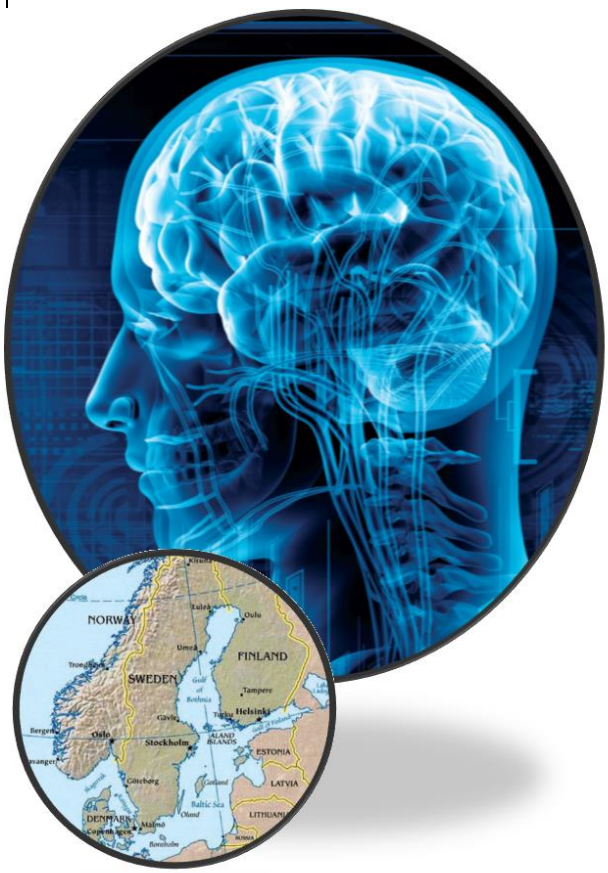
The Nordic Neuroscience 2015

JUNE 10-12, 2015

CONFERENCE BOOK



FIRST NORDIC NEUROSCIENCE MEETING

A scientific meeting for all neuroscientists arranged in Trondheim, Norway. A wish of achieving greater knowledge about the work done in Nordic institutes to open more interactions with neighboring countries and establish networks for future collaborations.

THE NORDIC NEUROSCIENCE 2015

JUNE 10-12 IN TRONDHEIM, NORWAY

We are delighted to welcome you to the first-ever gathering of Nordic Neuroscience, held in the city of Trondheim, Norway. This is a newly initiated conference with the goal of increasing our interaction with neighbouring countries and establishing networks for future collaboration. Our wish is simple and unambiguous: we want to increase our collective knowledge about the work being conducted at Nordic and Baltic institutes.

As the first event of this kind, we hope that speakers, poster presenters, participants and exhibitors will come away from the conference with the feeling they have met with success. We also have the larger ambition that this first conference in Trondheim will create a strong platform for the concept, so our goal of arranging this event biannually in various Nordic locations become a reality.

The programme committee has worked on the conference since the autumn of 2013. Members have met in Trondheim to look at locations and discuss the programme's composition. The result, we believe, is a stong line-up of excellent symposium speakers from Nordic institutes and top international plenary speakers from whom we hope you learn, are motivated by and enjoy. The programme committee also believes poster presentations are an essential aspect of the conference, and it is with great pleasure that we acknowledge the many individuals who have offered to present their work.

We hope you have a wonderful experience and find the conference a memorable one, both because of the great science and because of the future collaborations it helps create.

Welcome!

Bryndis Birnir	Sten Grillner	Poul Henning Jensen	Dan Lindholm
Cecilia Lundberg	Edvard Moser	Ragnhild Elisabeth Paulsen	Menno Witter

CONTENTS

SPEAKER LIST

PLENARY SPEAKERS

David Anderson – California Institute of Technology

Silvia Arber – Friedrich Miescher Institute

Michael Hausser – University College London

Nancy Kanwisher – Massachusetts Institute of Technology

Edvard Moser – Norwegian University of Science and Technology

May-Britt Moser – Norwegian University of Science and Technology

Christine Van Broeckhoven – University of Antwerp

PHOTO CREDIT: TRONDHEIM KOMMUNE

SYMPOSIUM SPEAKERS

Ernest Arenas – Karolinska Institute

Farrukh Chaudhry – University of Oslo

Gaute Einevoll – Norwegian University of Life Sciences

Abdel El Manira – Karolinska Institute

Ulrik Gether – University of Copenhagen

Poul Henning Jensen – Aarhus University

Zaal Kokaia – Lund University

Esa Korpi – University of Helsinki

Jeanette Hellgren Kotaleski – KTH Royal Institute of Science

Klas Kullander – Uppsala University

Marja-Leena Linne – Tampere University of Technology

Susanna Narkilahti – University of Tampere

Poul Nissen – Aarhus University

Agneta Nordberg – Karolinska Institute

Lars Nyberg – Umeå University

Vuk Palibrk – Norwegian University of Science and Technology

Jean-Francois Perrier – University of Copenhagen

David Rowland – Norwegian University of Science and Technology

Gilad Silberberg – Karolinska Institute

Heikki Tanila – University of Eastern Finland

Raimo Tuominen – University of Helsinki

Petrine Wellendorph – University of Copenhagen

Menno Witter – Norwegian University of Science and Technology

CHAIRS

Bryndis Birnir – Uppsala University

Abdel El Manira – Karolinska Institute

Sten Grillner – Karolinska Institute

Poul Henning Jensen – Aarhus University

Dan Lindholm – University of Helsinki

Axel Sandvig – Norwegian University of Science and Technology

Ioanna Sandvig – Norwegian University of Science and Technology

Heikki Tanila – University of Eastern Finland

Jonathan Whitlock – Norwegian University of Science and Technology

EXHIBITORS

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Federation of European Neuroscience Societies

Inter Instrument AS

Jackson ImmunoResearch Europe Ltd

Miltenyi Biotec Norden

Multi Channel Systems Gmbh

Nansen Neuroscience Network

Norwegian Neuroscience Society

Olympus Microscopy

Scientifica

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PARTICIPANTS

Abdel El Manira
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Agata Bochynska
Agneta Fahlgren
Agneta Nordberg
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Albert Gjedde
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Aliona Nacu
Amy Robinson
Anastasia Ludwig
Anders Victor Petersen
Andreas Kardamakis
Ane Charlotte Christensen
Angel Moldes-Anaya
Angela Cenci Nilsson
Anja Rose Strohmaier
Anna Dahl Myrvang
Anne Elisabeth Sølsnes
Anne Marte Sjursen Kvello
Anne Nagelhus
Annetta Redmann
Aphirak Juthajan
Arne Høibakk
Asgeir Kobro-Flatmoen
Ashraf Pakzad
Aurea Castilho
Axel Sandvig
Bartul Mimica
Belma Skender
Benjamin Dunn
Bente Gunnveig Berg
Bente Jacobsen
Birgitte McDonagh
Bjarte Bye Løfaldli
Bjarte Reve
Bjørnar Hassel
Boleslaw Srebro
Brita Robertson
Bryndis Birnir
C Ernesto Restrepo
Catherine Morgans
Cecilia Lundberg
Charlotte Hvaring
Chenglin Miao
Christian Broberger
Christian Clement
Christin Berndtsson
Christina Falk-Petersen
Christine Van Broeckhoven
Christoph Stangl
Claudia Battistin
Clive Bramham
Dan Lindholm
Daniel Lawer Egbenya
Daniel Tornero
David Anderson
David Rowland
Debora Ledergerber
Dennis Jensen
Dominika Chodlowska
Duda Kvitsiani
Edvard Moser
Eelke Snoeren
Eirik Nilssen
Elena Ian
Elham Jalalvand
Elisabeth Holm Diget
Emelie Cederholm
Emil Jakobsen
Emilie R. Skytøen
Emmy Rannikko
Emre Yaksi
Eric Hanse
Ernest Arenas
Esa Korpi
Ewa Blasiak
Farrukh Chaudhry
Flavio Donato
Gaute Einevoll
Gilad Silberberg
Grethe Mari Olsen
Gunhild Fjeld
Hamza Mousa
Hana Mala Rytter
Hanna Halme
Hanne Lehn
Hans Ekkehard Plesser
Hans Thorn

Harry Sedgwick
Haruna Muwonge
Hege Tunstad
Heidi Kleven
Heikki Tanila
Hiroshi Ito
Håvard Tangvik
Inger Lise Bogen
Ingrid Heggland
Ingvild Kruge
Ioanna Sandvig
Irena Loryan
Jan Haavik
Jan Hoeber
Jan Maka
Jan Sigurd Beddari Blackstad
Jan Wikgren
Jannike Mørch Andersen
Jeanette Hellgren Kotaleski
Jean-Francois Perrier
Jens Andersen
Jens Hjerling Leffler
Jianren Song
Johan Eliasson
Johan F. Storm
Johan Jakobsson
Johanna Huupponen
Johanna Mäkelä
Jonas Englund
Jonathan Whitlock
Josephine Malmevik
Juan Pérez-Fernández
Jørgen Sugar
Kam Sripada
Kamilla G. Haugland
Kang Zheng
Karin Wibrand
Karoline Hovde
Katja Scheffler
Kei Igarashi
Kirsten Kjelstrup
Klas Kullander
Kristian Kinden Lensjø
Kyle Cavagnini
Lars Kristiansen
Lars Nyberg
Lars Peter Engeset Austdal
Lene Christin Olsen
Li Lu
Linda Hildegaard Bergersen
Linda Veres
Liv Falkenberg
Lorenzo Ragazzi
Louise Thiesen
Ludivine Breger
Luis Quintino
Magnus Holth
Marco Barbariga
Margaret Veruki
Margarethe Bittins
Maria Hrozanova
Maria Jose Lagartos
Maria Mørreaunet
Maria Steene Eriksen
Marianne Fyhn
Marja-Leena Linne
Markus Michael Hilscher
Marthe Fjelldal
Martin Hägglund
Mattis Wigestrand
May-Britt Moser
Md Abdul Latif
Mehdi Djelloul
Mengliang Zhang
Menno Witter
Michael Hausser
Michela Pichereddu
Michele Gianatti
Miguel Carvalho
Mikkel Antonsen
Mikkel Vestergaard
Miriam Nokia
Mohammad Herzallah
Mona Gaarder
Mona Moisala
Nancy Kanwisher
Nathalie Jurisch-Yaksi
Nenad Bogdanovic
Nicola Bulso
Nils Borgesius
Nina Berggaard
Nora Gullbekkhei
Nouk Tanke

Oday Abushalbaq

Ola Huse Ramstad

Oleksii Nikolaienko

Osama Abu-Hadid

Patrik Frihlen

Paulina Pettersson

Pavel Uvarov

Pavla Jendelova

Per Arne Aas

Per Ludvik Brattås

Peter Petersen

Peter Wallén

Petra Aden

Petrine Wellendorph

Pia Osenbroch

Poul Henning Jensen

Poul Nissen

Prasanna Sakha

Pål Kvello

Qichen Cao

Rafal Czajkowski

Ragnhild Irene Jacobsen

Ragnhild Paulsen

Raimo Tuominen

Rajeevkumar Raveendran Nair

Ramunas Grigonis

Rannveig Eldholm

Rebecca Petri

Richard Gardner

Robertas Guzulaitis

Romina Macco

Roy Heijkoop

Roy Swain

Rune Berg

Rune Kleppe

Sabrina Zechel

Sadegh Nabavi

Saida Hadjab

Samer Siwani

Sanja Mikulovic

Sanna Lensu

Sarka Kubinova

Sergei Baryshnikov

Seth Agyei

Sharn Perry

Shreyas Mysore Suryanarayana

Silvia Arber

Sonchita Bagchi

Sonia Cavaliere

Stefan Blankvoort

Sten Grillner

Stephanie Balters

Stylianos Papaioannou

Sudarshan Patil

Susanna Narkilahti

Syed Ainul Abideen

Sylwia Lukasiewicz

Synne Steinsland

Tadiwos Mergiya

Tambudzai Jakobsen

Tammo Ippen

Tanja Wernle

Teri Sakshaug

Thanh Doan

Thomas Doublet

Tiina Parvainen

Timofey Tselykh

Tomi Taira

Tor Haugstad

Tora Bonnevie

Torben Helledie

Tore Ivar Malmei Aarsland

Torgeir Waaga

Torkel Hafting

Tsvetomira Atanasova

Tuce Tombaz

Ulrik Gether

Vegard Brun

Vuk Palibrk

Wei Wang

Yifan Zhou

Yu Hong

Zaal Kokaia

Zhe Jin

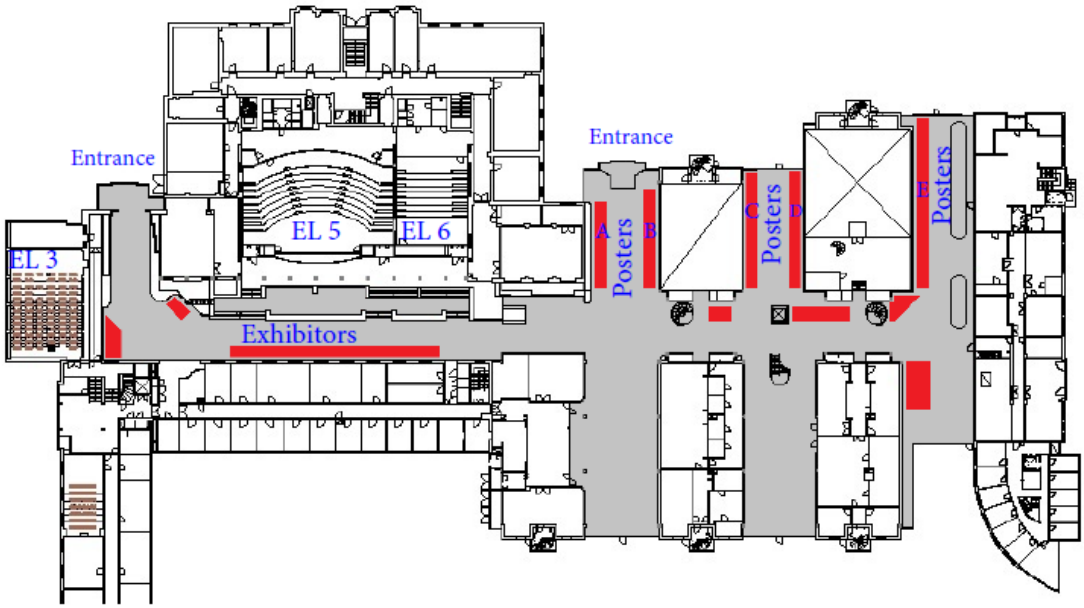
Øyvind Høydal

Øyvind W. Simonsen

POSTER PRESENTATIONS

Poster presentations are scheduled for June 11 with a standing lunch. Please feel free to visit the poster areas during other breaks and your spare time, as the posters will hang continuously throughout the conference.

We were delighted by the many participants who wanted to present posters, but this also required us to create a schedule for the presentations. A map and schedule follows:



Section A

A1	**A2**
Aliona Nacu	Angel Moldes-Anaya
Anne Marte Sjursen Kvello	Annetta Redmann
A3	**A4**
A5	**A6**
Axel Sandvig	Daniel Tornero
Hamza Mousa	Mohammad Herzallah
A7	**A8**
A9	**A10**
Oday Abushalbaq	Osama Abu-Hadid
Alaa Sharif	Pavla Jendelova
A11	**A12**
A13	**A14**
Luis Quintino	Sarka Kubinova
Jan Hoeber	Ioanna Sandvig
A15	**A16**
A17	**A18**
Birgitte McDonagh	Hanna Halme
Yu Hong	Ludivine Breger
A19	**A20**
A21	**A22**
Anne Nagelhus	Marco Barbariga
Tore Ivar Malmei Aarsland	Liv Falkenberg
A23	**A24**
A25	**A26**
Jens Andersen	Louise Thiesen
Irena Loryan	Emmy Ranniko
A27	**A28**

Section B

B1	**B2**
Bryndis Birnir	C. Ernesto Restrepo
Daniel Lawer Egbenya	Hiroshi Ito
B3	**B4**
B5	**B6**
Ingrid Heggland	Li Lu
Lorenzo Ragazzi	Nils Borgesius
B7	**B8**
B9	**B10**
Thomas Doublet	Jan Sigurd Blackstad
Samer Siwani	Hana Mala Rytter
B11	**B12**
B13	**B14**
Kamilla G. Haugland	Miriam Nokia
Jørgen Sugar	Josephine Malmevik
B15	**B16**
B17	**B18**
Jannike Mørch Andersen	Tambudzai Jakobsen
Eirik Nilssen	Kei Igarashi
B19	**B20**
B21	**B22**
Martin Hägglund	Torgeir Waaga
Øywind Simonsen	Bente Jacobsen
B23	**B24**
B25	**B26**
Heidi Kleven	Ane Charlotte Christensen
Maria Jose Lagartos Donate	Michele Gianatti
B27	**B28**

Section C

C1 Nina Berggaard	**C2** Pål Kvello
Anders Victor Petersen **C3**	Andreas Kardamakis **C4**

C5 Aurea Castilho	**C6** Christin Berndtsson
Elisabeth Holm Diget **C7**	Markus Michael Hilscher **C8**

C9 Pavel Uvarov	**C10** Ramunas Grigonis
Rune Berg **C11**	Sabrina Zechel **C12**

C13 Torkel Hafting	**C14** Christina Falk-Petersen
Johanna Huupponen **C15**	Prasanna Sakha **C16**

C17 Sylwia Lukasiewicz	**C18** Tsvetomira Atanasova
Zhe Jin **C19**	Eelke Snoeren **C20**

C21 Johanna Mäkelä	**C22** Linda Hildegaard Bergersen
Marthe Fjelldal **C23**	Emil Jacobsen **C24**

C25 Elham Jalalvand	**C26** Sharn Perry
Stefan Blankvoort **C27**	Yifan Zhou **C28**

Section D

D1 Rajeev Nair	**D2** Jens Hjerling Leffler
Sonchita Bagchi **D3**	Shreyas Suryanarayana **D4**

D5 Rune Kleppe	**D6** Christian Broberger
Juan Pérez-Fernández **D7**	Kristian Kinden Lensjø **D8**

D9 Karin Wibrand	**D10** Maria Steene Eriksen
Oleksii Nikolaienko **D11**	Rebecca Petri **D12**

D13 Haruna Muwonge	**D14** Jonas Englund
Rafal Czajkowski **D15**	Robertas Guzulaitis **D16**

D17 Sanna Lensu	**D18** Grethe Mari Olsen
Karoline Hovde **D19**	Lars Peter Austdal **D20**

Section E

E1 Peter Petersen	**E2** Mengliang Zhang
Abdolrahman Khezri **E3**	Emre Yaksi **E4**

E5 Jianren Song	**E6** Asgeir Kobro-Flatmoen
Agata Bochynska **E7**	Adrian Szum **E8**

E9 Anastasia Ludwig	**E10** Amy Robinson
Hans Ekkehard Plesser **E11**	Tammo Ippen **E12**

E13 Per Ludvik Brattås	**E14** Mona Moisala
Magnus Holth **E15**	Seth Agyei **E16**

E17 Kam Sripada	**E18** Anne Elisabeth Sølsnes

Schedule poster presentations – poster presenter is to be present at his/her stand at this hour on June 11 for presentation of poster:

Poster Session I, June 11 - 1100:

A: Nacu, Redmann, Abushalbaq, Jendelova, McDonagh, Breger, J. Andersen, Ranniko

B: Birnir, Ito, Doublet, Rytter, Mørch Andersen, Igarashi, Kleven, Gianatti

C: Berggaard, Kardamakis, Uvarov, Zechel, Lukasiewicz, Snoeren, Jalalvand, Zhou

D: Nair, Suryanarayana, Wibrand, Petri, Lensu, Austdal

E: P. Petersen, Yaksi, Ludwig, Ippen, Sripada

Poster Session II, June 11 - 1130:

A: A. Sandvig, Herzallah, Quintino, I. Sandvig, Nagelhus, Falkenberg

B: Heggland, Borgesius, Haugland, Malmevik, Hägglund, B. Jacobsen

C: Castilho, Hilscher, Hafting, Sakha, Mäkelä, E. Jacobsen

D: Kleppe, Lensjø, Muwonge, Guzulaitis

E: Song, Szum, Brattås, Agyei

Poster Session III, June 11 - 1400:

A: Moldes-Anaya, A. Kvello, Abu-Hadid, Sharif, Halme, Hong, Thiesen, Loryan

B: Restrepo, Egbenya, Blackstad, Siwani, T. Jakobsen, Nilssen, Christensen, Lagartos Donate

C: P. Kvello, A. Petersen, Grigonis, Berg, Atanasova, Jin, Perry, Blankvoort

D: Leffler, Bagchi, Eriksen, Nikolaienko, Olsen, Hovde

E: Zhang, Khezri, Robinson, Plesser, Sølsnes

Poster Session IV, June 11 - 1430:

A: Tornero, Mousa, Kubinova, Hoeber, Barbariga, Aarsland

B: Lu, Ragazzi, Nokia, Sugar, Waaga, Simonsen

C: Berndtsson, Diget, Falk-Petersen, Huupponen, Bergersen, Fjelldal

D: Broberger, Pérez-Fernández, Englund, Czajkowski

E: Kobro-Flatmoen, Bochynska, Moisala, Holth

Posters must be removed before 11.30 on June 12

PHOTO CREDIT: TRONDHEIM KOMMUNE

TRANSPORT AND VENUES IN TRONDHEIM

All scientific activities are held at Elektrobygget, NTNU Gløshaugen, while the two dinners are held at two different locations. The map below shows all relevant venues: Elektrobygget, Banksalen SMN, Café To Tårn and Scandic Lerkendal. Trondheim is a small, charming city with all facilities more or less within walking distance. Because of the short distances, there will be **no** arranged transport from Scandic Lerkendal to the conference venue, **nor** will there be any transport after the dinners.

We recommend that you walk between venues, but if you would like or need transport, Trondheim has two taxi companies as well as buses operated by AtB. The easiest way to use the AtB buses is to use a T-kort, which requires registration. Another easy way to use the buses is to download the AtB app, called **Mobillett**, which allows you to pay for individual tickets with your smartphone. Alternatively, you can simply pay cash on the bus, but this is the most expensive bus alternative (NOK 50).

A single bus ticket purchased using the Mobillett app costs NOK 38.50. If you create an account on the app linked to your credit card, the price for a single journey drops to NOK 30.80. You can also administer a T-kort online or show up at the AtB office located in Kongensgate 34 in Trondheim. Another simple alternative is AtB's "Svipp-kort", which can be used for unlimited trips during a specified period: a 24-hour card costs NOK 100, while a 72-hour card costs NOK 160. A "Svipp-kort" can be purchased at 7-Eleven, Narvesen, Deli De Luca and at the Tourist Information Office.

Trondheim's taxi companies are:

- Norgestaxi (+47 08000)
- Trøndertaxi (+47 930 07373)

While you are walking between venues or during your spare time, you might enjoy a stroll through the city centre, where you can experience some of the historical flavor of our more than 1000-year old city:

Nidaros Cathedral
The Trondheim cathedral is Norway's national religious sanctuary, built over the grave of St. Olav. Work on the cathedral first began in 1070, but the oldest extant parts of the structure date from the middle of the 12th century. An extensive restoration effort began in 1869, while work on the impressive west facade with its 57 statues and rose window began in 1901.

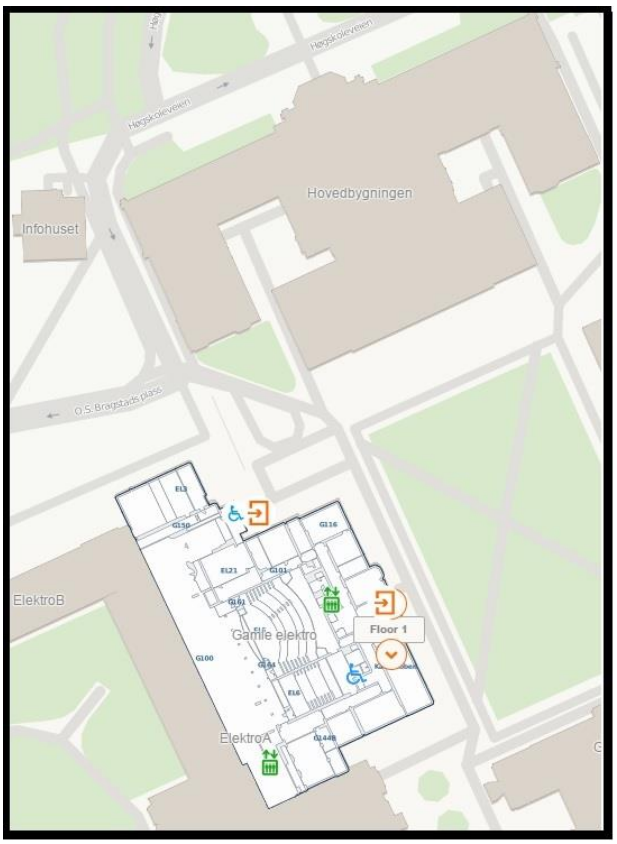
The Archbishop's Palace
The Archbishop's Palace is one of the best-preserved building complexes of its kind in Europe and is the oldest secular building in Scandinavia. Construction began in the second half of the 12th century. The building was the Archbishop's residence until the Reformation in 1537.

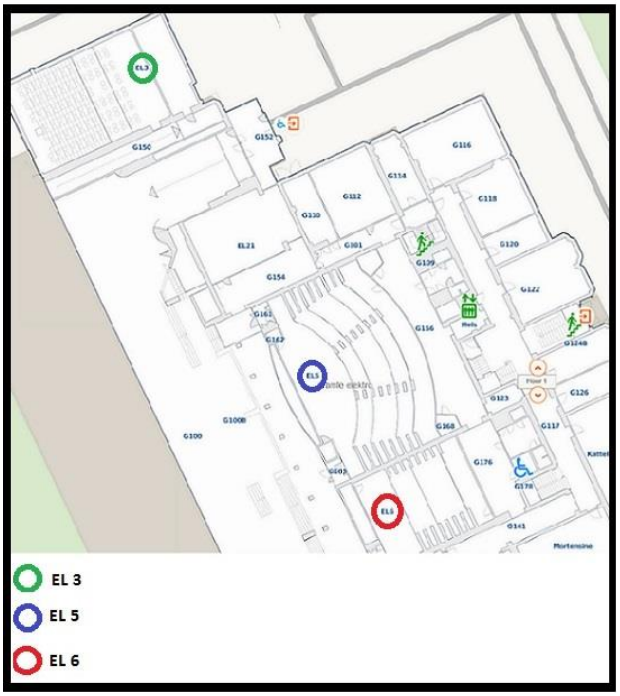
Bakklandet
Coffee shops, small clothing boutiques, antiques stores and more are the hallmark of Bakklandet, a cosy district with distinctive wooden architecture and cobblestone streets. You'll also get a view from the old city bridge (Gamle Bybro) of the colourful wharf buildings across the Nidelva River on Kjøpmannsgata in Trondheim proper.

The Trampe Bicycle Lift
Trondheim is the first city in the world with a lift specifically designed for cyclists. The Trampe Bicycle Lift, developed in Trondheim, goes up the hill at Brubakken near the Gamle Bybro (in Bakklandet), and takes you from the bridge and almost all the way up to the Kristiansten Fort. A sign explains how the mechanism works. If you rent a city bike, you can try it yourself!

Samfundet

The round red building on Elgeseter gate is the Student Association (Samfundet) building, constructed in 1927. It is home to concerts, cultural events, bars, a café and restaurants. Samfundet is owned and run by its approximately 10 000 members, whose goal is that the "Samfundet will be the natural meeting place for students in Trondheim".





WHAT AM I INVITED TO ATTEND?

All participants are free to attend the Nansen Neuroscience Network pre-programme, the conference opening, registration, reception, plenary talks, symposia, poster presentations, exhibitions and lunches/coffee breaks.

However, the dinners are only for individuals who requested and paid for dinner(s) as a part of the registration process. Two registration options allow you to attend dinners:

- payment of either the student or regular package price, or;
- the purchase of dinners separately in combination with either the student or regular basic price.

If you are uncertain as to whether or not you have purchased dinner(s), please contact either Paulina or Håvard.

The post-programme General Assembly of the Norwegian Neuroscience Society is only open to registered members of the society.

PRACTICALITIES

Shopping:
Grocery stores are usually open from 07:00 to 23:00 during weekdays. Grocery stores sell beer (but not wine) and related low-percentage alcoholic beverages until 20:00 on weekdays and 18:00 on Saturday. Most grocery stores are closed on Sundays.

Shopping centres are usually open from 10:00 to 21:00 on weekdays. Trondheim Torg, Mercur and Byhaven are three large shopping centres located in the city proper.

Wine and spirits can only be purchased at the Norwegian national alcohol monopoly, Vinmonopolet, which is open from 10:00-18:00 on weekdays and 10:00-15:00 on weekends. There are two stores in downtown Trondheim, one on Søndregate 8 and one on the ground floor in the Byhaven shopping centre on Olav Tryggvasons gate 28.

Bars typically close at 01:00 or 02:00.

Credit cards are widely accepted in Norway and are more commonly used than cash.

Scandic Lerkendal:
The hotel serves breakfast from 06:30-09:30 on weekdays and from 08:00-11:00 on weekends. The hotel's restaurant opens at 18:00 and closes at 22:00.

You may check in at Scandic Lerkendal beginning at 14:00. If you arrive earlier, the hotel has an area to store your luggage. If your room is ready before 14:00 the hotel will allow you into your room before check-in time. Checkout is at 12:00 noon.

The hotel has exercise/gym facilities, a sauna and a restaurant and bistro.

Exchange rates:
One euro (€) is roughly equal to NOK 8.5 – NOK 1 is roughly equal to €0.118.

One GBP (£) is roughly equal to NOK 11.7 – NOK 1 is roughly equal to £0.085.

One USD ($) 1 is roughly equal to NOK 8 – NOK 1 is roughly equal to $0.125.

One DKK is roughly equal to NOK 1.12 – NOK 1 is roughly equal to DKK 0.88.

One SEK is roughly equal to NOK 0.90 – NOK 1 is roughly equal to SEK 1.10.

Time zone:
Trondheim is located in same time zone as all of Norway, which is Central European Summer Time CEST (UTC+2)

Country code:
Norway's country code for telephone dialing is +47.

Wifi:
NTNU has two wireless Internet options:

1. *Eduroam*, which is accessible to those with a username and password for the Eduroam system.
2. *NTNUguest* is open to all and does not require a password. To connect, establish link to network, open a browser and submit your email-address. With the guest-network, you can use browsers and connect to a VPN.

Social media:
A Facebook group for attendees has been created to share posts, photos, discussions and network. The group name is "Nordic Neuroscience 2015".

Damage and loss liability:
The local and scientific organizing committees accept no liability for injuries and losses of any nature incurred by participants and/or accompanying individuals, nor loss of, or damage to, their luggage and/or personal belongings.

PHOTO CREDIT: VISIT TRONDHEIM

PROGRAM

SHORT

JUNE 10

1330-1530: Pre-Programme: Symposium with Nansen Neuroscience Network

1600: Opening and reception, Nordic Neuroscience – registration available from 1530

1645: Plenary Lecture 1

1755: Symposium Block 1 – Parallel sessions

1925: End of Day 1 conference proceedings

2000: Dinner at Banksalen for those who have prepaid – bus transport from conference venue to dinner
Dresscode: Casual

JUNE 11

06.30: Breakfast begins at Scandic Lerkendal

0900: Plenary Lecture 2

1000: Plenary Lecture 3

1100: Poster session I and II with (standing) lunch

1230: Symposium Block 2 – Parallel sessions

1400: Poster session III and IV

1515: Symposium Block 3 – Parallel sessions

1645: Break with snack

1715: Plenary Lecture 4

1815: Break

1830: Plenary Lecture 5

1930: End of Day 2 conference proceedings

2000: Dinner at Café To Tårn for those who have prepaid – bus transport from conference venue to dinner
Dresscode: Casual

JUNE 12

06.30: Breakfast begins at Scandic Lerkendal

0900: Symposium Block 4 – Parallel Sessions

1030: Break

1045: Plenary Lecture 6

1145: (Standing) lunch in hallway

1245: Chartered bus to airport from Elektrobygget for those who have prepaid – arrive at Værnes 1325

1300-1430: Post-Programme: General assembly of the Norwegian Neuroscience Society

LEGEND

Scandic Lerkendal:

Elektrobygget:

EL 3:

EL 5:

EL 6:

Banksalen, SMN:

Café To Tårn:

We kindly ask that you pay close attention to the schedule. Our programme is very full, and needs to be followed strictly so there is enough time for all the great talks and activities.

WEDNESDAY JUNE 10

1330-1530: Pre-programme – Symposium with Nansen Neuroscience Network

Innovation in Neuroscience: There is a growing demand for new and better ways to prevent, diagnose and treat brain diseases. How can we work to maximize patient benefit from neuroscience research?

Nansen Neuroscience Network invites to this symposium on collaboration across sectors and disciplines. Open for all.

