and paves the way for post-ischemic epileptogenesis

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Background:
Stroke is among the most common causes of death and disability in industry nations and one of the main reasons of acquired epilepsy in adults. About 3-25 % of patients develop seizures in the years following a stroke, depending on various factors, such as area of incident, type of stroke or age. However, little is known about the underlying molecular biological mechanisms that occur during epileptogenesis, so between the transient hypoxia and the first late seizure.

Some transcription factors drive gene-expression towards an epileptogenic phenotype by inducing various channelopathies. Channelopathies are one of the hallmarks of epilepsy and can be acquired through transient brain insults. A channel increasingly in focus in recent years is the T-type Ca2+ channel CaV3.2.

Upon hypoxia, hypoxia-inducible factor 1α (HIF-1α) is quickly activated and can bind to the promoter of T-type current Ca2+ channels. It could therefore induce an epileptogenic cascade in penumbra neurons. However, if HIF-1α underlies hypoxia-induced epileptogenesis is yet unknown. We therefore investigated if HIF-1α leads to an activation of pro-epileptogenic cascades in primary cortical neurons by a molecular biological as well as electrophysiological approach.
Methods:
We used a murine glioblastoma cell line (NS20Y) and overexpressed HIF-1α. To quantify the effect of HIF-1α on Cav3.2 we used the luciferase dual repor-

ter assay under the promoter of Cav3.2 as well as a fluorescent reporter under the Cav3.2 promoter. Furthermore, we performed the dual luciferase assay in murine primary cortical neurons, which were transduced with an AAV containing HIF-1α under a neuronal-specific promoter. To identify the effect of HIF-1α overexpression on the network activity of cortical neurons, microelectrode arrays (MEA) were performed and spike rate, burst rate and length, network burst rate and length as well as weighted mean firing rate was measured over one week for 20min every second day.

Results:

In NS20Y cells, we found a drastic upregulation of CaV3.2 upon overexpression of HIF-1α under normoxic conditions. This was shown by the luciferase dual reporter assay, where we observed a nearly 5-fold upregulation, as well as by the fluorescent reporter assay, where Cav3.2-mRuby was stronger expressed compared to the control group. In primary cortical neurons, we could confirm these results as we also found a significant upregulation of Cav3.2 upon HIF-1α overexpression by luciferase dual reporter assay. Interestingly, the MEA recordings showed that neurons transduced with HIF-1α developed an increased weighted-mean firing rate as well as higher numbers of network bursts. This could point towards pro-epileptogenic network activity.

Discussion:

Although PSE accounts for most acquired epilepsies in elderly, hardly anything is known about the underlying pathomechanism. Here, we provide first evidence, that transient ischemia could trigger pro-epileptogenic processes, which could together with other factors, such as gliosis and inflammation, lead to chronic seizures after a stroke. We could show that overexpression of HIF-1α not only leads to an upregulation of Cav3.2, but also causes network changes, as indicated by an increased weighted mean firing rate and more network bursts. Taken together, these results provide first insights into the molecular and electrophysiological mechanisms underlying the development of recurrent seizures after transient ischemia.

P-19 STAC proteins inhibit calcium and voltage dependent inactivation in L-type calcium channels

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Background:

Recently, we demonstrated that calcium-dependent inactivation (CDI) of L-type calcium channels (LTCC), an important negative feedback mechanism in calcium signaling, is inhibited by the co-expression of STAC proteins. However, this CDI inhibition could not be demonstrated for currents of the skeletal muscle channel CaV1.1, as CaV1.1 requires STAC3 for its functional expression. Interestingly, CaV1.1 calcium currents show negligible inactivation, which could be either an intrinsic property of the channel or the result of an inhibitory effect of STAC3 on the inactivation of CaV1.1.

Methods:
In order to discriminate between these two possibilities, we inserted a triple mutation in the linker region of STAC3 (ETLAAA), as the analogous mutation in the paralog STAC2 was shown to abolish the inhibitory effect on the CDI of CaV1.3. We performed patch clamp electrophysiology experiments in HEK cells with either calcium or barium in the extracellular solution to distinguish between CDI and VDI (voltage dependent inactivation).
Results:
We found that STAC3 inhibits the VDI rather than the CDI of CaV1.1 currents. To further investigate the effect of STAC proteins on VDI, we did experiments on other LTCC. We found that STAC proteins do not only inhibit CDI but also VDI of CaV1.2 and CaV1.3. Using the ETLAAA mutant, we could demonstrate that the inhibition of VDI relies on the same interaction, between STAC and the calcium channel, as CDI. Experiments co-expressing CaV1.3 with β2a and with or without STAC3 revealed STAC proteins inhibit VDI using a distinct mechanism than membrane tethered β subunits.
Discussion:
Here we show that STAC proteins play in important role by inhibiting not only the calcium-dependent CaV inactivation process but also the voltage-dependent one (VDI).

P-20 Acetaldehyde blocks the activation of BK channels by the ethanol metabolite acetate in GH3 cells

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Background:
Acetaldehyde and acetate are the two main metabolites of ethanol. They are known to produce their own effects, ranging from behavioural and physiological to pathological/carcinogenic actions. However, their physiological role is much less understood than that of ethanol itself. Even less is known about how these molecules interact with each other. For a better understanding of the physiological effects of ethanol, it is necessary to investigate not only the individual effects of each molecule of the ethanolic metabolic pathway, but also their co-combined impacts. In this study, we investigated the effects of acetate and acetaldehyde when applied co-combined on the large conductance calcium (Ca2+)-activated potassium (K+) channel also referred as BK or Maxi K+ channel which is very well known to be one of the main targets of ethanol in the brain.
Methods:
GH3 pituitary tumor cells were kept in MEM (minimal essential medium), supplemented with 7% fetal calf serum and 3% horse serum under standard cell culture conditions. Physiological standard Ringer solutions were used. Acetic acid was added to the bath solution at a concentration of 0.02% (3.49 mM), lowering the pH to 6.04. The pH was not corrected after the application

of acetic acid. Acetaldehyde was applied to the pipette solution in a nM to µM range. Electrophysiological experiments were performed with an Axopatch 200B. Data were recorded and analysed with pClamp 10.7 software. Single channel recordings were made in the excised outside out patch configuration. In silico models of the BK channel in complex with ethanol, acetic acid and acetaldehyde were obtained by protein-ligand docking calculations with the software tools AutoDock Vina [1] and UCSF Chimera [2].

Results:

Acetic acid at a concentration of 0.02% significantly increased the opening probability of BK channels when applied to the extracellular side of the channel. In the presence of acetaldehyde, on the intracellular side of the membrane, the effect of acetic acid on channel activity was blocked in a dose-dependent manner. These results were correlated with molecular modelling data.

Discussion:

In previous studies, we have already shown that both ethanol and acetic acid when applied alone increase BK channel activity [3,4]. In addition, we reported previously that acetic acid applied with uncorrected pH hyperpolarized the membrane potential of GH3 cells while a similar acidic solution depolarized the cells4. We also reported that acetaldehyde alone has only little effect on BK channels [5]. In a previous work, we showed that acetaldehyde blocked the activity-increasing effect of ethanol on BK channels in a dose-dependent manner. Here, we show the same for the co-combination of acetate and acetaldehyde. The present study establishes the important modulatory role of acetaldehyde in the physiological effects of alcohol and its metabolites on BK channel activity.

References:

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Cognition & Behavior

P-21 Decoding adaptive performance

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Background:

The plasticity in the sensorimotor system enables the brain to adapt its internal models of movements, according to sensory feedback signals, to refine motor performance. In the electroencephalography (EEG) signal, beta

band activity (BBA; ~13-30Hz) plausibly reflects sensorimotor processing in the cortex and therefore have been implicated in several aspects of motor learning and motor adaptation. Recent studies have demonstrated an increase in BBA during REM sleep and linked this increase to the sleep-mediated model updating processes. In this study, we elaborate on the role of sleep in the adaptation of fine-motor skills.

Methods:

To disentangle the role of sleep in motor adaptation, we employed a novel fine-motor task, i.e. typing on a mirrored keyboard. To this end, we trained human experts in touch-typing on the regular keyboard, to type on a mirrored keyboard and measured their performance before and after a retention interval of either a full night (~8h) of sleep with polysomnography (PSG) or a similar period of wakefulness. In total, we recruited 33 experts in touch-typing (N=16 for the sleep group and N=17 for the wake group) in a randomized, between-subjects design. Participants had to type eighteen five-letter, German words on a regular as well as a mirrored keyboard as rapidly and accurately as possible. We trained a linear discriminant analysis (LDA) classifier to decode i) correct from incorrect trials as well as ii) Regular from mirrored-typing trials based on pre- as well as post-movement EEG activity.

Results:

We show that brain activity in the 1s pre-movement period predicts typing performance. Specifically, the classifier was able to decode correct from incorrect typing trials in both regular and inverted typing conditions. Interestingly, following a period of sleep, the decoding accuracy for inverted typing increased significantly, with the change in decoding accuracy correlating with the change in the accuracy of typing on the mirrored keyboard, suggesting a role for sleep in optimizing adaptive behaviour. Confirming such observation, The accuracy of a classifier trained on BBA in the post-movement periods, presumably implicated in the process of model updating, increased significantly after a period of sleep but not wakefulness. However, sleep did not influence the decoding accuracy between regular and inverted typing trials.

Discussion:

Our results demonstrate a role for sleep in optimizing motor adaptation processes. Moreover, these findings suggest that post-movement BBA orchestrates memory processes essential for the adaptation of motor behaviour with such processes recommencing during subsequent sleep.

P-22 Drifting memories: spontaneous long-term evolution of memory representations in the hippocampus

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Background:

Sleep's fundamental role for the processing of memory and its consolidation has now received substantial experimental support. Nevertheless sleep can be

hardly considered as an homogeneous state: it consists of multiple stages that can be broadly classified in the two main categories of REM and non-REM (nREM) sleep. These two sleep states show widely different physiological characteristics both at the level of local activity and in terms of global brain dynamics. Importantly, their relative contribution to memory function is largely unknown and questions about their interaction during offline processing of newly acquired information have remained mostly untapped.

Methods:

In this study, we address these issues by combining a goal-directed learning task with long-term wireless electrophysiological recordings in the hippocampus of rats. After the acquisition of a novel episodic-like memory, place cell activity was continuously tracked for an extended period of time (>10hrs) while animals rested. We then combined multiple decoding approaches to obtain a time-resolved characterization of the evolution of a memory representation during sleep following its initial encoding.

Results:

Over the course of several hours, we could track a continuous drift in the reactivated activity patterns, as they progressively accumulated distance from the representation expressed at the end of learning. Intriguingly, the direction of drift was not constant: a closer inspection in fact reveals opposing effects for REM and nREM phases. While nREM sleep, pushed the reactivated activity away from the old representation, REM sleep coincided with periods of reversal, partially resetting the ongoing reconfiguration. We identified firing rate changes as the main driver of the observed drift. REM and nREM reactivations present otherwise only minor differences: while the reactivation content was largely overlapping in the two phases, activity patterns expressed during REM present a higher similarity to the awake ones, possibly due to REM slower temporal dynamics.

Discussion:

Together these results present a first-time detailed account of the effects of offline reactivations on the evolution of hippocampal memory representations. We show how the effect of REM and nREM stages integrate over the course of sleep in reshaping memory-related neural activity, a phenomenon relevant not only in understanding the nature of neural coding but also in establishing a link between memory transformation and homeostatic processes.

P-23 Innate sensorimotor processing deficits across mouse models of autism

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Background:

Disordered sensory processing is a widespread yet often overlooked feature across the autism spectrum (AS). Affected individuals frequently exhibit difficulties with gaze fixation, visual attention, and hyper- or hyposensitivity to visual stimuli, and mounting evidence suggests that the circuits involved in

visual information processing are disrupted. To directly study these phenomena, we took advantage of three different mouse models of autism and analysed their behavioural and electrophysiological responses to an innate, robust, and reproducible sensorimotor transformation – the looming avoidance response (LAR). Importantly, the circuits involved in LAR are well characterised and include the midbrain Superior Colliculus (SC), known primarily for its role in orienting visual attention, allowing functional circuit dissections.

Methods:

We characterised the behavioural properties of the LAR in Setd5, Cul3, and Ptchd1 mouse models (genes known for their strong penetrance in ASD) using a custom-built behavioural system. We then dissected the neural responses to the looming stimulus within the SC using 32 channel silicon probes in a head-fixed preparation while simultaneously tracking behavioural responses. The ability of the output pathways of the SC to pass information downstream was probed at the circuit level in Setd5 mice using optogenetics to activate excitatory cells within the SC.

Results:

We observed a marked and specific difference across the three genetic AS mouse models. Although animals can respond like their wild-type siblings and show no obvious behavioural differences, on average, they take longer to initiate defensive behaviour. This indicates a deficit in threat assessment. Moreover, despite displaying intact sensory transformations as probed by the spatiotemporal properties, the electrophysiological analysis identified divergent responses to looming stimuli within the SC. Together these results suggest a cognitive rather than sensory or motor deficit. We next probed the output neurons of the deeper layers of the SC that are known for their role in initiating the LAR. Preliminary experiments indicate that optogenetically activating the SC outputs in the Setd5 model mice differentially affects the behavioural responses, consistent with the LAR and measured physiological differences.

Discussion:

Our results show that genes associated to autism affect collicular dynamics and behavioural responses to threats, linking autism with a brain region known for its role in spatial attention and goal directed behaviours and opening a novel avenue to study visual attention deficits in autism.

P-24 A predictive processing framework for single arm use in octopuses

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Background:

Octopuses are highly intelligent animals with vertebrate-like cognitive and behavioural repertoires. Despite these similarities, vertebrate-based models of cognition and behaviour can be difficult to apply to octopuses, due to the vast

differences between structural and functional characteristics of vertebrate and cephalopod nervous systems. For instance, the octopus brain does not support a somatotopic or point-for-point spatial map of the body—an important feature of vertebrate nervous systems. Thus, while octopuses are capable of motor tasks whose vertebrate counterparts require detailed interoceptive monitoring, these movements are challenging to explain using motor control frameworks premised on internal spatial representation. One such motor task is the extension of a single arm.

Methods:

This conceptual study uses the analytical methods of philosophy to develop an explanatory framework for single arm use in octopuses.

Results:

This study accounts for single arm use in octopuses using a predictive processing framework.

Discussion:

Single arm use is driven by prediction error minimization, wherein the arm to be used is that which fulfils both visual and proprioceptive predictions generated as a result of the intention to perform a reaching and object retrieval task.

P-25 Neural signatures of contextual learning strategies

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Background:

Individuals need to track their position within an environment, and will learn to associate different behaviors with different locations. On the other side, the same location might trigger different behavioral responses depending on the contextual cues that are presented. This context-dependent behavior is known to rely on the hippocampus, an area in the mammalian brain which is famously known for its ability to encode precise spatial and contextual representations of the environment. Nonetheless, how these two levels of representation form and interact is still not entirely clear. Moreover, it is not known whether differences in learning abilities across individuals might influence the above mentioned representations. Here we investigate how memory representations in the hippocampal area CA1 evolve throughout this learning process to support performance.

Methods:

Five rats were chronically implanted with movable tetrodes unilaterally in the dorsal hippocampus area CA1. Local Field Potential (LFP), cell activity and animal position were recorded while the animals performed a memory task in an S-shape linear maze containing sand-filled holes along the track. During each trial animals had to correctly choose 2 out of 8 possible holes to dig for food. Location of one the rewards depended on the context presented (two possible contexts learned in parallel), defined by tactile and visual cues in the track. Animals performed 40 trials each day (20 per context, randomly

starting at either end of the track) until asymptote. We then analysed the data by extracting spikes from the multiunit signal, isolating putative units using an automated clustering algorithm and analysed the spatial tuning characteristics of the neurons involved in this task.

Results:

We first noticed that context-reward associations were learned by all animals in 3-4 days, yet individual learning curves differed considerably. To further investigate possible differences in learning strategy across individuals, we separately analysed trials belonging to one of 4 different categories, each corresponding to a different start-context combination. While some animals improved performance in all categories simultaneously over training days, others seemed to fully learn either just one or two categories initially, and only later learn the remaining ones. This led to the question of whether the formation of context representations in the hippocampus differ between animals. We currently found that, in days when animals achieve >80% correct, a portion of the cells show preference for either one of the two contexts; this does not affect the remaining neurons, which maintain their ability to code for the context-independent position within the environment. Moreover, preliminary qualitative results indicate that the context-independent reward is over-represented in comparison to the context-dependent ones.

Discussion:

As previously observed for humans and primates, individual rats seem to employ a variety of learning strategies when the complexity of the task allows for it. This might be a reflection of the animal's assumptions of task structure and rule. These behaviours we observe in our dataset point towards a possible differential representation of space and trial category for each animal. This corroborates the notion that learning includes features shared between animals as well as individualized ones, shedding light on the ability of learning contextual-dependent representations alongside with contextual-independent ones.

P-26 Role of the VIP/VPAC receptor system in the regulation of stress and anxiety reactions in the rodent brain

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Vasoactive intestinal polypeptide (VIP) is a 28-amino-acid amidated peptide isolated first from porcine duodenum. This peptide is not only present in gastrointestinal tissue but also in neural tissue, where it is highly distributed throughout the peripheral and central nervous system. Notably, within

the central nervous system VIP and its cognate VPAC receptors have been localized in high density in stress and anxiety-related brain areas such as amygdala, lateral septum (LS), bed nucleus of stria terminalis (BNST), some hypothalamic structures as well as cortical areas. Although of these neuroanatomical findings, the role of VIP in stress and anxiety is not well understood. Accordingly, the first aim of the present study was to examine whether the exposure to emotional stressors leads to alterations in the expression of VIP and VPAC1/2 receptors in selected brain areas of the stress/anxiety circuitry. Consequently, by using quantitative real-time PCR analysis gene expression levels of VIP and VPAC1/2 receptors in various stress-related brain regions were compared in Sprague-Dawley rats exposed to either repeated swim stress or chronic variable stress with respective unstressed control groups. Our results show that repeated swim stress or chronic variable stress significantly increased VIP transcript levels in limbic areas such as LS, BNST, medial and central nucleus of the amygdala, as well as in the hypothalamic paraventricular nucleus. Moreover, repeated swim stress significantly enhanced expression levels of VPAC1 and VPAC2 mRNA in the LS, BNST and basolateral amygdala compared to control animals. Second aim of the present study was to investigate the effects of central VIP infusions on anxiety-related behavior of rodents. Therefore, C57Bl6/J male mice implanted with an indwelling guide cannula were injected in the right lateral ventricle either with VIP (0.1 or 1 µg in 0.5 µL) dissolved in artificial cerebrospinal fluid (aCSF) or vehicle alone. For evaluating anxiety-related behaviors either the light/dark or elevated plus-maze test was performed 15 minutes after drug administration. The animals were sacrificed 2 hours after behavioral testing in order to evaluate c-Fos expression as marker for neuronal activation. Our data show that VIP-injected animals show a reduction of the time animals spent in aversive zones of the testing arena (e.g. the light-area of the light/dark test or open arms of the elevated plus-maze) compared to vehicle-injected controls, suggesting an anxiogenic-like effect. Taken together, these data implicate an upregulated central VIP/VPAC receptor system in response to aversive and stressful situations and highlights a potential important role of VIP signaling in the regulation of stress and anxiety functions.

P-27 Secretagogin marks amygdaloid PKCδ interneurons and modulates NMDA receptor availability

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Background:
The amygdala is a central hub for emotional processes and defensive behavior. It coordinates the avoidance response to dangerous stimuli, specifically upon conditioned fear. Central amygdaloid nuclei are pivotal in these processes through their local gamma-aminobutyric acid (GABA)-ergic interneurons. Protein kinase Cδ (PKCδ)-positive "fear-off" and somatostatin-positive "fear-on" neurons were identified as major regulators of danger-induced behavior. At the cellular level, glutamatergic neurotransmission through postsynaptic N-methyl D-aspartate (NMDA) receptors drives threat-induced changes in synaptic function. Calcium plays critical roles in synaptic neurotransmission by priming neurotransmitter release. Secretagogin is a calcium-sensor protein whose expression is activity dependent and specific to a hitherto undefined GABA interneuron subclass in the central amygdala. Even though a role of secretagogin in presynaptic integration is plausible, its specific contributions to modulating fear-responsive neurons remains unknown.
Methods:
We combined light-, superresolution and electron microscopy for morphological characterization, quantitative ribonucleic acid and protein analyses for expressional parallels, cell- and synaptic compartments were fractionated for subcellular assignment, while NMDA receptor subunit trafficking was charted by real-time fluorescence recovery after photobleaching microscopy in vitro. In vivo chemogenetics was deployed to control neuronal activity in behavioral paradigms. Secretagogin-dependent cellular processes were confirmed by in vitro loss-of-function analysis.
Results:
Neurons of the central amygdala that express secretagogin, are a subset of PKCδ-positive interneurons, likely "fear-off" cells. These cells were described to block fear-evoked behavior. Chemogenetic inactivation of secretagogin+/PKCδ+ neurons indeed augmented conditioned fear. Secretagogin is involved in danger response-related events but not in stress situations that lack a conditioning stimulus. Secretagogin was unusually localized to postsynaptic compartments. Herein, we found secretagogin to regulate the cell-surface availability of NMDA receptor 2B (NR2B) subunits. Accordingly, secretagogin loss-of-function reduced the cell membrane delivery of NR2Bs, at least in vitro.
Discussion:
Here, we characterized secretagogin-containing interneurons in the centrolateral amygdala across mammals. We identify secretagogin+ neurons as a subpopulation of PKCδ+ cells, which were classified originally as "fear-off" neurons to determine the balance between conditioned flight and fright responses. We show that secretagogin-positive neurons modulate the avoidance response to conditioned danger through the regulation of the postsynaptic surface availability of NMDA receptor 2B subunits. Inactivation of secretagogin-expressing neurons, or ablation of secretagogin itself, provided causality for the role of calcium-dependent feedback regulation at the cellular level.

P-28 Gender-affirming hormone therapy for transgender people makes sexual arousal in the ventral striatum more gender-congruent for lesbian scenes

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Background:

The term "sex" in both its meanings connects the act of coitus and the chromosomal sex, resulting in the stereotypical classifications of hetero-, bi- or homo-sexuality. While these relationships might be relatively clear for cisgender, they are much less so for transgender people. Still, differences in subjective and objective arousal are also known between cisgender men and women. Following persons with gender dysphoria through their transition process enables us to investigate the role of biological sex and preferred gender in sexual arousal [1]. Here, neuroimaging allows for the gathering of objective data and thus avoiding many of the difficulties that classical genital measures bring along. Therefore, we investigated how gender-affirming hormone therapy influences the neuronal response to erotic stimuli before and after 4.5 months of transitioning.