1600: Opening and Reception – registration available from 1530 – EL 5

1645: Plenary Lecture 1 – EL 5
Christine Van Broeckhoven – University of Antwerp
"Molecular Genetics of Neurodegenerative Dementia"

1755: Symposium Block 1 – Parallel Sessions

1. **Motor Systems – EL 3**
   Chair: Abdel El Manira – Karolinska Institute

   1755: Abdel El Manira – Karolinska Institute
   "Deconstructing the Spinal Locomotor Circuits"

   1825: Gilad Silberberg – Karolinska Institute
   "Neural Microcircuits Underlying Striatal Sensory Function"

   1855: Jean-Francois Perrier – University of Copenhagen
   "Serotonin Spillover onto the Axon Initial Segment of Motoneurons Induces Central Fatigue by Inhibiting Action Potential Initiation"

2. **Molecular Mechanisms of Drug Abuse – EL 6**
   Chair: Poul Henning Jensen – Aarhus University

   1755: Poul Nissen – Aarhus University
   "Structure and Mechanism of Neurotransmitter Transporters"

   1825: Petrine Wellendorph – University of Copenhagen
   "GHB (aka Fantasy) as a Signaling Molecule in the Mammalian Brain"

   1855: Esa Korpi – University of Helsinki
   "GABAergic Mechanisms in Addiction"

1925: End of Day 1 conference proceedings

2000: Dinner at Banksalen for those who have prepaid – bus transport from conference venue to dinner

Dresscode: Casual

THURSDAY JUNE 11

0900: Plenary Lecture 2 – EL 5
Michael Hausser – University College London
"All-Optical Interrogation of Neural Circuits"

1000: Plenary Lecture 3 – EL 5
Silvia Arber – Friedrich Miescher Institute
"Disentangling Descending Circuits for Motor Control"

1100: Poster session I and II

Lunch (standing) in hallway 1200

1230: Symposium Block 2 – Parallel Sessions

3. **Regenerative Neuroscience – Translating Stem Cell Technologies into Personalized Treatments for CNS Injury and Disease – EL 3**
Chair: Ioanna Sandvig and Axel Sandvig – Norwegian University of Science and Technology

1230: Vuk Palibrk – Norwegian University of Science and Technology
"Function of Promyelocytic Leukemia (PML) in Acute Brain Injury and Neurogenesis"

1300: Susanna Narkilahti – University of Tampere
"Differentiation of Human Neural Stem Cells and Induced Pluripotent Stem Cells"

1330: Zaal Kokaia – Lund University
"Clinical Translation of Stem Cell Based Therapies in the Treatment of Stroke"

4. **Cortex: Organization and Dynamics in Normal Brains and in Disease – EL 6**
Chair: Jonathan Whitlock – Norwegian University of Science and Technology

1230: David Rowland – Norwegian University of Science and Technology
"Towards a Functional Identification of Stellate Cells in Medial Entorhinal Cortex"

1300: Lars Nyberg – Umeå University
"Lifespan Cortical Dynamics in Relation to Changes in High-Order Cognition"

1330: Albert Gjedde – University of Copenhagen
"Cortical Mechanisms of Conscious Brain Activity"

1400: Poster session III and IV

1515: Symposium Block 3 – Parallel Sessions

5. **Neuronal Energy Crisis as a Key to Understanding Aging and Alzheimer's Disease – EL 3**
   Chair: Heikki Tanila – University of Eastern Finland

   1515: Agneta Nordberg – Karolinska Institute
   "PET Imaging of Amyloid Accumulation and Metabolic Failure in Early AD"

   1545: Menno Witter – Norwegian University of Science and Technology
   "The Specific Role of Entorhinal Neurons in Memory Disorders"

   1615: Heikki Tanila – University of Eastern Finland
   "The Role of Neuronal Hyperactivity in AD and Aging at the Network Level"

6. **Neurotransmitter Transporters in Pathophysiology – EL 6**
   Chair: Bryndis Birnir – Uppsala University

   1515: Ulrik Gether – University of Copenhagen
   "Novel Insight into the Role of the Dopamine Transporter in Disease: Association of Missense Mutations with Neuropsychiatric Disorders"

   1545: Klas Kullander – Uppsala University
   "Novel Aspects of Aminergic Signaling and Influence on Hippocampal Microcircuits and Memory"

   1615: Farrukh Chaudhry – University of Oslo
   "The Slc38 Family of Glutamine Transporters in Synaptic Plasticity and Pathology"

1645: Break with snack – posters available

1715: Plenary Lecture 4 – EL 5
David Anderson – California Institute of Technology
"Neural Circuit Modules for Social Behaviors"

1815: Break

1830: Plenary Lecture 5 – EL 5
May Britt Moser and Edvard Moser – Norwegian University of Science and Technology
"The Brain's Map of Space"

1930: End of Day 2 conference proceedings

2000: Dinner at Café To Tårn for those who have prepaid – bus transport from conference venue to dinner
Dresscode: Casual

FRIDAY JUNE 12

0900: Symposium Block 4 – Parallel Sessions

7. Molecular Mechanisms and Novel Targets in Parkinson's Disease – EL 3
Chair: Dan Lindholm – University of Helsinki

0900: Ernest Arenas – Karolinska Institute
"Novel Molecular Mechanisms Controlling Midbrain Dopamine Neuron Development"

0930: Poul Henning Jensen – Aarhus University
"Alpha-Synuclein Aggregates in Parkinson's Disease, any Prospect for Therapeutic Targets?"

1000: Raimo Tuominen – University of Helsinki
"Endoplasmic Reticulum Resident Neurotrophic Factor CDNF – Mode of Action and Efficacy in Experimental Models of Parkinson's Disease"

8. Computational Neuroscience Techniques to Explore the Structure and Function of the Brain – EL 6
Chair: Sten Grillner – Karolinska Institute

0900: Gaute Einevoll – Norwegian University of Life Sciences
"Brain Physics – Neuroscience with both Hands"

0930: Jeanette Hellgren Kotaleski – KTH Royal Institute of Science
"Reward Dependent Learning – Computational Investigation of the Read-Out Mechanisms"

1000: Marja-Leena Linne – Tampere University of Technology
"Modelling Neurons, Astrocytes and Synaptic Mechanisms to Explain Local Cortical Microcircuit Dynamics"

1030: Break

1045: Plenary Lecture 6 – EL 5
Nancy Kanwisher – Massachusetts Institute of Technology
"The Functional Organization of Human Auditory Cortex"

1145: Lunch (standing) in hallway

1245: Chartered bus to airport for those who have prepaid it – arrive at Værnes Airport 1325

1300-1430: Post-programme: General assembly of the Norwegian Neuroscience Society

The Norwegian Neuroscience Society will hold its first general assembly on June 12, from 1300 to 1430, in connection with the Nordic Neuroscience meeting in Trondheim. All members are invited to participate. The purpose of the general assembly is to present the activities, finances, and opportunities of NNS and to enlist the active participation of the membership in the further development of the society.

Open only for member of the Norwegian Neuroscience Society

1445: Chartered bus to Værnes Airport – estimated arrival 1525. Note that this bus is only for participators at the general assembly of NNS.

LEGEND

Scandic Lerkendal:

Elektrobygget:

EL 3:

EL 5:

EL 6:

Banksalen, SMN:

Café To Tårn:

We kindly ask that you pay close attention to the schedule. Our programme is very full and needs to be followed strictly so there is enough time for all the great talks and activities.

PHOTO CREDIT: TRONDHEIM KOMMUNE

ABDOLRAHMAN KHEZRI

NORWEGIAN UNIVERSITY OF LIFE SCIENCES

BEHAVIOURAL EFFECTS OF MULTISIZED GOLD NANOPARTICLES IN ZEBRAFISH EMBRYOS AND LARVAE

Abdolrahman Khezri[1*], Juan Germán Herranz Jusdado[2], Thomas Fraser[3], Ana Carolina Sulen Tavara[3], Erik Ropstad[3], Karin Zimmer[1]

1, Norwegian university of life science (NMBU), faculty of veterinary medicine, Department of Basic Sciences and Aquatic Medicine (Basam), Oslo

2, University of Oslo (UiO), Blindern, Oslo

3, Norwegian university of life science (NMBU), faculty of veterinary medicine, Department Production Animal Clinical Sciences (Prodmed)

Nanoparticles are particles with at least one dimension between 1 and 100 nm. Due to their unique properties, gold nanoparticles (AuNPs) are used for drug delivery, diagnostics and cellular imaging, but there are concerns they interfere with neuronal development.

In this study, we tested for potential neurotoxic effects of AuNPs by analyzing behavioral changes in zebrafish (*Danio rerio*) embryos and larvae. We injected three different sizes of AuNPs (20, 40 and 80 nm) into both embryos at 2-4 h post fertilization (hpf), and larvae at 72 hpf. The AuNPs were delivered into the yolk sack of the embryos and the duct of Cuvier (i.e. the blood stream) in the larvae. Five concentrations of each AuNPs were tested in triplicate (1000, 500, 100, 50 and 10 µg/ml), all of which were below the lethal dose. Changes in behavior are a good indicator of neurotoxicity. Therefore, we used an automatic tracking system to analyze the distance travelled and time spent active in 96 hpf zebrafish. These endpoints were tested under light-dark-light photo regimes. In addition, we monitored motorneuron development using whole-mount immunohistochemistry staining for α-AT.

We found that embryos injected with all three AuNP sizes showed reduced locomotor activity during the dark phase, but there was no effect in the light phase. These reductions were concentration dependent, whereby the highest doses resulted in the lowest movement. We did not find any significant interaction of AuNP size on the reduction in locomotor activity. In contrast, we found no clear response patterns in larvae injected with AuNPs at 72hpf. Here, some concentrations increased, whereas others decreased, activity levels. The results of the motoneurons development are forthcoming.

These results show that AuNPs have an effect on zebrafish behavior. As the behavioral effects were more apparent and consistent following the earlier exposure window, i.e. embryos, this suggests AuNPs may interfere with the early stages of neurological development.

ADRIAN SZUM

UNIVERSITY OF BERGEN

Imaging synaptic memory formation using a novel drug-controlled reporter

Adrian Szum (1) , Oleksii Nikolaienko (1) , Sudarshan Patil (1) , Michael Z. Lin (2) , Clive R. Bramham (1)

1 Department of Biomedicine and KG Jebsen Centre for Research on Neuropsychiatric Disorders, University of Bergen, Norway
2 Departments of Pediatrics and Bioengineering, Stanford University, USA

The immense capacity and specificity of memory storage in the mammalian brain is thought to depend on the plasticity of synaptic connections. Dysfunction of synaptic plasticity is implicated in a range of disorders from Alzheimer's disease to mental retardation and development of chronic pain states. Understanding how neural activity patterns are translated into lasting changes in synaptic connectivity is therefore one of the most important challenges in basic and clinical neuroscience. Long-term memory relies on lasting changes in synaptic connectivity, and these synaptic modifications depend on new gene expression and protein synthesis. Arc (activity-regulated cytoskeleton-associated protein), is an immediate-early gene that is rapidly induced upon neuronal activity. Arc transcription, translation, and protein function provides a finely-tuned system for converting neuronal activity patterns into protein synthesis-dependent synaptic plasticity and memory storage. By a biological logic that is not yet understood, Arc synthesis controls both long-term potentiation (LTP) and long-term depression (LTD) of synaptic connections.

A novel, drug-controllable tool for visualizing new protein synthesis, called TimeSTAMP, will be used to visualize and monitor local synthesis and trafficking of Arc in hippocampal neuronal cells undergoing functional changes such as LTP and LTD in the brain of rats. TimeSTAMP is the sole method that may allow us to study time-dependent functions of Arc in distinct subcellular compartments by allowing discrimination of different translated populations. It may further explain Arc's sites of synthesis and possible compartment accumulation. We expect to see that LTP and LTD stimulation induce different patterns of Arc accumulation and removal from synapses. Differences between LTP and LTD-induced Arc distributions would provide further evidence of a switch in Arc's function. Our approach will give fundamental new insights into how memory operates at the synaptic level.

In the TimeSTAMP reporter, the cassette encoding the protein of interest, e.g. Arc, is followed by a sequence-specific protease, an epitope tag and a yellow fluorescent protein called Venus. The protease in this cassette can be specifically inhibited by the cell permeant drugs. Upon translation of Arc, protease activity cleaves the epitope tag and a part of the Venus fluorescent protein effectively disallowing detection of signal. In the presence of inhibitor the entire fluorescent protein is preserved, so that it can mature and emit fluorescent light upon excitation, thus allowing selective detection of newly synthesized proteins both by fluorescence and epitope tagging. TimeSTAMP allows live
time-lapse imaging of new protein synthesis using the fluorescent protein signal in addition to detection of the epitope tagged protein in fixed samples. In this study, primary rat hippocampal neuronal cultures will be transfected with this drug-controllable reporter, followed by chemical LTP stimulation with 4AP/BIC or chemical LTD using DHPGLTD and imaging of the temporal and spatial characteristics of Arc by confocal microscopy.

AGATA BOCHYNSKA

NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

Re-enactment of eye movements' sequences facilitates retrieval of visuospatial information

Bochynska, A.[1,2], Laeng, B.[1]

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Abstract
Eye movements and fixations occur spontaneously during recall from long-term visual memory and recent research provides support for the hypothesis that executing these fixations plays a functional role in the retrieval of stored information (Johansson & Johansson, 2014; Laeng, Bloem, D'Ascenzo, & Tommasi, 2014; Laeng & Teodorescu, 2002; Mäntylä & Holm, 2006). However, evidence about whether the spacetime sequence (i.e., scanpath) of these eye fixations is also relevant for the accuracy of memory remains sketchy and ambiguous. In the current study, eye fixations were recorded by the means of a remote, infrared eye tracker while looking at a checkerboard-like pattern during an 8 seconds encoding. Two days later, in a recognition session, animations were shown where each square that formed the pattern was presented one by one, either according to the same, idiosyncratic, temporal sequence in which they were originally viewed by each participant or in a shuffled sequence, although the squares were always in their correct positions. Afterwards, participants judged whether they had seen the same pattern before or not (50% probability). Showing the elements according to the original scanpath's sequence yielded a significantly better recognition memory than in the shuffled condition. When participants maintained gaze on the center of the display so that gaze was restricted from enacting the sequence, the advantage of memory accuracy disappeared. Concluding, scanpaths (i.e., the order of fixations and not simply their positions) are functional to visual memory and that physical re-enacting of the original, embodied, perception can facilitate retrieval from long-term visual memory.

References:
Johansson, R., & Johansson, M. (2014). Look here, eye movements play a functional role in memory retrieval. *Psychological Science*, *25(1)*, 236–242. doi:10.1177/0956797613498260

Laeng, B., Bloem, I. M., D'Ascenzo, S., & Tomassi, L. (2014). Scrutinizing visual images: The role of gaze in mental imagery and memory. *Cognition*, *131*, 263-283. doi:10.1016/j.cognition.2014.01.003

Laeng, B., & Teodorescu, D. S. (2002). Eye scanpaths during visual imagery reenact those of perception of the same visual scene. *Cognitive Science: A Multidisciplinary Journal*, *26*, 207–231. doi:10.1207/s15516709cog2602_3

Mäntylä, T., & Holm, L. (2006). Gaze control and recollective experience in face recognition. *Visual Cognition*, *14*, 365–386. doi:10.1080/13506280500347992

Serotonin Transporter Genotype Modulates the Effects of Dopamine Transporter Genotype on Learning from Positive and Negative Feedback

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Abstract

In this study, we investigate the impact of interaction between dopamine and serotonin levels on feedback-based learning. A major modulator of dopamine levels in the brain is the dopamine transporter (DAT) that is coded by the DAT1 gene, while serotonin levels in the brain are modulated by the serotonin transporter (SERT) that is coded by the SLC6A4 gene. We examined the variable number of tandem repeats polymorphism (VNTR) in the 3' untranslated region (3'-UTR) of DAT1 the serotonin transporter polyadenylation polymorphism (STPP) of SLC6A4 in 120 racially homogenous healthy volunteers. We grouped the subjects according to DAT1 VNTR genotype into 9-repeat carriers (high dopamine) and 10-repeat homozygotes (low dopamine), and SLC6A4 STPP genotype into C allele homozygotes (high serotonin) and A allele homozygotes (low serotonin). All subjects were administered a probabilistic category-learning task that allowed for dissociation between the acquisition of positive feedback (reward) and negative feedback (punishment). Our results suggest that genes that modulate dopamine and serotonin levels affected reward learning but not punishment learning. When we held SLC6A4 constant and varied DAT1 genotypes, there was better learning from both reward and punishment with higher dopamine levels (9-repeat carriers) in the context of higher serotonin levels (C allele homozygotes). Conversely, there was no difference between DAT1 genotypes in learning from positive and negative feedback in the context of low serotonin levels (A allele homozygotes). When we held DAT1 genotypes constant, there were no difference between SLC6A4 genotypes in the context of high (9-repeat carriers) or low (10-repeat homozygotes) dopamine levels. These findings argue in favor of a modulatory role of serotonin on dopamine function. Future studies will investigate this gene-gene interaction in Parkinson's disease and Major Depressive Disorder as it relates to cognitive function and response to treatment.

ALIONA NACU

UNIVERSITY OF BERGEN

Early neurological worsening in acute ischaemic stroke patients

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Objectives: Neurological worsening in acute ischaemic stroke patients is common with significant morbidity and mortality. Aims: To determine factors associated with early neurological worsening within the first 9 hours after onset of acute ischaemic stroke

Materials & methods: The National Institute of Health Stroke Scale (NIHSS) was used to assess stroke severity. Early neurological worsening was defined as NIHSS score increase ≥4 NIHSS points within 9 hours of symptom onset compared to NIHSS score within 3 hours of symptom onset. Patients with early neurological worsening were compared to patients with unchanged or improved NIHSS scores.

Results: Of the 2484 patients admitted with ischaemic stroke, 552 patients had NIHSS score within 3 hours of symptom onset, and 44 (8.0%) experienced early neurological worsening. The median NIHSS on admission was 8.4 in both groups. Early neurological worsening was associated with low body temperature on admission (p=.01), proximal compared to distal MCA occlusion (p=.007) and with ipsilateral internal carotid artery stenosis >50% or occlusion (p=.04). Early neurological worsening was associated with higher NIHSS day 7 (p<.001) and higher mortality within 7 days of stroke onset (p=.005).

Conclusions: Early neurological worsening has serious consequences for the short term outcome for patients with acute ischaemic stroke and is associated with low body temperature on admission, and with extracranially and intracranially large vessel stenosis or occlusion.

AMY ROBINSON

NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

Intra-claustral connectivity in the rat measured with voltage sensitive dye imaging

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The claustrum is a small and highly conserved nucleus that extends rostrocaudally in the brain. In the rat, the claustrum is located lateral to the external capsule, bordering the insular and piriform cortices. The claustrum is comprised of two subregions, the (dorsal) claustrum and the endopiriform nucleus. Previous studies have shown that there are reciprocal connections with many areas of the cortex, however, little is known about the intrinsic connectivity and the function of the claustrum. One hypothesis is that the claustrum is able to synchronize and coordinate the activity of distant cortical areas based on this strong reciprocal connectivity. However, since there is a topographical arrangement of the reciprocal cortical connections of the claustrum, a strong intrinsic network within the claustrum must exist for it to be able to coordinate the activity of distant cortical regions. The current study aimed to investigate the potential intrinsic network within the claustrum and the dorsal endopiriform nucleus of the rat. Connectivity within the claustrum was observed by utilizing optical imaging of the voltage sensitive dye (RH-795) in brain slices from P23-P30 Long Evans rats (N= 6). Sections through the claustrum in both the horizontal and the sagittal planes were used to investigate the connectivity along the anterior/posterior or dorsal/ventral directions. Preliminary results indicate that there is connectivity in all directions tested, with bidirectional activation observed throughout the claustrum and dorsal endopiriform nucleus. More work in the future needs to be done to confirm the connectivity within and between the claustrum and dorsal endopiriform nucleus and to establish the origin and postsynaptic targets of this intrinsic network.

ANASTASIA LUDWIG

UNIVERSITY OF HELSINKI

KCC2 regulates actin dynamics in dendritic spines via interaction with βPIX

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Chloride extrusion in mature neurons is largely mediated by the neuron-specific potassium-chloride cotransporter KCC2. In addition, independently of its chloride transport function, KCC2 regulates the development and morphology of dendritic spines through structural interactions with the actin cytoskeleton. The mechanism of this effect remains largely unknown. Here, we show a novel pathway for KCC2-mediated regulation of the actin cytoskeleton in neurons. We found that KCC2, through interaction with the b isoform of Rac/Cdc42 guanine nucleotide exchange factor βPIX, regulates the activity of Rac1 GTPase and the phosphorylation of one of the major actin-regulating proteins, cofilin-1. KCC2-deficient neurons had abnormally high levels of phosphorylated cofilin-1. Consistently, dendritic spines of these neurons exhibited a large pool of stable actin resulting in reduced spine motility and reduced density of functional synapses. In conclusion, we describe a novel signaling pathway that couples KCC2 to the cytoskeleton and regulates the formation of glutamatergic synapses.

ANDERS VICTOR PETERSEN

UNIVERSITY OF COPENHAGEN

5-HT_{2C} receptors decrease the susceptibility of seizures in the dorsal subiculum by inhibiting Ca_V3 ion channels

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3: INMED, INSERM U.29, Parc scientifique de Luminy BP 13, 13273 Marseille Cedex 9, France

In this study, we investigated how serotonin (5-HT) modulates the activity of principal cells from the subiculum at the subcellular level. We recorded the electrical activity of subicular pyramidal neurons with the whole-cell patch clamp technique in a slice preparation from the hippocampus of mice (P7 to P36). In agreement with previous observations, we found that subicular neurons fired action potentials in bursts caused by the presence of a low threshold spike generated by T-type Ca^{2+} channels. A glass pipette filled with 5-HT was positioned near the membrane of the recorded cell and released either by pressure or microiontophoresis. A local puff of 5-HT inhibited the generation of action potentials. During voltage-clamp recordings performed after blocking currents mediated by Na^+ and K^+ currents, puffing 5-HT decreased the amplitude of a low-threshold voltage sensitive transient inward current sensitive to mibefradil. These results suggest that 5-HT inhibits a current mediated by T-type Ca^{2+} channels. To corroborate our findings, we monitored the variations in calcium concentrations by loading recorded cells with the calcium indicator FURA-2. We observed that a burst firing evoked by depolarizing current pulses induced an increase in calcium concentration. When 5-HT was puff-applied, the calcium signal was attenuated in all compartments of the neuron (AIS, soma, dendrites). Puffing the 5-HT_{2C} agonist WAY 629 instead of 5-HT had the same inhibitory effect on firing and Ca^{2+} current.

Our data suggest that 5-HT modulates the activity of subicular pyramidal cells by inhibiting T-type calcium channels through an activation of 5-HT_{2C} receptors.

ANDREAS KARDAMAKIS

KAROLINSKA INSTITUTE

Inhibition normalises multisensory integration on gaze controlling neurons in the optic tectum

Andreas A. Kardamakis, Juan Perez-Fernandez and Sten Grillner

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Movements towards and away from sensory stimuli in the environment are critical for survival. Neurons in the deep layers of the optic tectum (superior colliculus in mammals) that command gaze movements rely on the synaptic integration of diverse sensory inputs. These multisensory cells form a canonical circuit with afferent sensory input targeting the superficial and intermediate tectal layers. Although studies have shown that multiple sensory modalities can modulate their neural activity, the synaptic underpinnings of this multisensory integration have not been investigated.

Recently, we have shown that the local GABAergic system within the optic tectum can generate visual stimulus selection through a process of competitive inhibition in the lamprey - a conserved vertebrate system (Kardamakis et al., 2015). This is effectively carried out through long-range inhibitory connections across the tectal map of space that generate local excitation and global suppression onto gaze controlling neurons in the deep layer mediating orienting or avoidance movements. We now show that synaptic inhibition is not only critical for generating stimulus selection but also essential for multisensory integration.

Here, we studied the mechanisms underlying cross-modal integration of visual and electrosensory signals onto tectal gaze controlling neurons. Using whole-cell recordings in a midbrain preparation, we report that unimodal inputs generated direct (monosynaptic) fast excitation that was quickly followed by inhibition from local GABAergic interneurons. Notably, their distinctive intrinsic properties supported the temporal integration of excitatory and inhibitory synaptic currents during co-activation of both sensory afferent pathways, which resulted in the continuously scaling of their net excitatory responses. In particular, sensory-induced excitatory currents had a greater impact on their membrane depolarisation when they did not temporally overlap with feedforward inhibition, while synaptic depression during sustained activation ensured balanced activity in these neurons. This is was further corroborated in the intact animal where we demonstrate that extracellular neural activity in the deep layers during local visual and electrosensory activation increase sublinearly within a finite range of stimulus intensities. Pharmacological blockade of local GABAergic inhibition amplified responses with rigorous discharge of action potentials during unimodal and crossmodal stimulation, thus, cancelling any additive effects onto gaze controlling neurons. These results suggest that modality-dependent recruitment of inhibition could be an evolutionary strategy for providing a robust normalisation mechanism.

Reference: Kardamakis A, Saitoh K, Grillner S (2015) Tectal microcircuit generating visual selection commands on gaze-controlling neurons, *Proc Natl Acad Sci USA,* 112(15):1956-1965.

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ANE CHARLOTTE CHRISTENSEN

UNIVERSITY OF OSLO

The role of extracellular matrix molecules for spatial representations and plasticity in entorhinal cortex

Charlotte Christensen[1,2], Kristian Lensjø[2], Mikkel Lepperød[1,2], Marianne Fyhn[2], Torkel Hafting[1,2]
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The hippocampus and parahippocampal areas are key players in spatial memory processing. A substantial fraction of principal neurons in the medial entorhinal cortex (MEC) are grid cells which are characterized by their multiple firing fields forming a hexagonal pattern spanning the area visited by the animal. The grid cells form stable spatial representations that are reactivated any time the animal visits the same environment. The processes of preserving existing maps and encode novel representations require both stability and plasticity. Growing evidence suggests that GABA'ergic fast spiking parvalbumin-positive (PV+) inhibitory neurons, are essential for regulation of plasticity in sensory cortices. The PV+ cells in the adult cortex are to enwrapped in perineuronal nets (PNNs) which are condensed extracellular matrix molecules. Emerging evidence suggests that perineuronal nets (PNNs) stabilize synaptic connections and restrict plasticity in the adult brain. Enzymatic degradation of PNNs with Chondroitinase ABC (chABC) induces plasticity in adults and promotes some types of learning but it remains elusive if spatial processing of the hippocampus and medial entorhinal cortex is affected by degradation of PNNs.

In the present study, we investigated how enzymatic degradation of PNNs affects spatial representations in the MEC in awake and behaving rats. The superficial layers of MEC are densely filled with PV+ neurons, and we show that most of them are enwrapped by PNNs. In contrast to visual cortex, PNNs are also found around other types of cells in MEC, but their identity remains elusive. Bilateral injections of the enzyme chondroitinase ABC in the dorsal MEC were used to disrupt the nets. Extracellular recordings from single units in MEC were conducted in rats running in an open field (1x1m square box). When stable recordings of grid cells were obtained across successive recording sessions in a familiar environment, the rat was introduced to a similar box in a novel room. In animals with PNNs degraded we observed decreased spatial information of grid cells in a familiar environment. Also, the establishment of stable grid representations in novel environments was delayed, and continued to display decreased spatial correlation compared to control animals. Furthermore, analysis of local field potentials showed that disruption of PNNs lead to increased theta power in the MEC. Results from this study indicate that PNNs could be important for sustaining stable spatial representations of MEC in adult animals.

ANGEL MOLDES-ANAYA

UNIVERSITY OF TROMSØ

Targeting mnemonic pathways of stress by cerebral perfusion using ^{99m}Tc-HMPAO SPECT/CT

Angel Moldes-Anaya[1], Samuel Kuttner[2,4], Daniel Vincenz[3], Jürgen Goldschmidt[3], Vegard Heimly Brun[1,4], Kirsten Brun Kjelstrup[1,4]

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Generalization of aversive memories after a traumatic episode can lead to posttraumatic stress disorder (PTSD) and acute stress disorder (ASD). Little is known about the pathophysiology behind PTSD, and thus there is a lack of validated therapeutic targets for the development of an adequate treatment. The stress hormone cortisol (CORT) has, in humans, been associated with the formation and extinction of traumatic memories (Zohar et al., 2011). In addition, it has been suggested a central role of the amygdaloid complex and ventral hippocampus (VH) in processing traumatic stress and the retrieval of emotional memories (Pitkanen et al., 2000). We use predator odors to evoke a natural stress response in rats, measured by behavioral changes (e.g. flight, freezing) and a detectable increase in blood CORT. We are quantifying changes in regional cerebral blood flow (rCBF) that correlate with 1) the innate response to predator stress and 2) the memory for this event on a subsequent exposure 72 h after the aversive stimulation. For imaging the distribution of the cerebral blood flow at a certain time point, we make use of the radiotracer Technetium 99m-Hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO) administered intravenously during exposure to the aversive stimulus (Kolodziej et al., 2014). After injection, single photon emission computed tomography (SPECT) together with computed tomography (CT) scanning is performed to obtain a fused SPECT/CT dataset. The fused SPECT/CT image is further aligned to a magnetic resonance imaging (MRI) atlas using CT landmarks. The MRI atlas provides detailed anatomical information while functional SPECT data allows determining activation and inactivation patterns of different brain areas. The SPECT-scans of exposed animals are compared to intra-individual baseline scans and unstimulated controls.

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Cortical morphometry and IQ in VLBW children

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Background

Children born prematurely with very low birth weight (VLBW: bw ≤ 1500 g) has an increased risk of preterm perinatal brain injury, which may subsequently alter the maturation of the cerebral cortex. Deviant cortical development could have implications for the cognitive abilities in this population.

Objective

To assess cortical thickness and cortical surface area in VLBW children compared with term-born controls, and to investigate any relationship between cortical morphometry measures and full IQ and birth weight.

Design /Methods

In this cross-sectional study 37 VLBW and 104 term born children were assessed cognitively at mean age 8 years. We applied an automated method to reconstruct the cortical surface area and cortical thickness based on T1-weighted MRI images using the FreeSurfer software. Wechsler tests were used for cognitive assessment.

Results

The VLBW children had smaller cortical surface area bilaterally in frontal, temporal, and parietal lobes (figure 1). Thicker cortex in frontal and occipital regions and thinner cortex in posterior parietal areas (figure 2) were observed in the VLBW group. There were significant differences in IQ between groups (VLBW *M*=98, *SD*=9.71; controls *M*=108, *SD*=13.57; p <0.001). There was a positive relationship between IQ and surface area in both groups. In the VLBW group, reduced IQ was correlated with frontal cortical thickening and temporo-parietal thinning. In the VLBW group, the effect of birth weight was most pronounced in frontal and occipital regions on cortical surface area, and in frontal and temporal regions with regard to cortical thickness.

Conclusion

Deviations in cortical thickness and surface area seen in school aged VLBW children compared to controls and these differences was related to reduced birth weight and poorer cognitive function.

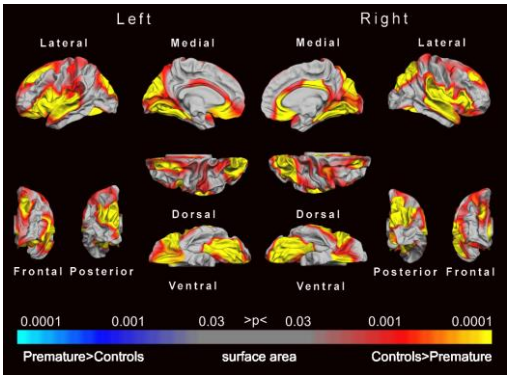

Figure 1: Surface area reduction in the VLBW group compared with controls.

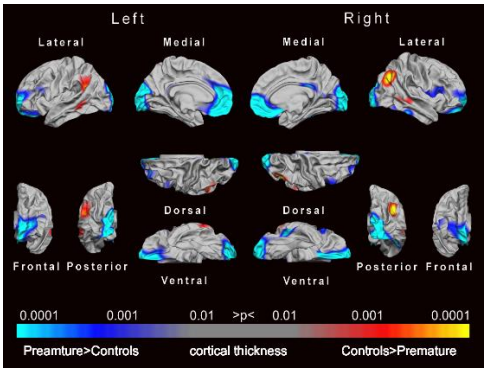

Figure 2: Areas with cortical thickening (blue) and thinning (red/yellow) in the VLBW group compared with controls.

ANNE MARTE SJURSEN KVELLO

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Preclinical studies of a heroin vaccine

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Passive drug vaccines are currently being studied as an alternative treatment strategy for drug overdose and addiction. The aim of such antibody therapy is to sequester the active drug in the blood and thereby prevent the psychoactive substance from entering the brain to exert its effects. The acute effects of heroin are mainly caused by its first metabolite, 6-monoacetylmorphine (6-MAM), which is rapidly formed in the blood, before crossing the blood-brain-barrier (BBB) and binding to brain opioid receptors. In a recently published study, we reported that a monoclonal antibody specific to 6-MAM (anti 6-MAM mAb) reduces acute heroin-induced effects in mice (Bogen et al., 2014). The aim of the present study was to characterize the time course of the interference of the antibody with heroin effects and to assess μ-opioid receptor binding after antibody treatment. Furthermore, we wanted to elucidate the efficacy of the antibody upon repeated heroin injections.

Mice were pretreated with anti-6-MAM mAb (10 mg/kg) or saline, followed by one or several injections of heroin (2.5 μmol/kg). Immediately after the last heroin injection, the behavioral drug effect was assessed in a locomotor activity test, which may be used as a measure of the psychostimulatory effects of opioids. Blood and brain levels of opioids were quantified by LC-MSMS. Finally, a radio ligand assay using [^{3}H]DAMGO was used to examine μ-opioid receptor binding *ex vivo*, comparing mAb treated animals and controls.

The behavioral effects of heroin, measured as locomotor activity, were reduced by approximately 60% in mAb-treated animals relative to controls. In mAb-treated animals, brain levels of 6-MAM at 5, 10 and 25 minutes after heroin injection were reduced by 27, 32 and 60% respectively. Furthermore, a single dose of anti-6-MAM mAb reduced the brain concentration of 6-MAM by approximately 60% even after daily repeated heroin injections for three consecutive days. We found a 36% increase in available μ-opioid receptors in the brains of mAb-treated mice compared to controls, indicating decreased levels of opioids in these animals. The results from the current study confirm that anti-6-MAM mAb treatment reduces the acute behavioral effects of heroin by blocking the entry of 6-MAM to the brain, and this is further supported by the low 6-MAM concentrations found in brain tissue of mAb-treated animals. Moreover, our results indicate that anti-6-MAM mAb reduces heroin-induced effects even after repeated drug exposure.

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Amyloid β-immunoreactivity in subicular interneurons in the McGill-R-Thy1-APP rat model of Alzheimer's disease

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The hippocampal formation (HF) is early affected by neuropathological changes in Alzheimer's disease (AD), and alterations in this area and the interconnected entorhinal cortex (EC) are likely to underlie the deficits in learning and memory associated with the disease. In the transgenic McGill-R-Thy1-APP rat model of AD, which shows AD-like progression of amyloid beta (Aβ) pathology, dorsal subiculum has the earliest and heaviest load of extracellular Aβ plaques, as well as strong intracellular Aβ-immunoreactivity (*Leon et al., 2010, J. Alzheimers Dis., 20:1; Heggland et al., 2015, Eur. J. Neurosci.*). Inhibitory interneurons, which make up approximately 10 % of cells in HF, regulate synaptic signalling of principal cells, and interneuron loss has been described in HF of transgenic AD mice models (*Loreth et al., 2012, Neurobiol. Dis., 47:1*). We did immunohistochemical double-labelling for human Aβ and glutamate decarboxylase 67-kDa (GAD67), a marker for GABAergic cells, on tissue from 1 month- and 6 month-old transgenic McGill-R-Thy1-APP rats, and quantitatively analysed the expression of intracellular Aβ in interneurons in subiculum. We found a significantly higher proportion of interneurons immunoreactive for Aβ in dorsal than in ventral subiculum (38.3% and 23.5%, respectively; $p < 0.01$). There were no significant differences between the age groups. Other areas that are heavily affected by both extra- and intracellular Aβ in this rat model include EC, more specifically it's most lateral and caudal portions. This part of EC is reciprocally connected specifically with dorsal subiculum. Our results emphasise the importance of dorsal subiculum and interconnected areas of EC in early AD. We further show that inhibitory neurons in subiculum express intracellular Aβ at early stages, similar to what has been shown for neocortical interneurons (*Mochizuki et al., 2000, Lancet, 355:9197*). This expression may result in network dysfunctions and synaptic alterations associated with AD.

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FGF21 affects inflammatory response of microglia cells

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Chronic neuroinflammation, a state of long lasting microglial activation and release of proinflammatory cytokines, affects the pathogenesis of many neurodegenerative disorders including Alzheimer's and Parkinson's disease[1]. Tumor necrosis Factor α (TNFα) is the key mediator of microglial inflammatory response with a dual role in neuronal protection and neurodegeneration[2]. Fibroblast growth factor-21 (FGF21) is a regulator of glucose and fatty acid metabolism and influences inflammation in the body[3,4,5]. FGF21 is expressed in glia cells in culture, can pass the blood brain barrier and exert protective effects on neurons[6,7]. However, studies on FGF21 in the central nervous system are up to date quite rare and preliminary. Therefore it is all the more important to study FGF21 more carefully in the brain. We have data supporting the expression of FGF21 in microglia cells and the involvement in the inflammatory reaction of murine microglia BV-2. Specifically, FGF21 decreases TNFα levels in lipopolysaccharide induced inflammation reaction. At the same time TNFα secretion seems to be increased. Altogether this study reveals new insights in the role of FGF21 in the brain.

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Reelin-Immunoreactive Neurons in Entorhinal Layer II Selectively Accumulate Intracellular Amyloid-β. Preliminary data in humans

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Research on subjects with a cognitive level ranging from mild impairment to severe Alzheimer's disease (AD) strongly implicate the entorhinal cortex (EC) at an early stage. In addition to EC-volume loss, EC layer II neurons undergo severe degeneration already before symptoms meet clinical criteria for AD. EC contains a population of Reelin-Immunoreactive (Reelin-IR) neurons in layer II, the majority of which project to the hippocampus. We utilized the McGill-R-Thy1-APP rat model for AD to test the hypothesis that intracellular Aβ in EC-layer II selectively express in Reelin-IR neurons during the early, pre-plaque stage. This model faithfully mimics the onset and distribution of amyloid plaque load seen in human AD subjects, and has an extended intracellular phase of Aβ-accumulation (*Heggland et al., 2015. EJN 12876*). By immunohistochemical double labeling, we obtained evidence that Reelin-IR neurons in EC-layer II selectively accumulate intracellular Aβ during the early, pre-plaque stage. Further, we found that neurons located towards the rhinal fissure have a higher burden of intracellular Aβ, which may have functional implications in view of the preferred reciprocal connectivity of this part of EC with the dorsal hippocampus. Further, using samples of human EC from three subjects with Braak stages I-III we observed that the Reelin-Aβ association directly translates to human AD-subjects. Our findings thus demonstrate that initial amyloid-related pathology in EC is restricted to the Reelin-IR neuronal population. Reelin may effect synaptic transmission of entorhinal-hippocampal projections, and interactions between Aβ and Reelin affect Reelin signaling. Synaptic plasticity in the entorhinal-hippocampal network may therefore initially change as a result of AD-related pathology emerging in Reelin-IR neurons.

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Inhibitory inputs to A17 amacrine cells in the rat retina

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Amacrine cells in the retina constitute perhaps the most striking exception to Cajal's law of dynamic polarization of a neuron, that the dendrites receive synaptic inputs from other neurons and the axons are responsible for transmitting output to other neurons. Instead, amacrine cells are characterized by synaptic inputs and outputs distributed throughout the dendritic tree. The A17 amacrine cell is a GABAergic wide-field amacrine cell that plays an important role in the scotopic pathway of the mammalian retina. It receives all of its excitatory input from rod bipolar axon terminals at varicosities located at the distal parts of the dendrites. The output is localized to the same varicosities and is directed exclusively back onto the rod bipolar terminals, forming reciprocal inhibitory synapses. From ultrastructural investigations, however, there is also evidence for synaptic input from other amacrine cells to A17 amacrines, but the input seems to be located at the proximal parts of the dendrites. This segregation between excitatory and inhibitory inputs challenges the hypothesis that signal processing in the A17 amacrine cells is confined to local microcircuits centered at each dendritic varicosity and suggests instead that spatial integration in these cells might occur over larger regions than previously thought. As a first step towards characterizing the inhibitory synaptic input to A17 amacrine cells, we have recorded spontaneous inhibitory postsynaptic currents (spIPSCs) in these cells by performing whole-cell patch-clamp recordings in rat retinal slices. A17 amacrines were visually targeted with IR-DIC video microscopy and spIPSCs were recorded in voltage clamp at a holding potential of -70 mV. The spIPSCs were completely and reversibly blocked by the GABAA receptor antagonist SR95531, but they were unaffected by the glycine receptor antagonist strychnine, indicating that the spIPSCs were GABAergic. The reversal potential of the spIPSCs was close to the equilibrium potential for chloride (ECl) and followed changes in ECl. The spIPSCs occurred at a relatively low frequency (1.1 ± 0.4 Hz, n = 16 cells) with an average amplitude of 23 ± 4 pA and a 10-90% rise time of 731 ± 20 µs (n = 10 cells). The decay phase could be well fit by a double-exponential function. These results suggest that A17 amacrine cells receive inhibitory synaptic from GABAergic amacrine cells.

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Inter-session reproducibility of diffusion tensor imaging tractography performed on cervical spinal cord in healthy subjects

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Background and objectives. Diffusion tensor imaging (DTI) is an MRI technique that provides information about the random thermal motion of water molecules *in vivo*. DTI tractography enables noninvasive image analysis of the structural integrity of axonal pathways at a cellular level. Here we assessed the reproducibility of DTI tractography performed on the cervical spinal cord in 14 healthy volunteers. Each subject was scanned twice and the inter-session reproducibility was analysed in terms of DTI volumetry, correlation between the T2 and DTI-based volumetric measures, and correlation between the number of fiber tracts and the number of voxels through which a tract projects.

Methods. MRI was performed on a 3 Tesla General Electric w750 scanner equipped with a 16 channel head-neck-spine array coil. High-resolution T2-weighted structural images were collected to measure the volume of examined 2D T2 FRFSE (35-43 slices according the size of the patient). The data used for calculation of volumetric axonal tracking were assessed with a DTI T2-weighted spin sequence, slice spacing 0mm, slice thickness 3 mm, field of view max. in average 18 -20 cm, B = 1000 s/mm^2; three B0 images were collected). Images were acquired with 18 independent directions. DTI data were acquired with an imaging sequence consisting of 35-43 transaxial slices. 5 slices were removed from the top and bottom of all the data sets to reduce the artefact effects at the edge of the images. Every voxel of the image was used as a seed-voxel for tractography, which eliminated user-involvement and reduced inter- and intra-observer variability. Tractography parameters were: FA threshold of 0.2, maximal angle change of 20°, minimal fiber length of 50 mm. The tractography results were visually checked for all the data sets. 8 data sets were excluded from the study due to low quality. To analyse the reproducibility of the various DTI tractography measures, Pearson correlation coefficients as well as the intra-class correlation (ICC) coefficient were used. The ICC measurement is considered highly reproducible for ICC > 0.9, moderately reproducible with values 0.7 < ICC < 0.9 and with low reproducibility with an ICC < 0.7. The study was conducted in accordance with ethical approval from the regional ethics committee (Dnr 2011-05-31M). Informed written consent was obtained from the volunteers.

Results and Discussion. The reproducibility of the T2 volume (mm^3) calculation was significant with a correlation coefficient of 0.979 ($p < 0.001$) and the ICC was 0.978 ($p < 0.001$). Additionally, there was a high correlation between the number of tracts and the number of voxels through which a tract projects for both measures of DTI volumetry. A high correlation existed between the DTI measures obtained using different tractography parameters. Thus, the results obtained were not affected by the specific choice of the DTI tractography parameters. The significant correlation that was observed between the T2-based and DTI-based volumetric measures for each subject at both time points is encouraging with respect to future routine clinical applications of this technology. This point is further supported by the fact that the statistical significance of the correlations was even higher when both time points were combined. As expected, a high correlation was also found between both measures of DTI volumetry, i.e. the number of tracts and the number of voxels through which a tract projects. This relationship enables fine tuning of the DTI data to include all the relevant fiber tracts that project through a particular section of the spinal cord.

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Monosynaptic tracing of inputs to PV interneurons in the medial and lateral entorhinal cortices

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Parvalbumin (PV) cells make up one of the largest populations of interneurons in the brain. Small and large basket cells as well as chandelier cells and several other morphological subgroups of interneurons have proved to be PV expressing. PV cells have fast-spiking electrophysiological profiles, and the basket cells in particular are known to have a profound effect on the local networks providing strong inhibition to the somata of surrounding principal cells. In the medial entorhinal cortex (MEC), it was shown that fast-spiking interneurons di-synaptically connect stellate cells in LII, and that these principal neurons do not contact each other directly (*Couey et al, Nat. Neurosci. 16:318*). Whether the same is true for the lateral entorhinal cortex (LEC) is unknown. In view of the unique properties of PV interneurons and their seemingly central role in the entorhinal local network, we have assessed the monosynaptic inputs of PV cells in both the medial and lateral subdivisions of the entorhinal cortex. We used monosynaptic retrograde tracing using a G-protein deleted rabies virus and a PV-cre transgenic mouse line.

The PV interneurons in the entorhinal cortex all seem to receive substantial input from the hippocampus and a number of neocortical areas, as well as from selected subcortical areas such as the thalamus, the amygdala and the medial septal complex. In general, the inputs resemble what has been observed in studies using conventional retrograde tracers. PV cells in the LEC receive stronger cortical inputs than those in the MEC, on average each LEC starter cell receives 37,3 cortical connections, while MEC starter cells on average receive 16,3 inputs per starter cell. On the other hand, PV cells in the MEC receive heavier input from the hippocampus than do LEC PV cells (6,2 inputs, and 3,6 inputs per starter cell respectively). The rabies tracing also provides a unique opportunity to look at interconnectivity within the LEC and MEC. The data indicate that LEC has substantially more connections between PV cells and the remainder of the cell population, compared to the MEC (26,3 connections per starter cell in the LEC, compared to 4,9 connections per starter cell in the MEC). In conclusion, the PV interneuron specific retrograde tracing corroborates previous retrograde tracer studies, and the neocortical and hippocampal inputs to PV cells resemble those of the total neuronal population in the two areas. Our results indicate that PV neurons are innervated proportionally similar to principal neurons in the LEC and MEC.

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L-DOPA-coated manganese oxide nanoparticles as dual MRI contrast agents and potential drug delivery vehicles

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Magnetic Resonance Imaging (MRI) is a powerful, non-invasive technique used for visualizing soft tissue. With MRI, it is possible to distinguish abnormal or compromised tissue structures, such as obstructed blood flow in heart vessels or potential malignant tumors. The contrast in an area of interest can be greatly enhanced by administrating positive contrast agents (CAs). Magnetic nanoparticles (NPs) are emerging as promising CAs in MRI, due to their large surface-to-volume ratios that offer an enormous contact area between magnetically active surfaces and water molecules. This in turn creates changes in the local magnetic field during MRI, and alters the contrast in that region[1]. The magnetic NPs can be functionalized with drugs, which makes them applicable both for diagnostics and therapy[2]. In the work presented here, we have synthesized multifunctional paramagnetic manganese oxide (Mn_2O_3) NPs that show both positive (enhanced/T_1) and negative (decreased/T_2) contrast. We functionalized the Mn_2O_3 NPs with L-DOPA which is a precursor for the neurotransmitter dopamine, and the current drug for Parkinson's disease. The effect on the contrast was determined *in vitro* in pig eyes (**Figure 1, left**) and *in vivo* in rats. Our results indicate that the Mn_2O_3 NPs are water degradable, which causes a time-dependent switch in the contrast. First, the Mn_2O_3 NPs give off a negative contrast, but as they degrade they release Mn^{2+} ions which enable manganese Enhanced MRI (MEMRI, **Figure 1, left**). In addition, the concentration of L-DOPA increased with time, which we interpret as release of L-DOPA from the surface of Mn_2O_3 NP (**Figure 1, right**).

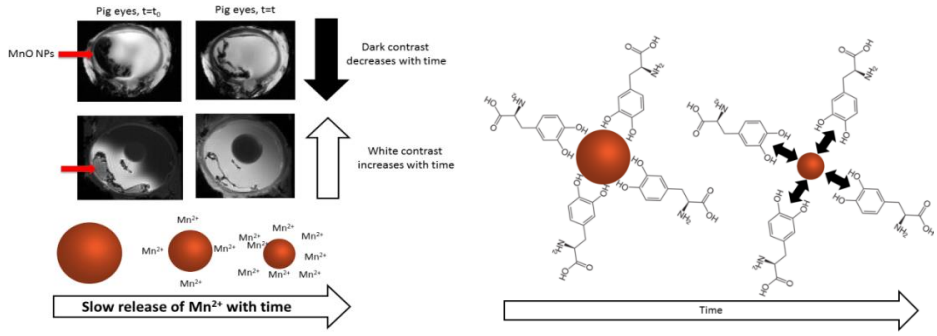

Figure 1: To the left, the time-dependent T1 and T2 contrast are shown in pig eyes. To the right, illustration of release of drug from the NPs.

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GLP-1, exendin-4 and diazepam modulate $GABA_A$ receptor-mediated synaptic and tonic currents in the rat hippocampus

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Glucagon-like peptide-1 (GLP-1) is a gut hormone that induces glucose-dependent stimulation of insulin release from β-cells and lowers glucagon secretion from α-cells in the pancreatic islets. GLP-1 receptors are found not only in peripheral tissues but also in many brain areas like the basal ganglia, septum, thalamus, hypothalamus, brain stem as well as in the hippocampus. The hippocampus is known centre for learning and memory formation and is important for cognition. Recently we have showed that GLP-1 and exendin-4 modulate γ-aminobutyric acid (GABA) signaling in the hippocampal CA3 pyramidal neurons. We studied how the GLP-1 receptor agonists GLP-1 and exendin-4 and diazepam, the positive allosteric modulator of the $GABA_A$ receptors altered $GABA_A$ receptor-mediated synaptic and tonic currents in the CA3 pyramidal neurons. Hippocampal slices from 16–20 days old Wistar rats and whole-cell patch-clamp technique were used to record $GABA_A$ receptor-mediated currents in the CA3 pyramidal neurons. GLP-1 transiently enhanced the amplitudes (at physiological concentration) and frequency of the spontaneous inhibitory postsynaptic currents (sIPSCs) as well as augmented the $GABA_A$ receptor-mediated tonic current. Diazepam induced increase in frequency and amplitudes of the sIPSCs and simultaneously sustained a potentiation of $GABA_A$ receptor-mediated tonic current. Continuous consecutive co-application of exendin-4 and diazepam did not evoke further enhancement in neither frequency, nor amplitudes of the sIPSCs but transiently increased the tonic current amplitude. The results demonstrate that GLP-1and its analogues enhance sIPSCs and a subpopulation of extrasynaptic $GABA_A$ receptors in hippocampal CA3 pyramidal neurons. These results further support the suggestion that metabolic hormones influence hippocampal function.

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Pyramidal cells-OLM interneurons network underlies theta activity and anxiety related behavior in the ventral hippocampus

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Theta oscillations in the dorsal hippocampus are described as one of the most prominent rhythms of the brain. This 4-12 Hz have been associated with multiple behaviors, especially with movement. Differentially, theta activity in the ventral hippocampus have been implicated in emotions related behavior. It was suggested that specific GABAergic interneuron subtypes play differential roles in driving hippocampal oscillations. Although different populations of hippocampal interneurons fire preferentially to specific phases of theta, phase-locking firing itself does not prove a causal role in theta generation. We found a specific subtype of oriens lacunosum-moleculare (OLM) interneurons expressing Chrna2 receptor differentially distributed along the dorso-ventral hippocampal axis. Using optogenetic tools in anesthetized and freely moving animals, we found that activation of this population induce prominent theta activity in the ventral but not in the dorsal hippocampus. This circuit includes pyramidal cells activity. Interestingly, the induced theta rhythm was not correlated with animals' movements. In addition, we found that this induced theta activity regulates anxiety related behavior. Taken together, our results provide the first evidence of a single morphologically defined cell population which in a network including pyramidal cells causally drives ventral hippocampal theta oscillations.

CHRISTIAN BROBERGER

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Dopamine autoregulation in tuberoinfundibular dopamine (TIDA) neurons

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Neuroendocrine tuberoinfundibular dopamine (TIDA) neurons in the hypothalamic arcuate nucleus regulate reproduction by providing tonic inhibition of pituitary release of the hormone, prolactin (see Ben-Jonathan & Hnasko, 2001). As in all neuroendocrine axes, feedback regulation at several levels is key to maintaining appropriate serum hormone concentrations. Yet, the effects of dopamine receptor activation have not been studied on the passive and active membrane properties of the TIDA network.

In whole-cell recordings from male rat hypothalamus slices, TIDA neurons can be readily identified by a robust oscillation between depolarized states crowned by action potentials and quiescent hyperpolarized states (Lyons *et al*., 2010, Neuron). Application of dopamine or the D2-like receptor (D2R) agonist, quinpirole increased the duration of the slow depolarizing phase of the hyperpolarized state, the duration of the depolarized state and decreased the frequency of the TIDA oscillation. Application of the D1R-like agonist, SKF81297 (10μM), did not affect TIDA oscillation properties. During application of the D2R antagonist, eticlopride (20μM), TIDA behaviour changed dramatically; the 0.05Hz oscillation was replaced by a faster fluctuation at a depolarized membrane potential (ca. -45mV) and action potential discharge ceased, possibly due to depolarization block. Similar effects were seen with the antipsychotic, haloperidol. Quinpirole application resulted in a hyperpolarization of TIDA cells, likely due to a shift in the balance of excitatory and inhibitory input, as well as attenuation of Ca2+ currents.

These findings identify a novel ultra-short feedback control of the TIDA network, where activation of D2 autoreceptors sets oscillation frequency that may determine dopaminergic tone in the pituitary. Our results suggest a system that homeostatically and continually tunes itself to ongoing activity reflected in ambient somatic levels of the neurons' own transmitter. Our data further indicate a novel mechanism for the sexual side effects of antidopaminergic antipsychotic drugs.

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CHRISTIN BERNDTSSON

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Monosynaptic inputs to cells in deep layers of the lateral entorhinal cortex revealed through cre-dependent rabies tracing

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The anatomy and function of the parahippocampal region (PHR) has been extensively studied. The PHR, and more specifically the entorhinal cortex (EC) was for a long time viewed as the input-output structure of the hippocampal formation (HF). This view of the EC has rapidly become antiquated as more and more functional and anatomical data suggest that the EC provides essential information and processing to the cortico-hippocampal circuit. Several types of spatially tuned neurons have been found in the medial entorhinal cortex (MEC), indicating its relevance in spatial navigation and memory processing. Conversely, less is known about the function of its lateral counterpart, the lateral entorhinal cortex (LEC). It has been shown to be involved in memory processes, but only weakly spatially modulated cells have been found.

The superficial layers of EC receive information from several brain regions and give rise to the main input to HF, the perforant path projection. In contrast, cells in deep layers receive input from the HF, have their dendrites extending up to the superficial layers and have also been shown to both receive input from, and project out to cortex. Therefore, the deep layers of EC have the potential to serve as an integrator, processing not only the information from the HF, but also the cortical and subcortical information coming into both superficial and deep layers of EC.

The majority of anatomical studies on EC have used conventional tract tracing techniques with a focus on rat anatomy. However with the emergence of transgenic mice and complementary viral techniques it is possible to provide a more targeted, cell-specific investigation of connectivity. Moreover, as more behavioral and functional studies are done using transgenic mice, knowledge of the mouse anatomy is essential. In this study we utilized the Ntsr1-cre mouse line in combination with a cre-dependent AAV helper virus and G-protein deleted rabies virus. This allowed us to specifically dissect out the inputs to cells in deep layers of the dorsolateral entorhinal cortex (DLE).

Our data show monosynaptic inputs to cells in deep layers of DLE from several regions, including piriform cortex, the hippocampal formation, putative auditory cortex, and several amygdaloid nuclei. This seems to be in line with studies using conventional tracers, indicating that our approach works.

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Time- and region-specific down-regulation of high-affinity GHB binding sites in SSADH knock-out mice

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γ-Hydroxybutyric acid (GHB) is a metabolite of γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the CNS. Normal levels of GHB in physiological fluids are less than 3 μM, whereas they are dramatically increased (500-1000 μM) in patients with the rare inheritable metabolic disorder succinate semialdehyde dehydrogenase (SSADH; *aldh5a1=aldehyde dehydrogenase 5a1*) deficiency. Such patients suffer from intellectual disability with deficits in expressive language, hypotonia, ataxia and in ~50% of patients epileptic seizures. The pathophysiological mechanisms for these symptoms are not fully understood. In the CNS, GHB is known to bind to both low- and high-affinity sites. GABA-B receptors are well-known low-affinity binding sites and mediate important pharmacological effects of GHB at mM concentrations. Highaffinity sites are highly expressed in the hippocampus and cortex. They are referred to as putative GHB receptors as their molecular identity is uncertain. Interestingly, in SSADH-deficient (knockout) mice, treatment with GABA-B antagonists and the selective GHB receptor antagonist, NCS- 382, significantly prolonged life span in these mice, supporting the concept that both GABA-B and putative GHB receptors are involved.

The aim of the study was to test the hypothesis that increased GHB levels, due to SSADH deficiency, causes a down-regulation of the high-affinity GHB binding sites. To test this, we performed autoradiography on mouse brain slices from PN13 and PN20 (postnatal age, days) SSADH knock-out mice and age-matched wild-type litter mates using the radioligand [^{3}H]3-hydroxycyclopent-1-enecarboxylic acid ([^{3}H]HOCPCA), which is highly specific for the GHB high-affinity binding sites (KD ~100 nM). Binding experiments were performed at pH 6.0 using 1 or 5 nM radioligand concentrations.

In brain sections from PN13 SSADH knock-out mice we found a significant decrease in binding levels in the hippocampal CA1 region compared to wild-type mice. This effect was not seen in P20 brain slices. By contrast, we found no differences in binding levels in the CA3 hippocampus, frontal cortex or striatum of the PN13 mice. Similarly, no differences were found in the binding levels of either frontal cortex, striatum or the CA3 region of hippocampus between PN20 SSADH knock-out and wild-type mice.

These data suggest a time- and region-specific down-regulation of GHB high-affinity binding sites in SSADH knock-out mice, and point to a potentially important role for these in SSADH-deficient patients. Our results are consistent with the temporal progression of seizures in the mouse model (absence, tonic-clonic, status epilepticus; Vogel et al 2013, J. Inherit Metab. Dis., 36; 401-10). To expand on these findings we are currently evaluating other GHB-specific radioligands and pH values.

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Decrease in synaptic AMPA receptor expression in the rat hippocampus in chronic medial temporal lobe epilepsy

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Neurodegenerative diseases induce brain cell death. However, surviving neurons may show synaptic plastic changes, which may either protect against further degeneration, or may further aggravate the condition. In any case, surviving synapses hold information crucial to understanding the diseased brain. In the present study, we wanted to detect changes in glutamatergic synapses in chronic epilepsy. Specifically, we have investigated the synaptic proteins GluA1, GluA2, PICK1 and synaptotagmin 1 in the rat hippocampus in chronic medial temporal lobe epilepsy (MTLE). Other synaptic proteins were used as controls: β-tubulin (as loading control), synaptophysin, PSD-95, NR2B, and GluK2. We used a kainic acid-induced rat model of chronic MTLE (eight weeks after first status epilepticus). Western blotting was used to examine relative concentrations of these proteins in synaptic fractions (synaptosomes) in chronic MTLE and control groups. In addition, immunogold electron microscopy was used for synaptotagmin 1, in glutamatergic synapses of the stratum radiatum of the CA1 and the CA3 areas of the hippocampus. We found a significant reduction in the AMPA receptor subunits GluA1 and GluA2 as well as the glutamate receptor interacting protein PICK1, and the Ca^{2+}-sensor and SNARE-interacting protein synaptotagmin 1 in the chronic MTLE groups compared to controls. No significant changes were observed for synaptophysin, PSD-95, NR2B and GluK2. Due to the highly significant reduction observed for synaptotagmin 1 as well as its role as a Ca^{2+}-sensor, quantitative immunogold electron microscopy analysis was carried out to determine the subsynaptic sites for this reduction. Synaptotagmin 1 was significantly reduced in the pre- and postsynaptic cytoplasm of CA3 area synapses. The reduction in synaptic concentrations of all four proteins (GluA1, GluA2, PICK1 and synaptotagmin 1) in the hippocampus point to adaptive or protective measures in surviving neurons and their synapses. These findings will be useful in understanding the molecular mechanisms underlying chronic MTLE.

DANIEL TORNERO

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Direct synaptic inputs from diverse host brain areas to grafted human iPSC-derived cortical neurons in stroke-injured rat cortex

AUTHORS: Daniel Tornero, Cristina Rodriguez, Somsak Wattananit, Olle Lindvall and Zaal Kokaia.

We have recently shown improved functional recovery after transplantation of human induced pluripotent stem cell (iPSC)---derived cortical neuronal precursors in a rat model of cortical stroke (Tornero et al., 2013, Brain). Grafted cells give rise to mature neurons that rebuild the damaged tissue and send fibers to several host brain structures. However, whether the grafted neurons receive direct synaptic inputs from the host brain and are functionally integrated into its neuronal circuitries is unknown.

Here we used a rabies virus (RV)-based strategy to explore whether host cells establish functional synaptic connections with the transplanted cells. Rabies-G Glycoprotein was replaced with red fluorescent protein (RFP) gene in the genome of the virus and the envelope was substituted with the foreign coat protein EnvA, generating replication incompetent virus that only can infect cells expressing TVA receptor. Human iPSC-derived long-term neuroepithelial like stem (lt-NES) cells were transduced to express Rabies-G glycoprotein, avian TVA receptor and histone-green fluorescent protein (HGFP) under the control of human synapsin I promoter (HTB construction). Two or five months after intracortical transplantation of HTB-lt-NES cells in a rat stroke model, we injected the RV in the location of the graft. Expression of TVA receptor in the mature neurons (synapsin I+) generated from grafted cells makes them suitable for infection with the RV. The presence of Rabies-G glycoprotein in these cells allows the virus to infect the cells that connect to them by functional synapses. Therefore, grafted and infected cells will express nuclear GFP and cytoplasmic RFP while the ones connected to them will only present RFP in the cytoplasm.

Immunohistochemical analysis of injured and transplanted brains one week after the infection with RV revealed the presence of RFP+ neurons in different areas, some of them located far away from the implantation site. The distribution of these cells corresponds with the proper anatomical locations of neurons projecting to the cortex in the intact brain. Comparison of early and later time-points (2 and 5 months, respectively) gives insight into the dynamics of lt-NES cell integration after transplantation in the damaged-brain.

We demonstrate for the first time that intracortical grafts of human iPSC-derived cortically fated neurons establish afferent synaptic connections with diverse areas in the stroke-injured brain. Further studies should evaluate how these connections may influence the maturation and function of the transplanted cells.

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The role of estrogen alpha receptors in sociosexual behavior in female rats housed in a seminatural environment.

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Estrogen is essential for the display of sociosexual behaviors. Females lacking estrogen receptors alpha (ERα) in the ventromedial nucleus of the hypothalamus (VMN) do not show paracopulatory behaviors, while ERα reduction in the preoptic area (POA) and medial amygdala (MePD) has no effect. The role of ERα in the bed nucleus of stria terminalis (BNST), on the other hand, has not been investigated before. Current knowledge comes from behavioral models that use only one or two individuals. In nature, however, rats copulate in groups and occupy a considerable amount of space.

Now, we used a semi-natural environment (2.8 x 2.4 x 0.75m) with burrows and open spaces, which is suitable to investigate the effects of estrogens in a group of rats in an environment where a substantial proportion of their behavioral repertoire can be expressed. We investigated the role of ERα in sociosexual behaviors and attractiveness in female rats. We used a shRNA encoded within an adeno-associated viral (AVV) vector directed against the ERα gene to reduce the number of ERα in the VMN and POA (Experiment 1), and BNST and MePD (Experiment 2) in female rats. Groups of four females and three males lived in the environment for 8 days. Behavioral observation was performed after hormonal injections.

In experiment 1, it was shown that ERα reduction in the VMN and MPOA diminished social interactions and attractiveness compared to controls. It also reduced the display of paracopulatory behaviors and lordosis responses. We suggest that the reduced attractiveness could be the cause of the diminished proceptivity. In experiment 2, ERα reduction in the BNST and MePD induced no differences in sociosexual behaviors compared to controls.

In conclusion, ERα in the BNST and MePD are not involved, while ERα in both the VMN and MPOA are essential for the display of sociosexual behaviors in a semi-natural environment. In a standard mating test, in contrast, only ERα in the VMN are necessary for these behaviors. These experiments show that it is essential to use the appropriate test paradigm to draw valid conclusions about the role of estrogens in sociosexual behaviors.

EIRIK NILSSEN

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Local connectivity and immunoreactivity of principal cells in layer II of lateral entorhinal cortex

E.S. Nilssen, G. Fjeld and M.P. Witter

Neurons in layer II of the entorhinal cortex provide a strong input to the dentate gyrus (DG) and CA3 of the hippocampal formation. In medial entorhinal cortex (MEC), DG-projecting cells are mainly, if not exclusively, stellate cells, and positive for the molecular marker reelin but not for calbindin. Stellate cells are connected with each other almost exclusively through inhibitory interneurons. In lateral entorhinal cortex (LEC), in contrast to MEC, all principal cell types (fan, pyramidal and multiform) project to the DG. Little is known about the local synaptic connectivity of these cells and their immunoreactivity.

The aim of the present study was to investigate whether the principal cell local network in LEC layer II is similar to the stellate cell network in MEC, or whether monosynaptic excitatory connections prevail. Furthermore, we aimed to check if there is a correlation between cell type identity and immunoreactivity for reelin or calbindin. We carried out simultaneous whole-cell recordings *in vitro* of clusters of up to four neurons in LEC LII, and filled neurons with fluorescent dyes during recording and subsequently immunostained for reelin and calbindin. Neuronal morphology and immunoreactivity were assessed with confocal laser-scanning microscopy and 3D-reconstructions. The data set includes recordings of 630 pairs of principal cells, of which 105 cells were selected for immunocytochemical analysis. Among 98 three-cell and 56 four-cell clusters, direct excitatory connections were observed in nine clusters, whereas indirect inhibitory connectivity was detected only in a single cluster. Most recorded clusters contained a mix of fan, pyramidal and multiform cells, however, fan cells were most abundant. Immunostaining of the recorded cells revealed a high degree of overlap with reelin, and clearly showed that reelin-positive cells are found among fan, pyramidal and multiform cells. Furthermore, no connections were detected between these reelin-positive cells. Given that the majority of our recordings were from fan cells, the results indicate that direct excitatory connectivity between these cells is sparse. The low number of disynaptic inhibitory connections is surprising, and might be due to weak connectivity with inhibitory interneurons. It is more likely, however, that the three principal cell types interact with separate populations of interneurons, making it difficult to detect disynaptic inhibitory connections in our clusters containing multiple principal cell types.