Methods:

To this aim, 20 trans male (female-to-male transgender, TM), 12 trans female (TF), 24 cis female (CF) and 12 cis male (CM) subjects were recruited. All subjects underwent two functional magnetic resonance (fMRI) scans with a median interval of 4.5 months. After the first MRI session, transgender subjects began their gender-affirming hormone therapy. During the fMRI paradigm subjects were shown sexually explicit heterosexual, lesbian and gay scenes. In light of the complex experimental design, a Bayesian multivariate mixed model was used to analyse the neuronal responses to all the images. We concentrated on the ventral striatum (VS) as this region was previously shown to specifically react to sexual rather than general arousal. A score calculated from the Klein Sexual Orientation Grid was used to correct for individual sexual preferences of the subjects. Finally, non-linear hypothesis testing was employed to infer whether the VS activation pattern of transgender subjects changed from that of their biological sex to their gender identity (i.e., generally moved from one to the other, only towards the desired, or away from the gender assigned at birth).

Results:

Posterior probabilities (PP) providing strong evidence for this assumption were found for lesbian scenes (and were considerably lower for heterosexual and gay ones). In detail, only TF markedly moved from a male to a female response pattern (PP = 98%). This was mainly driven by approaching a CF activation (PP = 99%). Such an approach tendency for lesbian stimuli was

also observed for TF and TM combined (PP = 95%). Furthermore, over all stimuli TF showed stronger changes in this direction than TM (response pattern moving from biological sex to preferred gender, as well as only approaching this, both PP = 96%).

Discussion:

These findings indicate that initial gender-affirming hormone therapy induces stronger changes in sexual arousal towards the gender identity measured as VS activation in TF than TM. The strongest effect found for lesbian scenes might be related to a more orientation-/gender-independent appeal of heterosexual and a previously shown often aversive nature of gay stimuli. Moreover, the relationship between sexual orientation and arousal, as well as the general level of induced sexual arousal were reported to be much weaker in CF than CM. If the neuronal response in TF is still generally stronger such as it is believed to be in CM, this might partly explain the effects in this group. However, also cisgender subjects did not show constant activation over time, which manifests as statistical discrepancies between transgender subjects' responses moving away from biological sex and towards gender. More targeted investigations based on these findings might help to further disentangle the complex links between sex, gender and sexual arousal.

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P-29 Co-treatment with aripiprazole and escitalopram reversed the schizophrenia-like behaviour and enhanced the BDNF mRNA expression in adult Sprague-Dawley rats induced by glutathione deficit during early postnatal brain development

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Background:

Schizophrenia is a chronic, one a most devastating psychiatric illnesses affecting about 0.5-1% of the world population. It develops progressively, remaining often undetected during childhood and adolescence, with the first episodes of psychosis that appear at early adulthood. Recently, clinical reports have demonstrated that glutathione levels are reduced in the cerebrospinal fluid and prefrontal cortex of schizophrenic patients as well as in the post-mortem striatum and prefrontal cortex of these treated earlier with antipsychotic drugs. The consequences of brain glutathione deficit during develop-

ment were studied in animal models in adulthood. Those studies demonstrated that chronic combined treatment of Osteogenic Disorder Shionogi mutant rats, which, like humans, cannot synthesize ascorbic acid, with the inhibitor of glutathione synthesis L-buthionine-(S,R)-sulfoximine (BSO) and the inhibitor of dopamine (DA) reuptake GBR 12909 during early postnatal life induced schizophrenia-like memory deficits during adulthood. These data have suggested that schizophrenia is associated with an impaired brain glutathione deficit. Moreover, some earlier studies indicated a low level of the serum brain-derived neurotrophic factor (BDNF) in schizophrenic patients compared to control subjects. In addition, clinical data have suggested that antidepressants are able to augment the activity of atypical antipsychotics, thus effectively improving treatment of some negative symptoms and cognitive impairment in schizophrenic patients.

Methods:

Between the postnatal days p5 and p16, male Sprague-Dawley pups were treated subcutaneously with the inhibitor of glutathione synthesis, BSO (L-buthionine-(S,R)-sulfoximine, 3.8 mmol/kg, daily) or the dopamine uptake inhibitor, GBR 12909 (5 mg/kg, every second day), alone or in combination. Control pups instead of the BSO or GBR 12909 were given vehicle. On postnatal day p23 the rats were weaned and housed in groups of four until p94. Aripiprazole and escitalopram were given repeatedly for 21 days before the tests. On p90-91 rats were tested in the social interaction and novel object recognition tests. The tissue (hippocampus and prefrontal cortex) for biochemical assays was dissected on p-92.

Results:

In the social interaction (p90) and in the novel object recognition tests (p91), BSO given alone and also BSO together with GBR 12909 induced deficits in both tests studied, and repeated aripiprazole administration (1 mg/kg) reversed these effects. Co-treatment with ineffective doses of aripiprazole (0.1 or 0.3 mg/kg) and escitalopram (5 mg/kg) also abolished the deficits in those tests and significantly increased the BDNF mRNA expression only in the prefrontal cortex.

Discussion:

The present study indicated that the inhibition of glutathione synthesis in early postnatal development induced long-term deficits in schizophrenia-like behaviour and decreased the BDNF mRNA expression in the prefrontal cortex in adult rats, and these deficits were reversed by repeated treatment with a higher dose of aripiprazole and also by co-treatment with ineffective doses of aripiprazole with escitalopram. The above data suggest that the neurodevelopment rat model of schizophrenia induced by glutathione deficit by repeated treatment with BSO alone and together with GBR12909 in early postnatal life may be very important for studies on the pathomechanism of schizophrenia.

P-30 Correlation between progesterone and word recall in a list-method forgetting paradigm in pregnant women

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Background:
Many women report about a subjective decrease in memory performance and cognitive performance during pregnancy. Previous studies showed an influence of hormone levels and cognitive performance in non-pregnant women.
Methods:
Therefore, we used a standardised directed forgetting paradigm and measurements of salivary hormones to assess the correlation between steroid hormone levels during pregnancy and forgetting. In the directed list forgetting paradigm, the instructor gives a cue either to remember or to forget the preceding list of words. Following a short distraction phase, subjects are asked to recall to-be-remember-cued and to-be-forgotten-cued list items. In this paradigm, fewer to-be-forgotten list items are recalled than to-be-remembered list items.
Results:
We observed that pregnant women recalled fewer forget-cued list items than non-pregnant women. In comparison to naturally-cycling women, first and third trimester pregnant women showed the highest rate of forgetfulness. The correlation between saliva progesterone concentration and number of recalled forget-cued list items followed an U-shaped distribution.
Discussion:
Our study demonstrates that the reported feeling of forgetfulness in pregnant women can be detected in a standardized forgetting paradigm and that is shows a biphasic correlation with endogenous free progesterone.

P-31 Dopamine drives extinction-promoting effects in deficient fear extinction

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Background:
The systemic administration of L-DOPA facilitates long-term fear inhibition after successful fear extinction, a central mechanism in exposure based therapy, and rescues fear extinction in the short, but not long-term (Haaker et al., 2013; Whittle et al., 2016; Gerlicher et al., 2018). In these cross-species studies we and others have proposed that the ventromedial prefrontal

cortex (the mouse homologue infralimbic cortex, IL) is an important node in mediating this fear extinction-facilitatory effect of L-DOPA. However, causal evidence is missing so far.

Methods:

Behavioural and neuronal responses to L-DOPA/dopamine or selective dopamine D1 and D2 receptor agonists in the IL were investigated in a mouse strain of deficient fear extinction (129S1/SvImJ), a characteristic of patients with an anxiety disorder, vs. extinction-competent C57Bl/6J mice using in-vivo microdialysis, GCaMP fiber photometry, catFISH and immunohistochemical markers of dopamine receptor signaling.

Results:

L-DOPA caused a sustained elevation of extracellular dopamine levels and increased neuronal activation in a subgroup of IL neurons in 129S1/SvImJ. Microinfusion of dopamine, but not a D1 or D2 receptor agonist, into the IL promoted fear extinction in 129S1/SvImJ mice in the long-term, an effect that did not involve α-/ß-adrenoreceptors. Systemic L-DOPA initiated fear extinction learning and promoted only short-term extinction retention in 129S1/SvImJ mice. In this non-extinguishing mouse strain the in-vivo activity of DA neurons in the ventral tegmental area, a brain area with dopaminergic projections to the IL, remained high during fear extinction training while it decreased in extinction-competent C57Bl/6J mice. This effect was not reflected in the dynamics of extracellular dopamine concentrations. Furthermore, dopamine-evoked prefrontocortical signalling in 129S1/SvImJ mice was disturbed that was most likely mediated via the D1 receptor in the IL.

Discussion:

We show that high local IL DA rescues deficient fear extinction. We further suggest that systemic L-DOPA can only mimic this effect in part causing a short-term, but not a persistent fear extinction memory that protects against the return of fear in a clinically relevant model organism. Thus, we are currently investigating approaches to further boost IL dopamine levels in order to overcome this insufficiency.

P-32 Dynamic and state-dependent switching of behaviour in response to competing visual stimuli in *Drosophila*

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Background:

Efficient behavioural analysis paired with a powerful genetic toolset in Drosophila has allowed the identification and functional characterization of numerous sensorimotor circuits. These circuits have classically been studied using defined stimuli that map to specific reproducible behavioural responses. However, stimuli rarely appear independently in the natural world. Individuals are faced with a complex environment filled with multiple stimuli. In order to traverse through this world, sensorimotor transformations in the brain need to be updated constantly depending on the behavioural needs of the animal.

Here we use a novel behavioural paradigm to study how the fly processes competing visual stimuli to generate coherent and efficient behavioural responses. We probe how sensorimotor circuits for different stimuli interact with each other, shedding light on mechanisms that allow a fly to perform tasks in a stimulus-rich environment.

Methods:

We developed a behavioural paradigm that takes advantage of two of the most well-studied behaviours in fruit flies: optomotor response and courtship tracking. A male fly that is engaged in tracking is presented with a global optic flow that, when presented in isolation, elicits a robust optomotor response. All behavioural experiments are performed in a custom-built setup that allows us to track and present visual stimuli to walking flies at 60 Hz in real-time under closed-loop conditions. For the experiments, we use either WT flies tracking blind [NorpA7] flies or Gal4-P1>UAS-csChrimson flies optogenetically induced to track a dark circular dot.

Results:

We report that flies are able to suppress optomotor responses while engaged in courtship tracking. When presented with the two stimuli simultaneously, male flies that are in a state of sexual arousal, switch between tracking and optomotor response in a state-dependent manner. The probabilities and dynamics of this switching also depend on the relative strength and position of the stimuli.

Discussion:

Our findings show that fruit flies dynamically suppress innate responses to stimuli that are irrelevant to the task that they are engaged in. This provides support for attention-like mechanisms in the fly brain. More importantly, by clearly defining the task and distraction in our behavioural paradigm, we show that this selective attention is directed to the most relevant stimulus which is defined by the task that the animal is engaged in at any moment.

Neurological Disorders & Regeneration

P-33 Behavioural characterization of the *Fmr1* knock-out mouse model of Autism Spectrum Disorder (ASD)

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Background:

Autism Spectrum Disorders (ASDs) have highly heterogeneous phenotypes including social interaction and communication deficits as well as repetitive and stereotypic behaviors. There have been more than 1,000 genetic mutations reported possibly linked to ASDs. An important syndromic autistic

form is Fragile X syndrome known as the most prevalent neurodevelopmental disorder with genetic origin. Thus, the overall aim of this study was to characterize the Fmr1 knock-out mouse model of ASD for behavioral deficits and to evaluate the efficacy of two compounds in this mouse model.

Methods:

45 male B6.129P2-Fmr1tm1Cgr/J (Fmr1 knock-out) mice at the age of six weeks were allocated to 3 different treatment groups of vehicle, LP-211, and R-Baclofen. In addition,15 male C57BL/6JRj mice were used as control group treated with vehicle only. The compounds were administered via intraperitoneal injections 30 minutes prior to each behavioral test. Compound effects were evaluated by Elevated Plus Maze, Self-Grooming, Three-Chamber Social Interaction test as well as ultrasonic vocalization recordings. Behavioral tests were conducted when animals were 6 to 10 weeks old. Ultrasonic vocalization was recorded at the age of 10 weeks.

Results:

Preliminary results suggest that Fmr1 knock-out mice are in good health up to an age of 10 weeks. Furthermore, Fmr1 knock-out mice seem to show reduced ultrasonic vocalizations at the age of 10 weeks old. Analyses of all other tests and the effect of both compounds is currently in progress and will be available at the time of poster presentation.

Discussion:

Our preliminary results suggest that Fmr1 knock-out mice present a very early phenotype already at the age of 6 to 10 weeks. The ultrasonic vocalization test is considered as an index of emotionality, social interest, and motivation. We have observed reduced number of emitted calls in Fmr1 knock-out vehicle-treated mice compared to control group. These results indicate impaired social communication and interaction of Fmr1 knock-out animals compared to C57BL/6JRj control group. Analyses of the remaining tests will further elucidate animals' behavioral phenotype and thus their value for ASD research.

P-34 Can predictive processing account for the spectrum of stereotyped repetitive behaviours in Parkinson's disease?

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Background:

Patients suffering from Parkinson's disease (PD) can show various aberrant and repetitive patterns of action and thought. In the symptomatic, dopamine-deprived OFF state, these include set-switching and action selection impairments, rigidity, freezing, perseveration, and obsessive traits. Conversely, dopaminergic therapy increases the risk for impulsive and compulsive

behaviours such as gambling and punding with a predilection for repetitive, ritualistic activities and restricted interests. These repetitive behavioral patterns appear to have a soothing effect linked to reducing perceived uncertainty rather than hedonic motivation [1]. Predictive Processing (PP) is a neurophysiologically grounded framework [2] that describes how brains actively generate models to make statistical inferences about the hidden causes of sensory inputs from inherently uncertain environments. Models emerge from a multi-level hierarchical architecture that dynamically compares top-down expectations with the upstreaming flow of unexplained sensory and proprioceptive data, coded as prediction errors (PE). A process described as active inference (AI) guides movements and behaviour to maximize model evidence and minimize PEs or surprise. Most importantly, several weighing mechanisms tune PE precision of relevant error units, estimating the importance of the sensory evidence (or its reliability). This serves to dynamically update relevant model predictions and strongly influences behavioural patterns. Dopamine participates in this process by modulating the post-synaptic gain of deep cortical layer neurons, thereby influencing expected PE precision, (sensory) salience and (motor) affordance [3]. We hypothesized that stereotyped repetitive behaviours might appear at both far ends of the spectrum between overweighted top-down models and abnormally high PE precision expectations.

Methods:

In this theoretical work, we outline a PP formulation to account for the functional role of dopamine in relation to stereotyped repetitive behaviours in both the OFF state and in dopaminergic overstimulation. Specifically, we propose that the former is characterized by abnormally precise models that represent strong attractor states, and the latter is characterized by overweighted PE precision expectations.

Results:

In this dual account of stereotypies, both overweighted models and overweighted PE precision expectations appear to impair dynamic agent-environment interactions, belief updating, and openness to experience. Both these extremes of PP information flow can result in stereotyped repetitive behaviours. In the first case, AI is biased towards repeated collection of model confirmation. In the second case, unsettling levels of perceived uncertainty elicit compensatory behavioural limitation to restricted and predictable contexts. Conceptually, this would make the OFF state more related to obsessive-compulsive disorder [4,5]; contrarily, compulsive behaviours like punding, evoked by dopaminergic overstimulation, would be functionally similar to repetitive behaviours seen in autism spectrum disorders [6]. This hypothesis is testable by how well it accommodates various neuropharmacological, neuropsychological, and electrophysiological findings. Furthermore, it allows for specific experimental and therapeutic predictions, and might support a refined nosology of different neuropsychiatric disorders.

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P-35 Silk fibroin-based conduits filled with native spider silk fibers successfully promoted nerve regeneration in a 10 mm sciatic nerve defect in rats

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Background:
The surgical repair of nerve transection injuries remains a challenging task and often results in unsatisfactory functional recovery. If a direct coaptation is not possible, the current gold-standard is the use of an autograft. However, the availability of autologous nerve tissue is limited and the harvest of a donor nerve entails functional loss and possible donor site morbidity. In the search for alternatives, different synthetic and biological materials are currently tested to bridge nerve gaps. Recent studies supported silk as promising material for tissue engineering and the development of artificial nerve conduits. In addition, nerve conduits that contain an internal framework as guiding structures could enhance a directed axonal re-growth. Spider silk possess excellent mechanical properties such as an adequate tensile strength, long-term degradability and a non-immunogenic nature, which support their use as promising conduit filling material. In this study, we investigated the performance of a silk fibroin-based conduit filled with spider silk fibers to bridge a 10 mm sciatic nerve defect in rats.
Methods:
In 18 male Sprague-Dawley rats, a 10 mm piece of the sciatic nerve was resected and immediately bridged with 1) autografts (control group, n=6), 2) empty silk conduits (experimental group one, n=6), and 3) silk conduits

filled with spider silk fibers (experimental group two, n=6). Walking track analysis was performed for each animal prior to surgical intervention and every 14 days over a course of 14 weeks. Functional recovery was evaluated by calculating the sciatic functional index (SFI) according Bain et al. At the endpoint, animals were sacrificed and the nerves were harvested to assess axon re-growth and myelination by histomorphometric as well as immunofluorescence analyses on paraffin sections.

Results:

The walking track results showed that there was no statistical difference in the mean SFI of animals treated with the autograft or the silk fiber containing silk conduits. Moreover, the immunofluoresence stainings of nerve sections illustrated a similar pattern of regenerated nerve tissue in sections of autografts and filled silk conduits, while a less advanced nerve regrowth was seen in the samples containing empty silk conduits. The histomorphometric parameters displayed a similar number of myelinated axons in the autografts and filled silk conduits. Additionally, the mean axon area was comparable between the autograft and the silk conduit filled with spider silk. However, the mean myelin area was the largest in the autograft group.

Discussion:

Taken together, our study demonstrated that the functional recovery of a 10 mm sciatic nerve defect bridged with silk conduits containing spider silk fibers as internal guiding structure was comparable to and autologous nerve grafts.

P-36 A rodent lumbosacral spinal cord injury model reflecting neurological and urological deficits of humans

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Background:

The lumbosacral spinal cord includes the lumbar enlargement and sacral segments of the spinal cord, containing critical motor centres, including the parasympathetic preganglionic innervating the bladder detrusor smooth muscle and Onuf's nucleus innervating the external urethral sphincter (EUS)[1]. In SCI patients with a lesion at the lumbosacral level, damage to these centres disrupts crucial bladder function, resulting in an underactive or flaccid bladder [2,3]. To test therapeutic strategies for restoring bladder function after motoneurons loss, consistent animal SCI model involving gray matter loss in lumbosacral spinal cord is required. Accordingly, we described a rat lumbosacral spinal cord contusion model with reproducible anatomical, histological, and functional outcomes similar to human lumbosacral injuries.

Methods:

Motoneurons innervating EUS was detected by using Fluorogold retrograde

labelling. A severe incomplete severe contusion SCI was conducted at the level of detected labelled neurons by using infinite horizon impactor. Two months after SCI, the lesion size and volume and also the sparing of white and gray matters were quantified by using an advanced µCT imaging. The urodynamic study was done to evaluate the bladder function and BBB score and semiautomatic gate analysis were also done to identify the locomotor function. The structure of bladder in control and SCI group was also evaluated by histology studies.

Results:

Neurons innervating the EUS are specially spread over ventrolateral portion of L 6 and S 1 spinal cord. The urodynamic study showed that severe contusion injuries at lumbosacral level results in an underactive bladder and provide relevant clinical content. The µCT analysis also showed that the disruption of bladder function is not due to differences in lesion size or to differential sparing of different portions of the white and gray matter. After SCI, in comparison to healthy control urinary bladder tissue, slightly increase in smooth muscle tissue and correlated significant increase of urothelial tissue was seen. Elastic fibers were reduced in numbers and diameter length. The results of locomotor function did not show a significant correlation between deficits in the bladder and locomotor function following lumbosacral SCI at the level of L6-S1.

Discussion:

In this study, we developed a consistent, reproducible contusion lumbosacral SCI model that mimics human urinary tract complications following lumbosacral SCI. Our experiments show that severe contusion at the L6-S1 spinal cord produces an underactive bladder with development of fibrosis. The animal model in this study can be used in subsequent translationally oriented studies aimed at preventing and restoring bladder function in lumbosacral spinal cord contusions.

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P-37 Modulation of microglia function via omega-3 polyunsaturated fatty acids in the context of Alzheimer's disease

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Background:
Alzheimer's disease (AD) and other neurodegenerative dementias affect millions of elderly people worldwide and so far, no therapy is available. Hence, there is great interest in the prevention of these diseases. Genome-wide association studies identified several genes, implemented in microglia phagocytosis and lysosomal degradation, which are associated with an increased risk for AD. Therefore, microglia and microglial phagocytosis are potential therapeutic targets in AD. Recent studies convincingly demonstrated that factors circulating in the blood can have profound effects on synaptic plasticity, neurogenesis and immune reactions thereby shaping brain aging and neurodegenerative diseases. Such factors include cytokines and growth factors as well as nutrients and food metabolites. Along this line, we investigated the effect of blood-born factors of AD patients on microglia function by using an in vitro parabiosis system.
Methods:
We had access to blood serum samples from two different cohorts. The first was provided from the University Hospital Graz and consisted out of samples from 30 patients, diagnosed with AD, and 30 samples from age-matched controls. We obtained the second data set through partnership in the EU D-CogPlast project, which conducted a case-control study (n=209 per group) embedded in the Three City (3C) study. From this study, we received not only blood samples taken before participants showed signs of cognitive deficit,

but also long-term data on diet and cognitive status. Consequently, a human microglia cell line was incubated for 24h with the serum and then encountered with pH sensitive fluorescence particles for another 24h. Afterwards, the fluorescence signal of the incorporated particles was measured by flow cytometry.

Results:

Interestingly, the serum of the AD patients led to an increased particle uptake in comparison to the controls. In addition, we found a significant association between the cognitive impairment over one year and the phagocytic value. To investigate a potential prognostic value of the phagocytosis assay we made use of the serum from the D-CogPlast case-control study. In this data set we found no measurable difference in phagocytosis between the groups, but the phagocytic value negatively correlated with the amount of eicosapentaenoic acid (EPA), one of the main omega-3 polyunsatturated fatty acids (n-3 PUFAs), found in the blood. Subsequent measures of EPA in the samples from Graz revealed the same correlation with phagocytosis and also EPA by itself was tested on the microglia cell line and indeed led to a reduction of phagocytosis.

Discussion:

For further validation of the effect of AD serum on microglia, we are not only planning to use another set of blood samples but also IPSC derived microglia as a second in vitro model. In addition, we will investigate the effect of n-3 PUFAs, especially EPA, on mouse primary microglia and conduct an animal experiment to reveal the impact of n-3 PUFAs on microglia in vivo.

P-38 Understanding the role of platelets in Alzheimer's disease

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Background:

In Alzheimer's disease (AD) platelets become dysfunctional. However, it remains unclear whether platelet dysfunction in AD is a consequence of the ongoing pathological events or a driver of the disease. To investigate platelet's contribution to AD pathology, we used an AD transgenic mouse model (i.e. APP Swedish PS1 dE9, APP-PS1) for cellular and molecular characterization of platelets and immune-mediated platelet depletion.

Methods:
We assessed the activation status (CD62P expression), ultrastructure and the proteome of blood isolated platelets in 14 months old APP-PS1 mice and wild type (WT) age-matched controls. To induce short-term immune-mediated platelet depletion in APP-PS1 mice (12-13 months old), we used intraperitoneal injections of an anti-CD42b antibody.
Results:
APP-PS1 mice showed significantly higher percentages of activated platelets in the brain but only a slight, non-significant, higher platelet activation in the bloodstream. Nevertheless, preliminary proteomics data revealed 77 differentially expressed proteins in APP-PS1 blood isolated platelets compared to WT mice. Interestingly, in the APP-PS1 mouse brain, about 20% of the platelets located extravascularly. Antibody-mediated depletion successfully induced thrombocytopenia (>99%) in APP-PS1 mice for five days. The withdrawal of the antibody treatment had a rebound effect on platelet production, resulting in thrombocytosis. Platelet depleted APP-PS1 mice showed an impaired microglia phagocytic capacity compared with controls. However, hippocampal and cortical amyloid loads did not differ between platelet depleted and control animals.
Discussion:
Platelets might present an altered cellular and molecular profile in AD, with implications to cerebral processes such as neuroinflammation. Even though the mechanisms underlying platelet's influence in the brain remain unknown, these findings provide a base for future developments.

P-39 Alterations of the leukotriene signaling pathway in aged and cognitively impaired rats

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Background:
Brain aging is associated with an age-related cognitive decline and increased neuroinflammation. Thereby, neuroinflammation is mainly driven by innate immune responses mediated throughout microglia cells and inflammatory molecules. One of these include the group of lipid-mediators of inflammation also known as leukotrienes (LTs). There is increasing evidence that microglia are the major source for LTs and harbor key components of the LT signaling

pathway in the brain. However, the exact contribution of microglia and altered leukotriene signaling to the age-related cognitive decline is less evident.
Methods:
The aim of this study is to determine alterations related to neuroinflammation and leukotriene signaling between young (Y), aged unimpaired (AU) and aged impaired (AI) rats. The hole-board spatial memory behavior test was used to assess cognition in a cohort of male Sprague-Dawley rats. Cellular and molecular changes within the aged brain were analyzed performing immunohistochemical (IHC) staining against microglia (Iba1) and the LT signaling related protein 5-lipoxygenase (5-Lox). Additionally, the LT signaling pathway was investigated by detailed mRNA gene expression analysis of hippocampal and cortical brain regions.
Results:
The herein presented data show that in old and in cognitively impaired rat brains LT signaling was significantly elevated. Overall 5-Lox expression was increased within the brain of old rats and this was even more pronounced in cognitively impaired animals. In old rats the number of microglia cells was increased and in cognitively impaired animals the highest number of 5-Lox expressing microglia was detected. Furthermore, lower cognitive scores of the animals correlated with higher numbers of 5-Lox positive microglia. Additionally, cognitively impaired rats had higher gene expression for the leukotriene receptor GPR17 in the prefrontal cortex. Our data indicate that enhanced 5-Lox expression in microglia of AI rats might contribute to the age-related cognitive decline in these animals.
Discussion:
With this work, new knowledge on potential inflammatory processes in the aged brain in particular on the role of microglia and the LT signaling pathway was generated. Further analysis on the LT signaling pathway must be performed to identify potential biomarkers for early determination of cognitive impairments also in the context of neurodegenerative diseases.

P-40 Galanin receptors 2 and 3 modulate the inflammatory response following experimental traumatic brain injury

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Background:
Traumatic brain injury (TBI) is a leading cause of death in young individuals. The clinical outcome still remains poor with a high mortality. Mechanisms underlying the associated neuroinflammation are barely understood.

The regulatory (neuro-)peptide galanin (GAL) and its three receptors (GAL1-3R) are involved in immunity and inflammation. Particulary, GAL2R participates in neuronal survival and neurogenesis. Of note, upon TBI in mice GAL and GALR expression were altered up to 7 days post injury (dpi). A single GAL injection into rat brain led to weaker motor deficits after TBI compared to controls. We hypothesize that GALRs participate in TBI recovery. By using GAL2 and GAL3 receptor KO mice (GAL2/3R-KO), we aimed to determine the role of GALRs in functional recovery and inflammatory responses following mild controlled cortical impact (CCI)-TBI.

Methods:
To evaluate acute inflammation, brains and cerebrospinal fluids (CSF) were collected 24 h after TBI. Functional recovery of animals was studied post TBI by performing a neurological severity score (NSS), elevated plus maze (EPM) and Morris Water Maze (MWM) tests. At 30 dpi, brains and CSF were collected. Numbers of TMEM119+ microglia, Iba1+ macrophages/microglia were counted, and the areas of GFAP+ astrocytes were determined in the cortex and hippocampus of paraffin-embedded brains 24 h post TBI by immunofluorescence. Hippocampus and cortex were dissected and used for mRNA expression analysis. Protein levels were determined in CSF by a multiplex assay.

Results:
Post TBI, neurological severity and memory/learning behavior were similar in WT and GAL2/3R-KO mice. At 4 dpi, TBI mice showed anxiolytic behavior in the EPM, but KO mice had less open arm entries than WT mice. At 24 h post TBI, brain mRNA levels of pro-inflammatory and nerve growth factors were increased in all mice at the ipsilateral compared to the contralateral side with higher induction in the hippocampus compared to the cortex. Ipsilaterally, KO mice showed a trend towards increased pro-inflammatory markers but reduced nerve growth factors compared to WT mice. In contrast, CSF protein levels of pro-inflammatory factors were higher and levels of Brain-derived neurotrophic factor were lower in KO compared to WT mice. At 24 h post TBI, no major differences were observed in the numbers of TMEM119+ microglia, Iba1+ macrophages/microglia, and areas of GFAP+ astrocytes between KO and WT mice in the hippocampus. In the cortex only the numbers of Iba1+ cells differed between KO and WT mice. In fact, WT mice showed a higher amount of Iba1+ cells than KO mice at 24 h post TBI. Furthermore, KO mice 24 h post TBI had less Iba1+ cells compared to sham.

Discussion:
We conclude that GAL2/3R are especially involved in acute inflammatory processes following TBI, whereas functional recovery is hardly affected. Future analyses will focus on the determination of immune neuronal cell types that might be affected by loss of GAL2/3R.

P-41 Development of adrenal gland hides the origin of neuroblastoma

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Background:

Neuroblastoma (NB) is a deadly childhood cancer with unmet clinical need. NB are often found in the vicinity of adrenal glands and are thought to start from the abnormalities in the neural crest cells (NCCs), which give rise to the peripheral nervous system, including chromaffin cells (main cell type in adrenal gland medulla), during development. Recently, we discovered that in mice, chromaffin cells originate from nerve-associated Schwann cell precursors (SCPs) instead of being directly derived from NCCs as it was thought previously. Hypothetically, one could suggest that in humans, SCPs may also be involved in genesis of chromaffin cells and possibly neuroblastoma. Therefore, we decided to analyze the cellular origin of chromaffin cells and sympathetic neurons in human adrenal gland region aiming to better understand the origin of the childhood disease.

Methods:
We have performed single cell RNA-sequencing (scRNAseq) of cells from human fetal sympathoadrenal region from weeks 6-14 (in total 11 samples) with 10x Chromium platform. As a result, we have generated the dataset with 74,401 cells, characterised the major cell types building human adrenal glands, and analysed the developmental trajectory between SCPs, chromaffin cells and sympathoblasts based on their transcriptional profiles. With IHC and in situ RNA hybridisation we have profiled bioinformatically predicted cell types in tissue and validated the predicted transitions between SCPs, chromaffin cells, and sympathoblasts. We also bioinformatically compared scRNAseq profiles of healthy adrenal glands with transcriptional profiles of neuroblastoma tumors.
Results:
We have found that in humans, chromaffin cells and a special population of intra-adrenal sympathoblasts originate from nerve-associated SCPs. Moreover, we discovered additional developmental transition from intra-adrenal sympathoblasts to chromaffin cells. Intra-adrenal sympathoblasts appeared highly proliferative, and are organised in large ganglia-like structures inside human adrenal medulla at weeks 6-14. Comparison of single cell transcriptomic profiles of embryonic adrenal gland with neuroblastoma samples revealed that the presence of extensive proliferative sympathoblast and chromaffin cell transcriptional signatures correlated with worse prognosis for patients with non-MYCN-amplified neuroblastoma.
Discussion:
The present study extends our current understanding of SCP potential to give rise to sympathetic neurons and chromaffin cells in humans. SCP transition to sympathoadrenal states spans over several weeks during human development and exceeds the short time window of NCCs migration. This transition includes previously unknown intermediate states, which are likely related to neuroblastoma origin. Overall, the discovered plasticity of nerve-associated progenitors and other intermediate cell types favors the conditions for neuroblastoma emergence in the human adrenal gland.

P-42 Activation of macrophages DRG (dorsal root ganglion) in Fabry disease

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Background:
Neuropathic pain in in the hereditary Fabry lipid storage disorder (FD) develops already in the earliest stages of the disease occurring as a consequence of alpha-galactosidase (GLA) deficiency. In addition to primary afferent neurons and Schwann cells, resident macrophages are located in dorsal root ganglia (DRG) with distinct function and phenotype. Here, we explored the morphology of macrophages in FD DRG during the progress of the disease in a genetic model of FD (GLA-KO).

Methods:
GLA-KO and GLA-KO::CX3CR1-GFP and littermate control mice (age 28 and 57 weeks) were explored by indirect immune fluorescence microscopy with antibodies staining for different monocyte populations (Cd68, Iba1, and Cd77). Fluorescent mages were acquired by multiphoton microscopy and 3D reconstruction was performed using Imaris 9.7.0. In addition, transwell migration assays were performed to assess the chemotactic potential of the main accumulating lipid Lyso-Gb3. For this, BV2 cells were incubated with a low (0.1 µM) or a high concentration (1µM) of Lyso-Gb3 for 24 hours and semi-automatic quantification was performed using Imaris
Results:
DRG macrophages of GLA-KO mice were shorter, more spherical with fewer branches and smaller surface volume. GLA-KO mice displayed a high expression of the phagocytic marker cd68 in DRG macrophages . Additionally, Gb3 accumulated in the GLA-KO DRG. These alterations were stronger in aged mice. However, the total number of macrophages was similar in GLA-KO and wt mice and no increase in migratory activity was observed for Lyso-Gb3.
Discussion:
Our results suggest that the accumulation of Gb3 triggers immune responses in the peripheral nervous system where macrophages altered their morphology and increased the expression of phagocytic markers (e.g. Cd68). These alterations may be causally involved in the development of sensory deficits and pain in FD.

P-43 The effect of low-energy extracorporeal shockwave treatment on the functional, morphological and molecular level in sub-acute and chronic phases of traumatic SCI

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Background:
Spinal cord injury leads to severe impairment of the patient´s physical, social and psychological well-being. Treatment options to improve symptoms are still very limited. Extracorporeal shockwave therapy (ESWT) has proven to be effective for treating various pathologies of soft tissues, bone and the peripheral nervous system. In this study, we investigated effects of ESWT in a sub-acute (2 weeks after injury) and chronic (5 weeks after injury) setting in a rat contusion model at the 11th thoracic vertebra, at clinically relevant time points.

Methods:
ESWT was applied weekly for three consecutive weeks (500 impulses, 0,11mJ/mm², 5 Hz). Animals were observed for 10 and 18 weeks after the last treatment session. Functional outcome was assessed weekly using BBB-Score and bi-weekly using Catwalk®-analysis. Excised spinal cords were stained with the contrast agent Lugol and assessed by µCT imaging (Scanco µCT50) in order to gain insights into morphological changes after ESWT. Subsequently, spinal cords were processed for histology analyzing axonal regeneration and glial scarring. Also, a large number of systemic microRNAs were screened throughout the observation period to explore therapeutic effects and underlying mechanisms of injury.
Results:
Basso, Beattie and Bresnahan (BBB) open field walking test revealed that animals in both treatment settings (subacute, n=12, BBB = 14.7 ± 2.6 ; and chronic, n=15, BBB = 15.6 ± 2.6) showed significant improvement in their functional outcome in contrast to untreated rats (n=24, BBB =12.9 ± 0.3). Results were reproducible in a long term observation group with treatment group reaching a mean BBB Score of 17 ± 1.1 and the control group reaching a mean score of 13.4 ± 0.9. Catwalk®-analysis for the long term observation group did show statistical differences between therapy and control group. Furthermore, we developed an automated computational algorithm to reliably analyze µCT images for changes in the injured spinal cord. Using this, significant correlation between higher BBB scores at the end of observation time and a greater rate of spared white matter compared to controls was revealed. Finally, we were able to identify several miRNAs showing significant treatment effects after ESWT (e.g.upregulation of miR-375 in the subacute group and downregulation of miR-382-5p in the chronic group).
Discussion:
We provide evidence for the safety and efficacy of ESWT in subacute and chronic spinal cord injury in a contusion model of the rat. Additionally, we established a novel contrast agent-enhanced 3D µCT imaging method for visualizing morphological changes upon spinal cord damage. Our results support the use of ESWT as a non-invasive treatment modality after spinal cord injury even after delayed onset of treatment.

P-44 MRI findings in atypical presentation of fibrous meningioma

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Background:
Meningiomas are extra-dural tumors and represent about 15% of intracranial tumors. The clinical presentation is usually related to persistent headaches

and episodes of paresis. Although most of these tumors are typically benign, there are a large number of histological variants with different imaging characteristics. The study aims to show an atypical MRI presentation of a grade I fibrous meningioma.

Case:

A 41-year-old female patient presented with a history of sudden holocranial headache refractory to common analgesics and opioids. During the initial and subsequent assessment, no focal deficits were evidenced. A neuroimaging test was requested for further evaluation, which showed an image suggestive of an expansive lesion of infectious origin, a probable bacterial abscess. Craniectomy was performed with excision of the lesion, in which the histopathological analysis suggested WHO grade I fibrotic meningioma.

Discussion:

Meningiomas are extra-axial tumors, considered non-glial, originating from meningocytes or cells of the arachnoid cap of the meninges and can be located anywhere there are meninges, being more commonly found in supratentorial regions. These tumors represent about 14-19% of intracranial tumors, being more common in women and the age group above 40 years. Clinical presentation is usually related to headache, paresis, and changes in the level of consciousness. They have multiple classifications, the most important being that of the World Health Organization (WHO), where they are divided into grades I, II, and III. Grade I representing benign lesions, grade II atypical lesions, and grade III anaplastic lesions. Fibrotic meningioma falls under WHO classification grade I.

Meningiomas usually appear as extra-axial masses with a broad dural base. They are generally homogeneous and well-circumscribed, although many variants are found. The signal intensity of meningiomas on T2-weighted images correlates with histological subtypes, usually exhibiting signal isointense with gray matter. In T1-weighted, gray matter is usually isointense or gray matter is hypointense, as in fibrotic and psammomatous variants. It tends to have a homogeneous and intense enhancement in the contrast medium. Spectroscopy has no relevant role, but an increase in alanine can be observed (1.3-1.5 ppm); increased glutamine/glutamate; increased choline (Cho); absence or reduction of N-acetyl aspartate (NAA); absence or reduction of creatine (Cr). Imaging findings that suggest atypical presentation are peripheral contrast enhancement, hypo and hyper signal halos on T2-weighted sequences, inferring the sign known as "dual kidney sign" and the sharp peak of Li/La, inferring necrosis/anaerobiosis, as well as reducing all other peaks. These findings led to the initial diagnosis of intracerebral abscess, and a surgical procedure for excision of the lesion was performed. However, the histopathological analysis of the lesion showed that it was a fibrotic meningioma (WHO grade I).

Conclusion:

Imaging findings in meningiomas are diverse, however, there are some imaging features typical of meningiomas. Although there is a certain pattern,

some meningiomas can have an unusual presentation and can often be confused with other intracerebral lesions. In these cases, histopathological analysis is essential in defining the diagnosis.

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Neuronal Networks & Activity

P-45 The anti-asthmatic drug Montelukast improves motor coordination and balance in the Line 61 mouse model of Parkinson's disease

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Background:
Parkinson's disease (PD) is a neurodegenerative disorder affecting over seven million people worldwide. Its major hallmark is an abnormal aggregation of the α-synuclein protein (α-syn) in neurons and/or glial cells of the substantia nigra and the deep brainstem, impairing movement. The complex pathomechanisms underlying α-syn aggregation and neurodegeneration are still not fully understood and available treatment options are limited.

Neuroinflammation, which is mainly driven by microglia and is also caused by a dysregulated leukotriene signaling pathway, might play a critical role in evoking neurodegenerative conditions. A previous study showed that blocking of leukotriene signaling via the leukotriene receptor antagonist Montelukast (MTK) improved learning and memory deficits in the α-syn transgenic D-Line mouse model.

This study aims to evaluate whether MTK treatment can additionally alleviate motor symptoms in the α-syn transgenic Line 61 mouse model of PD.
Methods:
Male Line 61 mice and non-transgenic (ntg) littermates were treated daily orally with 10 mg/kg MTK or placebo (vehicle) from 2 weeks of age for a total of 10 weeks. Behavioral tests for assessing motor functions, activity, anxiety and emotional learning were performed at different time points of the treatment period (2nd, 5th and 10th treatment week). Brains were collected for immunohistochemical and biochemical analyses of neuroinflammation and autophagy markers as well as α-syn load.
Results:
Significant motor deficits were already observed in 4-weeks old Line 61 mice compared to age-matched ntg littermates. In the beam walk test, motor coordination and balance were significantly improved in MTK-treated compared to vehicle-treated Line 61 mice. Additionally, moderate improvements of motor coordination were observed in MTK-treated Line 61 animals using the Rotarod test. Interestingly, muscle strength as well as activity, anxiety and emotional learning behavior of the mice were not affected by MTK treatment. Immunohistochemical and transcriptome analyses of the brain for detailed investigation on the MTK-mediated molecular and cellular mechanisms are currently in progress.
Discussion:
The results of this study demonstrate beneficial effects of Montelukast, specifically on motor coordination and balance, suggesting that Montelukast is a suitable drug for the treatment of motor impairments in Parkinson's disease and related neurodegenerative diseases.

P-46 Transcriptomic characterization of brain CD8+ T-cells identifies gene signature of tissue-resident memory T-cells in Alzheimer's disease transgenic mice

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Background:

Peripheral immune cell infiltration to the brain and their contribution to pathology is an important and rethought aspect in various neurodegenerative diseases such as Alzheimer´s disease (AD). With AD progression, that typically includes amyloid-beta plaque formation and microglia-mediated neuroinflammation, a population of CD8+ T-cells starts to infiltrate into the AD brain parenchyma. We recently demonstrated that CD8+ T-cells occur in the brain with increasing amyloid pathology and tightly associate with microglia and neuronal structures. A specific subpopulation of CD8+ T-cells is clonally expanded in the CSF of AD patients and by experimentally ablating CD8+ T-cells in transgenic AD mice (APP-PS1) we demonstrated that CD8+ T-cells regulate neuronal- and synapse-related gene expression in the hippocampus. However, so far a detailed transcriptomic profile and phenotypic characterization of brain CD8+ T-cells in transgenic AD mice is missing.

Methods:

In this study we isolated CD3+ CD8+ T-cells from the brain and blood of old APP-PS1 and WT mice (24-25 months old) using flow cytometry-based cell sorting and performed detailed mRNAseq analysis. We compared our transcriptomic data with available gene microarray data of brain isolated tissue-resident memory (Trm) CD8+ T-cells derived from an acute virus infection mouse model (Wakim et al., J Immunol, 2012). Additionally, detailed immunohistochemical analysis was performed on brain CD8+ T-cells in APP-PS1 mice.

Results:

Brain CD8+ T-cells from APP-PS1 animals had a more similar transcriptome to brain CD8+ T-cells from WT mice but substantially differed from blood circulating CD8+ T-cells. The genetic signature of brain CD8+ T-cells from APP-PS1 mice revealed the presence of Trm T-cells by specific regulation of core Trm T-cell genes including Klf2/3, Ccr7, Sell, Xcl1, Isg20, Litaf, Pdcd1 and

Cxcr6. Gene ontology enrichment analysis on the significantly up-regulated genes showed an overrepresentation of biological processes as "response to virus", "T-cell mediated immunity" and "response to interferon-beta". KEGG pathway analysis on up-regulated genes in APP-PS1 brain derived CD8+ T-cells demonstrated up-regulated pathways for various "virus infections", for "cellular senescence" and for "antigen processing and presentation". In brain CD8+ T-cells from APP-PS1 mice we observed a highly overlapping gene signature with genes from brain Trm CD8+ T-cells derived from a virus infection mouse model. Additionally, we detected the presence of Trm CD8+ T-cells (CD103+ and Cxcr6+) at sites of amyloid plaques.

Discussion:

The herein presented data give new insights on the transcriptome of brain CD8+ T-cells and demonstrate the presence of Trm CD8+ T-cells in the brain of transgenic AD mice. This might open the doors for immunotherapy as potential new treatment option for AD.

P-47 Insights into the development of a brain implant for local chemotherapy

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Background:

Aggressively growing brain tumors, such as glioblastoma multiforme (GBM) remain incurable due to limited surgical resection possibilities and hindrance of promising chemotherapeutics that are shielded by the blood brain barrier (BBB). We present an electrically-driven device able to deliver selected chemotherapeutics, such as Gemcitabine (Gem).