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Ciliated neurons lining the central canal sense both fluid movement and pH through ASIC3

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Cerebrospinal fluid-contacting (CSF-c) cells are found in all vertebrates but their function has remained an enigma over more than a century. Ideas regarding their function have varied from signaling fluid movements to sensing the chemical composition of the cerebrospinal fluid. We have recently characterized lamprey CSF-c cells according to their morphology, phenotype, and electrophysiological properties, and identified two distinct types. A class of CSF-c cells express active membrane properties. They co-express GABA and somatostatin and receive both GABA- and glutamate-mediated synaptic input. These CSF-c neurons have a bulb-like ending with a cilium that protrudes into the central canal and a lateral process, with collaterals to the gray matter, that extends to the lateral margin and form a plexus around the stretch-sensitive dendrites of mechanoreceptors (edge cells). To investigate the physiological role of the CSF-c neurons, we performed patch clamp recordings from these neurons in longitudinal sections of the spinal cord in which the central canal was exposed, while the cells were subjected to micro-jets of fluid and decreases of the extracellular pH. A short pressure pulse applied via a fluid-filled micropipette close to their cilia, reliably elicited receptor- or action potentials. These responses remained after application of GABA and glutamate antagonists. The next question was to identify which ion channel was responsible for the mechanotransduction. One candidate was the acid sensing ion channel 3 (ASIC3) that is expressed in sensory neurons and nerve endings. By bath-applying APETx2, an ASIC3-specific blocker, while testing for the mechanical response to fluid pulses, we could show that the response was eliminated in the presence of APETx2. To explore whether the presence of ASIC3 in lamprey CSF-c neurons would also make these cells pH-sensitive, we applied extracellular solutions of low pH (from pH 7.4 to pH 6.9 and 6.5), while performing patch recordings. The frequency of the spontaneous action potentials of CSF-c neurons reversibly increased upon exposure to decreases in pH. Following a blockade of ASIC3 with APETx2, also this response to lowered pH was eliminated. Lowering the pH also slowed down the locomotor burst frequency as recorded in the ventral roots (in 100 μM NMDA bath application). As CSF-c neurons express somatostatin and they are the only source of somatostatin within the lamprey spinal cord also examined the effect of somatostatin on the locomotor network. Somatostatin slowed down the fictive locomotor burst frequency in a dose-dependent manner (10nM, 100nM and 1μM). Moreover, administration of a somatostatin antagonist (CYN-154806, a selective somatostatin sst_2 receptor antagonist) reduced or abolished the effect of lowering of the pH, suggesting that these effects are mediated by CSF-c neurons releasing somatostatin while lowering the pH. To investigate if CSF-c neurons receive input from the spinal locomotor network, their activity was recorded during fictive locomotion. Our results showed that CSF-c neurons do not appear to receive input from the locomotor CPG under these conditions. Our results suggest that CSF-c neurons act both as mechanoreceptors and as chemoreceptors through ASIC3 channels, and when activated reduce the activity in the locomotor network. During active swimming movements, they may also suppress the sensory feedback provided by the stretch receptor neurons. The CSF-c neurons may thus serve as a negative feedback system.

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Can optogenetic stimulation of G-protein coupled receptor 81 rescue cognitive decline?

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In the present graduate program project, we use optogenetics to determine the physiological effects and molecular mechanisms of a G-protein coupled receptor, the lactate receptor GPR81, which we recently discovered to be active in the brain (Lauritzen KH et al. 2014, Cereb Cortex). By combining a photosensitive molecule with the receptor, we will make a chimeric receptor construct, OptoGPR81, that will be transfected *in vitro* and *in vivo.* (Airan RD et al. 2009, Nature). This procedure allows the receptor to be switched on and off instantaneously by photoactivation *in vitro* in brain slices or cultured cells as well as *in vivo* in behaving mice or rats. The optogenetic approach therefore will allow the function of the receptor to be studied with greater spatiotemporal resolution than possible with classical pharmacological methods. The novel tools (GPR81 optogenetics and antagonists) developed in conjunction with the project will be used to reveal whether the lactate receptor can ameliorate cognitive decline, as tested in a mouse model of Alzheimer's disease. In parallel, GPR81 agonists, and antagonists when available, will be used to explore the effects of the receptor on the intracellular signalling hub of dendritic spines by transcriptomics and proteomics in wild-type and GPR81 knock-out mice. The effects of GPR81 on metabolic flux will be investigated using SeaHorse approach. Canonical cAMP signalling (such as PKA, CREB) as well as non-canonical signalling (such as through β-arrestin, G-protein transactivation) and effects on neurotransmitter receptors (such as NMDA and AMPA type glutamatergic receptor subunits) will be explored.

EMIL JAKOBSEN

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Understanding brainpower: Role of glycogen phosphorylase isoforms for astrocyte bioenergetics

Emil Jakobsen, Ann-Kathrin Reuschlein, Anne B. Walls, Helle S. Waagepetersen, Lasse K. Bak

While the brain as an organ relies on glucose from the blood, astrocytes contain a small, albeit metabolically active amount of glycogen (1). Contrary to common beliefs, glycogen is not a static molecule but highly dynamic being degraded and rebuild continuously – a process that is important for neurotransmission and memory formation, and seems to be perturbed in some pathologies including diabetes and epilepsy (1-4). In astrocytes, two isozymes of glycogen phosphorylase, GPM and GPB, degrade glycogen (1). The differential regulation of these two isozymes in situ has been somewhat enigmatic; thus, we now ask if the two isozymes respond distinctly to energy-requiring tasks performed by astrocytes such as neurotransmitter or K^+ uptake. To begin to address this question, we have performed differential siRNA-mediated knock down of the two isozymes in cultured astrocytes, and investigated cellular bioenergetics employing the Seahorse Biosciences XFe96 instrument. The preliminary data suggest that GPB plays a distinctive role in supporting astrocyte bioenergetics since knock down of GPB increased both glycolysis and cellular respiration. It will be interesting to unravel the mechanistic underpinnings of this observation, both for our understanding of basic neurochemistry as well as for the possibility that some novel drug targets may be exposed.

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EMMY RANNIKKO

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Choline acetyltransferase as a potential PET tracer target for diagnosis of prodromal Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and greatly affects millions of patients and their families. Yet, our understanding of the molecular and cellular mechanisms of the disease is incomplete and no cure is available. The current clinical criteria of AD contain biomarkers of the disease, for example the decrease of $A\beta_{1\text{-}42}$ and increase of Tau in the cerebrospinal fluid or the increased tracer retention on amyloid PET[1]. However, there are still insufficient tools to diagnose prodromal AD. An early, preferably even preclinical, detection would enable an earlier start of therapy and thereby hopefully slow down the disease progression even before significant changes in the cognition would have occurred.

It is the cholinergic neurons that are the most affected neurons in AD and currently the only clinically used treatment of AD is increasing the acetylcholine levels by inhibition of the acetylcholine degrading enzymes. Choline acetyltransferase (ChAT) is the only known enzyme that synthesizes acetylcholine and our group has previously shown changes in ChAT levels during AD[2,3]. A long term goal of the work in our group is to develop a PET tracer molecule that could enable monitoring these changes in patients. We use docking based *in silico* screening using available chemical databases to identify molecules that potentially bind to ChAT. The candidate molecules are thereafter screened *in vitro* looking at their ability to decrease ChAT enzyme activity. Here we show more evidence that ChAT is present in extra cellular fluids like blood plasma and cerebrospinal fluid. Moreover we have confirmed that that 2-naphthoylethyltrimethylammonium, to date the only published inhibitor of ChAT[4], indeed decreases the activity of recombinant human ChAT. We further show the ability of a number of candidate molecules, which emerged in a docking screen, to inhibit the enzymatic activity of our recombinant ChAT.

Abbreviations: AD – Alzheimer's disease, ChAT – Choline Acetyltransferase
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EMRE YAKSI

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Sensory processing in zebrafish habenula

Suresh Kumar Jetti, Nuria Vendrell Llopis, Ewelina Bartoszek, Emre Yaksi

The habenula is a highly conserved brain region, which connects the forebrain and the limbic system to monoaminergic brainstem nuclei that regulates behavior. The habenula is organized into anatomically distinct sub-regions which innervate different brainstem target areas, releasing dopamine, serotonin and acetylcholine. Genetic ablation of habenula in zebrafish and habenular lesions in rats perturb experienced depended fear response. Thus understanding habenular computations are important for understanding the neural correlates of stress, fear and anxiety.

In my poster, I will present our findings on functional and topographically organization of habenular networks in zebrafish brain, by using two photon calcium imaging, electrophysiology and animal behavior. I will discuss about how habenula process sensory information, how sensory representations in habenula is modulated by animals' internal states and how habenula can contribute to regulating animal behavior.

GRETHE MARI OLSEN

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Thalamic and cortical connectivity of the posterior parietal cortex in the rat

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Abstract: The posterior parietal cortex (PPC) of the rat is a multimodal association area that has been proposed to participate in directed attention and spatial navigation. While the functional aspects of PPC have been widely studied, the anatomical aspects have not been agreed upon. In particular, the border of PPC with the neighboring visual domain has been discussed. We set out to define PPC based on architectural criteria and corroborated the delineations with thalamic connectivity patterns. Based on Nissl stained sections from an example brain we were able to distinguish PPC from its surrounding areas and to subdivide PPC into three distinct areas – a medial (mPPC), lateral (lPPC) and posterior (PtP) portion. We next investigated thalamic and cortical connectivity of these three areas.

Retrograde and anterograde tracers were injected across the parietal cortical domain, including somatosensory and visual cortices. PPC lacked connections with primary sensory ventral posterior and dorsolateral geniculate nuclei, but was found to be connected with the lateral posterior nucleus and posterior complex. Moreover, the connections were topographically organized such that mPPC was reciprocally connected with the medial portion of the lateral posterior nucleus, lPPC was reciprocally connected with the dorsal portion of the posterior complex, and PtP projected to a more ventral portion of the posterior complex.

Next, we investigated the connections of PPC with the parahippocampal region. In general, PPC received input from layer V cells in the entorhinal cortex as well as from neurons in deep layers of the peri- and postrhinal cortices. In turn, all areas of PPC provided a spatially restricted moderately dense input to the dorsal presubiculum layers I and III, but the projection from lPPC was weaker than the projections from mPPC and PtP. Fibers from all PPC areas reached layer VI of postrhinal cortex, entorhinal cortex, and parasubiculum. In addition, fibers from PtP reached layer V and I of posterior LEC.

Finally, we studied the connections of PPC with anterior cingulate and retrosplenial cortices. Connections were reciprocal and strongest with cingulate area 24b and anterior levels of retrosplenial area 30. Connections with anterior cingulate cortex differed between PPC subregions: mPPC showed reciprocal connections with the most anterior and most posterior portions, lPPC was only weakly connected with anterior cingulate cortex, and PtP projected to the middle and most posterior portions. In addition, PPC was found to be heavily interconnected with area M2: mPPC showed reciprocal connections with the entire extent while lPPC was reciprocally connected with the anterior and middle portions. PtP projected to the anterior and middle portions of M2.

In conclusion, we were able to distinguish PPC from its surrounding cortical areas, and to subdivide PPC into three different cytoarchitectonic areas. These areas differ in connectivity with thalamic nuclei, the parahippocampal region and the cingulate and retrosplenial cortices.

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AL QUDS

Depression Impairs Learning, whereas the Selective Serotonin Reuptake Inhibitor, Paroxetine, Leads to Overgeneralization in Treatment-Responsive Patients with Major Depressive Disorder

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Abstract

To investigate how medication status and task demands affect cognition in Major Depressive Disorder (MDD), we used between-subjects and within-subjects designs to evaluate patients with MDD and matched healthy controls. All subjects were administered a computer-based cognitive task with two phases, an initial phase in which a sequence is learned through positive feedback (which our prior studies suggest is striatal-dependent), followed by a generalization phase that involves a change in the context where learned rules are to be applied (which our prior studies suggest is hippocampal-region dependent). Using between-subjects design (Experiment #1), we tested medication-naïve patients with MDD, medicated patients with MDD receiving and responding to the Selective Serotonin Reuptake Inhibitor (SSRI) paroxetine, and healthy controls. Medication-naïve MDD patients were slow to learn the initial sequence but were normal on subsequent generalization of that learning. In contrast, medicated patients learned the initial sequence normally, but were impaired at the generalization phase. Experiment #2 utilized a within-subjects design, where we tested medicationnaïve MDD patients both before and 4-6 weeks after they were stabilized on SSRI regimen. We assessed response to SSRI administration 6 weeks after diagnosis. Healthy control subjects were also tested twice at the same time interval. Healthy subjects exhibited significantly better sequence-learning and generalization than patients with MDD at both baseline and retest. However, the effect sizes of these differences differed between baseline and retest. We argue that these data suggest (i) an MDDrelated impairment in striatal-dependent sequence-learning which can be remediated by SSRIs and (ii) an SSRI-induced exacerbation of impairment in hippocampal-dependent generalization of past learning to novel contexts.

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Typical environmental and specific social enrichment equally improve performance in a delayed alternation task after hippocampal injury in rats

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Abstract

Enriched environment (EE) has been shown to have a beneficial effect on cognitive recovery after brain injury. Typical EE comprises three components: i) enlarged living area providing physical activation, ii) sensory stimulation, and iii) social stimulation. The present study assessed the specific contribution of the social stimulation. Animals were subjected to either a sham operation or transection of the fimbria-fornix (FF) and randomly divided into groups of 1) a typical EE, 2) pure social enrichment (SE), or 3) standard housing (SH). The effect of these conditions on acquisition of delayed alternation task in a T-maze was assessed. The sham control groups were not affected by housing conditions. In the lesioned groups, both typical EE and SE improved the task acquisition, compared to SH. A baseline one-hour activity measurement confirmed an equal level of physical activity in the EE and SE groups. After delayed alternation testing, pharmacological challenges (muscarinergic antagonist scopolamine and dopaminergic antagonist SKF-83566) were used to assess cholinergic and dopaminergic contributions to task solution. Scopolamine led to a marked impairment in all groups. SKF-83566 significantly enhanced the performance of the lesioned group subjected to SE. The results demonstrate that housing in a typical as well as atypical EE can enhance cognitive recovery after mechanical injury to the hippocampus. The scopolamine challenge revealed a cholinergic dependency during task performance in all groups, regardless of lesion and housing conditions. The dopaminergic challenge revealed a difference in the neural substrates mediating recovery in the lesioned groups exposed to different types of housing.

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Optimizing classification of MEG responses to motor imagery

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Purpose: Motor imagery (MI) augmented with real-time feedback could be a useful technique in the rehabilitation of motor function in stroke patients. The challenge in providing reliable feedback is to detect relevant brain activity from noisy MEG/EEG measurements. In this study, we attempted to validate a robust feature extraction method for classification of hand MI in MEG. Multiple time–frequency decomposition and spatial filtering methods were compared in terms of their ability to capture relevant signal features for subsequent classification.

Methods: MEG was measured from 9 healthy subjects, who were visually cued to imagine left- or right-hand movements. Data were filtered to 8–30 Hz and segmented to 4-s epochs. Time–frequency features were extracted with short-time Fourier transform (STFT), Morlet wavelets, multitaper power spectral density and Fourier ICA[1]. 100 most relevant features were retained for the classification. Spatial filtering methods included Common Spatial Patterns (CSP, [2]), Filter Bank CSP [3] and Spatio–Spectral Decomposition (SSD, [4]), followed by calculation of averaged band-power features. A naïve-bayes algorithm was used for classification and its performance evaluated with 5-fold cross-validation.

Results: All subjects achieved > 65% single-trial accuracy in left-vs-right classification and > 70% in MI-vs-rest classification with at least one feature extraction method. CSP and FBCSP yielded best mean accuracy over subjects in left-vs-right classification: 70% and 69%, respectively. The combination of SSD and CSP yielded the best mean accuracy of 81% in MI-vs-rest classification. Features extracted with wavelet and STFT also yielded good accuracy.

Conclusions: Despite the significant inter-subject variability, good classification results were achieved for most subjects. These results suggest that both band-power features obtained by spatial filtering and carefully selected time–frequency features are efficient for characterizing untrained subjects' MI. However, individual calibration data is needed before the real-time neurofeedback can be used, which proposes a challenge for the clinical application. The intersubject generalization capability of each method will be evaluated in further studies.

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NEST: A Mature Brain Simulator

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Efficient and reliable simulation tools are essential for progress in brain research. Since the early days of neuronal computing [1], a wide range of simulators have been developed, each specialized on one or few spatial and temporal scales [2]. But the reliable and reproducible simulation of such complex systems as the brain remains a demanding challenge. But Computational Neuroscience is maturing: Just as our colleagues in electrophysiology, we begin to base our work increasingly on the use of standard tools, with modifications and adaptations for our particular research, instead of building home-brew solutions from scratch. This concentration was not least the result of several large-scale EU funded projects, such as FACETS and BrainScaleS.

From its humble beginnings as a PhD-student project 20 years ago, the Neural Simulation Tool NEST [3] saw its first incarnation as the SYNOD simulator in 1995 [4], leading to exciting results on synfire chains early on [5]. By tightly coupling software development with computational neuroscience research, simulator technology evolved steadily, facilitating new scientific insight at (nearly) every step. Some key examples were parallelization [6, 7], exact integration of model equations [8], precise spike times in a time-driven simulator [9, 10], spike-time-dependent [11] neuro-modulated plasticity [12], and a Topology module for spatially structured networks [13]. Streamlined data-structures allow NEST to efficiently exploit the capabilities of some of the largest computers on Earth for simulations on the brain scale, including a world-record simulation of a network of $1{:}86 \times 10^9$ neurons connected by $11{:}1 \times 10^{12}$ synapses on the Japanese K supercomputer [14]. Systematic quality assurance through testsuites [15] and continuous integration technology [16] ensure simulator reliability (within limits). With a user-friendly Python-based interface [17, 18], integration with PyNN [19] for simulator-independent scripting and MUSIC support [20] for integrated multi-scale simulation, NEST is a powerful simulation tool for brain-scale simulations today.

NEST is available under the GNU Public License and community engagement is actively encourage by the NEST Initiative as a non-for profit community organization managing NEST development and a public source code repository on GitHub. NEST is regularly taught at computational neuroscience summer schools such as ACCN, OCNC, and LASCON, allowing young researchers to become efficient NEST users quickly. Running on a wide range of computer architectures from laptops to leading-edge supercomputers, NEST is a widely used tool for simulating spiking neuron networks in computational neuroscience and neurorobotics, and will be the Network Simulation Component of the Human Brain Projects's UnifiedPlatform.

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Genetic ablation of Exchange factors directly activated by cAMP (Epac) causes altered stress responses in mice

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The hypothalamus pituitary adrenal (HPA) axis is fundamental for restoring homeostasis in response to stress. Along this axis, the second messenger cAMP plays essential roles at the cellular level, many of which have been assigned to cAMP dependent protein kinase. In the current study we present data implicating the exchange factors directly activated by cAMP (Epac1 and 2) in stress responses and HPA-axis activity. Male and female mice deleted for Epac factors were subjected to an acute stress regimen. Following different periods of recovery, the plasma corticosterone levels were determined.

Interestingly, female, but not male, Epac $1^{-}/2^{-}$ knockout mice exhibited altered corticosterone levels, suggesting a sex specific phenotype. Coupled to this, the expected increase in pituitary glucocorticoid receptor mRNA expression following restraint stress was delayed in the female Epac $1^{-}/2^{-}$ knockout mice. In the adrenal glands, the expression of the nuclear receptor nerve growth factor inducible clone B (Ngfi-B), which is a cAMP-inducible immediate early gene known to regulate central enzymatic reactions in steroidogenesis, was blunted in female mice lacking Epac following acute restraint stress, paralleling the decreased corticosterone production observed. In the hippocampus, although the glucocorticoid receptor, mineralocorticoid receptor, and nerve growth factor inducible clone A (Ngfi-A) mRNA expression was normal following acute restraint stress, mice lacking Epac exhibited a significant down regulation of miR-124. miR-124 is a highly expressed brain specific miRNA that is believed to regulate a number of neuronal processes, including neuronal differentiation, synaptic plasticity and physiological stress responses.

Taken together, the present study introduces the Epac proteins as potential regulators of HPA-axis activity, and suggests that these factors contribute to mediate the imperative regulatory functions that cAMP exerts on this neuroendocrine axis.

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Organization of entorhinal-hippocampal projections in the Egyptian fruit bat

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Spatially responsive neurons such as place cells and grid cells have been described in rodents, using two dimensional behavioral paradigms, as well as in bats, using two and three dimensional paradigms. Connections between the hippocampal formation (HF) and the entorhinal cortex (EC) play an important role and these have been studied in most detail in rodents, and to a lesser extent in monkeys and other species. Since information on entorhinal-hippocampal connectivity in bats is sparse, we aimed to determine this connectivity in the Egyptian fruit bat (*Rousettus aegyptiacus*).

We analysed the distribution of anterogradely transported tracers in HF following injections of anterograde tracers (dextran amines (DA), biotinylated DA and *Phaseolus vulgaris*-leucoagglutinin) in EC of bats (n = 12). We observed a clear dorsoventral organization of entorhinal-hippocampal projections. Injections in dorsolateral EC resulted in labeling of the dorsal HF and ventromedial EC injections labeled fibers ventrally in HF. Labeled projections from the medial (MEC) or lateral entorhinal cortex (LEC) revealed strikingly different terminal distributions, in that MEC labeled axons were seen in proximal CA1 and distal subiculum, whereas LEC projections were restricted to distal CA1 and proximal subiculum. In CA3 and dentate gyrus (DG), fibers showed a marked radial distribution in the molecular layer (ml). While the CA3ml pattern has yet to be analyzed in detail, in DGml we observed a difference along the transverse axis. In the exposed blade, MEC fibers distribute to the middle of DGml, while LEC fibers reach the superficial portion of DGml. In the enclosed blade however, projections from both MEC and LEC targeted the superficial two-thirds of DGml.
Our observations indicate that the organisation of entorhinal-hippocampal projections in the bat showed a topological distribution comparable to the rat, with the exception of the EC-dentate connectivity, which showed some resemblance to that described in the monkey.

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Temporal spike coordination in the prefrontal-thalamo-hippocampal circuit during trajectory decisions

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Phase synchrony of cortical oscillations is considered as a key mechanism for changing signal flow between regions (Singer, 1999). The connection from area CA1 of the hippocampus to the medial prefrontal cortex (mPFC) is an example of a circuit in which functional connectivity is thought to be modulated by cortical oscillations depending on behavioral demands. Temporal spike coordination of mPFC neurons relative to theta oscillations in CA1 local field potentials (LFPs) is enhanced when animals run down on the central stem of a T-maze, before they reach the choice point where they have to select one of two routes (Jones and Wilson, 2005; Benchenane et al., 2010). This increase of temporal spike coordination is considered a reflection of enhanced signal flow from CA1 to mPFC.

We here report that mPFC and CA1 are theta-coupled also in the reverse direction but with the midline thalamic nucleus reuniens (NR) as a link. In NR neurons, which project to both mPFC and CA1, spikes were found to exhibit enhanced phase-locking to theta oscillations in CA1 LFPs on the central stem, before the decision point, on a T-maze continuous alternation task. Phase-locking was weaker in other regions of the maze. There was also enhanced theta phase coherence between CA1 and NR LFPs on the stem. Our findings point to temporal spike coordination in NR as a potential mechanism for controlling signal flow from mPFC to CA1. This functional connection is essential for CA1 neurons to represent future action plans derived from mPFC (Ito et al., 2015). Thus, by reciprocally coupling mPFC and CA1 cells, theta-frequency spike coordination may enable trajectory decisions during spatial navigation.

We are currently exploring the role of the supramammillary nucleus (SUM) as a potential source of behavior-dependent modulation of theta oscillations in the mPFC-NR-CA1 circuit. SUM gives rise to inputs to mPFC, NR and CA2 of the hippocampus, which in turn projects to CA1. We found that many neurons in SUM exhibited rhythmic firing at theta frequency in behaving animals. Some of these neurons increased rhythmic activity on the central stem of the maze, pointing to a key role for SUM in behavior-dependent modulation of theta rhythms in the mPFC-NR-CA1 circuit.

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Electrophysiological characterisation of entorhinal layer II principal cells in pre-plaque McGill-R-Thy1-APP transgenic rats.

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The main hallmarks of Alzheimer's disease are intracellular neurofibrillary tangles of tau and extracellular amyloid plaques consisting of amyloid beta (A-beta), as well as neuronal loss. The McGill-R-Thy1-APP transgenic rat model displays a progressive amyloid pathology, which mimics the A-beta accumulation described in human patients. The first plaque pathology in this model is seen from 9 months of age, while deficits in spatial memory have been shown as early as 3 months of age, in the pre-plaque phase. This model also has intracellular accumulation of A-beta from as early as 1 week after birth. A-beta can affect synaptic transmission and basic neuronal physiology, including levels of excitability. Among the areas with early expression of intracellular Abeta in our rat model is the entorhinal cortex, which has been shown to be an area with early dysfunction and cell loss, also in human patients. The cells of entorhinal cortex layer II provide input to the hippocampus, and early electrophysiological changes in these cells could contribute to downstream dysfunction and pathology. In this study, we investigated in vitro electrophysiological properties of principal neurons in layer II of medial and lateral entorhinal cortex, stellate cells and fan cells, respectively. We used whole-cell patch clamp in acute slices from transgenic rats at 1 and 3-4 months of age to measure intrinsic electrophysiological properties, looking for changes in the homozygous (+/+) rats compared to control rats (-/-). Properties studied included input resistance, the membrane time constant, resting membrane potential, sag ratio, rheobase, action potential threshold, amplitude and half width, inter-spike intervals, firing rates and adaptation ratio. The neurons were filled with biocytin during recording and later stained with a fluorescent dye to assess their morphology. We also immunostained with the anti-A-beta antibody McSa1 to assess whether the patched neurons expressed A-beta intracellularly. Overall, there were only subtle changes in the physiology of the recorded neurons in the transgenic rats compared to the controls, although the majority of the patched neurons in the transgenic animals showed accumulation of intracellular A-beta. This suggests that the basic neurophysiology of the principal neurons in entorhinal cortex is not obviously altered at 3-4 months. The memory deficits seen in the rat model at this age might thus be due to changes either in other neuronal types or different areas of the hippocampal formation, or altered synaptic function.

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Cellular, anatomical, and functional effects of neural stem cell and olfactory ensheathing cell co-grafts in a rat model of transient cerebral ischemia

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Background: Ischemic stroke is caused by embolic or thrombotic obstruction of blood supply to the brain. An ischemic event triggers a complex molecular cascade including disturbance of neuronal circuitry, slowed cellular energy metabolism, cell depolarization, excitotoxicity, and disruption of the blood–brain barrier. The tissue where blood flow has been severely reduced comprises the ischemic core, which is characterized by rapid necrosis of multiple cell types. The core is surrounded by the ischemic penumbra, which represents salvageable tissue if reperfusion is restored. These pathophysiological changes lead to extensive neuronal and glial cell injury and death, and axonal loss and demyelination, resulting in severe functional deficits. The clinical management of stroke aims to restore the blood-flow in the affected areas, however, only 5%-15% of patients are eligible for this acute treatment. Cell replacement therapy can address key components of stroke pathophysiology and thus represents a promising avenue in stroke repair. Neural stem cells (NSCs) are self-renewing multipotent cells that can differentiate into both neurons and glia, however their survival post-engraftment in the ischemic brain is poor. Olfactory ensheathing cells (OECs) secrete a number of growth factors which are important for neuronal, glial and endothelial cells, and which promote axonal sprouting and neovascularization in the damaged CNS. The aim of this study was to investigate whether co-transplantation of NSCs with OECs improves NSC survival, differentiation, and function in an experimental model of transient cerebral ischemia.

Methods: Transient middle cerebral artery occlusion (MCAO) of 60 min duration was induced in the right brain hemisphere of adult Sprague Dawley rats (n=21). 12 days (d) after stroke stereotactic injections of a 3 μl suspension of 5×10^5 NSCs alone, or NSCs and OECs at 50:50 concentration were made into the ipsilesional globus pallidus. The animals were monitored on day 0, 10, 17 and 28 post-MCAO and assessed for neurological deficits using the Bederson scale. The Cylinder rearing test was performed at day 10, 11, 17 and 28 post-MCAO. MRI was performed at 10 and 38 d post-MCAO. Volumes of uninjured tissue in both cerebral hemispheres were estimated from T2w MRI images and the ratio of ipsilesional/contralateral hemisphere volumes calculated. Diffusion Tensor Images (DTI) was used to calculate mean diffusivity (MD) and fractional anisotropy (FA) in regions of interest in the corpus callosum, hippocampal fimbria, external and internal capsule in both hemispheres.

Preliminary results and discussion: There were no differences in hemispheric volume ratios, MD or FA values, or cylinder rearing test results between groups. However ongoing MRI analyses suggest improved MD and FA values in the graft area of individual subjects pre- and post-transplantation. Preliminary tissue analyses show surviving, undifferentiated (nestin+) NSCs at the graft site and partial migration towards the peri-infarct area. Relatively few OECs were found at the injection site of the co-grafted animals. Our findings suggest good survival and motility of the transplanted NSCs. Ongoing histological analyses aim to determine whether there is overall improved NSC survival or differentiation in the co-grafted groups and whether the relative absence of OECs is due to cell death or migration away from the graft site.

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Assessment of CNS regional unbound target-site concentration: Presentation of a novel approach and its application

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Aim: Very little is known about regional distribution of unbound drug in the brain. Influencing factors can be both local dissimilarities in blood-brain barrier (BBB) transport due to different expression of active efflux and influx transporters, as well as spatial differences in intra-brain distribution and binding. The Combinatory Mapping Approach (CMA) for evaluation of neuroPK properties of new drugs (Loryan et al., 2014) was, therefore, further developed to assess drug transport from blood to the CNS regions of interest (CMA-ROI). The aim of the current study was to develop CMA-ROI as a method for studying regional brain drug disposition using a group of well-described antipsychotic drugs.

Methods: The drugs (haloperidol, clozapine, quetiapine, olanzapine, risperidone and paliperidone) were administered to rats (n= 6 - 7 per drug) as a 4 h intravenous constant rate infusion. Blood, CSF and CNS regions of interest (frontal cortex, striatum, hypothalamus, hippocampus, brainstem, cerebellum and spinal cord) were sampled at the end of the infusion. Fraction of unbound drug in different CNS regions of interest, and unbound volume of distribution in cortex and striatum was assessed using the equilibrium dialysis and the brain slice techniques, respectively. Drug concentrations were analyzed with LC-MS/MS.

Results and Discussions: Significant spatial differences were observed in the extent of BBB and blood-spinal cord barrier transport of unbound antipsychotics. The dissimilarities were more pronounced for the strong P-glycoprotein substrates risperidone and paliperidone. There were also significant drug-specific regional dissimilarities in tissue binding and cellular uptake. As a validation of the CMA-ROI method, the striatal D2 and cortical 5-HT2A receptor occupancy was estimated from regional neuroPK parameters and compared to experimentally derived values from the literature. A good agreement was found between the estimated values using CMA-ROI and the experimentally observed values.

Conclusions: The suggested CMA-ROI methodology can be applied to any drug or drug candidate with potential CNS exposure aiming at in depth evaluation of CNS regional target site unbound drug concentration.

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HUMAN SPINAL CORD NEURAL PROGENITORS AND GROWTH FACTOR MIMETIC LOADED NANOPARTICLES ASSIST REGENERATION OF SENSORY FIBERS INTO THE SPINAL CORD AFTER DORSAL ROOT AVULSION

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Spinal root avulsion injuries result in permanent loss of sensory function and often cause neuropathic pain. We recently showed that human embryonic stem cells derived neural progenitors (hNP) transplanted to the site of avulsed dorsal roots assist regeneration of sensory fibers into the adult mouse spinal cord. Here, we explored the potential of human spinal cord neural stem/progenitor cells (hscNSPCs) and of growth factor mimetics loaded nanoparticles to repair spinal root avulsion injury. We found that hscNSPCs and to some extent mimetic loaded nanoparticles support regeneration of sensory axons into the spinal cord when they are applied separately, whereas hscNSPCs implanted together with mimetic-loaded nanoparticles failed to support sensory regeneration. These findings suggest that the positive effect of hscNSPCs may be eliminated by nanoparticle mediated release of neurotrophic factors due to changes in stem cell properties or surrounding cells at the place of avulsion, preventing growth of injured sensory axons into the spinal cord. Thus, hscNSPCs alone and mimetic loaded nanoparticles alone are able to assist restoration of sensory connections between the PNS and spinal cord, whereas their combination does not support regeneration.

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Hippocampal mossy cells in mink (*Neovison vison*)

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The hippocampal mossy cells are part of the entorhinal-hippocampal circuitry, which is essential for spatial orientation and memory function. Located in the polymorph layer of the dentate gyrus, the mossy cells are interconnected in a positive feedback loop with the subjacent granule cells, which convey information from the entorhinal cortex to the hippocampal pyramidal cells. Besides disynaptic input from the entorhinal cortex via granule cells, the mossy cells may also receive monosynaptic input from the entorhinal cortex via special dendrites (gm-dendrites) ascending to the granular and molecular layers. Such dendrites seem better developed in primates than in rodents. We have here studied mossy cell gm-dendrites in the *carnivore* mink, which has a more advanced brain than the much studied rat. The study is based on light microscopic observations of Golgi sections, including camera lucida drawings, computer based three-dimensional reconstructions, and metric-topological analysis (calculating dendritic ramifications, segments, and lengths). In mink as in other species, we find that the mossy cells are characterized by large complex spines, so-called thorny excrescences, on their proximal dendrites, postsynaptic to giant terminals of granule cell axon collaterals. Whereas proximal dendrites with excrescences are restricted to the polymorph layer, the remarkably slim gm-dendrites extend into the granular and molecular layers. In mink, 15 of the 17 sampled mossy cells had one or more gm-dendrites, on average 2.8 per cell (range 0-8). The total length of gm-dendrites per cell constitutes up to 29% of the total dendritic length (average 12%). Thus, mink have more gm-dendrites than rats, but fewer than primates. Via these dendrites the mossy cells may receive monosynaptic input from the entorhinal cortex prior to the disynaptic input to the proximal dendrites via granule cells.

†This work was initiated and to a large extent performed by Professor Theodor W. Blackstad, who passed away in 2003 before the project was completed.

JANNIKE MØRCH ANDERSEN

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Neurobiological mechanisms of synaptic plasticity in opioid-induced Conditioned Place Preference: changes in CamKII and β-actin in striatum and hippocampus in mice.

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Activation of Calcium/calmodulin-dependent protein kinase II (CamKII) is known to be essential for synaptic plasticity, a central mechanism for learning and memory in animals (1). This process is accompanied by structural modifications at the synapses, supported by changes in the organization of cytoskeletal microfilaments and, therefore, in actin polymerization and assembling (2). Drug abuse and addiction can be considered a form of (inappropriate) learning, where drug intake and effects are paired with environmental cues. The striatum, activated by dopaminergic neurons, is known to play an important role in reinforcement learning (3). Thus, neuronal mechanisms coupled to learning and synaptic plasticity may be crucial in the processes leading to development of drug abuse and dependence.

In the present study, we examined changes in mice brain levels of cFos, β-actin, CamKII, and phosphorylated CaMKII at Thr286 (pCamKII) using western blotting. The proteins were measured in dorsal and ventral striatum, and in hippocampus after s.c. administration of saline, 10 or 30 μmol/kg morphine or its active metabolite morphine-6-glucuronide (M6G). Drug administration was done in three different regimes: acute, subchronic (3 days) in combination with a Conditioned Place Preference (CPP) test, and subchronic mimicking the CPP administration regime without exposition to the CPP procedure. Brain samples were collected 30 minutes after the acute administration, immediately after the CPP test applied after saline injection on day 4, and 20 minutes after a saline administration on day 4 (corresponding to the length of the CPP test) in the subchronic group.

Acute morphine and M6G induced increases in the levels of cFos, CamKII, pCamKII and β-actin in both striatal areas, but not in the hippocampus. In mice subjected to the CPP procedure, these changes were observed in both of the striatal areas and in the hippocampus. After subchronic treatment without CPP, the only major change observed was a decrease in pCamKII in the three areas studied.

The present results show that opioids preferentially stimulate striatal neurons, as shown by the increase in the levels of cFos, CamKII, pCamKII, and β-actin. These changes are likely connected to activation of neuronal processes important for synaptic plasticity. Furthermore, when the opioid administration is coupled to a learning paradigm like CPP, other areas known to be related to learning are also recruited, as revealed by the increase in protein levels in the hippocampus. The decrease in pCamKII in hippocampus after the sub-chronic treatment without CPP can be related to the adverse cognitive effects observed after repeated opioid treatment.

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Type 2 diabetes mellitus is associated with alterations in cortical metabolism

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Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by an abnormal and persistent increase in blood sugar (1). A correlation between T2DM and the development of an Alzheimers-like dementia has been shown in animal models and human patients (2, 3). Impaired cerebral glucose utilization and mitochondrial dysfunction has been reported, but the specific roles of neurons and astrocytes in relation to T2DM remains to be elucidated. In these studies we utilized a frequently used animal model of T2DM; the db/db mouse. Acutely isolated cortical slices from 14 week old db/db mice were incubated with either [U-^{13}C]glucose or [1,2-^{13}C]acetate. In brain tissue, glucose is metabolized by both neurons and astrocytes, whereas acetate is primarily metabolized in astrocytes. The formation of ^{13}C-labeled metabolites was assessed by gas chromatography and mass spectrometry. From incubations with [U-^{13}C]glucose, a decrease in labeling was observed in double labeled glutamate and aspartate, in cortical slices from db/db mice in comparison to control mice. In contrast, from incubations with [1,2-^{13}C]acetate, the labeling of double labeled glutamate and aspartate was increased in cortical slices from db/db mice compared to control mice. The percent of ^{13}C labeled glutamate and aspartate, formed from the subsequent turns of the tricarboxylic acid cycle, was increased from [U-^{13}C]glucose and decreased from [1,2-^{13}C]acetate in cortical brain slices from db/db mice and comparison to control mice. Thus astrocytic tricarboxylic acid cycle activity seems to be increased in cortical slices from the db/db mice. In contrast, the cortical glucose metabolism seems to be decreased in slices from db/db mice. Our data confirm a neuronal decrease in glucose metabolism, and indicate an augmentation of astrocytic metabolism, in the db/db mouse model of T2DM.

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Neuronal cell types in the juvenile mouse telencephalon revealed by single-cell RNA-seq

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Abstract:

The mammalian cerebral cortex supports cognitive functions such as sensorimotor integration, memory, and social behaviors. Normal brain function relies on a diverse set of differentiated cell types, including neurons, glia and vasculature. Here, we have used large-scale (>3000) single-cell RNA-seq to classify cells in the juvenile (between postnatal day 21 and 30) mouse somatosensory cortex and hippocampal CA1 region. We found 47 molecularly distinct subclasses, comprising all known major cell types in the cortex. We identified numerous marker genes, which allowed alignment with known cell types, morphology and location. We found a layer I interneuron expressing Pax6 and a distinct post-mitotic oligodendrocyte subclass marked by Itpr2. Across the diversity of cortical cell types, transcription factors formed a complex, layered regulatory code, suggesting a mechanism for the maintenance of adult cell type identity.

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Endocannabinoids supplement a hardwired circuit to promote behavioral selection by switching between motoneuron pools in adult zebrafish

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Abstract: Animals constantly make behavioral choices to move efficiently through the environment. When faced with a threat, animals make decisions in the midst of other ongoing behaviors through a context-dependent integration of sensory stimuli. The mechanisms underlying these behavioral selections in vertebrates are not well understood. In this study, we examine the neural underpinnings of the context-dependent priority of escape behavior over swimming by recapitulating these two motor behaviors and their interactions in an in vitro preparation of adult zebrafish. We show that the selection of escape over swimming is mediated by switching between fast and slow motoneuron pools. The fast motoneuron pool underlying escape is engaged via monosynaptic excitation. In contrast, the slow pool underlying swimming is disengaged via indirect inhibition that decouples these slow motoneurons from the premotor swim circuit. The onset of the escape and the associated inhibition of swimming activity are determined by a hardwired fast circuit. However, the threshold for initiation of escape and the extent of inhibition of swimming relies on endocannabinoid retrograde signaling. Thus, our results reveal a novel mechanism involving a hardwired circuit supplemented with endocannabinoid modulation that shifts between slow and fast motoneuron pools and hence mediates behavioral selection in vertebrates.

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Developmental switch in the kinase dependency of long-term potentiation depends on expression of GluA4 subunit-containing AMPA receptors

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1. NVL and JH have contributed equally to this work

The AMPA-receptor subunit GluA4 is expressed transiently in CA1 pyramidal neurons at the time synaptic connectivity is forming, but its physiological significance is unknown. Here we show that GluA4 expression is sufficient to alter the signaling requirements of long-term potentiation (LTP) and can fully explain the switch in the LTP kinase dependency from PKA to Ca^{2+}/calmodulin-dependent protein kinase II during synapse maturation. At immature synapses, activation of PKA leads to a robust potentiation of AMPA- receptor function via the mobilization of GluA4. Analysis of GluA4- deficient mice indicates that this mechanism is critical for neonatal PKA-dependent LTP. Furthermore, lentiviral expression of GluA4 in CA1 neurons conferred a PKA-dependent synaptic potentiation and LTP regardless of the developmental stage. Thus, GluA4 defines the signaling requirements for LTP and silent synapse activation during a critical period of synapse development.

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Luchkina N V, Huupponen J, Clarke VRJ, Coleman SK, Keinänen K, Taira T, Lauri SE. Developmental switch in the kinase dependency of long-term potentiation depends on expression of GluA4 subunit-containing AMPA receptors. Proc Natl Acad Sci USA 111: 4321–6, 2014.

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Peroxisome proliferator-activated receptor-γ (PPARγ) agonist GW1929 is neuroprotective and stimulates the expression of PGC-1α and phosphorylation of cAMP responsive element-binding protein (CREB) in human dopaminergic neurons

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Mitochondria are the major source of energy in the cell, and neuronal survival depends on proper mitochondrial function. Mitochondrial dysfunction is thought to be involved in several neurodegenerative diseases, including Parkinson's disease. A deficient mitochondrial metabolism may generate reactive oxygen species (ROS) which contribute to neuronal loss in neurodegenerative diseases[1].

Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a transcriptional co-activator that regulates mitochondrial biogenesis and respiration as well as the defense system against reactive oxygen species (ROS)[2]. It is also thought to have a neuroprotective role in the brain by enhancing mitochondrial function[3]. PGC-1α expression and activation is tightly regulated in response to energy changes in neurons and modulating the activity or levels of PGC-1α could be beneficial for cell protection in various diseases.

Compounds that are involved in metabolic pathways, such as drugs for type 2 diabetes, could be useful in treatment of patients with neurodegenerative disorders by regulating brain metabolism. Peroxisome proliferator-activated receptor –gamma (PPARγ) is a member of the nuclear receptor superfamily of ligand-inducible transcription factors. PPARγ is thought to have a role in neuroprotection by regulating ROS and energy homeostasis in the brain. Therefore PPARγ might be a potential therapeutic target in the treatment of neurodegenerative diseases[4].

We show that treating human dopaminergic neurons with the nonthiazolidinedione PPARγ agonist N-(2-Benzoylphenyl)-O-[2-(methyl-2-pyridinylamino)ethyl]-L-tyrosine hydrate (GW1929) increases the expression and activation of PGC-1α. We also observed that GW1929 could activate CREB, and this activation might take part in the increased expression of PGC-1α. The mitochondrial function was also affected and showed an increase in mitochondrial biogenesis and the expression of antioxidant enzymes. GW1929 treated cells were also more resistant to H_2O_2 and Rotenone induced cell death, which may be due to the increase in antioxidant defense in these cells.

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Early life stress is associated with altered KAR expression and function in the amygdala

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Early-life stress is an important factor increasing the susceptibility for amygdala related mental disorders later in life[1]. However, very little is known on the synapse level mechanisms underlying these changes. Kainate-type ionotropic and metabotropic glutamate receptors (KARs) modulate neuronal excitability, transmission and plasticity in various areas of the brain, including the amygdala[2-7]. Increasing evidence supports a role for KARs in CNS disorders related to limbic system dysfunction[8-10]. However, very little is known on the functions of KARs in the developing amygdala, despite the apparent clinical importance.

To study the physiological mechanisms by which early experiences may lead to anxiety behaviors and the role of KARs in this process, we focused on the maternal separation (MS) model of early life stress in rats. The protocol consisted of daily 180 min separation of the pups and the dam from postnatal day (P) 2 until P14 (MS group), while littermate controls remained with the. Behavioral testing as adult (>P50) confirmed that MS resulted in an increase in anxiety – like behavior in open-field and elevated plus maze.

Gene expression of KAR subunits GluK1-5 and the auxiliary subunits Neto1,2 was assessed using RT-qPCR for hippocampus, basolateral amygdala (BLA), and central amygdala (CA) in control and MS rats, immediately after the MS (P14) as well as in the adult (P50). At P14, expression of GluK1 and GluK2 in the BLA was significantly higher in the MS group as compared to controls (1.5 and 1.6 fold-change, $p<0.05$). No significant changes were detected for other subunits or regions analysed. At P50, a trend towards lower expression of GluK1 and GluK5 was observed in the BLA and CA in the MS group, while expression of GluK2 in the BLA remained slightly elevated. Interestingly, MS effect on KAR gene expression was gender dependent. At p14, the MS associated increase in GluK1,2 expression was most pronounced in females, while the reduced expression of GluK1 and GluK5 at P50 BLA was predominant in males.

Given the observed changes in KAR expression, we went on to test whether MS also affected KARs regulating excitatory input to the lateral amygdala (LA). Whole cell patch-clamp recordings were done in acute slices from rats (>P50) to assess the effect of MS on eEPSCs (evoked excitatory post-synaptic currents) in LA principal neurons. Cortical and thalamic inputs were separately stimulated and the effect of GluK1 antagonist ACET (200nM) on the amplitude, the kinetics and the paired-pulse ratio was tested. Our preliminary results indicate reduced effect of GluK1 antagonist on EPSCs evoked by cortical input stimulation in the MS group.

Together, these results suggest that early life stress affects expression of KARs in the amygdala, in a gender, subunit and region specific manner. Concomitant changes in KAR function within the LA may influence transmission and associative plasticity within this main input region of the amygdala, implicated in generation of fear and aversive memories.

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Inhibition of individual microRNAs in the mouse hippocampus causes distinct behavioural phenotypes

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The small non-coding RNA population of microRNAs (miRNAs) are highly expressed in the central nervous system and have distinct patterns throughout the brain [1]. In each cell, miRNAs bind specific target messengerRNAs (mRNAs) and cause degradation and/or inhibited translation of the latter [2]. During the past few years, miRNA function has been implicated in the production, survival, branching, excitation and plasticity of neuronal cells [1, 3, 4]. Interestingly, inhibition of the miRNA biogenesis pathway through conditional deletion of the Dicer enzyme in the adult rodent forebrain, results in an increased cognitive capacity, suggesting miRNA regulation of learning and memory [5]. As Dicer exerts functions outside the miRNA biogenesis pathway [6], it cannot be fully asserted that miRNAs are fully responsible for the observed alteration in cognitive function. In this study, we use a more precise method of inhibiting specific miRNA families with subsequent analysis of behavioural phenotypes. Inhibition of four separate miRNA families through the use of miRNA sponges [7-9] in hippocampal neurons, we demonstrate distinct changes in learning and memory and related gene ontology networks. Together, these findings underline the importance of specific miRNAs in cognitive function of the adult rodent brain.

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DOPAMINE PROJECTIONS FROM SNc MODULATE TECTAL MOTOR REPONSES

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In the lamprey, as in mammals, dopamine plays a key role in movement control by modulating the excitability of projection neurons in the striatum. The direct "go" pathway neurons express the dopamine D1 receptor and mediate a net facilitation of motor actions desinhibiting the pallidal GABAergic output neurons, whereas the indirect "no go" pathway, acting through the D2 subtype, mediates motor suppression (Gerfen and Surmeier, 2011; Ericsson et al., 2013). This dopaminergic modulation of the striatum derives from the nucleus of the posterior tuberculum, the homologous region of the mammalian substantia nigra pars compacta (SNc). It receives pallial (cortex in mammals) input and shows the same connectivity with the other basal ganglia subnuclei observed in mammals. The SNc also receives input from the optic tectum as the evolutionary basis for salience/novelty detection (Pérez-Fernández et al., 2014). The importance of the dopaminergic innervation from the SNc in the lamprey is reflected in the fact that, when depleted, it gives rise to a marked hypokinesia, as in Parkinson's disease (Thompson et al., 2008). The lamprey SNc shows therefore striking similarities with its mammalian homologue. One important feature is that the SNc in lamprey, as in mammals (Takada et al., 1988), sends direct dopaminergic projections to different motor command centres, including the diencephalic and mesencephalic motor regions, and the output layer of the optic tectum (Ryczko et al., 2013, Pérez-Fernández et al., 2014). The nigral dopaminergic control of motor responses is thus likely to be more complex than generally assumed, and involves additional pathways to the widely studied striatal projection. Here, we explore how dopamine modulates motor responses in the optic tectum, the homologous region of the mammalian superior colliculus. This region shows in all vertebrates, including the lamprey, similar features, with a laminated structure, controlling eye, orienting and evasive trunk movements (Kardamakis et al., 2015). Neurobiotin injections combined with immunocytochemistry show that dopaminergic fibers from the SNc innervate the (inner) output layer premotor cells, which express D1 and D2 receptors as shown by *in situ* hybridization. Patch-clamp recordings of premotor cells show that D1 and D2 cells are separated populations, and dopamine increases the excitability of D1 cells, whereas it decreases the excitability of D2 cells. Dopaminergic modulation changes their responsivness to sensory inputs reaching the optic tectum, as shown by stimulation of the retinal afferents to the superficial layer. To analyze the effects of dopamine in the actual motor commands from tectum, we also performed electromyography (EMG) recordings in the eye muscles, showing that motor responses elicited by retinal stimulation can be modulated by locally injected dopamine agonists in the optic tectum. Our results indicate that dopamine directly modulates motor responses in premotor regions and, given the high degree of conservation of the basal ganglia and the presence of direct dopaminergic projections from the SNc to the superior colliculus in rats (Takada et al., 1988), this previously unexplored mechanism is likely to also be present in higher vertebrates including mammals. This mechanism may be involved in the problems observed in Parkinson´s disease to perform saccadic eye movements.

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Retrosplenial-parahippocampal projections in the rat are present and adult-like before eye-opening

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Retrosplenial cortex (RSC) is important for a variety of cognitive tasks, particularly within the field of spatial learning and memory. The presence of head-direction cells in rat RSC provides strong support for the notion that RSC plays a role in spatial cognition. Head-direction cells, and other spatially modulated cells are also found in the hippocampal-parahippocampal region (HF-PHR), suggesting a functional relationship. In the adult rat, RSC is connected with pre- and parasubiculum, medial entorhinal cortex, postrhinal cortex and subiculum, areas known to be part of a circuit important for spatial information processing. We have recently shown that sparse connections within the HF-PHR are present at birth, reaching adult-like features before eye-opening and before the first spatially modulated neurons appear at postnatal day (P)15 (Langston et al. 2010 Science 328:1576; O'Reilly et al. 2012 SFN Abstr.702.02). In this study, we aimed to investigate whether RSC connections to PHR develop in parallel with development of HF-PHR connections or in parallel with development of spatially modulated cells in HF-PHR. To examine development of RSC – PHR connectivity, rats aged P0-P26 were injected with anterograde tracers into RSC. Investigations of anterogradely labeled axons revealed that sparse connections were present at P0. Injections in all parts of RSC, across all postnatal ages, resulted in comparable topology of labeled fibers. During the first week, the connectivity developed gradually and reached adult like densities around P10. We therefore conclude that the topology of RSC – PHR projections is present at birth, while the density of the projections is fully developed during the second postnatal week. Thus, RSC – PHR connections develop in parallel with connections within HF-PHR.

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Subcortical volumes in children at early school age relate to preterm birth and very low birth weight

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BACKGROUND: Preterm birth is a worldwide problem, affecting 15 million newborns each year and burdening many survivors with lifelong physical, cognitive, and psychological challenges. Individuals born preterm with very low birth weight (VLBW: birth weight ≤1500 grams) are at an increased risk of perinatal brain injury and neurodevelopmental and cognitive problems. Cerebral white matter injury and neuronal and axonal abnormalities are considered the dominant neuropathologies in preterm-born infants and are believed to underlie many of these cognitive deficits.

OBJECTIVE: We investigated group differences in subcortical brain structure volumes between VLBW children and controls, as well as possible relationships between brain structure and IQ scores, birth weight, and gestational age.

DESIGN/METHODS: 103 term-born children participating in the Norwegian Mother and Child Cohort Study and 37 VLBW children born between 2001 and 2007 underwent 1.5 T MRI and age-appropriate cognitive testing with Wechsler tests (mean age=8 years). We used FreeSurfer software version 5.3.0 to extract volumes of subcortical structures and the general linear model for between-group analyses of subcortical volumes and partial correlations for morphometric data and IQ scores. All morphometry analyses were controlled for age at scan, sex, and estimated total intracranial volume.

RESULTS: Compared to controls, the VLBW group had reduced volumes of thalamus, right globus pallidus, right hippocampus, cortical white matter and brain stem, while the ventricular system was enlarged compared with controls. Uncorrected IQ scores were significantly lower (p<0.001 in ANOVA analysis) in the VLBW group (mean=98.6; SD 9.7) than in controls (mean=108.1; SD 13.6). Among all participants, IQ score correlated significantly with volumes in cortical white matter, both thalami, right hippocampus, and right ventral DC; only in right hippocampus among controls; and no correlations found in the VLBW group. Birth weight among all subjects correlated (p<0.05) to volumes of brain stem, cortical white matter, both hippocampi, both globus pallidi, both thalami, bilateral ventral DC, third ventricle, and lateral ventricles bilaterally. Gestational age among VLBW subjects correlated (p<0.05) with volume of cortical white matter, left accumbens area, and right cerebellum white matter.

CONCLUSIONS: Volumes of several subcortical structures in both hemispheres are associated with very low birth weight and preterm birth. These persistent structural differences may be due to perinatal brain injury, and their relationship to emergent cognitive, behavioral, and mental health outcomes merits further evaluation.

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The role of intrahippocampal growth hormone in spatial memory

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Low levels of growth hormone (GH) and insulin-like growth factor (IGF) are seen in ageing and endocrine disturbances, and are associated with cognitive deficits (Ashpole et al., 2014). Memory can be enhanced or impaired in animals by manipulating the GH/IGF axis (Chen et al., 2011), but the mechanisms behind are poorly understood. In the current study, we used viral transfections to reduce or enhance GH levels locally in the hippocampus. The hippocampus is a natural target as it contains receptors for several ligands along the GH/IGF axis, and also has local production of GH. Moreover, GH-stimulating receptor (GHSR) and IGF receptors can modulate long-term potentiation, neurogenesis and memory consolidation after local stimulation in the hippocampus. In our model, we transfected the dorsal hippocampus of Long Evans rats with recombinant adeno-associated virus (rAAV) expressing GH, mutated GH (mGH) or only green fluorescent protein (GFP). The AVV infection allows for chronic elevation (AAV GH) or inhibition (AAV mGH) of GH. The rats were tested in a displaced object task and a water maze task. The results indicate that the viral transfections affect learning and memory by acting locally in the hippocampus. Further experiments will be performed to explore which parts of the memory processing that are affected most severely by GH manipulation. Understanding actions of GH in brain can give insight to why cognitive decline often follows ageing, as GH levels are dramatically reduced in ageing across several species.

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Dynamic expression of long noncoding RNAs and repeat elements in synaptic plasticity

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Long-term potentiation (LTP) of synaptic transmission is recognized as a cellular mechanism for learning and memory storage (Bliss and Lømo). Although de novo gene transcription is known to be required in the formation of stable LTP, the molecular mechanisms underlying synaptic plasticity remain elusive. Non-coding RNAs have emerged as major regulatory molecules that are abundantly and specifically expressed in the mammalian brain. By combining RNA-seq analysis with LTP induction in the dentate gyrus of live rats, we provide the first global transcriptomic analysis of synaptic plasticity in the adult brain. Expression profiles of mRNAs and long noncoding RNAs (lncRNAs) were obtained at 30 minutes, 2 hours and 5 hours after high-frequency stimulation of the perforant pathway. The temporal analysis revealed dynamic expression profiles of lncRNAs with many positively, and highly, correlated to protein-coding genes with known roles in synaptic plasticity, suggesting their possible involvement in LTP. In light of observations suggesting a role for retrotransposons in brain function, we examined the expression of various classes of repeat elements (Erwin et al). Our analysis identifies dynamic regulation of LINE1 and SINE retrotransposons, and extensive regulation of tRNA. In sum, these experiments reveal a hitherto unknown complexity of gene expression in long-term synaptic plasticity involving the dynamic regulation of lncRNAs and repeat elements. These findings provide a broader foundation for elucidating the transcriptional and epigenetic regulation of synaptic plasticity in both the healthy brain and in neurodegenerative and neuropsychiatric disorders.

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Projections of the posterior parietal cortex to the orbitofrontal cortex in the rat

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The posterior parietal cortex (PPC) in the rat is a multimodal association area, implicated in spatial processing and directed attention. The PPC is commonly divided into a medial (mPPC), a lateral (lPPC) and a posterior (PtP) region, all reciprocally connected to specific parts of the thalamus. The orbitofrontal cortex is part of the ventral prefrontal cortex and is commonly divided into the medial orbital (MO), ventral orbital (VO), ventrolateral orbital (VLO), lateral orbital (LO) and dorsolateral orbital (DLO) cortices. The subregions of OFC have distinct connectivity patterns and are functionally different regarding spatial information processing, value-based decision making, and behavioural flexibility.

Reciprocal connections between PPC and OFC have previously been described, but variations in delineation of both cortical regions and difficulties in distinguishing PPC from the secondary visual cortex (V2) hampered a clear understanding of the connections. Moreover, no studies addressed PPC- OFC projections differentiating the origins in the three parietal subdivisions.

The aim of this study was therefore to describe the projections of PPC to the subregions of OFC, with a special focus on the differences in projection pattern arising from the three subregions of PPC. To this end, we injected the anterograde tracers 10 KD biotinylated dextran amine (BDA) and *phaseolus vulgaris*-leucoagglutinin (PHA-L) into the subregions of PPC. The retrograde tracers fast blue (FB) and fluorogold (FG) were injected into VO and VLO to study the layers origin of these projections. The brains were cut in the coronal plane and cortical areas were delineated based on Nissl stains with cresyl violet. Anterograde tracers were visualized using either 3.3'-diaminobenzidin tetrahydrochloride (DAB) or AlexaFluor® dyes, and their distribution, as well as that of the retrograde fluorescent tracers was analysed with conventional microscope techniques.

Anterograde tracing showed that the projections from PPC to OFC are not strong, which is supported by the retrograde cases that showed overall low numbers of retrograde labelled neurons in layers V and VI of PPC. Injections into mPPC resulted in labelling mainly in lateral VO and medial VLO, with some sparse fibres in MO. Injections into lPPC targeted medial VLO, while injections into the most lateral part of PPC, PtP, resulted in labelling in intermediate to lateral VLO. The results indicate that projections from PPC target OFC, showing a subtle topographical pattern within MO, VO and VLO, with a clear preference for VO and VLO and excluding LO and DLO.

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Spatial organization of gamma oscillations in medial entorhinal cortex

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Grid cells in the medial entorhinal cortex (MEC) provide the animal with a metric of the environment via regularly-spaced spatial firing patterns. We previously showed that grid cells are organized into discrete modules with different grid spacing (Stensola et al., *Nature* 492:72, 2012). Grid cells of different modules can respond independently to environmental change, whereas cells in the same module respond coherently. However, underlying mechanisms for the coherent spatial firing within modules are not clear. One possibility is that gamma oscillations promote communication between cells in the same module, and that different modules operate at different frequency bands. To test this hypothesis, we characterized gamma oscillations in local field potentials recorded from tetrodes with layer II and III cells belonging preferentially to a single module. Wavelet analysis was performed to characterize cross-frequency coupling of gamma oscillations against theta oscillations. The analysis showed that there are three distinct gamma bands: slow gamma (30-55 Hz), fast gamma (55-90 Hz) and very fast gamma (>100 Hz). The existence of the three gamma bands is similar to the frequency organization observed in the hippocampus (Colgin et al., *Nature* 462:353, 2009; Belluscio et al., *J Neurosci* 32:423, 2012). We used the slow and fast gamma bands for further analyses. Preliminary analysis suggests that tetrodes predominated by cells from modules with short grid spacing exhibit fast gamma, whereas tetrodes predominated by grid cells from larger-scaled modules show both slow and fast gamma oscillations. Tetrodes with large-scale modules had slow gamma oscillations that were more strongly coupled to theta oscillations than gamma oscillations on tetrodes with small-scale modules. Phase-locking analysis of spikes from grid cells further showed that spikes in large-scale modules are more strongly phase-locked to slow gamma oscillations than those in small-scale modules. No difference between large-scale and small-scale modules was observed for fast gamma oscillations in cross-frequency coupling and spike phase-locking. These results support the idea that modules with short spacing use only fast gamma whereas modules with larger spacing use both fast and slow gamma oscillations, and point to gamma oscillations as a mechanism for independent representation across grid modules in MEC.

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Removal of perineuronal nets in the visual cortex leads to a reduced inhibition and mimics critical period plasticity

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During postnatal development, the sensory cortices undergo a period of heightened plasticity. In primary visual cortex (V1), this critical period (CP) is initiated by the maturation of parvalbumin expressing (PV^+) inhibitory neurons. Towards the closure of the CP the PV^+ cells become enwrapped in a dense, specialized extracellular matrix called perineuronal nets (PNNs) which are believed to restrict plasticity in the adult. The PNNs are thought to act out this function by stabilizing synaptic connections, limiting the formation of new synapses, and facilitating the high spiking activity of PV^+ cells. Removing the PNNs in adult animals with the enzyme Chondroitinase ABC (chABC) restores plasticity levels comparable to that of juveniles. It remains unknown how the PNN degradation allows for plasticity, and how individual neurons change over time during activity-dependent plasticity.

We addressed this by degrading PNNs in V1 of adult rats (by local injections of chABC) and conducted extracellular recordings in freely moving animals of single-unit and ensemble activity using chronically implanted tetrodes.

Degradation of the PNNs caused a reduction in activity of putative inhibitory neurons and a subsequent increase in activity of putative excitatory neurons. This held true both in the spontaneous and visually evoked states. Preliminary data also suggests that removing the PNNs caused a significant increase in the number of inhibitory synaptic puncta within V1, while the number of excitatory synaptic puncta was no different from controls. No effects were found on tuning properties such as receptive fields and ocular dominance of individual neurons.

In order to induce activity-dependent plasticity we used monocular deprivation (MD) for five days. In chABC injected animals, this produced a shift in ocular dominance while no effect was seen in controls. Longitudinal recordings of the same neurons during MD revealed diverse effects of sensory deprivation, and a difference in time-course for plasticity between neurons in the ipsi –and contralateral hemispheres to the deprived eye. The effects on neuronal activity in chABC injected animals were comparable to previous work on CP animals, suggesting similar mechanisms are at play. Recordings in adult controls showed a period of homeostatic regulation of activity occurring within the first 48 hours of MD, before the activity stabilized at baseline levels.

We also investigated how the local field potential (LFP) was affected by MD. Sensory deprivation did not affect LFP in control animals, but immediately induced activity in the gamma-band (55-60 Hz) in chABC injected animals. This persisted for at least 30 minutes after eye closure, and could not be detected at any point later in the experiments.

In summary, degrading the PNN in adult rats mimics CP plasticity by allowing for increased synaptogenesis and shifting the inhibition-excitation balance in favor of excitatory activity. A brief period of sensory deprivation after PNN removal causes profound changes in neuronal activity, while adult controls display a brief period of homeostatic regulation of activity.

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Glucocorticoid dexamethasone reduces cerebellar size in chicken and induces accelerated maturation of cerebellar granule neurons with a transient increase in levels of PAX6 and MMP-9

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The developing cerebellum is known to be a vulnerable target organ for effects of glucocorticoids. In the present study we utilized a chicken model to study if prenatal exposure of the glucocorticoid dexamethasone (DEX) interfered with normal cerebellar development. We established the critical time window for cerebellar growth in chicken embryos to be E12-E19 and administered 0,1 µM DEX *in ovo* at E13 and E16 before isolation of tissue at E14 and E17 and preparation of cultures of granule neurons at E17. Exposure to DEX caused a significant decrease in cerebellar size and in the protein level of PCNA (proliferating cell nuclear antigen) indicating reduced proliferation in cerebellar tissue. Regulation of the transcription factor PAX6 and matrix metalloproteinase MMP-9 are critical for correct cerebellar development. PAX6 and MMP-9 are highly expressed in granule neurons that have yet to finish migration to the internal granule layer of the cerebellar cortex and are down-regulated when migration is completed. We report that DEX increased promoter activity of PAX6 and MMP9 *in vitro.* This was not mimicked by cytostatic ARA-C and was assumed not to be secondary effects caused by reduced proliferation. Prenatal exposure to DEX *in ovo* increased the protein level of PAX6 in a transient manner before the level was reduced earlier than in non-exposed embryos. Prenatal DEX also caused an earlier down-regulation of promoter activity of PAX6 and MMP-9. Together these findings indicate that prenatal DEX may disturb correct development of the cerebellum through several mechanisms both associated with reduced proliferation and accelerated maturation where PAX6 and MMP-9 play an important role.

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Topography of place maps along the CA3-to-CA2 axis of the hippocampus

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Abstract

The intrinsic structure of the hippocampus exhibits considerable diversity along the transverse axis, not only between but also within subfields. In CA3, cell morphology and connectivity change gradually from the proximal end (near the dentate gyrus) to the distal end (near CA2), with CA2 appearing in some respects as an extension of CA3 and in others as a distinct subfield with its own gene expression and connectivity patterns. We asked whether the heterogeneity of the CA3-CA2 axis is reflected in how space is mapped onto place cells in these subfields. We observed a transition from cells with small and sharp place fields in proximal CA3 to cells with large and dispersed fields in the most distal CA3 and CA2. The shift was accompanied by a progressive loss in the ability of place cells to distinguish different configurations of the same spatial environment. There was also a reduction along the CA3-CA2 axis in the extent to which place cells formed uncorrelated representations for different environments, in different places, but this transition was non-linear, with the sharpest drop within the distal CA3, 200 – 250 μm from the CA2 border. The functional changes along the CA3-CA2 axis mirror gradients in gene expression and connectivity that partly override cytoarchitectonic boundaries between the subfields of the hippocampus. The results point to the CA3-CA2 axis as a functionally graded system with powerful pattern separation at the proximal end, near the dentate gyrus, and stronger pattern completion at the CA2 end.

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The lactate receptor GPR81 / HCAR1: expression and action in brain

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We have proposed that lactate is a "volume transmitter" in the brain and underpin this by showing that the G-protein coupled lactate receptor, GPR81 (HCA_1, HCAR1), which promotes lipid storage in adipocytes, is also active in the mammalian brain. This includes the cerebral neocortex and hippocampus, where it can be stimulated by physiological concentrations of lactate and by the HCAR1 agonist 3,5-dihydroxybenzoate to reduce cAMP levels. Cerebral HCAR1 is concentrated on the synaptic membranes of excitatory synapses, with a postsynaptic predominance. HCAR1 is also enriched at the blood-brain-barrier: the HCAR1 densities at endothelial cell membranes are about twice the HCAR1 density at membranes of perivascular astrocytic processes, but about one seventh of that on synaptic membranes. In synaptic spines, as well as in adipocytes, HCAR1 immunoreactivity is located on subplasmalemmal vesicular organelles, suggesting trafficking of the protein between intracellular stores and the plasma membrane. The results indicate that, through activation of HCAR1, lactate can act as a volume transmitter that links neuronal activity, cerebral blood flow, energy metabolism and energy substrate availability, including a neuronal glucose and glial glycogen saving response to the supply of lactate. The actions of cAMP in brain function are characterized by a bell-shaped dose-response relationship, and the lactate receptor may contribute to optimizing the cAMP concentration. For instance in the prefrontal cortex excessively high cAMP levels have been implicated in impaired cognition in conditions such as old age, fatigue, stress and schizophrenia, and in the deposition of phosphorylated tau protein in Alzheimer's disease. It will be important to explore whether activation of HCAR1 can serve to ameliorate these conditions. Literature data from other organs indicate additional actions of HCAR1 through other down-stream mechanisms than cAMP that probably operate also in brain. In addition to locally produced lactate, lactate produced by exercising muscle, as well as exogenous HCAR1 agonists, e.g., from fruits and berries, might activate the receptor on cerebral blood vessels and brain cells.