Methods:

We observed Gem as drug in different cell cultures (GBM, neurons, astrocytes) and analyze the effect of the drug via cell-based assays (apoptosis, necrosis, viability) and via mass spectrometry (whole proteome analysis). Furthermore, we engineered Gemcitabine Ion Pumps (GemIPs), and monitored their performance via voltage-current measurements. The functionality of GemIPs was further tested in different cell culture models and effects were observed via flow cytometry analyzing induced apoptosis.

Results:

We show that Gem is a potent chemotherapeutic with special properties suitable for the application in the brain. We can state that Gem effectively kills GBM cells, is more potent than the gold standard temozolomide, but is at the same time harmless to neurons and astrocytes and does not alter protein expression in neurons after presentation of high Gem concentrations over

72h. The engineered GemIPs are able to administer Gem with pmol*min-1 delivery precision at currents in the nano-ampere range. The further application of this electrical and temporal control was done in the cell monolayer and 3D cell culture. Most noticeable was the disintegration of targeted GBM tumor spheroids among GemIP treatment.
Discussion:
The here exemplified electrically-driven chemotherapy has the potential to increase the efficacy of GBM treatment and represent a highly-targeted and locally-controlled drug delivery tool that is independent from BBB permeability of potent chemotherapeutics.

P-48 Unravelling the role of spinal astrocytes in nociception and pain

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Background:
Spinal astrocytes are at a prime position to control the processing of nociceptive information at the level of the spinal cord. Astrocytes release signalling molecules such as chemokines, cytokines and so-called gliotransmitters, which can all potently modulate synaptic transmission at nociceptive synapses in the spinal cord dorsal horn, and ultimately pain-related behaviour. Here, we tested whether selective activation of astrocytes in the lumbar spinal cord dorsal horn is sufficient to induce pain-related behaviour and changes in spinal nociceptive processing.
Methods:
Male Sprague Dawley rats were used in all experiments. The astrocytes were targeted selectively using cell-specific GqDREADDs (Designer Receptors Exclusively Activated by Designer Drugs). The DREADDs were activated using Clozapine-N-Oxide (CNO). To assess the effect of spinal astrocyte activation on pain-related behaviour, we performed the von Frey and the Hargreaves tests. We performed patch-clamp recordings from dorsal horn neurons in acute spinal cord slices with long dorsal roots attached to investigate the effect of spinal astrocyte activation on the cellular and synaptic levels. Additionally, we recorded C fibre-evoked field potentials in intact, deeply anaesthetized rats to evaluate synaptic plasticity in vivo.
Results:
In behaving animals, the selective activation of spinal astrocyte through DREADDs resulted in a significant hypersensitivity to mechanical stimuli. In contrast, thermal thresholds were not affected. Unexpectedly, activation of spinal astrocytes induced robust long-term depression at nociceptive C fibre synapses, both in-vitro and in vivo. Furthermore, activation of spinal astrocytes increased spontaneous inhibitory currents in neurons in superficial laminae in vitro.

Discussion:
Our study demonstrates that in behaving rats, a selective activation of spinal astrocytes is sufficient to trigger hypersensitivity to noxious mechanical stimuli. Unexpectedly and in striking contrast to other known forms of pain-hypersensitivity, astrocytogenic pain hypersensitivity did not come along with an amplification of synaptic strength at spinal C-fibre synapses, nor with an impaired spinal inhibition. This might point to novel cellular mechanisms of pain hypersensitivity after activation of spinal astrocytes.

P-49 Direct excitatory afferents onto hypothalamic tanycytes control metabolic states

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Background:
Tanycytes are a heterogeneous and specialized type of radial glia that reside at the medioventral border of the hypothalamic third ventricle. Tanycytes are classified based on their dorso-ventral location along the ventricular wall: α1, α2, β1 and β2. The position and interaction of tanycytes predisposes these cells to form a conduit between neurons and the brain's ventricular system, providing an important homeostatic link between the CNS and periphery. Tanycytes have functionally been implicated in the control of feeding, neuroendocrine secretion and adult neurogenesis via paracrine signaling [1]. However, information on whether tanycytes engage in other types of transcellular communication is elusive, even though synaptoid-like contacts between neuronal circuitry and tanycytes in the pars tuberalis were described more than 40 years ago [2].

Methods:
Confocal and electron microscopy, immuno-histochemistry, patch-clamp electrophysiology, calcium live imaging, trans-synaptic tracing, behavior.

Results:
Here, we addressed whether hypothalamic neurons directly innervate tanycytes and use them to translate synaptic signals into long-lasting metabolic code through the cerebrospinal fluid. We found that Vglut2-containing presynaptic terminals appose GluA2-expressing α1-tanycytes. By patch-clamp electrophysiology we recorded EPSCs in ~50% of tanycytes. Live calcium imaging in hypothalamic slices revealed that neuronal stimulation evoked calcium transients in tanycyte clusters, a response blocked by superfusion of an AMPA receptor blockers. To identify the neuronal origins of glutamatergic inner-vation, we used retrograde and trans-synaptic labeling [3] and found that tanycytes received inputs from neuronal clusters residing in the arcuate nucleus, dorsomedial nucleus, medial preoptic nucleus, as well as extrahypothalamic glutamatergic nuclei located at the parabrachial nucleus (PBN).
To identify the physiological stimuli that activate tanycytes through neuronal afferents, we studied PBN projections that control behavioral and metabolic

states in response to thermal challenge [4]. We found that tanycytes are selectively activated in response to acute heat exposure. Moreover, chemogenetic activation of the glutamatergic projections from the PBN was sufficient to provoke the activation of α1 tanycytes. These findings suggest that tanycytes are final transducers in a neuronal circuitry that controls temperature homeostasis. Notably, we find tanycytes to release angiotensin (AGT) and vascular endothelial growth factor (VEGFA) upon glutamate stimulation in vitro, which indeed implicates tanycyte-derived factors in general physiology through volume transmission.
Discussion:
Overall, our data identify a subset of tanycytes as end-organs of an excitatory neurocircuit to convert local into systemic long-range signaling.
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P-50 Fast and slow – precision tuning of spinal locomotor networks

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Background:
Locomotor behaviors in vertebrates are adapted to their way of life and the environments they inhabit. Control of the locomotor-related muscles is achieved through neural networks in the spinal cord (SC), called central pattern generators (CPGs). CPGs can govern a broad range of rhythm and frequency regimes – think, for example, about sloughs and cheetahs. However, such different motor behaviors require adaptations of the muscles exerting the motions as well as the muscle controlling central networks (i.e. the CPGs). But how are CPGs tuned to different motion regimes? A general problem when tackling this question is interspecies comparability. To overcome this pitfall,

we investigated motor CPGs in body and tail SC of western diamondback rattlesnakes (Crotalus atrox). While the respective CPGs most likely originated from a common predecessor network, they now work in fundamentally different frequency regimes. In thoracic and abdominal spinal segments, CPGs produce slow rhythmic muscle activity for locomotion, while shaker CPGs in the tail segments enable superfast, alternating muscle contraction for acoustic communication (i.e. rattling). Motoneurons (MNs) play an important role in transmitting CPG activity to the muscles. In this study, we therefore put a special emphasis on anatomical and physiological differences in MNs that might help to convey the vastly different frequency and precision regimes of body and tail movements.

Methods:

EMGs from tail and body muscles of rattlesnakes were recorded to visualize their respective neuromuscular activity patterns. To compare morphology and connectivity of MNs from locomotor and shaker CPGs, we performed in-vitro backfills of ventral root nerves with of fluorescent dextran dyes. The physiological differences of both MN types were investigated by performing patch clamp electrophysiology on slices of body and tail SC. Electrophysiological recordings were combined with pharmacological treatments to block specific ion channels that contributed to the differences in MN physiology. To verify the data gathered from patch clamp experiments, RNAseq and RT-qPCR were used to analyze ion channel expression and compare expression profiles of body and tail SC.

Results:

We show that MNs from locomotor and shaker CPGs do not differ in their general morphological characteristics. However, they do possess fundamentally different physiological characteristics which were noticeable in neuromuscular activity patterns recorded by EMG, as well as in single cell patch clamp recordings. Locomotor MNs displayed much higher input resistances, lower rheobase values and longer time constants than shaker MNs. Shaker MNs, but not locomotor ones, showed onset firing behavior upon stimulation. Pharmacological treatment as well as RT qPCR comparison of body and tail spinal cord expression profiles indicate that non-inactivating potassium channels from the KV7 family, in particular KV7.2 and KV7.3, largely contributed to the reported difference in precision tuning of MNs.

Discussion:

The evolution of novel motor behaviors can provide important contributions to increase a species' fitness, e.g. by enabling more effective foraging, defensive behaviors or by helping to occupy new ecological niches or even new habitats (e.g. water-land transition). However, the effective transition to a new motor behavior requires the co-adaptation of peripheral structures involved in locomotion (i.e. muscles, joints, tendons) and the central neural networks that produce and control motion patterns. We show that adaptations in the ion channel expression profile of MNs could provide an important

contribution in such a transition by changing the "filter characteristics" of MNs. Due to the role of MNs as transmitters between CPG and muscle activity, changes in their filter characteristics can reshape CPG output that reaches the muscles and thereby influence the motion execution. In combination with further adaptations of the CPG interneuron network, variations in the ion channel expression profile of MNs might therefore be an important factor in the evolution of novel motor behaviors.

P-51 Sex-dependent brain activity to anaesthetic ketamine exposure in mouse primary visual cortex

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Background:
Ketamine is a rapidly-acting dissociative drug that preferentially acts on NMDA receptors of cortical inhibitory neurons. Recent findings from the lab showed that repeated ketamine exposure results in a microglia-mediated clearing of perineuronal nets (PNN) - extracellular matrix components, which preferentially coat parvalbumin-positive neurons. We have also shown that the PNN removal through ketamine restored juvenile-like plasticity in adult primary visual cortex (V1) after monocular deprivation. However, the electrophysiological signatures, which gives rise to this ketamine-induced plasticity is not well-understood. Furthermore, potential sex differences in neural responses to ketamine remains largely unexplored.
Methods:
To reveal the effects of anaesthetic ketamine on neuronal activity, we implanted 2-shank 32-channel chronic silicone probes spanning across the cortical layers in V1 of male and female mice. We then performed an up-to 6 hours continuous in vivo electrophysiological recordings of the neural activity before and after saline or ketamine administration while the mouse is freely behaving inside a 55cm×38cm environment. Local field potentials were then extracted from low-pass filtered signals.
Results:
We observed that both male and female brains responded with an increase in mid-gamma band (55 Hz - 65 Hz) oscillation shortly after ketamine injection compared to baseline conditions. This increased activity is not observed in low- (30-45 Hz) and high- (70-100 Hz) gamma bands. Interestingly, we also observed a sex-dependent increase in the beta band which persisted during the recovery period.
Discussion:
Most studies that look at the effects of ketamine in neurons focus only on its immediate effect and never during recovery. Here, we have identified 60 Hz oscillation as a potential primary component of PNN clearing through ketamine. Indeed, we found that mice exposed with 60 Hz light flickering also had less PNN-coating in V1. Furthermore, the sex-dependent increase in beta os-

cillation during ketamine recovery suggests a sex-specific interaction between ketamine and somatostatin interneurons, which are also encased in PNNs.

P-52 Ca2+-mediated adaptation of neuronal metabolism to neuronal electrical activity

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Background:
Ca2+ entering the neurons during neuronal activity stimulates neuronal metabolism to cope up with the increased energy demand. Ca2+ can stimulate several enzymes of the tricarboxylic acid cycle after entering the mitochondria. It can also enhance the activity of malate aspartate shuttle (MAS) and glycerol phosphate shuttle (GPS) outside of mitochondria, resulting in an increased glycolytic activity and NADH shuttling to the mitochondria. Neurons can show a wide variety of electrical activities, from low frequency action potential firing to burst discharge. Such activities represent workloads of variable intensity, which are accompanied by proportional rises of cytosolic Ca2+. It can thus be envisaged that the dependency of neuronal metabolism on extra/intra-mitochondrial Ca2+ might vary with variable workloads. Hence, in the current study, we investigated the relative contribution of the aforementioned Ca2+ sensitive pathways in stimulating neuronal metabolism under variable workloads.
Methods:
For the experiments, electric field stimulation (EFS) of primary hippocampal neurons co-cultured with astrocytes was combined with live-cell metabolic imaging. Neuronal cultures at 14 DIV were stimulated using EFS of frequencies ranging from 1 Hz-10 Hz. The evoked neuronal activity was indirectly measured using calcium imaging (Fluo4-AM), while the "metabolic response" was monitored using the genetically-encoded fluorescent indicators Perceval (cytosolic ATP/ADP ratio sensor) and Peredox (cytosolic NADH/NAD+ ratio sensor). CEPIA2mt was employed to measure mitochondrial Ca2+. Pharmacological agents were used to modulate the activity of the aforementioned Ca2+ sensitive pathways, in order to study their particular role in the neuronal metabolic response. Recovery of the ATP/ADP ratio after the EFS-induced drop was evaluated as a measure of ATP production.
Results:
Our data demonstrate that ATP production in primary hippocampal neurons is Ca2+-sensitive and mitochondrial in nature. Interestingly, mitochondrial Ca2+ influx was only observed during higher stimulation frequencies (5 Hz and 10 Hz). Thus, stimulation of mitochondrial ATP production after lower stimulation frequencies did not rely on intra-mitochondrial Ca2+. Unexpectedly, we found that blocking any one of the NADH shuttles or mitochondrial Ca2+ uptake separately showed no obvious effect, while a combined inhibition significantly impaired ATP production. In our conference contribution we will present these

and follow-up investigations of the mechanisms by which neuronal mitochondria substitute for the loss of various Ca2+ sensitive pathways under variable workloads.

Discussion:

The results obtained suggest that neurons utilize both, cytosolic and mitochondrial Ca2+ to stimulate their metabolism during neuronal activity. The involvement of various Ca2+-sensitive components of neuronal metabolism changes with the intensity of the applied workload. In the absence of any one of the major Ca2+-sensitive pathways, the remaining pathways appear to increase their activity to compensate for the loss. The presented work shows how neurons maintain metabolic flexibility during electrical activity, thus avoiding potentially detrimental energetic crisis.

P-53 Parvalbumin-positive GABAergic neurons in the basal forebrain – role for neuropathic pain

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Background:

Chronic neuropathic pain is a serious public health problem that affects ~5 % of the European population and exerts major impacts on the patients' quality of life. However, the central mechanisms underlying the chronification of pain are only partially understood. The medial prefrontal cortex (mPFC), a brain region associated with emotional and cognitive aspects of pain, receives strong cholinergic innervation from the basal forebrain (BF) that is changed in neuropathic pain animal models. One neuron type that might be of special importance for this regulation are parvalbumin (PV)-positive GABAergic interneurons, which produce widespread inhibition of large brain areas with high firing frequencies and synchronized activity.

Methods:

In order to elucidate the influence of these neurons on the pain-associated changes in cholinergic signaling, we investigated the electrophysiological properties and synaptic strength of BF PV neurons using whole-cell patch-clamp recordings in acutely prepared brain slices. PV neurons were selectively labelled with enhanced yellow fluorescent protein (eYFP) by stereotaxic injection of a floxed viral vector into the BF of PV::cre mice and brain slices prepared one week after spared nerve injury (SNI) or sham surgery.

Results:

After SNI, BF PV-neurons exhibited reduced excitability and lower firing rates in response to ramp-shaped depolarizing current injections as well as an increased threshold current to reach a depolarization block as compared to sham controls. Consistently, the input-frequency (I-F) linear slope was reduced in SNI mice. In accordance with recent literature, we found three

different clusters of PV-neurons showing either high input resistance together with HCN channel currents, or with low input resistance with or without HCN channel currents.

Discussion:

Our findings suggest that the previously shown alterations in cholinergic synaptic transmission in neuropathic pain are associated with reduced inhibitory inputs mediated by local BF PV neurons. The underlying circuit modifications in the input regions to the BF are currently under investigation.

P-54 Retinal adaptation to natural luminance and contrast statistics

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Background:

Efficient neuronal representations of visual scenes require prior knowledge of its statistical properties, such as luminance and contrast distributions, to remove redundant components. This process begins in the retina, where center-surround receptive fields (RF) of retinal ganglion cells (RGCs) decrease redundancy in natural images uniformly across visual space. However, natural scene statistics are not uniform; they vary significantly across elevations.

Methods:

This work combines theory and experimental approaches to predict and test the uniformity of RFs across the retinal space. We use the efficient coding framework to make predictions about optimal RF structures at different elevations of natural images. To test these predictions, we developed new experimental methods to image high-resolution RFs of thousands of RGCs in individual mouse retinas. These methods improve the experimental throughput over previous state-of-the-art methods by more than an order of magnitude while reducing cost by a similar factor.

Results:

Theory predicts that optimal RFs strengthen their surround from the darker dorsal (groundward) to the brighter ventral (skyward) retina and have a marked asymmetry at the horizon, where a stark contrast change is observed. Consistently, we see that the RFs in the mouse retina adapt their surround strength across elevation and shift it asymmetrically ventral at the horizon. These effects were observed across RGC subtypes, suggesting a global principle in retinal architecture.

Discussion:

Our results suggest that mouse RGCs have efficiently adapted to the constraints imposed by statistics in different elevations of natural scenes. This adaptation appears to be an inbuilt prior that helps broaden the visual system's

dynamic range to cope with differences in light intensities between the sky and the ground. Moreover, the observed asymmetric surrounds at the horizon have the required properties for detecting upward motion and conveying it to the brain, consistent with the reported abundance of upward motion-sensitive cells in the mouse superior colliculus. Finally, given the position of the asymmetric streak at the horizon, it is tempting to speculate that its ethological role is to define the horizon's position for subsequent computations and behaviors.

P-55 Spinal locomotor circuits and their functionality in *Xenopus laevis*

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Background:
Locomotion is the ad hoc visible output of neuronal circuits and the underlying basis of behavior. While the molecular basis of locomotion in vertebrates is conserved from lampreys to humans, ongoing research continuously shows how much heterogeneity exists between the cells taking part in the circuitry of the spinal cord. To tie diversity of neurons to their functional role, my goal is to create a behavioral atlas of locomotion during Xenopus laevis development. This will not only give us insight into the development of locomotor strategies and kinematic abilities for swimming and walking but create a baseline to dissect the functional role of each motor circuit's cellular components. Towards this goal, using the CRISPR/Cas9 system, we have created half or full mutations of critical cell identity determinants by injecting guide RNA's in the two- or one- cell stage of Xenopus laevis. Comparing the effects on locomotion of single cell-type mutations against the wild type developmental behavioral series, will allow us to get high resolution functional output of manipulated locomotor circuits and the specific (developmental) timing of their activity. Our long-term goal is to discover the sufficient and necessary cellular components of the spinal cord circuitry that give rise to pre- and post-metamorphic behavior, and the transition between the two.
Methods:
Using SLEAP for marker-free pose estimation, we assign points for limb-and tail-features as basis for detailed kinematic analysis. This is combined with biased and unbiased analytical tools to detect the general development of locomotion in time spent moving, speed, patterns and rhythmicity as well as, inter- and intra-limb and tail/limb coordination.
Results:
Preliminary experiments and data show promising results towards the possible resolution of the behavioral assay as well as the effects of FoxP1 mutations. In foxp1 crispr mutants, we observe a loss of LMC identity (unpublished data) and a concurrent behavioral defect, which is restricted to the limbs.

Discussion:
Our results will shed light on the conservation of functionality across the diversity of neurons involved in spinal cord motor circuits.

P-56 NMDA receptor subunit GluN2B c-terminus orchestrates hippocampal left-right asymmetry

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Background:
Left-right asymmetries constitute a fundamental organizational principle of the vertebrate central nervous system. The hippocampal CA3-CA1 pyramidal cell synaptic connection shows an input-side dependent asymmetry where the hemispheric location of the presynaptic CA3 neuron determines the synaptic properties. Synapses made by left CA3 inputs on CA1 dendritic spines in stratum radiatum have higher densities of NMDA receptor subunit GluN2B, lower densities of AMPA receptor subunit GluA1 and smaller areas with less often perforated PSDs than right-input synapses. However, the mechanism of input-side dependent asymmetry formation remains elusive.
Methods:
We examined the role of GluN2B and its c-terminal domain for establishment of the input-side dependent asymmetry. To this end, we compared synaptic area, ratio of PSD perforation, and density of synaptic GluA1 between left- and right-input synapses in wild-type and two types of mutant mice. One is CA1-specific GluN2B conditional knock-out, and the other is GluN2B-2A swap mutant, in which the c-terminal domain of GluN2B has been replaced by that of GluN2A. PSD area and perforation was measured by reconstruction of serial electron microscopy images and GluA1 density was examined with SDS freeze-fracture replica labeling. We further performed electrophysiology to compare the sensitivity of NMDAR EPSCs to GluN2A- and GluN2B-selective

blockers between left- and right-input synapses. To identify the hemispheric origin of synaptic inputs we either injected a viral tracer into the left or right CA3 area, or transected the ventral hippocampal commissure to eliminate contralateral CA3 to CA1 synapses.

Results:

We found no significant left-right difference in GluA1 density or PSD area in either GluN2B conditional knock-out or GluN2B-2A swap mice. Surprisingly, the ratio of perforated synapses was higher in left- than right-input synapses in GluN2B-2A swap mice, an asymmetry that is opposite to that in wild-type animals. We found that asymmetric sensitivity of NMDAR EPSCs to the GluN2B-selective blocker Ro25-6981 was lost in GluN2B-2A swap mice, indicating that the GluN2B c-terminus is necessary for its asymmetric distribution. Wild-type NMDAR EPSCs showed a higher sensitivity to the GluN2A-selective blocker MPX-004 in right than left-input synapses and this asymmetry was also lost in GluN2B-2A swap mice.

Discussion:

Our results indicate that GluN2B c-terminus is necessary for normal formation of hippocampal asymmetry, including asymmetric allocation of GluN2B and GluA1, asymmetric PSD area, and asymmetric GluN2A function in CA3-CA1 pyramidal cell synapses. The opposite asymmetry for the ratio of perforated PSD in GluN2B-2A swap mice indicates an additional unknown factor controlling the input-side asymmetry. We demonstrate for the first time an asymmetry of GluN2A function which is opposite to that of GluN2B function in wild-type mice. This was unexpected, as synaptic GluN2A protein amount has previously been reported to be same between left- and right-input synapses. However, considering that GluN2A and GluN2B are the only GluN2 subunits expressed at this synapse, this result is consistent with the same total NMDAR EPSCs between left- and right-input synapses, indicating that GluN2A and GluN2B contributes more to the NMDAR response in right and left-input synapses, respectively.

P-57 Alterations in basal forebrain-to-medial prefrontal cortex cholinergic signaling in a mouse model of neuropathic pain

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Background:

Chronic neuropathic pain (CNP) constitutes a major public health issue, of which the underlying disease mechanisms are only partially understood. Although the involvement of the medial prefrontal cortex (mPFC) in CNP is well established, the role of cholinergic signaling from the basal forebrain (BF) to the mPFC for the processing of painful stimuli has so far not been addressed.

Methods:
We therefore investigated cholinergic synaptic transmission in acutely dissected mPFC brain slices from spared nerve injury (SNI) and sham treated control mice by multielectrode array (MEA) or patch-clamp recordings using pharmacological or endogenous optogenetic cholinergic stimulation. Furthermore, we investigated the functional and morphological properties of BF cholinergic neurons, which are the main source of acetylcholine in the mPFC.
Results:
Seven days after SNI, mPFC network activity in response to pan-cholinergic and muscarinic M1 receptor activation was reduced as demonstrated by MEA recordings, which was also observed in patch-clamp recordings of prelimbic (PrL) but not infralimbic (IL) layer 5 pyramidal neurons. Furthermore, cholinergic neurons in the BF showed more depolarized membrane potentials and decreased inhibitory postsynaptic current frequencies, as well as an increased dendritic length and complexity after SNI.
Discussion:
Our current findings suggest that during the development of CNP cholinergic signaling is altered in the PrL, and that these changes are based on pain-induced disinhibition and subsequent hyperexcitability of BF cholinergic neurons. The underlying mechanisms of this disinhibition are currently under investigation.

P-58 Neuroinflammation in pain affective disturbances: role of the parabrachial nucleus

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Background:
Chronic pain patients often suffer from complex affective disturbances, such as stress, depression and anxiety. The lateral parabrachial nucleus (LPBN) in the brainstem is thought to be crucial for the aversive dimension of pain. Neuroinflammation, resulting from neuro-immuno-glial interactions, may play a significant role as a driving force for both the sensation of pain and for its most prevalent comorbidities. At present, little is known about the occurrence and potential roles of neuroinflammation at supraspinal nociceptive brain areas such as the LPBN. Here, we hypothesized that parabrachial neuroinflammation may contribute to and aggravate emotional aspects of pain.
Methods:
In male Sprague-Dawley rats, immunohistochemistry methods were used to evaluate if chronic constriction injury of the sciatic nerve (CCI), a model for neuropathic pain, induced morphological and molecular adaptations of glial-neuronal network in the LPBN. Next, using whole-cell patch clamp recording, we tested parabrachial synaptic connectivity following an inflammatory insult via administration of lipopolysaccharide (LPS). Using electrical stimulation

and viral optogenetic targeting, we evaluated both ascending nociceptive connections from the spinal cord and descending modulatory connections coming from the periaqueductal grey (PAG). Finally, by means of a cannula implanted bilaterally in the LPBN, LPS was microinfused to selectively induce parabrachial neuroinflammation in awake animals. We assessed its contribution to pain-related aversive behaviour using a paradigm examining escape responses to a noxious thermal stimulus.

Results:

CCI of the left sciatic nerve lead to a bilateral increase in c-Fos expression, a neuronal activation marker, in the LPBN without changing neuroinflammatory markers. LPS application in brainstem slices had two distinct effects on neurons in the LPBN: It reduced the inhibitory postsynaptic currents evoked by photo-stimulation of optogenetically targeted PAG axons and it decreased the excitatory currents evoked via electrical stimulation of putative spinal axons. Induction of neuroinflammation in the LPBN by LPS microinfusion lowered heat thresholds for escape responses, suggesting a pro-aversive effect of neuroinflammation.

Discussion:

Neuroinflammation in the LPBN disrupted both inhibitory and excitatory synaptic connectivity in this brainstem area and potentiated the aversive component of pain. We suggest that neuroinflammation that accompanies many of the co-morbidities of pain may worsen the affective disturbances of chronic pain patients.

P-59 Medial entorhinal cortex integrates hippocampal offine inputs into independent, persistent memory representations

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Background:

Memory consolidation is usually understood as a progressive transfer of information from the hippocampus to other regions of the neocortex, following the acquisition of novel memory items. Nonetheless, the temporal dynamics required for memory consolidation are poorly understood both at the coarse-grained level (i.e. temporal offset and scale) and at the fine-grained level (i.e. activity patterns and their modulation). Here, we investigated the coordination between hippocampal CA1 region and the superficial layers of the medial entorhinal cortex (mEC) during learning and consolidation of a spatial navigation task.

Methods:

We trained rats to memorise three new reward locations every day on a cheeseboard maze, and allowed for rest sessions before and after the learning experience. We tracked the evolution of CA1-mEC interactions by means of tetrode recordings of the two areas. Data was collected both during the

acquisition of novel memory representations (40 learning trials) as well as during off-line periods; these occurred between learning trials, where animals waited for a few minutes in a start-box, and in sleep periods after learning. We focused on the responses and coherence properties of the two areas as a function of the strength of the underlying sharp-wave ripple (SWR) oscillations, quantified by the power of the oscillations in the 150~250 Hz band. We also investigated the presence of goal-related information within offline neural activity. This was quantified by the correlation of neural patterns in short time windows during offline periods and the average population activity measured around goal locations during locomotion.

Results:

We found that learning led to a specific increase of offline SWR power, event length and rate between trials, which in turn progressively elicited stronger responses in both populations of neurons. Offline SWR events were also found to increasingly perturb ongoing cell activity in the two regions; this was indicated by the diminished correlation between spike configurations immediately preceding and following SWR events. We also noticed that offline SWR activity patterns were similar to those of recently learned goal locations. This effect increased over learning in both areas, faster in CA1, and was carried over into sleep following learning. The correlation of SWR-induced goal reactivation increased between the two areas, peaking at the beginning of post-learning rest periods. HPC consistently led the reactivations by 10-20ms. Finally, we noticed that mEC activity reorganization happened on longer timescales as compared to the CA1 one. In particular, CA1 remapping happened mostly during learning, whereas entorhinal cell activity was restructured throughout the rest period after learning. MEC's slower timescale brought about a longer lasting representation; this was indicated by the increased correlation of MEC goal-reactivation during sleep with memory retention afterwards.

Discussion:

Our findings corroborate the idea that mEC does not only relay hippocampal information, but also possesses internal memory consolidation mechanisms, which are enhanced and influenced by HPC during SWRs. The end result of this process is a longer-lasting memory representation in the cortex, presenting a more reliable connection between past and future experience than the hippocampus. These results thus point to mEC acting as a temporal filter over the hippocampus activity: slower temporal reaction to novelty, longer integration times and continually morphing representation; but also larger correlations between events separated by large temporal intervals.

P-60 Presynaptic modulation by cAMP-PKA pathway at hippocampal mossy fiber synapses

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Background:
The mossy fiber synapse onto CA3 pyramidal neurons (MF–CA3) is a key synapse of the hippocampal trisynaptic circuit. This synapse exhibits various forms of synaptic plasticity. One characteristic form of short-term plasticity is post-tetanic potentiation (PTP), a transient enhancement of synaptic transmission that lasts tens of seconds following sustained high-frequency presynaptic activity. PTP at this synapse is primarily caused by an increase in the size of the readily releasable pool (RRP) of synaptic vesicles, leaving release probability (Pr) largely unaffected [1]. Furthermore, PTP appears to be mediated by the cAMP-PKA pathway. Although the activation of cAMP-PKA pathway is known to potentiate transmitter release at the MF–CA3 synapses [2, 3], the exact effect on quantal and vesicle pool parameters (RRP and Pr) have not been determined.
Methods:
To investigate the involvement of cAMP-PKA pathway in presynaptic potentiation at the unitary level, we performed paired recordings between mossy fiber terminals and postsynaptic CA3 pyramidal neurons in acute brain slice preparations, with selective non-invasive stimulation of single mossy fiber terminals in the tight-seal cell-attached configuration. We applied second messenger agonists to test how these pharmacological manipulations affect the quantal and vesicle pool parameters, which is estimated from cumulative excitatory postsynaptic currents (EPSCs) amplitudes during a 50-Hz train of 10 stimuli [4].
Results:
Forskolin (50 µM), which is thought to increase intracellular cAMP levels via directly stimulating the adenylyl cyclase, potentiated synaptic responses in CA3 pyramidal neurons, increasing EPSC amplitude to 429 ± 59% of the control value (n = 7 pairs). Cumulative release analysis indicated that the effect was mediated by an increase in RRP, whereas Pr was unchanged (n = 7 pairs). Isoproterenol (1 µM), an agonist of beta 2 adrenoceptors, also potentiated synaptic transmission (n = 4 pairs). Similar to forskolin, isoproterenol increased the RRP, but not the Pr. As beta 2 adrenoceptors are thought to activate adenylylcyclase via Gs proteins, these results provide further evidence for the hypothesis that cAMP-PKA pathway regulates the size of the RRP at hippocampal mossy fiber synapses.
Discussion:
Our results show that both activation of the adenylyl cyclase by forskolin and the more natural activation of the cAMP-PKA pathway via a beta 2 adrenoceptor agonist enhances transmitter release at the MF–CA3 synapse. This suggests that the cAMP-PKA pathway is not only involved in PTP [1], but also plays a role in neuromodulation of mossy fiber transmission.
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P-61 Functional asymmetry of medial habenula output in mice

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Background:
The habenula is a phylogenetically conserved bilateral brain structure known to modulate negative emotions. The functional and structural differences between bilateral brain areas are collectively summarized by the term "left-right asymmetry" and are hypothesized to play a key role in behavior and cognition. Although habenular asymmetry is prominent in several vertebrate species, such as zebrafish, there is currently no evidence for left-right asymmetry in the habenula of mammals.
Methods:
To investigate the asymmetry in synaptic transmission in the medial habenula (MHb) to the interpeduncular nucleus (IPN) pathway, we performed electrophysiological recordings using acutely cut brain slices. To test the importance of habenular asymmetry on behavior, we expressed inhibitory DREADDs in cholinergic MHb neurons using stereotaxic virus injection, and performed cued fear conditioning.
Results:
Using targeted electrical stimulation of left or right MHb-derived axons in slices, we discovered that the neurotransmitter release probability from left MHb synapses was significantly lower than that from right MHb synapses in the rostral IPN. Furthermore, the activation of GABAB receptors potentiated the release from left MHb terminals significantly stronger than that from right MHb terminals. To investigate the behavioral significance of our functional findings, we chemogenetically silenced either the left or the right MHb during recall of auditory-cue conditioned fear memory. Strikingly, activation of inhibitory DREADDs in the left but not the right MHb decreased the expression of cued fear memory in the retrieval.
Discussion:
Our study provides the first evidence for a functional asymmetry of the MHb-IPN pathway in mammals and reveals a regulatory role of this asymmetry for emotion-related behaviour. Physiologically, the right MHb dominates neurotransmission at rest, whereas following the GABAB receptor-mediated presynaptic facilitation, both sides have similar levels of synaptic strength

because this potentiation is much stronger in the left MHb synapses. The left side-specific silencing effect on the fear expression indicates hemispheric laterality in the emotional memory regulation in mice habenula. The mechanism underlying the observed asymmetry and the in vivo activity profile of the habenulae neurons are yet to be studied.

P-62 Electrical synapses improve motion extraction in a natural visual environment

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Background:
In the fly, wide-field motion-sensitive horizontal system (HS) cells extract optic flow by integrating the output of arrays of elementary motion detectors in the optic lobe. This visual transformation facilitates prompt corrective responses to course deviations, e.g., due to a gust of wind. However, natural environments are ambiguous and non-uniform, which challenges the accuracy of the system. Theoretical studies predict that electrical synapses (gap junctions) between HS cells correct for such natural fluctuations by sharing information among them. To test this hypothesis experimentally, we generated and assessed a new inducible mutant of shakB, the gene which encodes transmembrane proteins that form gap junctions in the fly visual system.
Methods:
Shaking-B mutant flies were generated by integrating FlpStop cassette into shakB[MI15228] line (shakB-FlpD). The coupling of neurons via gap junctions was visualized using intracellular neurobiotin injections. Visual response properties of HS cells were examined using in vivo whole-cell patch-clamp recordings. Visually induced behavioral responses of freely-walking flies were tested using an arena for simultaneous animal tracking and stimulus presentation.
Results:
ShakB-FlpD flies show a reduction in Shaking-B protein expression, as well as the loss of neurobiotin coupling between HS cells. Similar to an older shakB mutant [shakB2], the membrane potential of HS cells in mutant flies has a strong oscillatory component at 10–20 Hz; however, their direction-selective responses are preserved. Preliminary results suggest that their responses to more challenging visual stimuli (low-contrast and higher motion noise) are attenuated, indicating a reduced efficiency under naturalistic circumstances. In line with these results, ShakB-FlpD flies also show deficits in loom-induced escape and optomotor response.
Discussion:
Classical optomotor responses and directional tuning of HS cells are largely preserved in ShakB-FlpD flies. However, when challenged with more complex

and naturalistic visual stimuli, HS cells and animal locomotion responses exhibit evident deficits. Our results suggest that gap junctions are not essential for establishing motion selectivity in HS cells but rather refine their output and improve behavioral performance of the animal in a complex natural visual environment.

P-63 The role of hippocampal VIP-expressing interneurons in the pathophysiology of temporal lobe epilepsy

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Background:
Temporal lobe epilepsy (TLE) is the most frequent focal epilepsy and affects almost 30 % of all epilepsy patients. TLE is characterized by spontaneous recurrent seizures arising from limbic brain structures, in particular, the hippocampus. Compelling evidence obtained in human TLE and animal models suggests that malfunctioning of hippocampal GABA interneurons is related to epileptogenesis. In the hippocampus, two groups of vasoactive intestinal peptide (VIP)-expressing interneurons (cholecystokinin (CCK)-expressing basket cells and/or interneuron-selective interneurons) control GABAergic transmission and pyramidal cell activity. The aim of the current study is to assess the possible role of VIP-expressing interneurons in the pathophysiology of epilepsy.
Methods:
To demonstrate a possible causative role of defunct VIP interneurons in the generation of TLE, we permanently inhibited GABA release selectively from VIP interneurons of the ventral subiculum by injecting a viral vector expressing tetanus toxin light chain (TeLC; fused with a green fluorescent protein [GFP] tag) in male epileptic as well as non-epileptic VIP-cre mice. Mice were then subjected to telemetric EEG recording for 4 weeks. In addition, to assess possible effects of TLE on memory function, spontaneous alternation Y-maze and novel location recognition test were conducted to evaluate spatial memory and learning, respectively. Double labeling immunohistochemistry was conducted to evaluate the sensitivity and specificity of virus injection.
Results:
According to immunohistochemistry, sensitivity and specificity of viral infection was 61 and 80 percent, respectively. In epileptic mice, the mean of the total daily number of spikes trains in the control group was significantly higher than in the experimental group (15.34 and 8.05 respectively, $p=0.0039$). In addition, the experimental group showed a dramatic decrease in the mean of the total daily duration of spike trains ($p<0.0001$), mean of the total daily number of spikes in trains, ($p<0.0001$), and the mean of total daily number of spikes outside trains ($p<0.0001$). In non-epileptic animals, there was no

significant difference in the mean of the total daily number of spike trains between control and experimental groups (3.609 and 3.493 in the same order, p= 0.9336). However, the mean of the total daily number of spikes (outside trains) was increased significantly in the experimental group ($p<0.0001$). No significant difference was observed between groups in epileptic as well as non-epileptic animals in both behavioral tests.

Discussion:

Interneuron-selective interneurons mainly target parvalbumin- and somatostatin-expressing GABAergic interneurons; therefore, silencing them would increase inhibition in pyramidal cells, which is compatible with the result of the experiment in epileptic animals. In non-epileptic animals, the increased spiking activity could be the result of silencing CCK-expressing basket cells, which needs further assessment by triple immunohistochemistry labeling for GFP, CCK and VIP.

P-64 Ponto-genicular waves dynamics in the sleeping rat brain

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Background:

Sleep has various physiological functions, including the consolidation of memories. Current theories establish that memories are transiently stored in the hippocampus and transferred to the neocortex for long-term storage during sleep. These processes are associated with plastic changes in distinct brain circuits, heralded by different types of macroscopic electrical activities that occur upon changes in the neuromodulatory activity of the brainstem. The brainstem prompts periods of both enduring and transient changes of neuronal excitability that affect the activity of other sub-structures in a precise manner. Thus, brainstem activity is likely critical for long-range coordination of several brain structures underlying memory formation. However, the specific neural mechanisms that allow these subcortical regions to participate in memory formation remain poorly understood.

Methods:

Wild-type Long Evans rats (N = 4) were chronically implanted with recording micro-drives incorporating three bundles of movable recording electrodes targeting (either unilaterally or bilaterally) the dorsal hippocampus, the dorsal lateral geniculate nucleus (dLG) of the thalamus, and the parabrachial nucleus (PBn) of the brainstem, respectively. The neural responses of the PBn, dLG and hippocampal circuits were then probed during periods of natural sleep. To this end, each animal was recorded for a period of 3 to 5 hours of daily unrestrained sleep from their home cage using a radio-telemetry recording system (Triangle BioSystems International, TBSI; n = 15 to 20 recording days per animal).

Results:
We show that global changes in neuronal activity associated with the animals' states of vigilance (waking and sleep stages) were accompanied by the occurrence of transient, coordinated high-synchrony neural events in the PBn-dLG electrical activity. These episodes displayed electrical characteristics consistent with pontogeniculooccipital (PGO) waves [1]. In particular, in our recordings, PGO activity was characterized by the emergence of simultaneous negative deflections in the ponto-thalamic local field potentials (LFPs). Consistent with a previous study in anaesthetized macaques [1], we show that the brainstem transiently modulates hippocampal high-synchrony events through PGO waves. These highly selective interactions spanned both non-rapid eye movement (NREM) and rapid eye movement (REM) sleep phases, influencing hippocampal SWR episodes and REM-associated high-amplitude phasic theta waves. When analysed separately, these two types of PGO episodes were associated with opposite profiles of brainstem population firing, and opposite hippocampal spike-field coupling. Crucially, learning of a spatial memory task resulted in an increase in the coupling between PGO waves and hippocampal events, observed during extended post-learning sleep periods.
Discussion:
The preliminary results of this investigation indicate that the control of hippocampal ensembles by PGO waves might be a phylogenetically well-conserved neural mechanism. These episodes may correspond to windows for promoting hippocampal-cortical communication and plasticity during NREM and REM sleep, likely promoting memory consolidation and synaptic homeostasis. In addition, our data open, for the first time, the possibility to determine the correlates, neuronal mechanisms and physiological significance of the PGO phenomenon in memory formation and long-term consolidation in awake, behaving rodents.
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P-65 The role of hippocampal cholecystokinin-expressing interneurons in spatial coding

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Background:
Given the importance of inhibitory cell types in shaping hippocampal activity, we addressed the role of a subpopulation which remains poorly studied in freely moving conditions: the cholecystokinin-expressing interneurons (CCKIs).

Methods:
We expressed the ArchT opsin in CCK inhibitory cells using double transgenic mice and an adeno-associated virus (AAV) whose activity depended on the presence of both Cre and Flp recombinases. Therefore, via extracellular recordings and intersectional genetics in mice, we tested the effect of CCKI optogenetic silencing on the network activity.
Results:
There was an increase in pyramidal cell firing during and after the laser-mediated silencing of CCKIs, indicating a disinhibitory effect. We then evaluated the network activity during network oscillations. First we tested sharp wave ripples (SWRs): an increase in the firing rate around the SWR events was observed not only for the case of pyramidal cells but also of non-CCK interneurons. In addition, we studied the place cell activity and observed changes in the spatial coding properties: specifically, a decrease in place field similarity upon laser-mediated CCKI silencing. Furthermore, when analyzing the activity changes in relation to the behaviorally relevant theta oscillations, we detected a shift in pyramidal cells' preferred theta phases. Congruently, we further analyzed theta phase precession on a linear track running task, revealing that the laser application led to a disruption of theta phase precession and this impairment remained in the session after the laser delivery.
Discussion:
Such an effect in the theta precession properties of pyramidal cells suggests that CCKIs coordinate neural ensembles in natural conditions. In conclusion, these findings point to a crucial role of CCKIs in modulating the activity of place cells and their involvement in relevant temporal coding schemes.

P-66 Visualising priority maps: attentional modulation of neuronal population dynamics in the superior colliculus

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Background:
During selective visual attention, distractors are ignored in favour of behaviourally important visual stimuli. This process requires an assessment of "top-down" priority signals resulting from executive function and associations. Although several brain regions work jointly to enable such computations, the Superior Colliculus (SC) has received a prominent role due to its topographic representation of space. It appears to encode a priority map representing the location of important objects in the visual environment and guides attention to these objects. However, changes in neuronal population dynamics that coincide with learning new priority information have yet to be disentangled, and no shifts in attention have been directly explored at a population level.
Methods:
I aim to directly study the executive function and associations at a population level in the mouse SC. I am currently exploring changes in sensorimotor trans-

formation by combining functional Calcium imaging of population responses of the mouse SC with specific training paradigms designed to assess selective visual attention.

Results:

Here, I present results of successful learning of detection and discrimination tasks during brain imaging, enabling the study of (i) sudden shifts of attention and (ii) cue-related shifts of attention. Successful allocation of attention coincides with increases in the successful hit rate and reduced reaction time of the animal.

Discussion:

Currently, I am leveraging these specific training paradigms designed to assess selective visual attention in the study of the executive function and associations at a population level in the mouse SC. I explore changes in sensorimotor transformation as behavioural training progresses. I further investigate the neural correlates of allocating attention to locations based on memory or specific cues. Together, these results will deepen our knowledge on (i) the role of "top-down" modulations of visual processing, (ii) relate these to animal performance, and (iii) improve our understanding of attentional processes in general.

P-67 Spontaneous intrinsic spatiotemporal dynamics in superior colliculus

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Background:

The brain receives myriads of sensory signals, any of which could signify the presence of danger to be avoided or an opportunity to be grasped. To ensure survival, relevant stimuli should be represented accurately by sensory systems. At the same time, the relevance of different signals may vary given the animal's behavioral goals and internal state. For example, the same sensory input might require different computations, depending on whether the animal is relaxed or aroused. Such interactions between changing internal demands and encoding of sensory signals are believed to contribute to response variability – a phenomenon which is prevalent across the brain, yet not well understood. Here we explore interactions between internal state and sensory processing in the superior colliculus (SC), a midbrain region instrumental for immediately survival-relevant sensorimotor transformations such as threat detection or hunting. Because it combines direct sensory input from the retina as well as other brain areas (e.g., visual cortex, basal ganglia), the SC is an ideal model system to study the interactions of sensorimotor processing and internal states.