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Auditory Hallucinations in Schizophrenia – Aberrant Association Between Glutamate Levels and Resting-State Functional Connectivity

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Introduction

Resting-state functional connectivity is generally found to be aberrant in schizophrenia, but more specifically it is hypothesized to be a driving force behind auditory hallucinations [1]. Functional connectivity is partially modulated by glutamate through the excitation/inhibition balance [2], and glutamatergic abnormalities are consistently found in schizophrenia [3]. However, little is known about how glutamate possibly influences the functional connectivity and auditory hallucinations in schizophrenia.

Methods and Results

39 schizophrenia patients (SZ) and 39 healthy controls (HC) were subjected to resting-state MR spectroscopy (^{1}H-MRS; left temporal lobe) and resting-state fMRI, and patients were assessed with the Positive and Negative Syndrome Scale (PANSS). After applying an independent component analysis/dual regression approach, individual spatial maps of the auditory resting-state network and left temporal lobe glutamate levels were used for the group analysis. The functional connectivity analysis indicated a significant interaction effect between *group* (SZ vs. HC) and *left temporal glutamate* in the left thalamus and right frontal pole, with follow-up correlation analyses showing significant correlations in the left thalamus (HC: r=-.39; p<0.5; SZ: r=.32; p<0.5) and frontal pole (HC only; r=-.56; p<0.5). When splitting the SZ group into a *frequent* (≥4) and *less-frequent* (<4) hallucinations group based on PANSS, the correlation analysis revealed a significantly positive correlation between left temporal glutamate level and left thalamus functional connectivity in the frequent hallucination group only (r=.60; p<0.5).

Discussion and Conclusions

The present results show that left temporal lobe glutamate level is positively associated with functional connectivity of the auditory resting-state network in SZ, particularly within the left thalamus, and that this effect is mainly driven by SZ experiencing frequent auditory hallucinations. These results show for the first time the relevance of left temporal lobe glutamate levels for auditory resting-state network connectivity in patients with frequent auditory hallucinations. Moreover, this effect was found in the left thalamus, pointing towards an involvement in the thalamocortical loop. This could perhaps be interpreted as a glutamatergically mediated disinhibition of the thalamocortical loop in SZ with severe hallucinations, possibly 'opening the gates' to ongoing neuronal activity in the auditory network [1], and thus creating a perception of external auditory input that is actually internally generated.

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Ventral hippocampal encoding of non-spatial information: preliminary results

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While precise spatial firing fields is a hallmark of pyramidal cells in the dorsal part of the hippocampus, it is still unresolved whether the large-scale place fields in the ventral cell population are modulated by visuospatial input, or if the cells respond to something else (Kjelstrup, 2008). To answer if the ventral cells encode non-spatial information, as spatial selectivity is lost, we recorded cells along the longitudinal axis of the hippocampus in rats that experienced changes in contextual valence rather than visuospatial changes. Several lines of evidence suggest emotional valence to be represented in the ventral hippocampus. Gene expression, anatomical connections, electrophysiological recordings and functional data in unison suggest a ventral hippocampal involvement in stress responses. While the dorsal hippocampus is required to solve vision-based navigation tasks, the ventral hippocampus seems to have a role in innate fear responses (Wang, 2013). Dorsal hippocampal place fields respond and re-map to non-spatial stimuli, such as odors. One hypothesis is that the ventral hippocampus is responsible for remapping even of dorsal firing fields. The ventral cells could
also help generalize learned experiences to similar situations (Komorowski, 2013).
Larger place fields in the intermediate hippocampus could be a mere reflection of increasing grid spacing in the MEC (Stensola, 2012), but we do not know if this applies to the very ventral part of the hippocampus.

In the current experiment, we investigate how dorsal and ventral hippocampal cells change their coding when emotional valence of the environment is changed. Single hippocampal units were recorded in Long-Evans rats while an emotional change was evocated by applying white noise (90 dB) or the scent of a natural predator to a costume made environment (a 70 cm x 70 cm ventilated box).

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The GHB analogue HOCPCA is a substrate for the monocarboxylate transporter MCT1 in vitro and in vivo

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ϒ-Hydroxybutyric acid (GHB) is a recreational drug, a clinically prescribed drug in narcolepsy and alcohol dependence, and an endogenous substance binding to both high- and low affinity binding sites in the brain. Ligands with high affinity and specificity are attractive compounds for studying the molecular mechanisms pertaining to the specific GHB high- affinity binding sites that may hold promise as therapeutic targets. The conformationally restricted GHB analogue 3-hydroxycyclopent-1-enecarboxylic acid (HOCPCA) is one such compound.

After exogenous intake, GHB readily enters the brain. The drug is a known substrate for monocarboxylate transporters (MCTs) subtypes 1, 2 and 4. Specifically, MCT1 is suggested to be involved in the transport of GHB across the blood-brain barrier. The objective of this study was to investigate HOCPCA, as an MCT substrate in vitro and in vivo. The study is the first to characterize GHB and GHB analogues at recombinant MCTs.

For in vitro uptake studies, MCT1, 2 and 4 were recombinantly expressed in Xenopus laevis oocytes and the in-house developed radioligand [^{3}H]HOCPCA used. HOCPCA could inhibit the uptake of the endogenous MCT substrate L-[^{14}C]lactate and [^{3}H]HOCPCA was shown to act as substrate for MCT1 and 2 with estimated K_m values in the low millimolar range. Introducing single amino acid mutations into positions known to be essential for MCT function and substrate specificity further confirmed that HOCPCA binds to the endogenous substrate pocket of MCTs. Additionally, HOCPCA transport into the brain was confirmed by assessing the effects of the potent MCT inhibitor AR-C141990 on brain distribution of HOCPCA (10 mg/kg s.c.) *in vivo*. Concomitant administration of increasing doses of AR-C141990 to mice inhibited brain penetration of HOCPCA in a dose-dependent manner (ID_{50}=4.6 mg/kg), thereby providing proof of concept that HOCPCA enters the brain viaMCT-mediated transport. This adds to our knowledge about the *in vivo* pharmacokinetics of HOCPCA as an investigational tool compound and qualifies for future *in vivo* studies.

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Retrogradely transported lentiviral vectors for down-regulation of the ERK pathway.

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Parkinson's disease is the second most common neurodegenerative disorder, affecting over 6 million people worldwide. Currently, L-DOPA treatment remains the best pharmacological approach to relive motor symptoms in patients. Unfortunately, this medication is associated with disabling side effects, called dyskinesias that develop after few years of treatment. These abnormal, involuntary movements have been shown to correlate with an increase in the ERK (extracellular signal-regulated protein kinase)[1]. The up-regulation of the ERK pathway occurred primarily in dopamine receptor 1 expressing medium spiny neurons (D1 neurons)[2-4]. We have developed a lentiviral vector for expression of a dominant negative version of MEK (Mitogen-activated protein kinase kinase), a protein implicated in ERK phosphorylation, in an attempt to normalise this pathway in a rat model of the disease. In more details, the dominant negative protein binds the endogenous MEK and forms an inactive heterodimer, unable to phosphorylate ERK. Because ERK plays an important role in numerous cell functions, it is paramount to specifically target cells that are affected by the treatment. In order to achieve D1 neuronal cells specificity, we took advantage of the FuG-B pseudotype lentiviral vector, which is retrogradely transported[5]. Striatal D1 neurons project in the substantia nigra, consequently, FuG-B lentiviral vectors injected in the substantia nigra will be transported to the cell bodies of D1 neurons in the striatum, thus allowing specific targeting of the direct pathway. Using a reporter gene, we showed that, as expected, injection of the vector in the rat substantia nigra leads to expression of the fluorescent protein in the nigra, as well as the striatum, thanks to retrograde transport of the vector. Better coverage of the striatum was observed 8 weeks after stereotaxic injections, when compare to 4 weeks time points. We showed that it was possible to target striatal medium spiny neurons using MEK-dominant negative FuG-B vector and observed decreasing trend in striatal ERK level. However, the injection of viral vector led to an increased immune response in the substantia nigra and the striatum. Further experiments aiming at improving this system in order to avoid immune response against the vector are currently undergoing.

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Advanced inducible system to regulate neurotrophic factor gene therapy in PD.

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Glial Cell Line-derived Neurotrophic Factor (GDNF) and associated factors are the only therapeutic proteins able to protect damaged dopaminergic neurons, regenerate axons and improve dopamine release both in animal models and patients suffering from Parkinson's Disease. However, continuous high expression of GDNF in the brain has also significant side effects. To address this issue, we have used a novel inducible system based on Destabilizing Domains (DD) to regulate GDNF protein expression in vivo. The DD system operates by fusing a protein of interest to a DD that will lead the cellular machinery to target the fusion protein for proteasomal destruction. In the presence of an inhibitor, the fusion protein will be stable and the protein of interest will be expressed [1]. We have previously shown that induction of GDNF expression by DD (GDNF-DD) was able to protect neurons in animal models of PD [2] and wanted to validate the system further.

To determine if the effect of GDNF-DD was long-lasting, we repeated the experimental designed used previously[2]. Amperometry assessment of dopamine release in striatum showed a clear 2-3 fold increase in dopamine release of GDNF-DD when compared to controls even after expression of GDNF-DD has been turned OFF for 13 weeks.

To evaluate how fast GDNF-DD induction could be turned ON and OFF, viral vectors were delivered to the striatum of animals and expression was turned on over a period of 5 weeks. After expression has reached maximum levels, GDNF-DD expression was progressively turned off. Assessment of biological activity of GDNF-DD used phosphorylation of ribosomal protein S6 (p-RPS6) as a biological activity marker. Maximum biological activity of GDNF-DD was achieved after 3-4 weeks of induction and biological activity of GDNF-DD reverts to basal levels 1 week after system has been turned OFF.

To further validate if the DD system is immunogenic, we have established an ELISA to detect presence of antibodies against DD and showed that expression of DD does not elicit an immune response. Taken together, these results indicate that the DD system shows great promise as a viable system to regulate therapeutic gene expression in the brain. The preclinical data generated from these studies show that the DD system seems to be especially suited to regulation of gene expression in the brain.

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Successful interceptive timing: Two experiments combining findings from gaze, joystick movement and EEG

Many behavioural studies on prospective control have been carried out, but little is still known about the neural correlates for successfully intercepting a moving object [1,2]. New EEG analysis software allows researchers to correct for and analyse recordings contaminated by behavioural artefacts. Even actively incorporating behavioural events as time-locking triggers for the EEG recording has been proposed [3].

The first study actively combined gaze and EEG recordings in a visual tracking task to study whether human adult participants differentiate between three deceleration conditions [4]. Adult participants followed with their gaze a small car moving horizontally on a large screen, where the final approach of the car was temporarily occluded, and pushed a button to stop the car at the reappearance point. The participants initiated a prospective gaze shift (eye jump) prior to the push button response over the occluded area. Parietal activity observed during this time period showed that participants differentiated between the three car decelerations, but only when the averaged EEG was time-locked to the eye jump event and only when the participants managed to stop the car successfully. This study supports an active incorporation of behavioural data into the EEG analysis, revealing neural correlates of prospective control that otherwise would not be apparent in traditional analysis.

The second study was similar to the visual tracking task [4], but this time the participants controlled a vertically moving car with a joystick. The task was to move the joystick car, and crash it into the horizontally moving stimulus car in the target area. The behavioural joystick data showed that participants varied between early and late initiation of the joystick movement, indicating different control strategies. Neural correlates between perception and action in the second study is still unclear, but the first study hint at promising findings that are worth following.

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MARCO BARBARIGA

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The restorative effect of PDGF-BB in Parkinson disease therapy

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Background: Parkinson disease (PD) is a progressive neurodegenerative disorder characterized by degeneration of dopaminergic (DA) neurons. The therapies now available for PD are only symptomatic treatments, not able to stop the progression of the disease. We have recently concluded a phase I/IIa study using PDGF-BB in PD patients[1], based on previous animal studies which demonstrate that two weeks intracerebroventricular (icv) administration of PDGF-BB restores DA neurotransmission and provides functional recovery in several PD models, with an increased periventricular cell proliferation, an increased number of tyrosine hydroxylase-positive nigral DA neurons, a partial restoration of striatal DA transporter (DAT) levels and a behavior recovery in PD models[2]. Our first clinical results indicate that patients receiving the highest PDGF-BB dose show a significant improvement in DAT-binding, a marker of DA fibers, whereas the signal declines in placebo and low dose patients indicating ongoing neurodegeneration[1]. The mechanism underneath this effect is still not known. In the mouse model, the effect is abolished by inhibition of cell proliferation with a mitosis inhibitor, which suggests that the effect is mediated via dividing cells[2]. Since there are no newborn DA neurons after PDGF-BB treatment, the yet unknown dividing cell type most likely secretes molecules that have a protective and restorative effect on the DA system. Candidate cell types likely express PDGFRα and/or β, like pericytes, the perivascular cells embedded in the blood microvessels basement membrane of the whole body that control neurovascular functions necessary for neuronal homeostasis in the brain.

Hypothesis: We hypothesize that the beneficial effect of PDGF-BB is mediated by pericytes, most probably triggering the secretion of molecules (growth factors, cytokines, interleukins) that can modulate/regulate the activity and/or the differentiation of other cell types, (e.g. neuronal stem cells).

Aims: The results from this study could help to identify the PDGF-BB treatment mechanism of action, uncovering the cell population target of the treatment *in vivo* and discovering molecules downstream the PDGF-BB signaling that can be exploited for new innovative therapies in neurodegenerative disorders.

Methods: 6-hydroxydopamine (6-OHDA) PD mouse model is used as established by us[3], performing functional behavioral test before/after PDGF-BB/vehicle treatment (14 days icv using an Alzet mini-pump[2]) 5 weeks after lesion. Mice are then sacrificed directly or 6 weeks after the PDGF-treatment and brains are processed fresh frozen for gene and protein analysis. To identify molecules and/or cell populations target of the treatment, gene expression and protein level are analyzed in different brains regions (striatum, substantia nigra, subventricular zone) with an array system (MouseWG-6 v2.0 Expression BeadChip, ILLUMINA), qPCR and ELISA assay. In parallel, different pericyte cell lines are analyzed *in vitro* for gene and protein (in the medium) expression after PDGF-BB stimulation (20 ng/ml), to find common molecular changes as in the *in vivo* treatment.

Results: RNA/protein extraction has been performed on samples collected from treated mice (CSF and brain regions). We have already assessed the good concentration/integrity of the extracted RNA (Agilent 2100 Bioanalyzer), and the gene expression analysis is now ongoing.

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Postnatal development of postrhinal projections to medial entorhinal cortex

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The postrhinal cortex of the rat, which is considered homologous to the parahippocampal cortex of the primate, supplies main visual and visuospatial input to the entorhinal cortex, mainly to its medial component (MEC). The postrhinal cortex is strongly implicated in coding of the physical and temporal context of the animal. In view of the differential postnatal emergence of various types of spatially modulated neurons in MEC (*Langston et al, 2010, Science 328: 1576; Wills et al, 2010, Science 328: 1573*), it seemed relevant to study the postnatal development of this projection. In the adult, this projection is topographically organized and at least in part, targets principal neurons in layer II, which project to the dentate gyrus and CA3 of the hippocampus.

By combining anterograde tracing, and intracellular filling of retrogradely identified projection neurons in MEC, we studied the postnatal development of postrhinal cortex projections to MEC in rats. Our preliminary results showed that an adult-like topography of the postrhinal cortex to entorhinal cortex projections is present by postnatal day 4. From this early postnatal stage, postrhinal projections to MEC originate in layers II/III and V and terminate preferentially in layers II and III of MEC following a dorsoventral topographical organization. Thus, dorsal postrhinal cortex projects ventrally in MEC while ventral postrhinal cortex projects to more dorsal parts of MEC.

The density of these projections increased from P4 to P23, but always stayed in line with the adult topography. Our data further revealed that, like in the adult, principal neurons in LII of MEC, which project to the hippocampal formation, formed potential targets of postrhinal inputs. Ongoing experiments will address when these anatomically defined projections will become electrophysiologically active.

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Regulation of Arc by phosphorylation – a molecular switch for synaptic plasticity and memory

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Arc is an immediate-early gene which has emerged as a key player in synaptic plasticity. Local Arc protein synthesis at the synapse is thought to contribute to both synaptic strengthening and synaptic weakening. These opposing consequences might be regulated through post-translational modifications (PTMs) of Arc, serving as a molecular "switch" on Arc function. One hypothesis is that phosphorylation of Arc by ERK (extracellular signal-regulated kinase) could act as such a modulator.

Not much is known about Arc PTMs, but experiments performed in our group has shown Arc to be phosphorylated *in vitro* by ERK, and GST pull down analysis shows enrichment of ERK to GST-rArc (Nikolaienko et.al 2015, in progress). My PhD-project builds on these findings and focuses on confirmation and identification of Arc protein-protein interactions and work on the functional consequence of Arc post-translational modifications.

Recently conducted experiments using surface plasmon resonance show WT-ERK binding to Arc. This method allows for calculation of binding kinetics between proteins, and Arc shows binding affinity to ERK in the µM range. A mutated form of ERK which prevents substrates with DEF-domains from binding shows reduced Arc binding affinity. This indicates a binding region on Arc in its flexible "hinge region", recently described in Myrum et al. (2015). Identification of the direct binding site for ERK on Arc will strengthen the theory that the two proteins have a functional relationship also *in vivo.*

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Martinotti cells defined by Chrna2 coordinate layer V pyramidal cell activity

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GABAergic interneurons in the mammalian cortex possess a broad variety of morphological, neurochemical and electrophysiological properties. The somatostatin+ Martinotti cells (MCs), are the most prominent slow-inhibitory interneurons targeting distal dendrites of pyramidal cells (PCs). We identified the cholinergic nicotinic receptor alpha2 (Chrna2) as a marker to label layer V/VI MCs ($MCs^{\alpha 2}$). We used a Chrna2-cre mouse line crossed with a fluorescent reporter (tdTomato) confirming that the layer V/VI $MCs^{\alpha 2}$ exhibit the defining characteristic of a long axonal projection to layer I and extensive ramifications in layer IV. Immunohistochemistry showed that the vast majority of Tomato+ cells comprised a subpopulation of somatostatin+ interneurons. Whole-cell current- and voltage-clamp recordings were performed in the auditory cortex and confirmed that passive and active electrophysiological properties of $MC^{\alpha 2}$ resemble the classical low-threshold spiking patterns of MCs. Recorded $MC^{\alpha 2}$ usually exhibited spike frequency adaptation and burst discharge when depolarized from hyperpolarized potentials. In paired recordings, layer V PCs showed long inhibitory postsynaptic potential rise times and synaptic depression upon electrical stimulation of the presynaptic $MC^{\alpha 2}$. Moreover, optogenetic manipulation of channelrhodopsin-activated $MCs^{\alpha 2}$ demonstrated that layer V PC spiking can be controlled by MCsα via oscillatory inhibition of PC distal dendrites suggesting that MCs^{α} may play a key role for PC network coordination.

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Chick forebrain neural cells are useful as an *in vitro* model to screen drugs aimed at glutamate receptor ion channels

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The NMDA receptor ion channel is activated by the excitatory neurotransmitter glutamate and is present in virtually every nerve cell in the CNS. It is essential to all neurophysiological processes and is implicated in pathological neurodegeneration after stroke, epilepsy, Alzheimer's, Parkinson's and other CNS diseases. The so called glutamate "excitotoxicity" is also associated with brain toxic insults, e.g. resulting from warfare nerve agents and organophosphate pesticides.

Rodent neural cell cultures is a widely used model to characterize drugs aimed at the NMDA receptor and recently there has been significant interest in the relative involvement of NR2A and NR2B NMDA receptor subtypes in neurodegeneration and in their localization, synaptically or outside the synapse (extrasynaptically). We used a model with cultures of chick forebrain neurons as an alternative to mammalian neural cell cultures in order to screen for anticonvulsant and neuroprotective drugs aimed at NMDA receptor subtypes. We hypothesized that chick forebrain neurons, mainly containing cortical cells, are a possible alternative to rat or mouse cerebellar granule neurons to study NMDA receptor drugs. The advantages compared with the rodent model are that a) cells are dissected from 10 day old embryos (eggs) and such "young" embryos are not subject to EU regulations for animal experiments b) the model is economical and does not require animal house facilities c) dissection or handling of chick embryo is less likely to give allergy d) embryos may be injected with drugs prior to dissection to test for neurotoxic or developmental effects. A disadvantage is that the forebrain cells from 10 day embryos are still in the growth phase and therefore typical inhibitors of glial cell proliferation cannot be used.

We examined the NMDA, kainate and depolarization (K+) induced Ca2+ responses and found that the potencies of inhibitors at NMDA receptors have a lower but the same relative potency as in rodent cells. We further developed a model to screen drugs specifically aimed at the NR2B containing subtype, derivatives of ifenprodil and RO 04-5595, and use the "switch" in expression from NR2B to NR2A subtype from DIV1 to DIV4 in culture to compare the effects and selectivity of the drugs. The *in vitro* model was also used to characterize the inhibitory effects of poly-unsaturated fatty acids on glutamate induced excitability and we conclude that chick forebrain neural cell cultures is an excellent alternative platform in preclinical screening and drug development.

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Local deformation in the entorhinal grid cell pattern

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Grid cells are spatially modulated cells that fire in a hexagonal pattern when an animal moves across the environment. Recent studies demonstrated that the orientation of the grid axes is influenced by the geometry of the environment {1, 2}. In square enclosures, one of the grid axes is oriented with a small but consistent offset from one of the cardinal axes of the box. The orientation offset was distributed bimodally around plus or minus 7.5 degrees, with only very few cells oriented at 0 or 15 degrees from the cardinal axis.

The orientation offset is accompanied by distortions of the grid structure, defined by fitting an ellipse to the innermost hexagon in the autocorrelogram of the rate map of the grid cell. When the grid is transformed by shearing the original data in either the x or y axis, aimed at minimizing the ellipticity, the orientation offset is abolished and grid instead aligns to the cardinal axis of the environment. The offset is smaller in novel than familiar environments, suggesting that the deformation and rotation depend on experience.

The close relation between the ellipticity and the orientation suggests that there is an endogenous grid structure with minimal elliptic distortion and alignment with the wall. In a typical recording box, however, the interaction between grid and environment is not limited to one wall. It is therefore possible that the overall deformation of the grid stems from a compromise between the internal structure of the grid system and its interaction with local features of the environment. Whether this default grid is lost with experience, and whether experience-dependent deformations occur locally or globally, remains unknown.

In this study we investigated the interaction between grid cells and the geometry of the environment as animals foraged in differently sized and shaped environments. In large recording boxes, the grid was often fragmented so that the grid took on distinct characteristics in different parts of the environment. Using a sliding window autocorrelogram, we were able to measure features of the grid locally, so that possible sources for the deformation and rotation could be determined.

Animals displayed variability in their grid structure throughout the environment. Typically, the grid had one or more basins in the environment where it displayed almost no ellipticity. In these locations, the grid axis closest to one of the walls tended to align with the wall, with almost no rotational offset, or the axis aligned with the diagonal of the box at 45 degrees. Furthermore, the tilt of the ellipse tended to be oriented towards one of the axes of the box (90 or 180 degrees). These findings corroborate the model established earlier that the grid first aligns to the box, but it extends this model by showing how the grid might still retain its default structure in parts of the environment.

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Some cells in the spinal cord could produce monoamines after spinal cord injury

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Serotonin (5-HT), an important modulator of both sensory and motor functions in the mammalian spinal cord, originates mainly in the raphe nuclei of the brainstem. However, following complete transection of the spinal cord, small amounts of 5-HT remain detectable below the lesion. It has been suggested, but not proven, that this residual 5-HT is produced by intraspinal 5-HT neurons. Here, we show by immunohistochemical techniques that cells containing the enzyme aromatic L-amino acid decarboxylase (AADC) occur not only near the central canal, as reported by others, but also in the intermediate zone and dorsal horn of the spinal gray matter. We show that, following complete transection of the rat spinal cord at S2 level, (AADC) cells distal to the lesion acquire the ability to produce 5-HT from its immediate precursor, 5-hydroxytryptophan (5-HTP). Our results indicate that this phenotypic change in spinal AADC cells is initiated by the loss of descending 5-HT projections due to spinal cord injury. By *in vivo* and *in vitro* electrophysiology, we show that 5-HT produced by AADC cells increases the excitability of spinal motoneurons. The phenotypic change in AADC cells appears to result from a loss of inhibition by descending 5-HT neurons and to be mediated by 5-HT1B receptors expressed by AADC cells. These findings indicate that AADC cells are a potential source of 5-HT at spinal levels below a spinal cord injury. The production of 5-HT by AADC cells, together with an up-regulation of 5-HT2 receptors, offers a partial explanation of hyperreflexia below a chronic spinal cord injury.

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Projections of calbindin expressing neurons in layer II of the entorhinal cortex

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The hippocampal formation and the parahippocampal region have been implicated in learning, memory and spatial processes. In the parahippocampal region, the entorhinal cortex (EC) is considered the gateway between the hippocampal formation and the neocortex. The entorhinal cortex can be divided on the basis of function and cytoarchitectonic structure into two subregions, the medial (MEC) and the lateral (LEC) entorhinal cortices. Cells in MEC are spatially modulated, whereas cells in the LEC convey among others olfactory and object-related information. Layer II of EC comprises at least two distinct populations of principal cells. The first expresses reelin and projects to the dentate gyrus (DG). The second population expresses calbindin and was claimed to project commissurally to the contralateral MEC in the rat. However, this is in contrast with previous findings, as MEC commissural cells are mainly located in layer III (Steward et al., 1996). Recently, a study in the mouse found that the calbindin layer II neurons project to stratum lacunosum of hippocampal field CA1. The aim of this study was therefore to investigate the projection targets of the calbindin population in layer II of the EC in rodents. Fluorescent retrograde tracers were injected dorsally in CA1 of rats, whereas in mice dorsal CA1 and the contralateral MEC were targeted with different tracers. Immunohistochemistry for calbindin was performed on horizontal sections in order to verify the presence of colocalization with retrogradely labelled cells. All sections were analyzed with fluorescence and confocal microscopy.

Results showed that 10% of CA1 projecting cells were positive for calbindin in MEC layer II of rats, whereas in LEC layer II, 6% of CA1 projecting cells were positive for calbindin. However, the majority of EC layer II calbindin cells was not retrogradely labelled. In mice, 11% of CA1 projecting cells were positive for calbindin in MEC layer II, while in LEC layer II, 7% showed colocalization. Regarding commissural projections, we observed that in MEC, 3% of cells where positive for calbindin, whereas in LEC layer II, 2% were positive for calbindin. Comparable to the results in the rat, the majority of EC layer II calbindin cells was not retrogradely labelled. The results indicate that the calbindin population in EC layer II has heterogeneous projection targets, where the projection target of the majority of cells is currently unknown.

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Eyeblink classical conditioning contingent on hippocampal fissure theta phase

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Hippocampal theta (3-12 Hz) oscillations (Buzsáki, 2002) are implicated in learning and memory, but their functional role remains unclear. According to a computational model by Hasselmo and colleagues (Hasselmo et al., 2002; Hasselmo and Stern, 2014), the phase of hippocampal theta oscillation determines specific and separate time windows for the efficient encoding and retrieval of memories. We studied the effect of the phase of local theta oscillation on hippocampal responses to a neutral conditioned stimulus (CS) and subsequent learning of classical trace eyeblink conditioning in adult female New Zealand White rabbits. An 80-dB, 4-kHz, 200-ms tone was used as the CS. During conditioning, the tone was followed by a 500-ms stimulus-free trace period and then by a 100-ms airpuff towards the eye (unconditioned stimulus). Sixty trials per session were presented. All rabbits were trained for 10 sessions with the CS timed to start at either the peak (Peak group) or trough (Trough group) of the fissure theta cycle. Yoked control animals were trained simultaneously and received trials irrespective of their neural state. In the Trough group, high-amplitude, regular hippocampal theta-band responses (that predict good learning) were elicited by the CS. Regardless, learning in this group was not enhanced compared to the yoked control group, possibly due to a ceiling effect. However, in the Peak group hippocampal theta-band responding was less organized and learning was retarded. In well-trained animals, the hippocampal theta phase at CS onset no longer affected performance of the learned response, suggesting a time-limited role for hippocampal processing in learning. To our knowledge, this is the first study to demonstrate that timing a peripheral stimulus to a specific phase of the hippocampal theta cycle produces robust effects on the synchronization of neural responses and affects learning at the behavioral level. Our results support the notion that the phase of spontaneous hippocampal theta oscillation is a means of regulating the processing of information in the brain to a behaviorally relevant degree.

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AL QUDS

Cognitive Assessments Prospectively Differentiate Future Responders and Non-Responders to Selective Serotonin Reuptake Inhibitor Treatment among Medication-Naïve Patients with Major Depressive Disorder

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Abstract

Short cognitive assessments of learning from positive feedback have the potential to prospectively differentiate future SSRI responders from non-responders in patients with Major Depressive Disorder (MDD). We evaluated a group of medication-naïve patients with MDD before and after 4-6 weeks of treatment with SSRI, as well as a matched group of controls tested across the same time interval. All subjects were administered a probabilistic category-learning task that allowed for the dissociation of learning from positive versus negative feedback. At the time of retesting, 65% of our sample responded to SSRI treatment, while 35% were non-responders. We compared the cognitive profiles of these two subpopulations at both baseline and retest against that of healthy subjects. While SSRI non-responders show balanced learning between positive and negative feedback at both baseline and again at retesting following SSRI administration, the SSRI-responders in their medication-naïve state show a strong bias for negative feedback, which was later altered by SSRI therapy to become more balanced during the subsequent post-treatment retesting. The findings in SSRIresponders replicate our earlier results (Herzallah et al., 2013a) using a within-subject design. These cognitive differences between responders and non-responders assessed prospectively prior to any SSRI treatment have the potential to inform the development and validation of a pre-treatment cognitive assessment to predict future clinical response to SSRIs. No such assessment currently exists and, if successfully developed and validated, would have the the potential to significantly impact clinical treatment as well as inform the search for new antidepressant medications that would provide symptomatic relief to those who do not benefit from SSRIs. The ability to differentiate, *a priori,* the SSRI-responders from the non-responders prior to initiating antidepressant treatment, would also shed much-needed light on the complex and poorly understood underlying heterogeneity of MDD.

MONA MOISALA

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Differences in brain activations during divided and selective attention related to ICT user profiles

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As the use of modern digital technology has become an increasingly integral part of our everyday lives, concerns have been raised about whether the continuous fast-paced influx of information offered by modern media affects our ability to focus (Carr, 2010). These concerns are especially relevant when it comes to the generation of "digital natives" (Prensky, 2001). We investigated whether different information and communications technology (ICT) user profile groups show differences in performance or brain activity during an attentionally demanding task. Participants were 160 healthy adolescents and young adults in three age cohorts (14-, 17- and 20-year-olds), with each cohort divided into three ICT profile subgroups: computer gamers, social media actives, and controls with as little ICT use as possible. We measured brain activity with event-related functional magnetic resonance imaging (fMRI) in participants performing a sentence congruence judgment task in the auditory or visual modality (selective attention conditions), or both (divided attention condition).

Performance was significantly deteriorated during divided attention compared with selective attention, and performance accuracy improved significantly with age in all conditions. There were no performance differences between the groups with different ICT profiles, or interactions between age and ICT profile. Whole-brain analyses revealed a main effect of ICT group in the left temporoparietal junction during all conditions, with the gamer group showing more activity in this area than the other groups especially during divided attention. Region of interest (ROI) analyses revealed that blood-oxygen-level-dependent signal increases in lateral and medial frontal and parietal areas that were activated specifically by divided attention correlated positively with task performance. The social media group showed significantly smaller activity increases than the other two ICT profile groups within these ROIs during all conditions, but only in the youngest age cohort.

The results suggest that the type and extent of ICT use may have an impact on the functioning of brain networks involved in divided attention and language processing. More specifically, increased activity in the left temporo-parietal area was observed in the gamer group, possibly reflecting increased demands on linguistic processing during the experimental task. In addition, lower activation in fronto-parietal regions (which was associated with poorer task performance in general) was observed in the youngest social media group. This suggests that in this ICT user group, performing the experimental task placed more demands on brain networks related to attention and executive functioning. Possible factors explaining the relationship between ICT use and brain activity will be discussed.

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NILS BORGESIUS

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Inactivation of CA2 principal cells increases MEC LII-CA1 coherence in the high gamma frequency range (90-140Hz).

Authors

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Abstract

The long overlooked hippocampal subregion CA2 possesses a number of unique qualities. It is connected differently (Rowland, 2013; Kohara, 2013; Cui, 2013), has different physiology (Chevaleyre, 2010; Mankin, 2015), has different gene expression (zhao, 2001) and it is involved in different behavioral functions than CA3 and CA1 (Hitti, 2014; Pagani, 2014). However, the influence on the (para)hippocampal network of CA2 has not been reported on yet. The CA2 region provides an alternative route for the classic trisynaptic pathway (entorhinal cortex layer II (ECLII)-dentate gyrus-CA3-CA1) as it directly and reciprocally connects the ECLII to CA2 (Rowland, 2013; Kohara, 2013). In contrast to the EC layer III (ECLIII)-CA1 connections the ECLII-CA2 are very strong and plastic (Chevaleyre, 2010). Importantly, the neurons in EC that receive the CA2 projections are stellate cells and putative grid cells. Finally, CA2 has strong projections to the deep layers of CA1 (Kohara, 2013). CA2 is ideally positioned to and thus expected to coordinate activity between CA1 and ECLII. This led us to the following hypothesis, CA2 modulates phase-phase coherence between CA1 and MEC.