Methods:

With two-photon Calcium imaging, we simultaneously recorded the activity of thousands of SC neurons in mice. The animals were presented with visual

stimuli on a dome screen while they ran freely on a spherical treadmill and their behavior was monitored. We parameterized the neuronal activity using dimensionality reduction techniques (Nonnegative Matrix Factorization - NMF). To capture interactions of neural activity, sensory stimuli, internal states and network dynamics, we developed a simple encoding model based on logistic regression.

Results:

In addition to sensory responses, we observed a previously unknown intrinsic population dynamic - anatomically localized groups of neurons activate strongly even without sensory stimulation. Using NMF, we decomposed these complex population dynamics into a set of components i.e. systematically occurring patterns of neural activity. The majority of identified components are spatially localized, have similar spatial size and uniformly cover the entire SC surface. Using encoding models we demonstrated that, while weakly influenced by sensory inputs, these localized components are most likely to be active when the animal is in a low-arousal state (as measured by the pupil size). Simultaneously, strong activation of one local activity pattern decreases the probability of others becoming active. This interaction is reminiscent of lateral-inhibition dynamics. Localized activity patterns persist in animals with removed visual cortex, suggesting a local or subcortical origin.

Discussion:

We identified a novel type of intrinsic network activity in superficial layers of the SC, which were presumed to be mostly involved in sensory coding. This previously unobserved activity is dominated by local patterns of synchronized neural activity, which are predominantly controlled by arousal - an important internal state of the animal. Taken together, our results demonstrate that even in early brain regions, sensory responses are not merely relayed, but combined with complex neural activity related to internal states of the brain.

P-68 Anti-aversive drugs modulate inputs to the lateral parabrachial nucleus

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Background:

The brainstem lateral parabrachial nucleus (LPBN) is an important centre for the emotional processing of aversive stimuli including pain. It receives direct input from the dorsal horn of the spinal cord and forms reciprocal connections with brain regions critical for adaptive behaviour in potentially harmful situations, such as the periaqueductal grey matter (PAG). Mounting evidence suggests that the neuronal net within the LPBN is subject to plastic changes possibly contributing to the development of chronic pain. Here we investigated whether and how LPBN neurons are involved in processing aversive

stimuli such as noxious stimuli. We specifically tested the impact of drugs with potent analgesic and/or anti-aversive properties on distinct inputs to neurons in the LPBN. We focused on: (i) opioids, which affect the affective component of pain more potently than the sensory component, (ii) diazepam, a potent anxiolytic devoid of anti-nociceptive effects and (iii) cannabinoids, which are suggested to modulate pain processing as well as general aversion.

Methods:

We aimed at neurons in the LPBN that receive synaptic input either from the spinal cord or from the PAG. In young Sprague-Dawley rats (40-100 g) we injected adeno-associated viral vectors carrying the genetic information of mCherry-coupled Channelrhodopsin-2 under the control of the CamKIIα-promoter (AAV2/DJ-CamKIIα-hChR2(H134R)-mCherry) into either the dorsal horn of the spinal cord or the PAG. After at least six weeks, acute coronal brain slices containing the LPBN were prepared. We performed whole-cell patch-clamp recordings from LPBN neurons that displayed postsynaptic currents upon photo-stimulation of axons originating either from the spinal cord or PAG. We then evaluated the effects of DAMGO (10 µM; µ-opioid receptor agonist), diazepam (10 µM) and WIN 55,212-2 (5 µM; cannabinoid-receptor agonist) on neuronal excitability and synaptic strength.

Results:

Spino-LPBN projections were all excitatory, while the PAG sends both excitatory as well as inhibitory projections to LPBN neurons. µ-Opioid receptor activation strongly reduced the excitability of LPBN neurons with either spinal or PAG input and decreased excitatory as well as inhibitory synaptic strength. Diazepam failed to affect strength at spino-LPBN synapses but enhanced inhibitory PAG-LPBN synaptic signalling. WIN 55,212-2 had no effect on neuronal excitability, but reduced inhibitory as well as excitatory PAG-LPBN synaptic transmission. Spino-LPBN synapses were not affected by WIN 55,212-2.

Discussion:

This is, to the best of our knowledge, the first study investigating the effect of anti-aversive drugs on LPBN neurons with identified spinal or PAG input. Our study demonstrates opioids and benzodiazepines but not cannabinoids exerted strong effects on LPBN neurons which may underlie the anti-nociceptive and / or anti-aversive effects of these drugs. Interestingly, the effects of the cannabinoids might be explained at a brain area other than the LPBN.

P-69 Acute anesthetic ketamine triggers sexual dimorphic microglia response in the mouse and human cortex

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Sex-specific differences in the brain network activity has been observed in the context of various neurological diseases such as schizophrenia [1], Alzheimer's [2], or Parkinson's disease [3] but the underlying mechanism is less well understood. Recently, we reported that repeated ketamine-induced anesthesia causes the loss of the perineuronal net (PNN), a physical extracellular barrier that surrounds interneurons, and allows reestablishing juvenile ocular dominance plasticity in the adult visual cortex [4]. We show that the PNN dismantling is enabled by microglia, the resident immune cells, which survey their local surrounding and respond to changes in neuronal activity. Interestingly, we noticed with our ketamine-regime that the first dosage already affected microglia, however more severely in females.

Here, we followed up on this result, and demonstrate that only female microglia turn into a reactive state after acute ketamine dosage that is reflected in an upregulation of engulfed perineuronal nets fragments within the microglia lysosomes-endosomes. Strikingly, we replicated the same results upon ketamine exposure in acutely isolated human brain slices obtained from temporal lobe neurosurgeries. When we performed ex-vivo electrophysiological recordings of excitatory projection neurons in the adult mouse primary visual cortex, we found that ketamine anesthesia promotes sex-specific metaplasticity. Depletion of microglia prevented this plasticity underlining their critical impact for plasticity induction. In line with this result, female excitatory projection neurons increased their dendritic spine number. To obtain the broader consequences of this sex-specific differences, we are currently performing in-vivo imaging and in-vivo electrophysiology of awake mice of both sexes, and plan single cell RNA-sequencing. Our results suggest that a sexual dimorphic microglia response exist that interfere with brain network activity.

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P-70 Cell-specific synaptic wiring within the hippocampal CA3 network

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Background:
The hippocampus is crucial for learning and memory, and the distinct wiring of its CA3 area appears uniquely configured for memory storage and retrieval [1]. CA3 pyramidal neurons form a network of reciprocal connections, which could allow storage of unique experiences as ensembles of interconnected cells. However, while the overall route of information flow through the hippocampus has been long understood, little is known about the wiring of CA3 on an individual cell level. In particular, how diverse subtypes of both excitatory and inhibitory neurons are connected will dictate the rules of information processing in the network. Therefore, whether the properties of this circuit are sufficient or optimal to support memory formation remains unclear.
Methods:
We determined the connectivity, synaptic properties, and arrangement of the CA3 network using multi-cellular whole-cell patch-clamp recordings from acute slices of mouse hippocampus. Up to 8 neurons were simultaneously recorded to identify and characterize synaptically connected pairs. Cells were filled with intracellular biocytin during recording, and post-hoc imaging was performed for neuron subtype identification, localization of synaptic contacts, and cell reconstruction.
Results:
We recorded 33 synaptic connections between morphologically identified CA3 pyramidal neurons (978 tested connections; total connection probability 3.4%). The apparent synaptic conductance was small, with a peak value of only 312 ± 29 pS. However, the circuit arrangement was non-random, containing sub-networks of highly interconnected cells, generating a potential mechanism for memory storage within synaptically coupled cell ensembles [2]. Unique subtypes of excitatory pyramidal neurons can be identified based on both functional and anatomical properties. We demonstrate that these subtypes have specific wiring rules with respect to one other, adding a further layer of complexity to the CA3 network. Connectivity between pyramidal neurons and local fast-spiking interneurons was more abundant than principal neuron interconnectivity, with 33 % of tested connections demonstrating synaptic transmission from pyramidal neurons to interneurons (18 recorded, 54 tested connections), and 11 % connectivity from interneurons to pyramidal neurons (6 of 54 confirmed connections). This wiring appears less cell type-specific, yet with unique synaptic properties that will differentially process excitatory activity in a frequency and spatially dependent manner.
Discussion:
Together, our results begin to provide insights into the microcircuit arrangement of the CA3 network, which is essential for understanding information processing in the hippocampus. Non-random principal neuron connectivity and cellular heterogeneity may increase the processing capacity of the network, and suggest the possibility of distinct pyramidal cell types having specific roles in encoding life experiences within neuronal ensembles.

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P-71 Does the kappa-opioid receptor derived DREADD have a therapeutical potential in temporal lobe epilepsy?

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Background:
With a prevalence of 0.5 – 1% epilepsy represents one of the most common neurological diseases. Among the different types of epilepsy, temporal lobe epilepsy (TLE) is the most frequently diagnosed. The fact that up to 80% of TLE patients do not achieve seizure-freedom with currently available pharmacotherapies underlines the urgent need for novel treatment strategies.

Targeting anatomically restricted regions such as epileptic foci with gene therapy tools has shown encouraging results. Designer receptors exclusively activated by designer drugs (DREADDs) may be well-suited candidates for such a gene therapy approach offering the additional advantage of suppressing neuronal excitability in a controllable way.

In this study we investigated the therapeutic potential of a viral-vector delivered DREADD based on the kappa-opioid receptor (KORD) in the kainic acid mouse model of chronic TLE.

Methods:
Using mice after unilateral intrahippocampal kainic acid application, we injected an AAV6 vector construct encoding the KORD under the control of the neuron-specific hSyn promoter into the epileptic focus in the hippocampus. Upon selective activation of the KORD by Salvinorin B (SALB) an inhibitory Gi-cascade is initiated. To assess if epileptic activity is attenuated in response to SALB administration, we analyzed in-vivo EEG recordings regarding spike trains and hippocampal paroxysmal discharges (hpds, representing drug-resistant focal seizures).

Results:
SALB did not show a statistically significant effect on spike trains and hpds in comparison to the vehicle DMSO, whereas DMSO alone, intriguingly, resulted in a significant reduction of spike trains and hpds compared to saline. Regarding the spreading of the AAV6-KORD, we observed a remarkable discrepancy between non-epileptic animals robustly expressing the AAV6-KORD in large

parts of the hippocampus and epileptic mice with pronounced hippocampal sclerosis showing only limited spreading.
Discussion:
In conclusion, the observed effect of the solvent DMSO on epileptic activity interferes with the evaluation of the KORD/SALB pressing the quest for alternative solvents.

P-72 Using Xenopus to define tetrapod motor circuit cell types across evolution

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Background:
A hallmark of the nervous system is its rich cell-type diversity, its intricate connectivity and its coordinated patterns of activity. Behavior largely is an emergent property of this complexity. Thus, to understand behavior, we must parse neurons' molecular, cellular and functional properties. This task has proven especially challenging for motor circuits which exhibit readily apparent output in motor activity but astonishly heterogenous populations of neurons.
Methods:
In the Sweeney lab, we apply a novel approach harnessing the unique behavioral switch during Xenopus frog metamorphosis and the evolutionary transition of vertebrates from swimming to walking to parse motor circuit complexity.
Results:
I will present on our ongoing efforts to dissect motor circuit cell-type composition in the developmental and evolutionary context utilizing single-cell sequencing and immunohistochemistry.
Discussion:
Our intra- and inter- species approach has the potential to deepen our understanding of the origin of motor complexity and its relationship to motor circuit composition and output in tetrapods.

New Methods & Disease Models

P-73 MorphOMICs: a new algorithm to unravel region- and sex-dependent microglia morphological plasticity in health and disease

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Background:
Morphological characterization of neuronal shapes has provided important insights into the diversity of cell types, which pinpointed to shared genetic and functional features. Microglia, the resident immune cells of the brain, have been exempted from this success, although this would provide insights in their interaction with their local environment under distinct conditions. Neuronal network interferences trigger microglia to alter cell-cell connections resulting in unknown behavioral consequences that can severely impact disease onset and progression. Thus, establishing a reliable brain region-specific microglial signature is critical to obtain a baseline signature and track plastic changes as deviations from it.

Methods:
Microglia morphology is commonly determined with classifiers that extract from a three-dimensional (3D) reconstructed branching tree user-selected morphological features such as total dendrite length, branch number, or number of terminal points. The drawback of this approach is that the number of implemented classifiers and the types of extracted features biases the biological readout. Classically, microglial morphology is determined based on classifiers, the distribution of which is statistically compared across conditions. However, this approach is often not optimal for detecting small morphology changes. The reason for this is that the user-driven selection of one or few morphology classifiers leads to an incomplete description of microglial morphology signature. Moreover, microglia's dynamic nature introduces high variability of the data sets already within a condition, further inhibiting data extraction. Thus, strategies are needed to optimize the extraction of morphological information from microglia. Here, we developed the MorphOMICs algorithm, combining the topological morphology descriptor (TMD) with bootstrapping, dimensionality reduction, and data visualization techniques allowing an unbiased identification of microglial signature across the brain.

Results:
We show a brain region-specific microglial signature in adults with sex as a confounding factor. Remarkable, the signature of ovarectomized females matched neither the corresponding brain regions nor males or females underlining that sex impacts microglia plastic signature. This was confirmed when

we looked during postnatal development. Here, we found an early microglial sexual dimorphism, which gradually declines towards adulthood. In contrast, the sex-specific signature diverged over time following the environmental changes during disease progression in two distinct Alzheimer's disease models, 5xFAD and CK-p25. Interestingly, females foreran males in the disease trajectory. When we aligned the trajectory of development and degeneration, we observed a spectrum of plastic microglial signatures indicating that, compared to classifiers, MorphOMICs resolves better differences in microglial morphology. Finally, we identified that the contribution of primary processes is critical for the observed sexually dimorphic disease trajectory in the microglia of the frontal corte.

Discussion:

In conclusion, MorphOMICs provides an unbiased, unsupervised strategy to map signatures for microglia across conditions and proves that microglia are morphologically similar within their local environment and that sex influences the microglial response to environmental cues.

P-74 Plasma, brain and spinal cord pharmacokinetic profile of tetrahydrocannabinol from cannabis sativa extract or THC administration

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Background:

Cannabis sativa contains over 100 bioactive substances, such as cannabinoids, flavones and terpenes. Of these, the cannabinoids delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are predominant and generally accepted therapeutics in nausea, epileptic disorders and pain. Other constituents of the cannabis plant remain insufficiently explored and there is insufficient understanding of potential additive, synergetic or antagonistic interactions or effects on absorption and bioavailability of THC or CBD. Therefore, we aimed to explore and compare the pharmacokinetics of THC in two sets of plant extracts with semisynthetic (THC).

Methods:

Oral bioavailability of THC and its metabolites Hydroxy-THC (OH-THC) and 11-nor-9-carboxy-THC (THC-COOH), were evaluated following administration of medical marihuana extract rich in THC (THC+), THC-depleted extract (THC-), and THC at corresponding concentration to THC+. Formulations were prepared in sesame oil and administered by oral gavage in a single-dose to C575BL/6J mice. Concentrations of THC and metabolites were evaluated with a limit of detection of 1 ng/ml in plasma, brain and spinal cord up to six hours after application using triple-quadrupole mass spectrometry.

Results:
60 min after administration of THC+ extract, THC concentrations were three times higher in plasma and brain tissue as compared to THC single compound (167.5 vs. 21 ng/ml in plasma; 96.1 vs. 24.6 ng/ml in brain). Faster bioavailability of THC following THC+ administration was further supported by the pharmacokinetic profile of its metabolites, THC-OH and THC-COOH. In case of THC+ extract, peak concentrations of both metabolites occurred at 120 min in plasma and brain while for THC alone they were measured after 240 min. The psychoactive metabolite, THC-OH, reached significantly higher concentrations in brain after THC+ administration compared to THC alone (Cmax 104.3 vs. 61.6 ng/g).
Discussion:
Our results support enhanced bioavailability of psychoactive THC and metabolites from THC+ medical marihuana extracts indicating a potentially higher risk of unwanted psychoactive effects. Importantly, the presence THC in the spinal cord after oral application is reported for the first time.

P-75 Maternal high-fat diet during pregnancy and lactation provokes epigenetic modifications in the offspring brain

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Background:
Clinical and preclinical data indicate the period of intrauterine development and early childhood as extremely sensitive to external factors, such as an improper diet consumed by mothers, which may contribute to the occurrence of molecular and functional abnormalities in the offspring brain. Maternal diet through the establishment of the epigenetic profiles can influence susceptibility to numerous neurodevelopmental and mental diseases later in life. The results indicate that exposure to maternal high-fat diet (HFD) during pregnancy and lactation leads to a reduction in social interactions and an increase in repetitive behavior in male offspring in adolescence. Therefore, we decided to investigate the effect of maternal HFD on changes in DNA methylation in selected brain structures of the offspring.
Methods:
TWistar rat dams were maintained ad libitum on a standard diet (SD; 13% energy from fat, Special Diet Services, VRF1) or HFD (60% energy from fat, Altromin, C1057 mod.) during gestation and lactation. After weaning, offspring at postnatal day 22 were switched to SD. At postnatal day 28 (adolescence) male and female offspring were sacrificed, and the brain areas (prefrontal cortex, frontal cortex and hippocampus) were promptly dissected. Next, global DNA methylation, mRNA and protein expression of DNA methyltransferases (Dnmt1a, Dnmt3a, Dnmt3b) and DNA methylation of CpG islands of the Nlgn3, Setd1b and Taok2 were assessed.

Results:
Our findings indicate that exposure to maternal HFD during pregnancy and lactation significantly increases the level of global DNA methylation in the prefrontal cortex in male offspring. At the same time, these animals showed an elevated protein level of DNMT3B. No changes in the DNA level of methylation of CpG islands of the studied genes were observed within the prefrontal cortex. Maternal HFD also did not significantly affect the level of global DNA methylation in the frontal cortex and hippocampus of the offspring of both sexes.
Discussion:
These results emphasize that maternal HFD in the early stages of individual development can lead to disturbances in the DNA methylation pattern in the offspring's brain and thus contribute to disturbances in the offspring's behavior. However, the epigenetic changes induced by exposure to the maternal HFD are gender and brain-region-specific. Further research is needed, focusing on the role of maternal diet in the regulation of other epigenetic mechanisms, such as histone modification and microRNAs.

P-76 Effect of modified maternal diets on expression of autism spectrum disorder-related genes in the offspring limbic areas

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Background:
Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder, whose prevalence has increased rapidly over the last few decades. The development of ASD is caused by a complex interaction between genetic and environmental factors. Among numerous environmental factors, such as older age of parents, maternal infections, imbalance macro-and micronutrients, and exposures to air pollution and heavy metals, maternal overweight or obesity increases the risk of ASD development in offspring by 36%. To better understand the role of maternal diet on the risk of ASD in offspring, in this study we assessed the effects of maternal high-fat diet (HFD), high-carbohydrate diet (HCD; rich in sucrose), and mixed diet (MD; rich in carbohydrate and fat) on expression of genes and proteins associated with the ASD.
Methods:
The pregnant Wistar rats were assigned to one of the four groups: standard diet (SD; 65% carbohydrate, 13% fat, 22% protein; VRF1) or modified diets: HFD (24% carbohydrate, 60% fat, 16% protein; C1057 mod.), HCD (70% carbohydrate: sucrose – 40%, 12% fat, 18% protein; C1010) or MD (56% carbohydrate, 28% fat, 16% protein; C1011). Dams were fed these diets during the pregnancy and lactation. After weaning (postantal day 22), offspring were

switched to SD. Transcriptome sequencing was done in the frontal cortex of male and female offspring rats sacrificed at postantal day 28. Moreover, the protein levels of Ankyrin Repeat Domain-Containing Protein 11 (ANKRD11), Eukaryotic Translation Initiation Factor 4E (EIF4E), Neurofibromin 1 (NF1) and Serine/Threonine-Protein Kinase TAOK2 (TAOK2) were measured using ELISA within the offspring frontal cortex and hippocampus at postnatal day 28.
Results:
We identified 48 ASD-related genes (SFARI Gene database) whose expression was significantly increased in adolescent offspring in the frontal cortex after exposure to maternal HFD, e.g.: Gabbr2, Nf1, Shank1, Syn1, Taok2 (involved in synaptic function), Ankrd11, Ash1l, Crebbp, Setd1b, Kmt2e (involved in chromatin regulation) and Ankrd11, Btaf1, Med13l, Spen, Taok2 (involved in transcription regulation). In the frontal cortex, after exposure to maternal HFD, there was a significant increase in the protein levels of ANKRD11 and EIF4E in male offspring compared to the control group. In females from the HFD group, an increase in the protein level of NF1 was observed, with a simultaneous decrease in the protein level of TAOK2. Within the hippocampus, exposure to maternal HFD decreased level of TAOK2 in females, as well as level of EIF4E in male and female offspring.
Discussion:
The presented results indicate a key role of maternal HFD during pregnancy and lactation in the disturbance of normal brain development, which may predispose to the development of ASD symptoms in offspring. Exposure to maternal HFD alters the transcription and translation of ASD-related genes mainly in male offspring. Further research on the role of the maternal diet in the risk of ASD is needed to fill the existing knowledge gaps.

P-77 Gemcitabine Ionic Pump (GemIP) brain tumor treatment on the chick embryo chorioallantoic membrane

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Background:
Glioblastoma multiforme (GBM) is the most aggressive of all brain tumors, with a median survival of only 15 months after diagnosis. Treatment typically comprises maximal safe resection, followed by chemotherapy with BBB-passing alkylating agents (e.g. Temozolomide) and radiotherapy. Full resection is, however, rarely feasible due to tumor cell infiltration of surrounding normal tissue. Residual tumor cells are, moreover, in 50% of cases, resistant to the standard chemotherapeutic drug used to treat GBM patients. There has only been little progress in the past few decades to find new strategies for treatment.

Methods:
Different human GBM cell lines were seeded onto the chorioallantoic membrane (CAM) and treated with Iontronic Pumps. The grown tumors were removed and histologically and immunohistochemically analysed.
Results:
GBM cell lines generate solid tumors with high vascularization. We showed that GemIP tumor treatment is feasible, also combined with irradiation.
Discussion:
The main important goal is the optimization of GemIP treatment on CAM, for observing the chemotherapeutic tumor effects in vivo.

P-78 Generation of a human neuronal in vitro model for Rett Syndrome by non-viral expression of lineage factors

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Background:
Rett Syndrome (RTT) is a severe, X-linked neurodevelopmental disorder predominantly affecting females. Clinical features including loss of acquired language and motor skills emerge in early childhood. The main cause for this rare disease are mutations in the human methyl-CpG-binding protein 2 gene MeCP2 encoding for methyl-CpG-binding protein 2 (MeCP2), an global transcriptional regulator with highest expression levels in mature neurons. MeCP2 is not only important for neuronal development, but is also required for proper nervous system function and maintenance. The main goal of our study is the development and characterization of a patient-derived neuronal cell model for further in vitro studies including potential treatment options.
Methods:
The reprogramming process was started with the transfection of MeCP2-deficient and wildtype human dermal fibroblasts using plasmid DNA of the transcription factors PAX6 and SOX2. Culturing transfected cells under reprogramming conditions for several weeks resulted in induced neuronal progenitor cells (iNPs), which are then differentiated into neuronal cells following a two-step differentiation protocol [1]. Bright-field microscopy was used to monitor morphological changes during the reprogramming and differentiation processes. Additionally, the transfection efficiency, iNPs and neuronal cells were analysed via immunocytochemistry. Further characterization of the presented in vitro model was performed on the RNA level comprising of quantitative real-time PCR and total RNA sequencing.

Results:
The comparison of iNPs to untransfected fibroblasts via quantitative gene expression analysis has revealed higher gene expression levels of progenitor-associated genes such as NES, NEUROG2, NCAM1 or FOXG1. Immunocytochemical staining carried out on differentiated neuronal cells showed expression of neuronal-lineage markers TUJ1, NeuN and MAP2 in addition to glutamatergic (vGLUT1) and GABAergic (GAD65/57) markers. Pathway analysis of differentially expressed genes between MeCP2-deficient and wildtype neurons exhibited distinct differences in axon guidance, calcium signaling pathway and cAMP signaling pathway.
Discussion:
In conclusion, our patient-derived cell model for RTT is a promising tool to study phenotypic differences between MeCP2-deficient and wildtype neuronal cells as human brain tissue is not readily available. Moreover, this in vitro model could be used to evaluate novel treatment possibilities.
Reference:
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P-79 Long-term intranasal application of alarin is safe with no effects on food intake and body weight

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Background:
Over the last years, obesity has become a severe worldwide epidemic with a continuing upward trend. Underlying neurohormonal processes that regulate hunger, satiety and formation of body fat are scarcely known but essential for the development of new anti-obesity drugs. Our laboratory has identified a novel regulatory neuropeptide called alarin, which alters acute food intake upon intracerebroventricular injection. In the present study, we investigated the effects of minimal invasively applied alarin in obese and lean mice on food intake, body weight, body fat formation as well as neuronal activation in the brain. Moreover, we were interested whether long-term intranasal application of alarin is safe with no or minor side effects and whether the blood brain barrier can be bypassed.

Methods:
To elucidate whether intranasally applied alarin can bypass the blood brain barrier and subsequently, which brain regions are activated, lean mice were treated with 3 different concentrations of the peptide (10, 20 and 30nmol in 10µl 5% alpha cyclodextrin saline) or vehicle (10µl 5% alpha cyclodextrin saline) and compared to untreated naïve counterparts (over 2 hours; short-term). Following transcardial perfusion, brains were extracted from the skull, hemispheres were separated and sagitally sliced. Immunohistochemistry was performed on free-floating tissue sections using an anti-c-fos antibody indicating neuronal activation. Fluorescence images were acquired using a confocal microscope and positive neurons were counted using the FIJI/ImageJ software. To examine whether long-term application of alarin is safe and whether it affects food intake and body weight in lean and obese mice, mice were either treated with a 60% fat diet to induce obesity or a 10% fat control diet over 13 weeks. After habituation, alarin or the vehicle was intranasally applied in a daily routine (over 4 weeks). Changes in behaviour, appearance and social interaction as well as alterations in body weight, composition of adipose tissue and other toxic effects were monitored.
Results:
Short-term application of alarin did not lead to changes in body weight and social behaviour. However, alarin bypassed the blood brain barrier upon intranasal application and led to a dose dependent activation of c-fos in various brain regions. Long-term application of alarin had no effects on behaviour, appearance and social interaction of mice. Furthermore, it did not significantly alter body weight and adipose tissue composition compared to control mice, neither in 60% nor in 10% fat diet-treated mice. Additionally, alarin did not lead to any toxic side effects.
Discussion:
Short-term and long-term application of alarin has proven to be safe with no major side effects on appearance and behaviour of mice. Here, we could demonstrate that intranasal application of alarin is suitable to bypass the blood-brain barrier as shown by neuronal activation of brain regions upon treatment. Further studies will be performed to uncover the modes of action of intranasally applied alarin.

P-80 Dipeptide repeat protein toxicity and its contribution to DNA damage, nucleolar stress and heterochromatin dysregulation in ALS patients with underlying C9orf72 repeat expansions

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Background:
A repeat expansion in C9orf72 is the most common mutation leading to familial Amyotrophic lateral sclerosis (ALS). ALS is a rare neurodegenerative disease characterized by a progressive loss of upper and lower motor neurons. The hexanucleotide repeat expansion in the intron of C9orf72 is bidirectionally translated into five different dipeptide repeat proteins (DPRs): poly-PR, poly-PA, poly-GA, poly-GR and poly-GP. These DPRs were shown to have various cytotoxic effects and localize differentially within the cell.

In recent years, especially their maleficial effects on processes within the nuclei and consequently, on genomic stability were granted more attention. Various mechanisms have been reported via which DPRs exert toxicity in the nucleus, including their effects on nucleolar integrity, chromatin structure and DNA damage.

This project aims to enlighten the exact roles of DPRs in interfering with genomic stability and to unravel possible mechanisms underlying these dysregulations in a time depending manner that can then be targeted with rescuing therapies.

Methods:
50 repeats of DPRs were synthesized from Thermo Fisher Geneart and cloned into mCherry tagged plasmids using gateway strategy. Successful cloning of the sequence was validated by restriction enzyme digestions and Sanger sequencing. DPRs were transfected using Lipofectamine into N2a cells and expression of mCherry was verified by fluorescence and confocal microscopy. Evaluation of DNA damage, heterochromatin structures and nucleolar stress was mainly conducted using molecular techniques including immunocytochemistry, western blot analysis and FACS.

Results:
Firstly, we show that when we transiently transfect N2a cells with constructs harboring DPRs (poly-PR, poly-PA, poly-GR, poly-GA) we can recapitulate morphological phenotypes, lower DAPI counts and enriched p62 levels. Concerning DNA damage and nucleolar stress, Our data additionally show that the two nuclear localized DPRs seem to have enriched DNA damage at earlier time points compared to other DPRs. Cells were then co-stained with nucleolar markers presenting nucleolar stress in both DPRs at different timepoints. This result suggests that nucleolar stress might be differentially affected depending on the DPR. Furthermore, we investigated heterochromatin markers, which were downregulated in our systems and decreased with the time that DPRs were in culture, suggesting disruption of heterochromatin structures is due to increasing toxicity of DPRs. We aim to validate these findings in C9-ALS patient fibroblasts as well as patient iPSC-derived motor neurons. Finally, critical observations will be confirmed in vivo in postmortem ALS patient brains.

Discussion:
Genomic instability is recognized as a key feature of C9ORF72-related neurodegeneration, however many studies focus on the cytoplasmic pathologies more attention has to be granted to impaired mechanisms on a genomic level. While there are several publications describing the effects of single DPRs on known molecular pathologies, increasing evidence supports the importance of unraveling the difference between these DPRs and investigate their divergent effects on molecular pathways.
Our study, along other studies, shows different localizations of DPRs and, consequently distinctly affected cellular pathways. In our system, poly-GR and poly-GA are nuclear localized and seem to present enriched DNA damage at earlier time points compared to other tested DPRs. This could be explained by their direct interaction with DNA and chromatin structures due to their localization. At later time points and with increasing toxicity of DPRs, DNA damage is enriched in all conditions compared to empty vector, suggesting that also cytoplasmic localized DPRs have indirect effects on genomic regulations. This hypothesis was highlighted even more when investigating the expression of heterochromatin markers. All tested DPRs show decreased heterochromatin markers at late time points, proposing that although localization might have an effect on direct surrounding, toxicity of DPRs can lead to impaired mechanisms through yet unknown interactions. Last but not least, when analysing nucleolar stress in DPR conditions we were able to see a significant difference of nucleolar impairment over time depending on the DPR.
All in all, this data suggests the importance of unraveling the time line underlying DPRs pathologies and their various toxic functions within the cell.
The ultimate goal of our translational efforts is to understand DPR-induced genotoxic stress in hopes of identifying novel therapeutic avenues.

P-81 Spreading of P301S aggregated tau investigated in organotypic mouse brain slice cultures

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Background:
Alzheimer´s disease (AD) is a debilitating brain disorder, characterized by extracellular beta-amyloid plaques and intraneuronal tau depositions. Tau is a microtubule-associated protein and can be hyperphosphorylated leading to the formation of aggregations and neurofibrillary tangles. Tau spreads throughout the brain in a stereotypical manner, starting from the entorhinal cortex to the hippocampus and subsequently, the surrounding regions. Emerging evidence shows that misfolded forms of tau can spread from cell to cell and seed aggregation of native tau, which is reminiscent of the pathological

spread of prion proteins. However, the details of the underlying mechanisms and general spreading characteristics are not entirely understood. The aim of the present study is to investigate the spreading of tau using organotypic slice cultures.

Methods:

We prepared coronal hippocampal organotypic brain slices (170 µm) from postnatal (day 8-10) C57BL6 wildtype mice. Collagen hydrogels loaded with different tau proteins (full length tau, K18 PHF tau, Δ306-311 mTau and P301S aggregated tau) were applied to slices and the spread of tau was assessed by immunohistochemistry using the tau-5 antibody after 8 weeks of incubation. Western Blot and release experiments were performed to support the data.

Results:

Collagen hydrogels prove to be an effective protein delivery system subject to natural degradation in 14 days and release tau proteins up to 8 weeks. From all 4 tested tau proteins, only P301S aggregated tau loaded in collagen hydrogels was detectable by immunoblotting. Slices with collagen hydrogels loaded with P301S aggregated tau demonstrate significant spreading of tau to the ventral parts of the hippocampal slices compared to empty collagen hydrogels after 8 weeks

Discussion:

We illustrate that spreading of tau can be investigated in organotypic slice cultures using collagen hydrogels as a protein delivery system. P301S aggregated tau appears to spread to the ventral parts of the slices, suggesting that the disease relevant aggregated tau form possesses more spreading potential compared to the other tau proteins. Thus, the results offer a novel insight, which would enhance understanding of tau spreading in AD.

P-82 A fibrous nature of hydrogels causes directed migration in Schwann cells

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Background:

Hydrogel fillings have been successful in enhancing artificial conduits, but the ideal hydrogel is yet to be found. Its discovery is impeded by an overflow of available products as well as a lack of in vitro experiments that fully analyze the effects of these hydrogels on cells crucial for nerve regeneration.

Methods:
This study investigated various hydrogels advertised for peripheral nerve regeneration. Schwann cells (SCs), fibroblasts and dorsal root ganglia neurons were seeded on the hydrogels and their morphology, viability, proliferation and migration were examined in vitro.
Results:
Our results demonstrated that the basement membrane extract hydrogel Cultrex® 3D Culture Matrix® (TrevigenTM) promoted elongated morphology and directed migration in SCs. To elucidate the possible reasons behind this, detailed material characterizations of the hydrogels were conducted and identified highly bundled networks of fibres in Cultrex® 3D Culture Matrix® as well as strain-stiffening. In a next step, we further analyzed two more hydrogels consisting of solely one component of Cultrex® 3D Culture Matrix® respectively namely laminin and collagen and found that laminin is the cause of increased elongation in SCs, but not oriented migration. It also seems to be the reason behind strain-stiffening in Cultrex® 3D Culture Matrix®.
Discussion:
These experiments provide a unique systematic assessment of various hydrogels in respect to their material properties and their effect on cells thereby paving the path for the future manufacturing of tailored biomaterial.

P-83 The influence of Cox-1 on microglia reactivity after optic nerve crush

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Background:
Prostaglandins play a key role in modulating the inflammatory response in the central nervous system. They are synthesized from arachidonic acid via the enzyme cyclooxygenases (COX, prostaglandin-endoperoxide synthase PTGS), which is the target for several Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) commonly used to relieve pain or fever. Interestingly, in the central nervous system the Cox-1 isoform is highly enriched in microglia, the resident immune cells of the brain. In response to infection, tissue damage or neurological diseases, microglia become reactive which is associated with phagocytosis and pro-inflammatory function. Concurrently, selective Cox-1 inhibition has been shown to ameliorate pathological effects in several neurodegenerative diseases with an inflammatory component. So far, it is not known which impact Cox-1 expression has on the regulation of microglia activity.
Methods:
We use the optic nerve crush (ONC) as a model to address both acute injury and inflammation in in the injured and the contralateral eye, respectively. ONC causes retinal ganglion cell loss due to damage of their axons, and an activation of nearby microglia. In the non-injured, contralateral eye, ONC triggers a similar but milder response that is thought to be driven by inflammatory signals from the injured eye.

Results:
First, we established an inducible microglia-selective Cox-1 knockout mouse line and confirmed the selective depletion 30 days after induction. Second, we performed ONC and as expected, we found a robust microglial reactivity in wild type mice 7 days after ONC in the injured eye, which was less prominent in the contralateral eye. Remarkably, when we selectively knocked out Cox-1 in microglia we observed a clear reduction in microglia reactivity. Moreover, we show that this effect is not exclusive to the eyes but also occurs in the dorsal lateral geniculate nucleus and the superior colliculus, which receive direct input from the optic nerve, and the primary visual cortex. We are currently investigating how the reduction in microglial activation caused by the knockout of Cox-1 affects the disease progression of ONC.
Discussion:
Overall, our data suggests that Cox-1 expression plays an important role in microglia activation in the ONC model and indicates a potential role in neuronal degeneration.

Stem Cells & Development

P-84 Using synthetic biology to dissect G protein-coupled receptor signaling in microglia

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Background:
Microglia are tissue-resident macrophages of the central nervous system that maintain homeostasis during physiological conditions and trigger inflammatory responses upon tissue damage. G protein-coupled receptors (GPCRs) are critical for this function as they allow fast adaption to local tissue environment perturbations. An example is β2-adrenergic receptor (β2AR/ADRB2), which can modulate inflammation in various immune cells. So far, over 800 GPCRs are identified but many of their ligands are either still unknown, suffer from off-target effects, or have poor bioavailability. Additional challenges exist to dissect cell-type specific responses when a GPCR is expressed in several cell types within the same tissue..
Methods:
Here, we overcome these limitations by engineering chimeric GPCRs based on DREADDs (Designer Receptor Exclusively Activated by Designer Drugs). Stimulation with the DREADD agonist clozapine-N-oxide (CNO) selectively activates these chimeric DREADD-GPCRs and mimics the signaling cascades of a GPCR-of-interest.
Results:
First, we validated our approach with β2AR and designed chimeric DREADD-β2AR. Upon CNO stimulation, DREADD-β2AR triggered the same second messenger cascade, kinase activity, post-translational modifications, and

protein-protein interaction as non-chimeric β2AR. Additionally, DREADD-β2AR successfully mimicked the effect of β2AR on primary microglia motility by rapidly inducing filopodia formation. Subsequently, we applied our strategy to dissect the role of individual GPCRs during inflammation. We focused on β2AR, GPR65, and GPR109A/HCAR2 which are highly enriched in microglia. We expressed our chimeric DREADD-GPCRs in the human microglia cell line HMC3 and studied their impact on inflammatory gene expression. DREADD-β2AR and DREADD-GPR65 both modulated the inflammatory response in the same way as endogenously expressed β2AR by upregulating interleukin 6 (IL6) and downregulating tumor necrosis factor (TNF). DREADD-GPR109A did not impact this response.

Discussion:

Our data show that chimeric DREADD-GPCRs recapitulate distinct signaling pathways and provide a new strategy to investigate cell type-specific GPCR responses with a well-known ligand and limited off-target effects.

P-85 Dissecting the hormonally controlled loss of regenerative potential using a cell-based approach

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Background:

Most species, including vertebrates, are capable of regenerating tissues of the central nervous system during early stages of development. However, this ability is gradually lost with development and age in almost all animals we know. We reason that animals in which this transition is experimentally accessible will be highly suited to investigate the molecular mechanisms responsible for this change in capacity.

Methods:

We explore the marine bristle worm Platynereis dumerilii, whose axial central nervous system exhibits significant molecular similarities to the vertebrate spinal cord. The capacity of the animal to regenerate the central nervous system is controlled by brain-derived physiological cues, exhibiting a dramatic reduction when sexual maturation is initiated. By combining systematic single-cell RNA sequencing, visualisation of gene expression, pulse-chase labelling of proliferating cells, and immunohistochemistry, we aim to dissect the molecular and cellular processes underlying posterior growth and regeneration, and identify changes brought about by distinct physiological cues changing in the regenerative-reproductive transition.

Results:

By modifying a protocol for simultaneous fixation and cell dissociation, we were able to obtain single cells from multiple stages throughout worm regeneration to be used for single-cell RNA sequencing. Analyses of a pilot dataset revealed that advanced regenerates contain a multitude of cell types including

several neuronal sub-types. Along with a time series of transcriptomes, these data also allowed us to infer differentiation trajectories for regenerating neurons. Combined in-situ hybridization and EdU pulse-and-chase assays confirmed the inferred differentiation of neurons in normally regenerating animals, and form a basis for the interrogation of the system under conditions in which physiological cues are systematically modified.

Discussion:

Our results support the notion that Platynereis dumerilii is an accessible model for studying the transition of regenerative to non-regenerative developmental states. By analyzing the proliferation and differentiation dynamics of stem cells and neuronal progenitors and their receptor expression profiles, we are working towards elucidating the mechanisms by which changing hormone levels affect regenerative growth on the stem cell and differentiation level. We expect that this approach will yield mechanistic insight that is also relevant for other animal branches.

P-86 Modeling α-synuclein spreading in whole brain sagittal organotypic slices

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Background:

Accumulation of α-synuclein (α-syn) in the brain is found in a group of neurodegenerative disorders named synucleinopathies. It is hypothesized that α-syn pathology spreads in a prion-like fashion between connected brain regions. Numerous in vitro and in vivo models were established to elucidate the mechanisms of α-syn spreading and its neurotoxic effects; however, both types of models have various drawbacks. In this study, we establish a sagittal organotypic whole brain slice model with preserved tissue architecture for evaluating the spreading of α-syn pathology. The effects of exogenous α-syn were compared between slices from wildtype (WT) and transgenic mice overexpressing human α-syn under the promoter of proteolipid protein (PLP) in oligodendroglia.

Methods:

Sagittal organotypic whole brain slices with 200 µm thickness were obtained from C57BL/6 or transgenic PLP mouse pups (postnatal day 9-11). Collagen hydrogels were loaded with monomers of human α-syn, as well as human and mouse pre-formed fibrils (PFF) to allow local application and slow release. Release profile of the hydrogels was evaluated with ELISA. After 1 week of culturing, these hydrogels were produced and applied onto striatum and slices were cultured for further 8 weeks. Spreading of α-syn was evaluated in different brain regions by immunohistochemistry for total α-syn and α-syn phosphorylated at serine129 position (α-syn-P). Time-dependent changes in the levels of α-syn and α-syn-P were evaluated by Western Blotting. Additi-

onally, changes in endogenous α-syn expression in response to exogenous α-syn were evaluated by qRT-PCR.
Results:
Organotypic brain slices showed a good survival that did not change with exogenous α-syn. The release profile of collagen hydrogels for human α-syn PFFs was evaluated as an acellular release system, and revealed complete release of α-syn within 2 weeks. WT or PLP organotypic brain slices did not show aggregation in response to human α-syn monomers. On the other hand, human and mouse PFFs of α-syn induced aggregation and spreading of α-syn-P in all the brain regions evaluated, being most pronounced at the region of hydrogel application and surrounding striatum as well as over the median forebrain bundle. Brain slices from PLP mice showed significantly more α-syn pathology compared to WT mice. Levels of α-syn-P increased time-dependently in both WT and PLP slices. Endogenous α-syn expression did not change in WT slices as a response to exogenous α-syn monomer or PFF application.
Discussion:
Collagen hydrogels can be used for local administration of α-syn PFFs onto brain slices. Our study demonstrates that seeding with α-syn PFFs but not monomers induces an α-syn pathology. This effect was significantly more prominent in brain slices with α-syn overexpression.

P-87 Improved grid-glued method of freeze-fracture replica labeling for molecular and structural identification of neuronal profiles

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Background:
Sodium dodecyl sulfate-digested freeze-fracture replica labeling (SDS-FRL) technique is recently widely used to study cell membrane protein localization at the EM level. Because of complete exposure of the targeted epitope, which makes membrane penetration of antibodies unnecessary, and SDS treatment, which makes the epitope readily available, immunolabelling efficiency of SDS-FRL can be close to 100%. However, one major challenge of SDS-FRL is to keep large replica samples intact throughout the procedure for complete tissue orientation in the grid. The grid-glued replica methods have been developed to solve this problem but not widely used yet because of technical difficulties.
Methods:
To improve the previous grid-glued method, we used paraformaldehyde-perfused CA1 hippocampal mouse slices. After high pressure freezing and freeze-fracture replication, the thawed slice with replica was attached to a calibrated grid via a UV-sensitive glue (Norland Optical Adhesive 61) and subsequently treated with SDS. We then performed labelling for AMPA receptors using an antibody reactive to GluA1-3 subunits on the E-face in one replica, and that for GluA1 subunit on the P-face in the corresponding replica (mirror method).

The labelled replicas were carbon coated and treated with Dynasolve 711 solvent to dissolve the glue and the sample was observed under transmission electron microscope.

Results:

The improved grid-glued method provided a 90% success rate in retaining the whole replica intact on its initial finder grid. Furthermore, no negative impact on the immunolabeling efficiency was observed. We compared the distribution patterns of pan AMPA receptors and GluA1 subunits on the E- and P-face, respectively, in the same synapses using mirror replicas covering all layers of the CA1 field.

Discussion:

Performance of the conventional free-floating replica method often suffers from fragmentation of replica samples during SDS treatment and immunolabelling procedures. The modified grid-glued replica technique allows easy handling and robustness of freeze-fracture replica samples. It is particularly useful for samples that contain complex sub-regions (Bhandari et al., 2021), which need a good histological orientation. Furthermore, modified grid-glued replica facilitates finding of the corresponding mirror profiles in replica pairs via the coordinate system on the finder grid.