We recorded single-units and LFP simultaneously in CA1 and medial ECLII (MECII) in *Amigo2-Cre* mice that express Cre specifically in the principal cells of the CA2 region (Hitti, 2014) injected with AAV-flex-PSAM-Chr2-HAtag. These mice express the inhibitory pharmacogenetic PSAM receptor allowing us to specifically inhibit the CA2 region by an i.p. PSEM injection (Magnus, 2011). Animals were allowed to freely explore a one by one meter box before and after i.p. injection of either PSEM or saline. We analyzed the phase-phase coherence of the LFP oscillations between MEC and CA1 as well as the temporal aspects of the firing patterns before and after injection.

The coherence between CA1 and MECLII is increased by injection of PSEM and unaffected by control saline injections. The effect is very specific for the 90-140 Hz band (fast gamma or epsilon band) (Belluscio, 2012). Theta, slow and medium gamma are not affected. Moreover, it is only seen between MECLII and CA1 stratum pyramidale, not stratum radiatum. Surprisingly, the temporal aspects of the firing in CA1 and MECLII are not affected. Although the LFP signal in the epsilon band can be contaminated with spikes (Belluscio, 2012) our data suggests that the effect is truly oscillatory. We do not see changes in phase locking or firing frequency of the single-units simultaneously recorded with the LFP. The lack of effect on spiking could be due to insufficient amounts of activity reduction, both at the level of the individual CA2 neurons and the percentage of transfected neurons. Taken together, our data shows that CA2 modulates the coherence between CA1 and MECLII in the 90-140 Hz frequency band. This suggests that CA2 could control the information flow between CA1 and MECLII.

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Afferent input on parvalbumin-positive interneurons in medial entorhinal layer II: fine structural investigations

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Spatial representation in the brain arises from a complex interplay between different functionally defined neurons within several brain regions. Among these neurons are grid cells, which have a hexagonal firing pattern spanning the entire environment that an animal explores (1). It is known that most of these cells can be found within layer II (LII) of the medial entorhinal cortex (MEC); however, empirical evidence for the morphology of these cells is still lacking, as well as their precise connection in a microcircuit. It has nevertheless been proposed that parvalbumin-positive (PV^+) interneurons, the most abundant inhibitory cell type in the MEC LII, are involved in the generation of grid cell firing patterns (2,3). Reasons for this include functional evidence that show these interneurons control the firing of several spatially modulated cell types, among them grid cells, and also because grid cells have been found to be responsible for most of the input into PV^+ interneurons (3). Additionally, the most likely grid cell candidates, stellate cells, are disynaptically connected via such fast-spiking PV^+ interneurons (2–4). It is therefore clear that increasing our knowledge about these interneurons is of interest. In order to do so, we are combining different immunocytochemical techniques on rat vibratome sections to both investigate these cells, as well as their interplay with other cells in the MEC LII with confocal and electron microscopy. Particularly the input organisation and synaptic morphology will be inspected at the ultrastructural level along the dorsoventral axis, with the aim to provide valuable insights into how grid maps arise in the entorhinal cortex.

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Learning from Positive, but not Negative, Feedback is Modulated by Dopamine Transporter Genotype in Patients with Major Depressive Disorder and Healthy Subjects

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Abstract

Understanding the impact of genetic variations in the dopaminergic system is key for clarifying how such variations contribute to individual differences in cognition, as well as to risk factors for mental disorders and differential responses to therapy. To examine the influence of the 3' variable number of tandem repeats (VNTR) polymorphism in the dopamine transporter gene (DAT1) on cognitive function, we used a probabilistic category-learning task that allowed for dissociation between the acquisition of positive and negative feedback. Of note, the DAT1 polymorphism influences expression of the DAT protein and ultimately dopamine levels in the striatum, and previous research has shown that variations in dopamine levels can influence whether one learns more from positive or negative feedback. We tested racially homogenous, healthy volunteers as well as SSRI-treated and responding patients with Major Depressive Disorder (MDD) and grouped them according to DAT1 VNTR genotype into 9-repeat carriers and 10-repeat homozygotes. Both healthy and MDD carriers of the 9-repeat allele, who should express less DAT1 and thus have higher levels of dopamine, were more efficient in learning from positive feedback. On the other hand, among healthy subjects, there was no difference between genotypes in learning from negative feedback. Overall, patients with MDD learned significantly less well than healthy subjects from both positive and negative feedback. These results contribute to a growing body of data that implicates the dopaminergic system in striatal-dependent feedback-based learning and the pathogenesis of MDD, and add weight to the proposition that individual differences in cognition have a strong genetic basis. Future work is needed to focus on studying the effect of this polymorphism on cognitive function in medication-naïve patients with MDD, and link that to future response to SSRIs.

OLEKSII NIKOLAIENKO

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Post-translational regulation of Arc/Arg3.1 by Erk2

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The immediate-early gene ARC (aka ARG3.1) is required for long-lasting synaptic plasticity and memory formation. Arc expression is precisely regulated in space and time by activation of extracellular signal-regulated kinase (Erk/MAPK). Here, we found that Erk2 also acts as a post-translational regulator of Arc. Using *in silico* tools Arc was predicted to bind Erk family kinases and to contain several potential Erk phosphorylation sites. GST-fused Arc of rat origin was able to pull down endogenous Erk1 and Erk2 from rat hippocampal lysates. Using a cellulose-bound peptide array covering the Arc/Arg3.1 sequence, we mapped the binding site of purified Erk2 on Arc. Activated Erk2 phosphorylated bacterially expressed Arc *in vitro* at different sites, as confirmed by phospho-specific protein staining and subsequent LC-MS/MS analysis. We also raised rabbit polyclonal antibodies that specifically recognize S206-phosphorylated Arc and show that this residue is modified *in vivo* and that treatment with the mitogen-activated protein kinase kinase (MEK) inhibitor U0126 affects Arc S206 phosphorylation. The results identify Arc as an Erk substrate and further suggest a dual role for Erk signaling in regulating Arc expression and function.

DAT1-COMT Gene Interaction Modulates Learning from Reward in Healthy Individuals

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Abstract

Sensitivity to positive feedback varies considerably among individuals. One possible reason for this can be attributed to genetic variability in regulatory components of the dopaminergic system (DA). These regulatory components include catechol-o-methyl-transferase (COMT) and the dopamine transporter (DAT). COMT, which functions particularly in the prefrontal cortex (PFC), plays a role in tonic DA signaling and hence phasic DA signaling; while DAT, mainly functioning in the striatum, regulates phasic DA signaling. Studies show that the PFC regulates DA striatal signaling via a top-down mechanism. The genetic variability results from functional polymorphisms in both the COMT and DAT encoding genes. Namely, the Val158Met substitution of the COMT gene gives rise to a 4-fold reduction in COMT activity, whereas a variable number of tandem repeats polymorphism (VNTR) in the DAT gene is thought to regulate phasic release of dopamine by affecting DAT density. To study the cognitive correlates of these physiological processes, we tested 53 healthy subjects using a computer-based probabilistic feedback-based category-learning task that allows for the dissociation of learning from positive versus negative feedback. We grouped subjects according to their COMT/DAT1 genotype into four groups: (1) Val/Val-9/9, with high COMT activity, low DAT density, resulting in high tonic and medium phasic dopamine, (2) Val/Val-10/10, high COMT activity, high DAT density, resulting in high tonic and low phasic dopamine, (3) Met/Met-9/9, with low COMT activity, low DAT density, resulting in low tonic and high phasic dopamine, and finally, (4) Met/Met-10/10, with low COMT activity, high DAT density, resulting in low tonic and medium phasic dopamine. Subjects with genotypes reflecting medium phasic dopamine signals learned significantly better from positive feedback than other groups, going hand in hand with the previously proposed inverted U-shape curve of optimal DA related cognitive functioning. There was no difference between groups in learning from negative feedback. These findings suggest the importance of looking into collections of genes that work together in mediating the role of dopamine in learning from positive feedback. New insights in the interaction between various dopamine-regulating pathways may be useful in future research to better understand individual differences in brain disorders where dopamine dysfunction is implicated, including Parkinson's disease and schizophrenia.

PAVEL UVAROV

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Optogenetic probing of the KCC2 role in mouse brain.

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Epilepsy is a complex disease, mechanisms of which are not fully understood. In general, epilepsy is characterized by the impaired inhibition, and the neuronal inhibition in adult brain is mainly controlled by GABAergic system. Hyperpolarizing responses of $GABA_A$ receptors require a low intracellular chloride concentration that is known to be maintained by the neuronal potassium chloride cotransporter 2 (KCC2). KCC2 cotransporter demonstrates an exclusively neuron-specific expression, which is strongly upregulated during postnatal development, reaching its adult levels only in about one month old mice. *In vitro*, KCC2 knockdown in mature hippocampal cultured neurons leads to the depolarizing instead of hyperpolarizing responses of the $GABA_A$ receptors, while KCC2 transient overexpression in immature cultured neurons results in the hyperpolarizing GABA responses. In addition to the cotransporter function, KCC2 also controls neuronal migration as well as plays an important role in the formation and maintenance of small dendritic protrusions – dendritic spines.

KCC2 null pups die within 15 minutes after birth due to the impaired respiration, thus significantly precluding analysis of the KCC2 role in adult mouse brain. To overcome the problem, we have started developing of a novel approach, where KCC2 gene is to be rapidly inactivated exclusively in small spatially restricted subpopulations of adult mouse cortex by optogenetics tools, namely by the light-controlled activity of Cre recombinase. Multiple optogenetics systems that are able to control an activity of Cre recombines have been first tested in immortalized cell lines, and two of them, Cry2-CIBN and LightON, were chosen. On the second step, the two optogenetics systems were compared between each other in hippocampal cultured neurons derived from KCC2-flox mice. Our analysis showed that the LightON system is better suited for the light-controlled inactivation of the KCC2 expression in cultured neurons. To accomplish the analysis of the KCC2 role *in vivo*, components of the LightON optogenetics system, cloned into pJAZZ® Linear Cloning System, can now be directly used for production of the transgenic mice, in which the activity of Cre recombinase is controlled by blue light. Subsequent crossing of the LightON line and KCC2-flox transgenic lines will provide us with a tool to study KCC2 role *in vivo* in adult mouse CNS.

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A comparative study of different stem cells for treatment of rat spinal cord injury

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A comparison of stem cells of different origins in the treatment of spinal cord injury has not been performed in one experimental setting. We compared human bone marrow mesenchymal stem cells (hMSCs), and two types of neural precursors (NPs); human conditional fetal spinal line (SPC-01), and human induced pluripotent derived neural progenitors NP-iPS), in the treatment of balloon-induced spinal cord compression in rats. One week after lesioning, rats received either intrathecal application of hMSCs or saline or were implanted intraspinally with SPC-01 or NP-iPS or saline. Rats were assessed for their basic and advanced locomotor skills (BBB, flat beam test, rotarod). Analyses of white/gray spared matter, axonal sprouting, glial scar modulation, qPCR and cytokine levels were performed to detect host tissue response to stem cell therapy. The highest locomotor recovery was observed in NP-iPS treated animals followed by rats treated with bone marrow MSCs and SPC-01 cells. In all cell treated groups white matter sparing was observed, while gray matter was preserved only in the NP-iPS treated rats. Both NPs significantly increased the number of GAP43+ axons, reduced astrogliosis downregulated Casp 3 expression and increased levels of IL-6 and IL-12. hMSCs transiently decreased levels of inflammatory IL-2 and TNF-α. These findings correlate with the hMSCs 2 week span of survival, while NPs survived 2 months and matured slowly. We conclude that the NP-iPS treatment of SCI provided the highest recovery of locomotor function due to robust graft survival, tissue sparing, reduction of glial scarring and increased axonal sprouting.

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PER LUDVIK BRATTÅS

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The epigenetic co-repressor Trim28 regulates retroelements in human neuroepithelial stem cells.

Retroelements comprise about 45% of the human genome and are since not so long ago known to play an important role in evolution as well as being responsible for creating genomic mosaicism by mobilization in the genome during development as well as in adult stem cells of the brain [1]. That mobilization can influence the expression of genes by insertional mutagenesis within coding or regulatory sequences. Furthermore retroelements can influence the expression of nearby genes by serving as enhancers and promoters.

In most somatic tissues, retroelements are silenced by CpG-methylation to minimize potential disease-causing insertions. However, in embryonic stem cells (ESCs) and neural progenitor cells (NPCs) they seem to be regulated more dynamically by heterochromatin formation [2]. Those processes are induced by TRIM28 (KAP1 or TIF1βwhich is known to be an epigenetic co-repressor of gene transcription.

Recently we were able to show that deletion of *Trim28* in mouse NPCs causes an upregulation of endogenous retroviruses (ERVs). Therefore we are currently investigating Trim28 depletion in human neuroepithelial stem cells (hNESCs). We have performed knockdowns using shRNAs against *Trim28* in hNESCs, and qPCR as well as RNA-seq analysis indicate an upregulation of hERVIP10FH, AluY5 and L1. We are currently doing additional analysis on Trim28 deficient cells.

Our current data suggests that TRIM28 plays a crucial role in regulation of multiple classes of retroelements in hNESCs.

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Skewed participation of neuronal populations during spinal motor activity is governed by a fluctuation-driven regime

Authors

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Abstract

Every aspect of behaviors consists of orchestrated activity of populations of neurons. Populations of neurons, in which the individual neuron plays a smaller role, but as a group is capable of producing a multitude of spatio-temporal patterns in a reliable yet versatile manner. Through spiking activity, individuals in the population are able to interact, yet we know little about how this activity is distributed in the population. Here we investigate the distribution of firing rates in different motor behaviors in the spinal cord. We implanted silicon probes in the ventral horn of turtles and recorded single unit activities while inducing different motor behaviors. We find that the neuronal populations in the spinal cord have highly skewed distributions of firing rates. The distribution is lognormal-like and remains robust across different scratch behaviors. Individuals within the population retain their weight in the distribution across trials of different behaviors, but a redistribution of weights are observed when the behavior is altered. At shorter timescales we observe skewed time-dependent firing rates distributions. Spiking is mainly irregular, yet the activity becomes more regular with increasing firing rates. Our data suggests that synaptic fluctuations result in a nonlinear expansive f-I transfer function in neurons, that gives lognormal-like distribution of firing rates within spinal networks.

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Regulation of synaptic development and function by low and high affinity presynaptic kainate receptors

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Kainate type of glutamate receptors (KARs) are highly expressed during early brain development and influence the activity-dependent fine-tuning of the neuronal circuitry by contributing to the mechanisms of synaptic plasticity[1, 6]. In addition, accumulating evidence suggests a direct role for KARs in morphological maturation of the neurons [2-5]. However, the role of different types of KARs in synaptogenesis is not well understood. Using microfluidic chamber to asymmetrically isolate axons[7-9] we show that irrespective of subunit composition, presynaptic KARs stimulate the early stages of synaptogenesis by promoting formation of filopodial protrusions in isolated axons. However, in subsequent steps of synaptogenesis involving clustering of presynaptic vesicles and formation/ stabilization of synaptic contacts, the homomeric and heteromeric KARs had distinct roles. Presynaptic expression of any of the subunits capable of producing homomeric receptors (GluK1-3) lead to enhanced vesicle clustering, increase in the synaptic density and transmission efficacy manifested as an increase in the probability of glutamate release (Pr). These effects were associated with widened synaptic active zone detected in transmission electron micrographs. In contrast, expression of the high-affinity subunits (GluK4, GluK5) had no effect on synapse density on their own, but when co-expressed, prevented the synapse-promoting effects of GluK1 or GluK2 and lead to a strong inhibition of the presynaptic efficacy (i.e. decrease in the Pr). The presynaptic effects of GluK1 on synaptic vesicle clustering involved both PKA and PKC pathways while GluK2 and GluK5 selectively encompass PKA and PKC signalling cascades, respectively. The data supports distinct subunit-independent and subtype-specific roles of presynaptic KARs in different steps of synaptic differentiation, the low-affinity subunits having a strong growth, differentiation and function promoting effect restricted by the inclusion of the high-affinity subunits to the receptor complex.

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PÅL KVELLO

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Connectivity and Molecular Identity of Principal Neurons in Layer Three of the Lateral Entorhinal Cortex

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Convincing evidence points to the importance of the hippocampus for spatial navigation and memory formation. Furthermore, there is ample evidence pointing to the respective relevance of the two main input sources to the hippocampus, the medial (MEC) and lateral entorhinal cortex (LEC). Due to intensive studies of the MEC over the last decade, much of its function and connectivity has been elucidated. Information about the LEC however, is limited. In this ongoing study, we focused on LEC layer III, where three morphological and three physiological types of principal neurons have been described, but data on intrinsic connectivity and molecular properties are lacking. In the present study, we aimed to analyze the local connectivity of these neurons as well as the expression of previously used molecular markers such as reelin and calbindin. We performed whole-cell patch clamp recordings from clusters of up to four neurons simultaneously in brain slices, combined with fluorescent dye injections, immunolabelling and subsequent visualization by confocal laser-scanning microscopy, as well as 3D-reconstructions. Our main results so far showed three populations of principal neurons: reelin positive (N = 8), calbindin positive (N=1) and reelin/calbindin negative (N =52). Each of these populations, except calbindin, consisted of a mixture of the previously described morphological or physiological types of principal neurons. We further observed that three of 66 neuron pairs (4.5 %) tested for connections were monosynaptically connected, and only one way, by excitatory synapses. All three pairs showed a regular spiking pattern. One neuron was characterized morphologically and immunolabelled. It showed a pyramidal shape and was negative for both reelin and calbindin. These results indicate a lower connectivity in layer three of the LEC compared to layer three of the MEC where the probability of finding connections between principal cells was 9 %.

Strategy-specific patterns of Arc expression in retrosplenial cortex during T-maze learning

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Spatial memory depends on several interconnected brain regions with spatially tuned neurons. One of the elements of this network is retrosplenial cortex (RSC). It harbors head direction cells and damage to this structure impairs spatial navigation based on environmental cues. T-maze is one of the most commonly used behavioral tasks that allow testing rodent spatial memory and navigation. Its most popular version is based on spontaneous tendency of rodents to explore novel areas during foraging for food. In the first phase of each training trial the animal is forced to make a turn into selected arm and collect a reward. In the second phase, both arms are open and only the previously unvisited arm is rewarded. Animals quickly learn the rules of the task, but they tend to utilize several strategies in order to solve it. We modified the T Maze design in order to increase the dependency on allothetic, extramaze cues. Opaque walls were replaced by ones made of transparent plexiglass and a large projection screen was placed in front of the maze. Animals were trained to follow two sequences (right-left or left-right) based on two different images projected onto the screen (context A or B, respectively). Almost all animals (13 out of 14) learned to perform both versions of the task within two weeks (8 trials per day). During the test phase, the context was replaced between forced and choice phase. In half of the animals (7 out of 13) this resulted in revisiting the arm, consistently with the changed spatial cues. On the next day the animals were subjected to one regular trial in each of the contexts with 18 minutes interval between trials. 2 minutes after second trial animals were sacrificed and brains were processed for in situ hybridization (catFISH) with a probe for immediate early gene Arc. The number of Arc-positive nuclei (activated only in trial 2) and double positive cells (nucleus and cytoplasm, activated in both trials) was counted for agranular and granular RSC, and hippocampal regions: dentate gyrus, CA1 and CA3. The overall number of Arc positive nuclei was significantly higher in the animals that did not follow spatial cues in both agranular RSC (6.82% vs 3.25%, $p<0.5$) and in granular RSC (4.43% vs 1.86%, $p<0.05$). There were no differences observed in dentate gyrus, CA1 or CA3. No differences in the level of overlap between cytoplasm and nuclei labeling was observed in any of the regions. Since Arc is commonly associated with learning – dependent plasticity, these results suggest that the non-spatial animals were still acquiring the spatial strategy. These observations also confirm the participation of RSC in spatial memory formation.

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Transcriptomic profiling of projection neurons to anterior cingulate cortex

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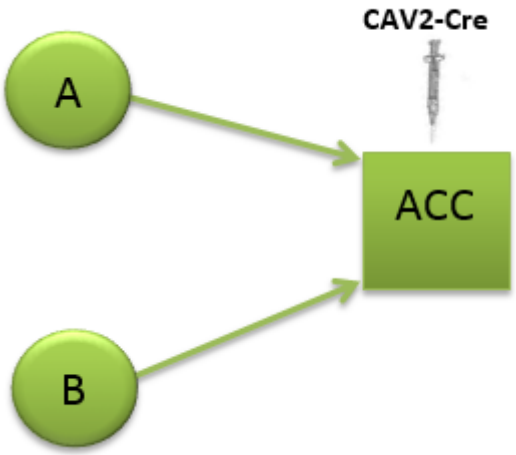
Deciphering the neural circuitry principles requires systematic identification and analyses of the connectivity between neurons in participating brain regions. Transcriptomic profile of neurons projecting 'to and from' an interconnected cortical region is not known. Anterior cingulate cortex is implicated in remote memory storage processes, is connected to distant brain regions and easily accessible by stereotaxic surgery. We are trying a combinatorial approach to characterize the projection neurons using 4-Thiouracil (TU)-tagging. 4-TU-tagging is an *in vivo* genetic and chemical intersectional method for covalent labelling and purification of cell-specific RNA. We utilized a retrograde transducing Cre-expressing virus, a transgenic mouse line (GFP-*flox-stop-flox*-UPRT) expressing UPRT in a Cre-dependent manner and transcriptomic analyses to this regard. Recombinant canine adenovirus (CAV2) having high retrograde transduction efficiency expressing Cre is stereotaxically injected to ACC in GFP-*flox-stop-flox*-UPRT mice for Cre-dependent expression of UPRT at projection neurons to ACC (for eg. A and B in the diagram). Only cells that express UPRT will create thio-RNA upon 4-TU injection. Spatial specificity is provided by distinct expression of UPRT in projection neurons, while the temporal specificity is given by 4-TU injection. Dissection of projected regions, biochemical purification of labelled RNA and subsequent RNAseq will provide sufficient information on the transcriptomic characterization of the neurons projecting to ACC. Identification of cell type specific gene markers, shedding light to candidate molecular mechanisms could be supplementary information from the data.



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Increased membrane conductance increases the magnitude of spike frequency adaptation but does not change the gain and the threshold potential

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The pattern of action potential generation in spinal motoneurons depends on synaptic input and intrinsic response properties. The synaptic activity of the premotor neural network not only directly excites and inhibits motoneurons, but it may also modulate intrinsic properties. During functional spinal neural network activity motoneurons receive massive synaptic excitation and inhibition, and their membrane conductance increases considerably [1]. This can substantially alter response properties of motoneurons.

It is straightforward to expect that increased conductance will increase the rheobase and decrease the firing frequency of the neuron. However, it is not clear how increased membrane conductance will influence spike frequency adaptation, the gain (i.e., the slope of frequency-current relationship) and the threshold for action potential generation.

In the present study we investigated this issue by using intracellular recordings from adult turtle motoneurons in spinal cord slices. Membrane conductance of spinal motoneurons was increased pharmacologically by extracellular application of $GABA_A$ receptor agonist muscimol.

Our findings suggest that an increase of about 40% in membrane conductance increases the magnitude of spike frequency adaptation, but does not influence the threshold for action potential generation and causes a subtractive rather than a divisive effect on the frequency-current relationship of motoneurons.

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REBECCA PETRI

LUND UNIVERSITY

Neuron-specific analysis of microRNA targets in the adult mouse hippocampus using AGO2-RIPseq

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microRNAs (miRNAs) are small, non-coding, single stranded RNAs that act as important regulators of gene expression. By associating with the Argonaute2 (AGO2) protein in the RNA-induced Silencing Complex (RISC), they bind to mRNAs and induce their translational repression or degradation. Although miRNAs were suggested to influence cellular processes such as synaptic plasticity and dendritic branching in neurons, very little is known about which genes and functions are regulated by miRNAs in distinct neuronal populations.

To analyse the role of miRNAs in hippocampal neurons, we have developed a neuron-specific AGO2-RNA interacting immunoprecipitation (AGO2-RIP) that allows for the isolation of active miRNAs and their mRNA targets. We injected adeno-associated viral (AAV) vectors encoding a GFP-AGO2 fusion protein under the control of a Synapsin1 promoter into the adult mouse hippocampus. After eight weeks, we dissected the hippocampus and by using antibodies targeting GFP, we isolated miRNAs and mRNAs bound to the AGO2 protein in the RISC. Sham-injected mice served as control.

Poly-A enriched RNA sequencing of the RIP samples (RIP-seq) identified over 2000 miRNA-target genes bound to AGO2 in hippocampal neurons which we found to regulate essential neuronal function such as cell signalling, transcription and axon guidance.

Moreover, we have used AGO2-RIPseq to analyse changes in miRNA-mRNA target interaction upon the depletion of two in the hippocampus highly expressed microRNAs, miR-124 and miR-125, using miRNA-sponges. We found that the inhibition of miR-124 and miR-125 led to distinct changes in AGO2 binding of target mRNAs, resulting in the upregulation of multiple miRNA targets.

Our data represents an important step forward for understanding the role and function of miRNAs in specific neuronal populations.

ROBERTAS GUZULAITIS

UNIVERSITY OF COPENHAGEN

Patterns of excitatory, inhibitory and intrinsic conductances in motoneurons during rhythmic motor behaviour

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Intrinsic properties of motoneurons were proposed to play a key role in rhythmic motor behavior (Grillner, 2003). The importance of intrinsic properties was challenged by the demonstration that motoneurons enter a high conductance state during rhythmic network activity (Alaburda et al., 2005). A dramatic increase in synaptic conductance may shunt intrinsic properties (Berg et al., 2008). The assumption that intrinsic properties are shunted by synaptic conductance during network activity enabled extraction of inhibitory and excitatory patterns from the total conductance. Contrary to the generally accepted reciprocal inhibition/excitation model it was implied that motoneurons receive concurrent patterns of inhibition and excitation (Berg et al., 2007).
Here we tested the assumption that intrinsic properties are shunted by synaptic conductance during network activity. We found that substantial conductance increase during rhythmic network activity is voltage dependent. Voltage dependence suggests that some of conductance increase is due to activation of intrinsic ion channels. We demonstrate that the current-voltage relation of motoneurons at rest is nonlinear and rectifies at depolarised membrane potentials. By application of selective antagonists we show that this outward rectification is due to activation of K_V and K_{Ca} channels. In addition, using whole-cell patch clamp technique, patterns of inhibitory and excitatory input was measured directly during rhythmic network activity. We found that motoneurons receive alternating inhibitory and excitatory synaptic input. We conclude that neglecting voltage dependent conductance led to overestimation of inhibition and the level of concurrent excitation and inhibition.
Our findings justify reciprocal inhibition/excitation of motoneurons during rhythmic motor behaviour. We suggest that outward rectification observed in our study may serve as dynamic mechanism to stabilize the membrane potential near the threshold for action potentials during the depolarizing waves that characterize rhythmic motor activity.

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RUNE BERG

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Skewed participation of neuronal populations during spinal motor activity reveals dominant fluctuation–driven regime

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Abstract:
Motor patterns such as chewing, breathing, walking and scratching are primarily produced by neuronal circuits within the brainstem or spinal cord. These activities are produced by concerted neuronal activity, but little is known about the degree of participation of the individual neurons. Here, we use multi-channel recording (256 channels) in turtles performing scratch motor pattern to investigate the distribution of spike rates across neurons. We found that the shape of the distribution can be described as “log-normal”, i.e. normally shaped on logarithmic frequency-axis. Such distributions have been observed in other parts of the nervous system and been suggested to implicate a fluctuation driven regime (Roxin et al J. Neurosci. 2011). This is due to an expansive nonlinearity of the neuronal input-output function when the membrane potential is lurking in sub-threshold region. We further test this hypothesis by quantifying the irregularity of spiking across time and across the population as well as via intra-cellular recordings. We find that the population moves between super- and sub-threshold regimes, but the largest fraction of neurons spent most time in the sub-threshold, i.e. fluctuation driven regime.

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Models of tyrosine hydroxylase feedback regulation – implications for dopamine homeostasis

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The dopamine D2 receptor (D2R) is the prominent autoreceptor that regulate excitability and transmitter release from dopaminergic neurons. This autoregulatory function is implicated in several neuropsychiatric disorders. In addition, the D2-autoreceptors regulate the activity of tyrosine hydroxylase (TH), a key regulatory enzyme of dopamine biosynthesis. TH is feedback inhibited by dopamine binding to its active site, but can be reactivated by phosphorylation on serine40. The cAMP signaling pathway is a major mediator of TH Ser40 phosphorylation and this pathway is also inhibited by D2-autoreceptors. These feedback regulatory mechanisms are particularly prominent in the striatum, a major site for dopamine transmission in the brain. Presently, two prevailing mechanistic models exist of TH control by the two linked feedback mechanisms. Based on reported experimental data, we use mechanistic modeling to investigate the consequence of these TH-models on dopamine homeostasis of the striatal terminals. The implications for regulation by the two nested feedback mechanisms are discussed, as well as the implication for dopamine neurotransmission and disorders linked to dopamine function.

SABRINA ZECHEL

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Radial dispersion of neocortical GABAergic interneurons controlled by thalamo-cortical axons

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Neocortical GABAergic interneuron migration and thalamo-cortical axon (TCA) pathfinding follow similar trajectories and timing, suggesting they may be interdependent. Here we report that interneurons derived from the medial ganglionic eminence (MGE) reached the neocortex in normal numbers in the absence of TCAs. However, disruption of TCA innervation, or TCA-derived glutamate, affected the laminar distribution of MGE-derived interneurons in primary cortices in newborn mice, resulting in abnormal accumulation in deep cortical layers of interneurons that failed to switch from tangential to radial migration. This phenotype resembled the normal distribution of interneurons in secondary cortices, which lack afferents from specific thalamic nuclei. Expression of the KCC2 cotransporter, a known inhibitor of cortical interneuron migration, was prematurely upregulated in interneurons of denervated cortex, and KCC2 deletion rescued the interneuron phenotype caused by lack of TCAs. Pharmacological inhibition of NMDA receptors, or calpain, also led to increased KCC2 expression and defective radial dispersion of interneurons. Thus, although TCAs are not required to guide tangential migration of MGE-derived interneurons to the neocortex, they provide crucial signals, such as glutamate, that limit interneuron KCC2 levels, allowing normal radial dispersion and laminar distribution of interneurons in the cortex.

SAMER SIWANI

UPPSALA UNIVERSITY

Optical modulation of hippocampal OLM interneurons affects memory related behaviors

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It is widely accepted that the hippocampus has a crucial role in declarative memory formation, however, the knowledge about the functional role of specific hippocampal cell types and circuitries is limited. In this study we aimed at further elucidating the role of the oriens lacunosum-moleculare (OLM) cells in memory function. These cells are a group of inhibitory interneurons in the hippocampus that have recently been shown to regulate sensory input to the hippocampus. OLM cells are located in the stratum oriens (SO) layer and target the distal dendrites of pyramidal cells. Activation of OLM cells in the CA1 region leads to inhibition of the temporoammonic pathway and disinhibition of the schaffer collateral pathway. In accordance with the cholinergic input from the medial septum, OLM cells specifically express the acetylcholine receptor alpha-2 subunit (Chrna2). We therefore used a chrna2-cre mouse line to genetically target OLM cells and combined optogenetic tools, light stimulation and a novel object recognition behavioral paradigm to investigate memory function. We detected behavioral impairments in exploration of novel objects, which leads us to suspect that OLM cells are involved in context specific memory acquisition via modulation of sensory input to the hippocampus.

SANNA LENSU

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Does Intrinsic, Inherited Difference in Running Capacity Affect Learning, Memory and Neural Cell Proliferation?

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In recent years, growing number of studies have shown positive correlation between physical activity and brain health. In addition to improve and maintain metabolic health physical activity seems to enhance and support learning and memory, and increase neurogenesis. Thus, western lifestyle with diminished physical activity and fitness brings about a complex public health risk. It has been difficult to make causal inferences between aerobic fitness and cognition, because the process of acquiring aerobic fitness (i.e. exercise and a physically active lifestyle) involves factors, such as social interaction and enriched living environment, known to affect cognition positively. To study the neurobiological factors through which aerobic fitness influences cognition, we utilize a heterogenic animal model that is genetically determined for the intrinsic aerobic fitness: High and Low running Capacity Rats (HCR and LCR, Koch & Britton 2001). Our group has shown that in this model, intrinsic aerobic fitness is related to learning that requires flexible cognition (Wikgren et al. 2012).

In the current project, Active, Fit and Smart (AFIS, funded by the Academy of Finland, Grant no: 274098), we seek to determine the mediating factors for the difference in cognition. In the first stage, we shall determine the baseline difference between the rat lines in temperament, all levels of cognitive processing and neurobiological factors, especially neural cell proliferation, as all of these factors might play a role in learning. Manifestation of the possible differences will also be studied as a function of age, to see if cognitive deficits develop together with deterioration of other biomarkers of health that are found in this model (e.g. Kivelä et al. 2010, Wisloff et al. 2005). Finally, we also aim to study whether aerobic exercise helps to alleviate the possible adverse effects of inherited low aerobic capacity.

Here we show the first results of the AFIS-project in sedentary, ~6 months old male rats. In addition, behavioral data of young (less than 2 months) will also be shown. The clearest differences were seen in prepulse inhibition (PPI) of the startle response, a test for sensory processing, the HCR being more efficient than the LCR. In the open field arena (old rats), the differences were not significant, although there was a trend of increased locomotion in the HCR rats compared with the LCR. Furthermore, tentative results on hippocampal cell proliferation (immunohistochemistry of Ki67) indicate differences between the rat lines.

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Extracellular Matrix Hydrogels as Scaffolds for Spinal Cord Injury Repair

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Restoration of lost neuronal function after spinal cord injury (SCI) still remains a big challenge for current medicine. An important repair strategy is tissue reconstruction by bridging the SCI lesion with a supportive and stimulatory milieu that would enable axonal rewiring. Injectable extracellular matrix (ECM) derived hydrogels have been recently reported to have neurotrophic potential *in vitro* [1]. In this study, we evaluated the presumed neuroregenerative properties of ECM hydrogels *in vivo* in the acute model of SCI.