References:

Bhandari P, Vandael D, Fernández-Fernández D, Fritzius T, Kleindienst D, Önal C, Montanaro J, Gassmann M, Jonas P, Kulik A, Bettler B, Shigemoto R, Koppensteiner P (2021) GABAB receptor auxiliary subunits modulate Cav2.3-mediated release from medial habenula terminals. Elife 10:e68274.

P-88 Developmental regulation of coupling between presynaptic Ca2+ channels and release sensors at basket cell–Purkinje cell synapses in cerebellum

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Background:

GABAergic synapses undergo substantial changes during development. In the cerebellar basket cell (BC) to Purkinje cell (PC) synapse, synaptic efficacy is markedly reduced during the first three weeks of development [1]. However, the underlying mechanisms are unclear.

Methods:

To address this question, we combined functional measurements (paired recording from synaptically connected neurons, presynaptic recording from BC terminals, and patch pipette perfusion) and structural analysis (freeze-fracture replica immunolabeling, FRIL and transmission electron microscopy, TEM). To probe developmental changes, we compared synapses between postnatal day 7 (P7), 14 (P14), and 21 (P21).

Results:

Nonstationary fluctuation analysis of inhibitory postsynaptic currents (IPSCs) revealed that release probability (Pr) decreased during development (0.52 ±

0.06 at P7, 0.27 ± 0.04 at P14, and 0.09 ± 0.02 at P21; 6, 6, and 5 pairs; P = 0.013), whereas the number of functional release sites increased (12.8 ± 2.9 at P7, 14.3 ± 2.4 at P14, and 43.3 ± 6.5 at P21; P = 0.013); in parallel, quantal size significantly decreased. Furthermore, the dynamics during repetitive stimulation changed from depression to facilitation. Thus, BC–PC synapses may undergo substantial changes in the coupling between Ca2+ channels and release sensors.

To functionally probe the coupling configuration, we examined the shape of the presynaptic action potential (AP) by direct presynaptic recording. Presynaptic APs showed substantial shortening during development, suggesting reduced efficacy of Ca2+ channel activation. Furthermore, we probed the coupling distance between Ca2+ channels and release sensors by application of the Ca2+ chelator EGTA via patch pipette perfusion. At P7, release was moderately sensitive to EGTA, and at P21 it became almost insensitive [2, 3], indicating a tightening of the coupling.

To structurally analyze the coupling, we combined FRIL and TEM. FRIL analysis revealed that presynaptic Ca2+ channels formed clusters within active zones. Whereas the number of Ca2+ channels per cluster was relatively constant (15.4 ± 1.0 at P7, 16.6 ± 1.1 at P14, and 17.6 ± 1.1 at P21; 73, 69, and 82 active zones, P = 1) the total estimated number of clusters per connection significantly increased during development (33.1 ± 2.1 at P7, 47.4 ± 2.7 at P14, and 81.5 ± 3.9 at P21; $P < 0.001$). Furthermore, serial section TEM analysis indicated that the estimated number of docked vesicles per connection became markedly larger during synaptic maturation (77.5 ± 9.2 at P7, 116.3 ± 10.5 at P14, and 428.47 ± 36.3 at P21; $P < 0.001$). The ratio of number of docked vesicles to Ca2+ channel clusters increased from 2.34 at P7 to 5.26 at P21, approaching the stoichiometry of a hexagonal lattice.

Discussion:

Our results indicate that BC–PC synapses undergo substantial changes in the coupling configuration during development. Shortening of the presynaptic AP, together with a constant number of Ca2+ channels per cluster, may underlie the observed reduction of Pr. An increase in the number of Ca2+ channel clusters could explain the increase in the number of functional release sites. Finally, the observed increase in the vesicle–Ca2+ channel cluster ratio during synaptic maturation may help to ensure reliable transmission at the BC–PC synapse during repetitive BC activity.

References:

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P-89 Linking the moonlight interpreter L-Cry to the circalunar clock in the marine annelid *Platynereis dumerilli*

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Background:

Endogenous oscillators are internal timing machineries, fundamental for the coordination of metabolism, physiology and behavior across different species. Marine breeders, such as the bristle worm Platynereis dumerilii, are well-documented to possess a particular endogenous timer, - a monthly (circalunar) oscillator set by moonlight, in order to synchronize their sexual reproduction. My lab identified L-Cry, part of the cryptochrome/photolyase family, as the first molecule linked to the circalunar timing and its function of recognizing and distinguishing moon- from sunlight. My work aims to identify how the L-Cry signals are transmitted to the circalunar clock. For this, identified putative interactors are of interest, particularly the ones with monthly regulation of expression - YTHDF1/2/3 and G3BP1/2.

Given their roles in transcript regulation, I aim at deciphering if L-Cry, together with these proteins, is involved in the post-transcriptional regulation of circalunar clock-related processes. Potential downstream targets of this complex were discovered by comparing the transcriptomes of wildtype and l-cry homozygous mutant worms and contain genes involved in neuronal differentiation and cell division, particularly considering another putative interactor of L-Cry – Prospero, marker of asymmetric cell division.

Methods:

Using immunohistochemistry together with biochemical assays for interaction studies, I want to verify and visualize the interaction of L-Cry, YTHDF1/2/3 and G3BP1/2.

The protocol for simultaneous detection of in situ hybridization chain reaction, immunohistochemistry and EdU staining, which I helped establish, allows the visualization of proliferating cells together with gene expression of candidate neurogenesis factors and L-Cry for an implied biological relevance.

For the functional characterization of G3BP1/2, I am utilising a described pharmacological inhibitor. Additionally, using CRISPR/Cas-9, I am generating a knockout animal strain for side-by-side comparison and verification of the drugs' inhibitory effect. Changes in the output of the circalunar clock, as a possible consequence of the involvement of G3BP1/2 in the circalunar oscillator and subsequent loss of function, will be analysed on both molecular (via circalunar phase markers) and physiological level (reproductive timing).

Results:
Whole-mount in situ hybridization together with L-Cry protein staining depicts co-localization of both g3bp1/2 and ythdf1/2/3 in L-Cry positive cells, implying a spatial connection of the relevant factors in the posterior domain of the forebrain.
On the level of biochemical verification of interaction - glycerol gradient centrifugation and size-exclusion chromatography show the co-migration and elution of L-Cry and G3BP1/2, hinting at their interaction a large complex of proteins.
For the functional analysis of the role of G3BP1/2 in the circalunar clock pathway, I performed functional inhibition and was able to provide evidence for an impact of G3BP1/2 inhibitions on circalunar clock oscillations, i.e. complete downregulation of the oscillator on the molecular level.
Finally, to get a first idea on the localization of the potential downstream targets of the L-Cry complex and their expression relative to L-Cry, I have performed multiplexed stainings of HCR for the candidates together with L-Cry protein staining, showing co-localization in the same cells.
Discussion:
With my work, I want to provide in-depth understanding of the circalunar timing machinery in Platynereis dumerilii. More importantly however, is its relevance for the integral and ecologically widespread phenomenon of monthly oscillators synchronized by the moon. L-Cry is the first functionally characterized molecule in the context of the monthly oscillator. Furthermore, the importance and influence of the lunar cycle on humans cannot be disregarded. Recent data brings the lunar cycle and the gravimetrical forces of the moon in context with human physiology, as well as the menstrual cycle and sexual reproduction in women.
Analyzing the players and their role in the circalunar clock machinery is thus critical for understanding the much-debated lunar influences on humans. For this, Platynereis dumerilii, with its well-examined reproductive behavior and established techniques for molecular and physiological analysis of the circalunar clock, is the perfect model organism.

P-90 Exploring a novel model of neurogenic plasticity as a function of endogenous timing mechanisms

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Background:
In various vertebrate and invertebrate species, the central nervous system does not only grow continually throughout the adulthood, but is capable of remodeling its architecture in response to cues such as periodic light and temperature fluctuations, as well as reproductive maturation. We explore the marine annelid worm Platynereis dumerilii as a model system for studying the

link between adult neurogenesis, ambient cues and endogenous timing mechanisms. Distinct structures of the adult Platynereis central nervous system grow at various stages of the animal's life cycle before it completes sexual maturation, which precedes mating and death. Maturation, like numerous physiological functions and behavior, is governed by the worm's endogenous monthly timing mechanism, the circalunar clock, which is entrained by nocturnal photoreception.

Methods:

To map differences in neurogenesis throughout the life of the animal, as well as over the lunar month, we are employing a combination of proliferation detection, single cell RNA-sequencing and in situ hybridisation. To assess the links between the neurogenic potential and the monthly timing, we also intend to study variations after disturbing regular circalunar timing, as well as after inhibition of neurogenesis in the brain and retina of the worm.

Results:

First data obtained on the proliferation of the worm's brain and retina imply that while immature adults exhibit proliferation predominantly in medial brain domains, individuals that have begun maturing, display additional growth in the posterior lobes, posterior medial brain and retina. Sexually mature individuals, in turn, present an arrest in growth and regeneration. Additionally, the highly proliferative structures of the maturing worm overlap with gene expression domains of genes implied in circalunar timing.

Discussion:

Our data are in agreement with a non-linear progression of neurogenesis in the brain and retina; this coincides with transitions between phases of the life cycle of the bristleworm, which are synchronized according to the monthly cycles by the moonlight-dependent circalunar clock. Our future analyses will target underlying causative mechanisms, with the aim of dissecting the coupling between neurogenic brain plasticity and endogenous timing mechanisms, as well as the vastly unexplored role of neurogenesis in sexual maturation.

P-91 Molecular design of hypothalamus development

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Background:

A wealth of specialized neuroendocrine command systems intercalated within the hypothalamus control the most fundamental physiological needs in vertebrates. Nevertheless, we lack a developmental blueprint that integrates the molecular determinants of neuronal and glial diversity along temporal and spatial scales of hypothalamus development.

Methods:
Here we combine single-cell RNA sequencing of 51,199 mouse cells of ectodermal origin, gene regulatory network (GRN) screens in conjunction with genome-wide association study-based disease phenotyping, and genetic lineage reconstruction to show that nine glial and thirty-three neuronal subtypes are generated by mid-gestation under the control of distinct GRNs.
Results:
Combinatorial molecular codes that arise from neurotransmitters, neuropeptides and transcription factors are minimally required to decode the taxonomical hierarchy of hypothalamic neurons. The differentiation of γ-aminobutyric acid (GABA) and dopamine neurons, but not glutamate neurons, relies on quasi-stable intermediate states, with a pool of GABA progenitors giving rise to dopamine cells. We found an unexpected abundance of chemotropic proliferation and guidance cues that are commonly implicated in dorsal (cortical) patterning in the hypothalamus. In particular, loss of SLIT–ROBO signalling impaired both the production and positioning of periventricular dopamine neurons.
Discussion:
Overall, we identify molecular principles that shape the developmental architecture of the hypothalamus and show how neuronal heterogeneity is transformed into a multimodal neural unit to provide virtually infinite adaptive potential throughout life.

P-92 Two light sensors decode moonlight versus sunlight to adjust a plastic circadian clock to moon phase

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Background:
Animals possess endogenous clocks to anticipate cyclic environmental changes driven by astronomical cycles in order to optimally adapt their physiology and behaviour. Environmental light is one of the main entrainment cues that is used by animals to synchronize their internal clock(s) with these environment cycles. While moonlight is thought to typically entrain monthly clocks, which are well known to exist in many marine species such as the marine annelid Platynereis dumerilii, sunlight is considered as the main entrainment cue for the daily circadian clock. However, an increasing amount of evidence suggests

that moonlight also affects daily timing in various species, ranging from invertebrates to humans. Nevertheless, how moonlight is perceived and is discriminated from sunlight on a molecular level and how it affects the circadian clock remains largely elusive, also due to a lack of suitable model species.
Methods:
We developed a novel behavioural paradigm for the bristle worm Platynereis dumerilii using an automated locomotor video tracking system to assess the exact time when these animals initiate their reproductive behaviour. We mimic naturalistic moonlight, sunlight and darkness to assess if swarming onset is controlled by a circadian clock and if this clock is moonlight-sensitive. Furthermore, we test Platynereis mutant lines that are deficient in candidate moonlight receptors to identify which photoreceptors mediate the effect of moonlight. Using immunohistochemistry, we visualize the light receptor L-Cry to assess if its levels and localization differ under moonlight and sunlight conditions. To test for evolutionary conservation of our findings, we perform comparative experiments in Drosophila melanogaster.
Results:
We show that the onset of reproductive behaviour in Platynereis is governed by a moonlight-sensitive plastic circadian clock that times reproductive behaviour to the respective portion of the night when no moonlight is present. Furthermore, we identify two photoreceptors, L-Cry and r-Opsin1, that in a non-redundant fashion mediate the effects of light on the circadian clock. Whereas r-opsin1 is genetically required for advancing swarming onset in response to a naturalistic waning moonlight regime, we find a dual function of L-Cry in adjusting the plastic circadian clock to light: it entrains the circadian clock to naturalistic sunlight, and also advances circadian period length under prolonged moonlight exposure. Furthermore, we provide biophysical, biochemical and behavioural evidence that L-Cry engages in two distinct signaling pathways that encode sun versus moonlight valence. We extend our analysis to insects, where we show that fruitfly Cry is also required to distinguish moonlight from sunlight, effectively shielding the fly circadian clock from disturbance by moonlight.
Discussion:
The discovery that moonlight modulates the circadian period length in Platynereis hints at significant effects of moonlight on circadian timing that might be of broader relevance. To answer global conservation challenges, it is important to know how widespread the effect of moonlight on circadian timing is, as anthropogenic light pollution can easily interfere with these fundamental timing mechanisms.

P-93 Onecut transcription factors in the developing hypothalamus

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Background:
The family of Onecut (OC) transcription factors includes members with a highly conserved pattern of expression and function across different animal species. Three homologues found in mammals (OC1, OC2 and OC3) are sequentially expressed during embryonic development with a partially overlapping pattern in both the peripheral and central nervous systems (CNS). Their expression is particularly significant in the developing retina and spinal cord, as well as in a dopaminergic cell contingent in the hypothalamus. The most recently discovered member, OC3, has not been studied at depth, particularly by using mouse genetics. Based on a single-cell sequencing study of the fetal and infant mouse hypothalamus, we have identified OC3 as a marker of immature neuronal sets. Nevertheless, the spatiotemporal expression of OC3 along with its function in cellular fate determination remains unknown.
Methods:
A combination of multiple fluorescence immunohistochemistry, in situ hybridization and fluorescent reporter mice was used to characterize OC3-containing cell populations in the developing mouse hypothalamus. The function of OC3, particularly its effects on cellular differentiation, was tested by in vitro overexpression in a Neuro-2a neuroblastoma cells in combination with bulk RNA-sequencing.
Results:
Our data show that OC3 is present exclusively in post-mitotic neuroblasts that have detached from their respective proliferative zones by embryonic (E) day 10.5. The pattern of OC3 expression is terminally established by E14.5 and maintained into adulthood. Anatomical mapping of OC3-containing neurons in the embryonic mouse hypothalamus reveals two distinct populations of neurons, marked by their respective GABA/tyrosine hydroxylase (TH+) and glutamate/thyrotropin releasing hormone (TRH+) contents. GABA/OC3+/TH+ neurons concentrate in the medial hypothalamus. In contrast, glutamate/OC3+/TRH+ neurons are positioned predominantly in the lateral hypothalamus. Based on our findings that OC3 is expressed from a very early developmental time-point onwards yet exclusively in differentiation zones, we hypothesized that OC3 could contribute to neuronal fate determination. Indeed, overexpression of OC3 in Neuro2a cells promotes neurite outgrowth. RNA-sequencing in vitro identified neuronal navigator 2 (Nav2) as a preferred OC3 target with relevance to neurite outgrowth. NAV2 accumulates in neurites and localizes at the intersection of stable (mature) and mobile (developing, dynamic) neurite segments. Inhibition of the NAV2-interacting partner TRIO, a Rho guanine nucleotide exchange factor, indeed occludes neurite outgrowth, phenocopying the effects of OC3 loss-of-function.

Discussion:
Here, we provide substantial anatomical and functional information on OC3, a transcription factor newly identified in hypothalamic neurons. Herein, OC3 appears to promote neuronal migration and differentiation, thus underpinning the timely specification of hypothalamic neurons whose intrauterine differentiation is critical to control pituitary hormone secretion throughout life.

P-94 Long and short RNA transcriptomics giving insight into human iPSC-derived sensory neuron development

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Background:
The transduction process giving rise to the sensation of pain is achieved by primary afferent nociceptor neurons which are affected by numerous disorders causing chronic pain including hereditary neuropathies and migraine. Thus, understanding the regulatory pathways critically implicated in sensory neuron differentiation is crucial for the detection of disease onset, pathogenesis as well as optimal treatment windows. Unfortunately, studies exploring pathophysiological mechanisms are often performed in rodents thus suffering from interspecies differences or in human post-mortem samples without temporal information.
Methods:
To overcome this translational paresis, we established a human induced pluripotent stem cell (iPSC)-derived nociceptor (iDN) culture, largely sharing characteristics of mouse dorsal root ganglion (DRG) neurons as indicated by canonical immunoreactivity markers and functional attributes assessed with microfluorometric calcium measurements and whole-cell patch-clamp recordings. Since iDNs can be used to reconstitute major sensory neuron related developmental processes, we explored gene and miRNA signatures throughout sensory neuron differentiation using paired long and small RNA sequencing, immunofluorescence microscopy, qPCR validation and electrophysiology of three iPSC clones at six timepoints. We applied differential gene expression analysis and WGCNA, with the aim to identify putative hub genes/microRNAs (miRNA) and miRNA::mRNA interactions critically implicated in sensory neuron development.
Results:
Several modules and stages of highly correlated genes and miRNAs emerged, and stage-specific hub genes/miRNA were identified. Thereby, multiple well described rodent sensory neuron development markers such as SOX10, PRDM12 and PAX3 were found conserved in all three human iPSC lines. We further identified neuropeptide related genes such as somatostatin, galanin and CGRP highly enriched at D16 with putative functions in phenotype identity regulations documenting that iDNs resembled peptidergic sensory neurons. Since gene expression is tightly post-transcriptionally regulated by miRNAs,

we performed complex bioinformatic analyses integrating miRNA::mRNA predictions, validations as well transcriptomic signatures, which revealed that miRNAs target-spaces were highly enriched for neuron related gene pathways critically implicated in synapse development, neurite outgrowth and ion channel regulation during early differentiation stages. Finally, we developed NOCICEPTRA as a publicly available tool to explore mRNA and miRNA signatures, disease onset and miRNA target-spaces throughout iDN development.
Conclusion:
In summary, our paired RNA and small RNA sequencing approach allowed us the complex mapping of a developmental gene and miRNA expression atlas discovering major hub genes and miRNAs significantly contributing to the differentiation of iDNs which lay the grounds for mechanistic research towards chronic pain disorders in a human model system.

P-95 Modeling rare neurodevelopmental disorders using human iPSC-derived neurons

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Background:
Spastic Paraplegia and Psychomotor Retardation with or without Seizures (SPPRS) is an ultra rare complex autosomal recessive neurodevelopmental disorder, typically shows an infantile-onset, starting with hypotonia at birth, followed by severely impaired global development, intellectual disability and notable motor disability. Mutations in the HACE1 gene, which encodes for HECT domain and ankyrin repeat-containing E3 ubiquitin-protein ligase (HACE1) have been identified to be causative of SPPRS. Previous studies have implicated HACE1 as a tumour suppressor gene involved in multiple cancers. However, HACE1 is ubiquitously expressed in the brain and targets proteins for proteasomal degradation. Best described target for HACE1 E3 ligase activity that is highly expressed in the brain is Rac1, a member of the Rho GTPase subfamily that is known to be involved in patterning cerebellar development by controlling cell morphogenesis and migration. Studies on Hace1 knockout (KO) mice and SPPRS patient-derived fibroblasts reported elevated levels of the RAC1 leading to the neuronal pathology including an increase in synaptic transmission at CA3-CA1 synapses, and fewer synaptic spines at CA1 pyramidal neurons. The HACE1 pathology was never studied before in human neuronal context, therefore, we aim to understand the cellular and molecular mechanisms leading to the abnormal synaptic plasticity using HACE1 patient-derived stem cells.
Methods:
The HACE1 patient-derived iPSCs and neurons was characterized using various stem cells, neuronal progenitors (NPs) & neuronal markers. Automated image analysis is carried out using a custom CellProfiler pipeline to identify and

segment individual cells to capture morphological features of the cell. Finally, intracellular calcium imaging is being performed to understand the functional impact of the molecule's underlying HACE1 mutation in matured neurons. Additionally, a novel gene-editing technology termed prime editing is being used to correct the disease-causing mutations in HACE1 patient-derived iPSCs and neurons.

Results & Discussion:

HACE1 patient-derived iPSCs are differentiated into cortical neurons using an inhibitory cocktail combination LDN-193189 and SB-431542 to yield a homogenous population of PAX6 neuroectoderm cells. Later, the iPSCs were characterized using SSEA3, OCT4 & Tra-1-60 stem cell markers, Nestin & PAX6 NP markers, MAP2, Synapsin & PSD95 mature neuronal makers, and TBR1 & vglut1 to confirm glutamatergic-cortical neuronal lineage. A tight balance between excitation and inhibition (E/I balance) in synaptic inputs is important for correct brain development & function and has been previously implicated in numerous neurodevelopmental disorders. Therefore, to understand the impact of HACE1 mutation in E/I imbalance we measured intracellular calcium responses in mature neurons using a dual excitation ratiometric calcium indicator fura-2 acetoxymethyl ester (fura-2/AM). Cells are stimulated with different glutamate (DHPG, AMPA, Kainate, NMDA) or GABA receptor agonists and our preliminary results suggest alterations in the intracellular calcium responses to activation of receptors. To prime edit iPSCs, the spacer and RTT template sequences of the pegRNAs were designed and cloned with intermediate pegRNA scaffold sequences by Golden Gate Assembly. Cloned pegRNA constructs and prime editor 2 Cas9 are delivered together into iPSCs by nucleofection. After 72 hours, genomic DNA will be collected from the pool of edited cells, we will PCR across the editing site, sequence, and estimate editing by CRISPResso. The prime edited iPSCs will be further differentiated into cortical neurons and compare with control neurons. Altogether, the whole project helps to identify fundamental mechanisms causing the HACE1 phenotype and also opens doors for investigating new therapeutic interventions for patients impacted with SPPRS. Besides, the novel genome editing technology has a great potential for neurodevelopmental disorders that are currently suffering from a paucity of targeted therapeutics.

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