ECM hydrogels were prepared by decellularization of porcine spinal cord (SC), brain or urinary bladder (UB) tissue. The hydrogels were characterized *in vitro* in terms of their biochemical composition and adhesion, as well as the proliferation and migration of human mesenchymal stem cells (hMSC). In an *in vivo* study, SC- and UB-ECM hydrogels were acutely injected into the spinal cord hemisection and evaluated after 2, 4 and 8 weeks. Histological analysis showed that both hydrogels integrated into the host tissue and stimulated neovascularization and nervous tissue ingrowth into the lesion. On the other hand, massive infiltration of macrophages into the lesion and rapid hydrogel degradation did not prevent cyst formation, which progressively developed over 8 weeks. Gene expression analysis at 2 weeks post-SCI revealed significant down-regulation of genes related to immune response and inflammation in both hydrogel types, whereas this effect diminished at later time points.

In conclusion, ECM hydrogels are biocompatible and promote *in vitro* the proliferation and migration of hMSCs. When injected into SCI, UB- and SC-ECM hydrogels modulated the innate immune response and provided a stimulatory substrate for *in vivo* neural tissue regeneration. However, fast hydrogel degradation resulting in cyst formation may be a limiting factor for the use of ECM hydrogels in the treatment of acute SCI.

ACKNOWLEDGMENTS

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SETH AGYEI

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Functional Brain Development of Full-term and Preterm Infants: Longitudinal EEG Study

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Electroencephalogram (EEG) was used to investigate brain electrical activity of full-term and preterm infants at 4 and 12 months of age as a functional response mechanism to structured forwards optic flow, structured reversed optic flow, and random visual motion. Analyses of visual evoked potential (VEP) and temporal spectral evolution (TSE, time-dependent amplitude changes) were performed on EEG data recorded with a 128-channel sensor array. VEP results showed a significant improvement in full-term infants with age in latencies to forwards and reversed optic flow, but not to random visual motion. In addition, full-term infants at 12 months significantly differentiated between the three motion conditions. They showed the shortest latency to forwards optic flow and the longest latency to random visual motion. On the other hand, preterm infants did not improve their latencies with age, nor were they capable of differentiating between the three motion conditions at 12 months. When the TSE of the motion conditions were compared with the TSE of a static non-flow dot pattern, infants at 4 and 12 months showed significant differences in induced activities with desynchronisd theta-band activities observed in both term and preterm infants. Induced synchronised activities at alpha-beta frequencies were, however, observed only in the term infants at 12 months. It appeared that full-term infants at 12 months with a substantial amount of self-produced locomotor experience and accompanying neural maturation, rely on the perception of structured optic flows to move around in the environment efficiently, and that they are negatively affected by the lack of structure in random visual motion. It was concluded that the preterm infants' poorer performances are related to impairment of the dorsal visual stream which is specialized in processing visual motion. To better understand the fundamentals of early neurodevelopment and how it differs in prematurity, more research comparing data on the changes in brain activity in response to visual motion perception during infancy is suggested.

SHARN PERRY

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Dmrt3 derived neurons are "Gait-keepers" in spinal locomotor circuitry

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Coordinated stereotypical locomotor patterns are generated by networks of spinal cord neurons known as central pattern generators (CPGs). A subset of the dI6 class of spinal cord interneurons differentiate into a distinct neuronal population through the transcription factor Doublesex and mab-3 related transcription factor 3 (DMRT3). DMRT3 has been found to have major implications in the gait and locomotion of both horses and mice - a non-sense mutation in the horse *DMRT3* gene has major effects on gaiting ability, and mice lacking *Dmrt3* display impaired CPG activity affecting locomotion. Whilst these findings demonstrate a requirement for the *Dmrt3* gene in normal spinal neuronal network formation and function, the role of *Dmrt3* derived neurons remains unknown. For the first time, this study selectively targets the Dmrt3 subpopulation of the dI6 interneurons and gathers detailed electrophysiological, morphological, functional and behavioural information on the Dmrt3 population. The glycinergic commissural *Dmrt3* neurons receive extensive synaptic inputs from several sources. Removal of inhibitory neurotransmission in the Dmrt3 population in $Dmrt3^{Cre}$;$Viaat^{lx/lx}$ mice, resulted in a severely disturbed CPG output *in vitro* and consequently led to impaired limb coordination in neonatal mice whilst adult mice displayed impaired fine motor coordination and gait abnormalities. Electrophysiological recordings revealed that Dmrt3 neurons comprise of accommodating and regularly spiking neurons, some of which displayed hyperpolarization-activated cation currents (I_h). Furthermore, two-photon Ca^{2+}-imaging confirmed that Dmrt3 neurons were rhythmically active during fictive locomotion and fired at individual and separate frequencies. Taken together, these findings uncover a physiological significance and an unexpected character of the Dmrt3 subclass of dI6 interneurons in the neural circuitry controlling locomotion.

SHREYAS MYSORE SURYANARAYANA

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The lamprey blueprint of the mammalian motor projections from cortex – *the lateral pallium*.

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The lamprey homologue of the mammalian cortex, the lateral pallium, shows remarkable conservation in terms of its efferent connectivity (Ocaña et al., 2015). Electrical stimulation of circumscribed regions in the lateral pallium evokes eye and orienting movements, movements of the mouth, and in some cases, locomotion. Partially overlapping yet distinct groups of projection neurons in the lateral pallium have glutamatergic, monosynaptic projections to different downstream motor centers like the optic tectum (superior colliculus), mesencephalic locomotor region, reticulospinal neurons and the rostral spinal cord. Collaterals from the pallial projections (pyramidal tract-like, PT) to the downstream motor centers also target the ipsilateral striatum. Moreover, intratelencephalic (IT) pyramidal-like neurons with axons to the contralateral pallium also specifically target the striatum. Projections are also present to other basal ganglia nuclei like the nucleus subthalamicus (hyperdirect pathway) and to the dopaminergic substantia nigra *pars compacta* (Pérez-Fernández et al., 2014). Interhemispheric pallio-pallial projections are present, mirroring interhemispheric cortico-cortical projections, which are a crucial aspect of bilateral sensory integration. Thus, the efferent projection pattern is identical to that in mammals. Membrane and firing properties of pallial neurons resemble those of pyramidal and non-pyramidal cells recorded in the cortices of higher vertebrates. The morphology of pallial cells are pyramidal-like with spiny dendrites extending into the outer molecular layer which is devoid of neurons, like the outer molecular layer of the mammalian cortex. In terms of afferent sensory input, the lateral pallium receives visual input via a thalamic relay, from the lateral part of the dorsal thalamus, which is also known to receive retinal afferents, similar to the lateral geniculate nucleus. In addition, there are afferent projections to the lateral pallium from the more medial part of the dorsal thalamus, which is known to send projections to the striatum. The lateral pallium also receives olfactory input which bypasses the thalamus as in higher vertebrates - directly as well as via a relay nucleus - the *dorsomedial telencephalic nucleus* which receives intense “mossy fiber”-like projections from the olfactory bulb and may represent a novel pallial structure for olfactory processing. Taken together, our data shows that the basic efferent and afferent *bau-plan* of the cortex is ancient; it had already evolved when the lamprey diverged from the main vertebrate line 560 million years ago. It also highlights what the primordial cortex has evolved for – an integrator of information important for goal-directed motor behaviour.

SONCHITA BAGCHI

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High multiplicity of neuronal glutamine transporters; why so many?

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Introduction

The well-studied sodium-coupled neutral amino acid transporters (also known as SNATs) in the SLC38 family are classified into system A (SLC38A1, SLC38A2 and SLC38A4) and system N (SLC38A3 and SLC38A5). Functional characterizations of these transporters have shown that they have similar substrate profile, with all being able to transport glutamine. Our lab have recently shown that SLC38A7 (*Hagglund et al, 2011, JBC*) and SLC38A8 (*Hagglund et al, 2014, submitted)* have a similar transport profile as well. All transporters from the SLC38 family have also been shown to be expressed, in various degrees, in the brain. We are currently investigating what the functional relevance is to have this high number of transporters with very similar substrate profiles in the brain. Are there differences regarding their sub cellular localization, expression regulation, cell-type specificity, details in the substrate profile, ion coupling or dimerization patterns?

Methods and Preliminary Results

Our present study indicates an interesting localization for SLC38A6, being expressed primarily in the excitatory neurons and significantly minor or no expression in the glial cells. Moreover, we have used the unique method of *in situ* proximity ligation assay (PLA) to demonstrate presence of SLC38A6 in the synaptic membrane. We are performing functional characterization of it by over-expression in Xenopus laevis oocytes to identify the exact substrate profile. We have knocked it down in neural cell line using RNAi and also have created clones to over-express the gene in order to compare levels of uptake of different amino acids. We have also documented interesting interaction pattern between the transporters. For example, in situ PLA study reveals that SLC38A10 interacts more frequently with SLC38A8 compared to SLC38A7, which is very intriguing as SLC38A7 and SLC38A8 have very similar structure, localization and substrate profile. We are using bioinformatics to predict interaction partners of some of the transporters. We will further experimentally investigate how these proteins might be interacting in the brain to execute specific functions.

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Enhancer driven expression of transgenes in neuronal subtypes

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Types of neurons differ from each other in their gene expression pattern. Many of the existing transgenic mouse lines are based on making a phenocopy of expression of a specific protein. Generally, these transgenic mouse lines are not able to target specific neuronal subtypes as defined by their morphology, connectivity and physiological properties. We are making novel mouse lines based on enhancer driven expression of transgenes. This may give access to specific types of neurons that are homogenous in their gene expression, morphology, connectivity and physiological properties.

We identify putative enhancers that are active in cell types present in specific brain regions. Putative enhancers are identified by Chromatin Immuno Precipitation and massive parallel DNA sequencing (ChIP-seq) on microdissected tissue. The ChIP is carried out against H3K27ac, which binds active enhancer regions in the genome. The precipitated sequences are aligned to a reference genome, and thus give a quantitative readout of promoter and enhancer activity. Sequences (of the putative enhancer and promoter regions) obtained from this read-out are graded based on evolutionary conservation and other parameters to identify the most promising candidates (Cotney, Leng et al. 2013). Furthermore all putative enhancers are graded on the expression of genes they are likely to control (based on *In Situ Hybridization* data from atlas (AllenBrainAtlas 2014)).

The most attractive putative enhancers are cloned into constructs for pronuclear injection (enhancer-HSP68 min promoter- tetracycline TransActivator (tTA)). We have made several mouse lines based on putative enhancers specifically active in microdissected tissue from the medial entorhinal cortex (MEC). Several of these lines show expression of transgenes specifically in the MEC of adult mice. Furthermore, in some lines the expression is limited to single layers of the MEC, supporting the notion that enhancers drive the expression of specific cell types. With this method, we can identify enhancers that drive expression in specific brain regions and presumably in specific neuronal subtypes.

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STUDIES OF POTENTIAL OF SERETONIN RECEPTORS TO FORM HETEROMERS

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It is well established that the serotonin (5-HT) receptors play an essential role in neurotransmission. The interaction between 5-HT receptors with dopamine receptors may have a key significance since alterations in serotonin and dopamine neurotransmission have been implicated in many human neurological and psychiatric disorders [1,2]. Currently, the idea of G protein-coupled receptors (GPCR) oligomerization has become widely accepted**.** Formation of heteromers among serotonin receptors or between serotonin receptors and other GPCRs enables signaling diversification. Receptor heteromers may possess biochemical and pharmacological characteristics and functional properties that are distinct from those of mono- and homomers [3]. Moreover, the existence of a hetero-interaction is especially promising since heteromers can be formed only on neurons expressing receptors which are engaged in formation the complex. Thus a higher selectivity of the appropriate compounds may be achieved. Evaluation of dimerization process among these receptors is very important in answering the question whether some neuroleptics influence these processes. The complexes formed between the serotonin 5-HT_{1A}, 5-HT_{2A} and dopamine D_2 receptors may serve as potential targets for novel compounds for treatment of schizophrenia [4,5]. Therefore, in the present study we focused on the evaluation of the interaction between these receptors. Experiments were conducted in HEK 293 and CHO cell lines. Two various strategies based on the Förster resonance energy transfer (FRET) phenomenon, HTRF and FLIM techniques, were adopted to the study. Furthermore, the effects caused by specific receptor ligands as well as commercially available drugs used for schizophrenia treatment on the protein complex formation were estimated. We observed different influence on dimerization depending on the type of investigated heteromers as well as on whether homo- or hetero-complexes were present. Moreover, activation of signal transduction pathways as a result of antipsychotic action on investigated heteromers were determined. The release of secondary messengers like cAMP and IPOne as well as ERK activation were measured. We postulate that selective action of pharmacological compounds on heteromers formed among serotonin and dopamine receptors may have better therapeutic properties, what may have a key significance in novel therapy of schizophrenia.

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Arc regulates translation initiation complex formation during LTP consolidation in the dentate gyrus of live rats.

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The immediate early gene product, Arc, has emerged as a key regulator of protein synthesis-dependent synaptic plasticity, postnatal cortical development, and long-term memory. In the dentate gyrus (DG) of adult anesthetized rats, sustained Arc translation during a critical time window is necessary for LTP consolidation and stabilization of new F-actin at synaptic sites. The function of F-actin regulation in dendritic spines during LTP is unclear, but one possible function is regulation of protein translation. Translation of Arc requires activation of MAP kinase-interacting kinase (MNK1). MNK1 phosphorylates the mRNA cap-binding protein eIF4E and allows formation the eIF4E-eIF4G translation initiation complex during LTP (Panja et al., J Biol Chem, 2009, 284:31498-31511). Recently, we found that MNK activation triggers release of the translation repressor complex, CYFIP1/FMRP, from cap-bound eIF4E. (Panja et al., Cell Reports 2014, 9:1430-1445) Here, we show that local infusion of Arc antisense oligodeoxynucleotide (Arc AS) during in vivo DG LTP not only disrupts synaptic Arc protein expression and nascent F-actin (FITC-phalloidin staining), but also rapidly inhibits MNK phosphorylation, initiation complex formation, and eIF4E phosphorylation. mTORC1 signaling to rpS6 is not disrupted by Arc AS treatment. Infusion of the F-actin stabilizer, jasplakinolide, after LTP induction but prior to Arc AS infusion, rescued LTP consolidation and phosphorylation of eIF4E. The results suggest that Arc synthesis is required for regulation of CYFIP1/FRMP and initiation complex formation. Furthermore, the effects of Arc on translational control are mediated at least in part through Arc-dependent stabilization of nascent F-actin.

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Massively Parallel Neuronal Network Model Construction

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Biological neuronal networks models can be investigated with the NEST simulator (Gewaltig and Diesmann, 2007). Being a hybrid OpenMP and MPI parallel application, NEST is already capable of simulating neuronal networks of spiking point neurons of the size of 1% of the human brain (Kunkel et al., 2014). Beside efficient parallel simulation of these networks, their construction becomes more relevant. Current neuronal network sizes span multiple orders of magnitude and future investigations of the brain will require more complex and larger networks. While Kunkel et al. (2014) presented highly optimized data structures that allow the representation and simulation of neuronal networks on the scale of rodent and cat brains, the time required to create these networks in the simulator becomes impractical. Hence, efficient parallel construction algorithms, which exploit the capabilities of current and future compute hardware, are necessary to perform these large scale simulations. We present here our ongoing work to provide efficient and scalable algorithms to construct brain-scale neuronal networks.

The number of cores on single compute nodes are constantly increasing. When using MPI-based parallelization only, each rank has to store MPI-related data-structures, which entails an overhead compared to a shared memory (OpenMP) parallelization. However, previous implementations of parallelized neuronal network construction did not scale well when using OpenMP. We find that this is caused by the massive parallel memory allocation during the wiring phase. Using memory allocators specialized for thread-parallel memory allocation (Evans, 2006, Ghemawat, 2007, Kukanov, 2007) makes thread-parallel wiring scalable again.

Constructing neuronal networks in large compute-cluster- and supercomputer-scenarios shows suboptimal wiring performance as well. We find that most of the wiring time is spent by idling none-local target neurons. By refactoring the algorithms to enable the iteration over local target neurons only, we achieve good wiring performance in these scenarios.

With these optimizations in place, we gain scalable construction of neuronal networks from single compute node to supercomputer simulations. On concrete network models we observed twenty times faster neuronal network construction. These performance enhancements will allow computational neuroscientists to perform significantly more comprehensive in silico experiments within the tight limits of available supercomputer resources. Studies on the relation between network structure and dynamics will benefit especially, since these typically require the randomized instantiation of large numbers of networks. Experiments scanning network parameter space will benefit equally. Finally, by exploiting energy-hungry supercomputer resources more efficiently, our work also helps to reduce the overall energy consumption and thus the carbon footprint of computational neuroscience.

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Can rats create a cognitive map through observation only?

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In humans and other mammals, the hippocampus is critical for episodic memory. Hippocampal "place cells" are neurons that are selectively activated when the animal occupies a particular location in space termed "place fields". Therefore, the hippocampus has been proposed to function as a cognitive map of space, with "place cells" being the basic element[1]. Different environments have distinct place cell maps which are generally stable (i.e. the same cell retains the same place field), and the reorganization of place fields associated with environmental novelty is called "remapping". Thus remapping refers to the formation of a distinct hippocampal place cell representation following environmental novelty, making it a compelling electrophysiological model of memory encoding[2].

Earlier research shows that rats are capable of learning by observation[3,4], and that temporal sequences of firing of place cells expressed during a spatial experience occurred regularly during the resting or sleeping period before and after the spatial experience[5]. These findings, although not directly indicative, are compelling reasons to believe that rats may generate a cognitive spatial map of a space they did not physically experience, but that in some way interests them.

However, how a rodent's experience of a space is coded into stable place fields remains largely unknown. As an animal should physically explore an environment to demonstrate which place cells are firing there, we still do not know if observation of space is sufficient to generate a hippocampal cognitive map. Rowland et al suggested that rats could not create a cognitive map through observation only combining an environment containing both directly experienced and purely observed areas with pharmacological blockade of NMDA receptor dependent plasticity, which destabilizes newly formed place fields (CPP; [(±)-3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid])[6]. Rowland et al injected CPP to the animals prior to the first direct experience of the observed environment. The hippocampic spatial map recorded was then compared with a second direct exposure (without CPP effect). The remapping of cognitive maps suggested that the animals did form them during the first direct exposure and not during extensive observations (the cognitive map would then have been stable during posterior exposures).

A possible explanation for this result could be that the rats were not paying sufficient attention to the observed space to warrant the formation or consolidation of stable place fields. Hence, we propose to offer the animals a more attentionally salient space using food and conspecific animals. We will present during the 2015 Nordic Neuroscience conference the first behavioral and electrophysiological results.

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Serum concentrations of kynurenines in patients with attention-deficit hyperactivity disorder (ADHD): A case-control study

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Abstract

Background: The essential amino acid tryptophan is mainly metabolised through the kynurenine pathway. Altered circulatinglevels of kynurenines have been reported in chronic inflammatory conditions and in several neuropsychiatric disorders, including depression and schizophrenia. Candidate gene studies suggest that genes related to the kynurenine metabolism are associated with attention-deficit/hyperactivity disorder (ADHD). ADHD patients often report comorbid depression or anxiety. In this study we investigated serum levels of kynurenines in Norwegian adult ADHD-patients and adult controls.

Methods: We compared serum levels of tryptophan and seven tryptophan metabolites, kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, 3-hydroxyanthranilic acid and quinolinic acid, in 133 adult patients with ADHD and 131 non-ADHD adults (age 18-40 years). Riboflavin (vitamin B2), total vitamin B6 and the nicotine metabolite cotinine were also measured. Serum samples were analysed using stable isotope dilution liquid chromatography-tandem mass spectrometry. ADHD patients were diagnosed according to DSM-IV criteria. In addition, patients and controls reported comorbid disorders and past and current ADHD symptoms using the Wender Utah Rating Scale (WURS) and Adult ADHD Self-report Scale (ASRS), respectively. Logistic regression was used to calculate odds ratio for ADHD for each metabolite. In addition, we used Spearman's correlations to investigate the associations between serum levels of tryptophan and kynurenines and ADHD symptom scores.

Results: Logistic regression analyses showed that lower serum concentrations of tryptophan, kynurenic acid, xanthurenic acid and 3-hydroxyanthranilic acid, and higher levels of cotinine, were significant predictors for ADHD. When adjusting for tryptophan levels, only 3-hydroxyanthranilic acid and cotinine remained significant. Lower levels of tryptophan, kynurenic acid, xanthurenic acid, 3-hydroxyanthranilic acid and total vitamin B6, and higher levels of cotinine, were also found to correlate with higher total ASRS score and higher total WURS score. In the ADHD group, lower levels of tryptophan were correlated to higher total ASRS score, higher score in ASRS inattentive and hyperactive/impulsive subscale and higher total WURS score.

Conclusions: Our results suggest that there may be differences in serum levels of tryptophan and kynurenines between adult ADHD-patients and non-ADHD adults. However, the mechanism and clinical implication of these findings should be further explored.

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Development of grid cells is influenced by early experience

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The brain's internal metric for space is expressed in the repetitive firing fields of grid cells in the medial entorhinal cortex (MEC). Fields of individual cells tile the environment to form a tessellating grid. The hexagonal pattern of the firing fields is maintained independently of running direction and speed, suggesting that the periodicity arises from network activity within the MEC itself. Developmental studies in rat pups demonstrate that adult-like grid cells appear two weeks after pups first exploration outside the nest. However, other spatially modulated cells, i.e. place, head-direction, and border cells, express adult-like features already within 48 hours after the eyelids unseal at postnatal day 14. We asked whether the late appearance of grid cells implies that their development depends on experience with salient spatial features, or if maturation of complex wiring in the MEC-network is what delays development. Rats were born and raised in three different environments: a sphere, a cube, or multilevel cages. Sphere-raised rats were deprived of stable, vertical reference boundaries and distal cues, while cube-raised rats were deprived of distal cues. All pre-surgery handling of sphere- and cube-raised animals was done in complete darkness. Control rats were raised in spatially enriched environments. Adult animals were implanted with microdrives, and screened for grid-like cells and theta band EEG in their home cage. Cell screening was performed in either darkness or light, allowing different degrees of exposure to external spatial cues. Perfectly symmetric grid cells appeared immediately in the control group, and developed within three days in the cube group. In the sphere group, animals screened in darkness did not develop reliably hexagonal grid fields in the square arena, even after one week of recording with room lights on. The results point to early spatial experience as a contributing factor to the functional maturation of grid cells.

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Temporal processing in the visual cortex in the awake and anesthetized rat.

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Anesthesia has profound effects on the brain by suppressing neuronal and glial cell activity, reducing energy metabolism and change the dynamics of large-scale neuronal networks. However, much of what we know about sensory information processing is from experiments in animals under anesthesia. How anesthesia affects the activity of individual neurons and their temporal dynamics during information processing remains undetermined.

In auditory and somatosensory cortices, Luczak and co-workers (2007) described a structured sequence in neuronal activity which ocurred spontaneously during UP-states and the sequence was retained in responses to different types of stimuli, suggesting a stereotyped mode of information flow in a local cortical population. However, it remained elusive if the same principle also applied for visual cortex.

Using chronically implanted tetrodes into primary visual cortex of rats we conducted extracellular recordings of single units and followed the same units in the awake and anesthetic states. We provide evidence that the transition from awake to anesthesia involves a change in temporal response characteristics of units in the primary visual cortex of the rat. Anesthesia induced a profound overall decrease in firing rates, but there was large individual variation among the recorded units. Measuring the response times to visual stimulation it was clear that the visual evoked responses were delayed during anestesia. The latency to the peak response were increased both for single unit measurements and at the population level, in the local field potential. Recordings from the same populations in awake and anesthesia enabled direct comparisons of sequence in firing activity within a population and across states. Within populations of recorded units there was a specific temporal sequence of firing in response to visual stimulation which was preserved between awake recording sessions. Moreover, a clear temporal structure within the populations in response to visual stimuli was observed when comparing separate time points in anesthesia. Thus, units in the visual cortex appear to share the trait discovered by Luczak and co-workers (2007) in the auditory and somatosensory cortex. However, the unit firing sequence changed between states indicating a less degree of hard wiring in the visual cortex, compared to previously investigated cortices. This was supported by an increase in the pair wise correlations between unit firing under anesthesia indicating a change in temporal dynamics between states. Altogether, the discribed changes in temporal dynamics and information processing between the anesthetic and awake state has implications for the interpretation of results recorded in different states.

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Role of kainate receptors in the activity-dependent development of hippocampal neuronal network

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Abstract

The development of neuronal networks passes through a period of activity dependent fine-tuning and maturation of the synaptic connectivity. Temporal patterns and the degree of synchrony of the electrical activity in neuronal networks are critical determinants of the activity-dependent synaptic development. Developing hippocampus displays glutamate-driven recurrent synchronous bursts of neuronal activity known to be essential for synaptic development (Huupponen et al. 2012). Kainate-type glutamate receptors (KARs) play a critical role in modulating the early hippocampal activity (Lauri & Taira 2011) and are also directly involved in the hippocampal synapse formation (Vesikansa et al. 2007).

Here we have investigated the role of GluK1 KARs in the development of hippocampal synaptic circuitry and network activity using multi-electrode array (MEA) recordings and organotypic hippocampal cultures. We found that the electrical activity in the hippocampus between 2 days *in vitro* (DIV) to 10 DIV displayed characteristic developmental patterns. Typically, there was a transition from uncorrelated random spiking to highly synchronous, stereotypic bursting behavior towards the 10 DIV in the hippocampal areas dentate gyrus, CA3 and CA1. Chronic application of the specific GluK1 antagonist ACET (200 nM) interfered with the developmental phase shifts and delayed the appearance of synchronous activity patterns. GluK1 KARs are thus important for the proper maturation and functional integration of the intrahippocampal circuitries.

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NMDA receptors in rod amacrine cells in the mammalian retina

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Fast excitatory synaptic transmission in the CNS is generally mediated by ionotropic glutamate receptors, typically by both NMDA and non-NMDA receptors contributing to a dual-component excitatory postsynaptic current. However, in some systems, there is an absence of a synaptic NMDA receptor-mediated component. In the scotopic pathway of the mammalian retina, rod bipolar cells form glutamatergic dyad synapses with two postsynaptic rod amacrine cells, the AII and the A17 amacrine cell. The AII amacrine is a narrow-field, glycinergic neuron that carries the rod signal to ON- and OFF-cone bipolar cells. The A17 amacrine is a wide-field, GABAergic neuron with outputs exclusively directed back onto rod bipolar axon terminals, forming inhibitory reciprocal synapses. There is evidence that the synaptic input from rod bipolar cells to these postsynaptic targets is mediated via ionotropic, non-NMDA type glutamate receptors. The potential involvement of NMDA receptors in the signal transmission to AII and A17 amacrine cells is unknown. To investigate this, we performed whole-cell patch-clamp recordings from these amacrine cells in a rat retinal slice preparation. In dual recordings between presynaptic rod bipolar cells and postsynaptic amacrine cells, we did not detect an NMDA receptor-mediated component of the postsynaptic current. However, both cell types responded to application of NMDA, suggesting the presence of extrasynaptic receptors. The NMDA-evoked responses displayed J-shaped I-V curves with negative slope conductance between -70 and -30 mV in the presence of normal [Mg^{2+}] extracellularly. NMDA-evoked currents were blocked following application of the specific antagonist CPP extracellularly or the open-channel blocker MK 801 intracellularly. Pharmacological analysis revealed a differential expression of specific NMDA receptor subunits on these cells, with AII amacrines predominantly expressing the GluN2B subunit and A17 amacrines predominantly expressing the GluN2A subunit. As the specific subunit composition confers unique biophysical and signaling properties to NMDA receptors, this differential expression of NMDA receptor subunits at extrasynaptic sites on these two postsynaptic targets of the rod bipolar cell is likely related to a diversity in glutamatergic signaling and could be an important mechanism for differential signal processing between rod bipolar and rod amacrine cells.

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Acetylcholine receptor and titin antibody status in Chinese myasthenia gravis patients

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Introduction

Myasthenia gravis (MG) is an autoimmune disease caused by antibody-mediated destruction at the neuromuscular junction. Autoantibodies against acetylcholine receptor (AChR) and muscle specific kinase (MuSK) have been found to play roles in the pathogenesis of MG[1, 2]. Besides, some MG patients also have autoantibodies against proteins inside the striated muscle cells[3]. The AChR and titin antibody status of Chinese MG patients is still not well elucidated.

Subjects and methods

522 MG patients followed in neurology department of Qingdao University Affiliated Hospital were included in the study. All patients were Han Chinese origin. MG subgroups were classified into: 1 Juvenile: onset age<15 years, no thymoma; 2 Early-onset: generalized, 15≤onset age<50 years, no thymoma; 3 Late-onset: generalized, onset age≥50 years, no thymoma; 4 Thymoma: thymoma diagnosed by CT and/or pathology; 5 Ocular: Ocular symptoms only, onset age≥15 years. AChR antibody and titin antibody were tested by ELISA methods.

Results

399(76%) patients have AChR antibody, 125(24%) have titin antibody. No significant differences were found between female and male MG subjects ($P>0.05$). The distribution of AChR and titin antibody showed significant differences among different subgroups ($P<0.05$). The positive percentages of titin antibodies were higher in thymoma(51%) and late onset MG subgroups(41%) ($P<0.05$), and were lowest in juvenile MG subgroup (1.3%). The positive percentage of AChR antibody was highest in thymoma MG (97.6%) and lowest in ocular MG(60.2%). The positive rates of titin antibodies showed an increasing trend as the onset age increases in nonthymomaous MG patients. The titin, AChR antibody positive rates were correlated with Osserman classification.

Conclusions

Titin antibody distributed mainly in elderly and thymoma MG patients; AChR antibody positive rate was higher in thymoma MG subgroups; Titin and AChR antibody were correlated with maximum Osserman classification of MG patients.

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ZHE JIN

UPPSALA UNIVERSITY

Insulin regulates $GABA_A$ receptor-mediated inhibition in rat hippocampal and amygdala neurons

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The pancreatic islet hormone insulin, in addition to its critical role in glucose regulation, has actions in brain modulating neuronal excitability and memory functions. GABA (gamma-aminobutyric acid) regulates neuronal excitability and network activity by activating $GABA_A$ receptors that generate phasic and tonic currents. The hippocampus and amygdala, two medial temporal lobe structures, both participate in memory formation. We study the insulin action on $GABA_A$ receptors-mediated inhibition in the hippocampus and amygdala. Quantitative RT-PCR was run on samples from rat and human post-mortem brain samples. Immunohistochemistry for insulin receptor and electrophysiological recordings were performed on rat hippocampal and amygdala brain slices. Our results show that the insulin receptor mRNA is present in both rat and human hippocampal and amygdala samples. Immunostaining of the insulin receptor was observed in rat hippocampal and amygdala neurons. Insulin enhanced the $GABA_A$-mediated tonic conductance in rat hippocampal CA1 neurons by turning on high-affinity $GABA_A$ receptors (Jin *et al*, 2011). In rat amygdala neurons, acute application of insulin increased $GABA_A$-mediated tonic and synaptic currents and reduced the action potential firing frequency. These data demonstrate that insulin regulates the inhibitory GABAergic transmission in both the hippocampus and amygdala, and provide a putative cellular mechanism through which insulin modulates cognitive function in the brain.

Reference

Jin Z, Jin Y, Kumar-Mendu S, Degerman E, Groop L, Birnir B (2011). Insulin reduces neuronal excitability by turning on GABA(A) channels that generate tonic current. *PLoS One* **6**(1): e16188.

ØYVIND W. SIMONSEN

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Convergence of retrosplenial and subicular inputs on principal neurons of deep medial entorhinal cortex

Øyvind W. Simonsen and Menno P. Witter

As part of our ongoing investigations into neuronal networks underlying spatial processing, we are interested in clarifying the interactions of the retrosplenial cortex (RSC) with the hippocampal region. The RSC provides a dense projection to the deep layers of the medial entorhinal cortex (MEC), whose superficial layers provide the main cortical input to the hippocampal formation. The subiculum provides a major output from the latter, targeting the deep layers of MEC. Using a triple neuroanatomical tracing technique together with confocal laser scanning microscopy (CLSM), we characterized principal neurons of the deep MEC that receive input from both RSC and subiculum, and in addition project to the superficial layers of MEC. A retrograde tracer, fast blue, was injected into the superficial layers of MEC and anterograde tracers PHA-L or BDA were injected into the RSC and subiculum. After transcardial perfusion and sectioning, retrogradely fast blue labelled neurons in deep layers of MEC which were located within both the anterogradely labelled plexuses were intracellularly filled with Alexa 568 fluorescent dye. Three-D-reconstructions of 27 superficially projecting neurons in layer V displayed putative contacts with both retrosplenial and subicular inputs. Further analyses indicated that the main proportion of putative synaptic contacts for both inputs intermingle on the same parts of single dendrites. Currently, we endeavour to corroborate the existence of functionally convergent input using electrophysiological techniques. We inject AAV virus expressing channelrhodopsin and eYFP into the RSC allowing us to stimulate retrosplenial axonal fibers in deep MEC optogenetically *in vitro,* while conducting whole cell patch clamp recording from deep MEC neurons. In addition, we stimulate the subiculum using an electrode while recording from the same neurons.

Connecting European Neuroscience

Founded in 1998 at the first Forum of European Neuroscience, the Federation of European Neuroscience Societies (FENS) is the main organisation for neuroscience in Europe. FENS currently represents 42 European national and mono-disciplinary neuroscience societies with close to 23,000 member scientists from 32 European countries.

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- **European Journal of Neuroscience (EJN).** FENS official journal. It publishes papers on a wide range of topics.
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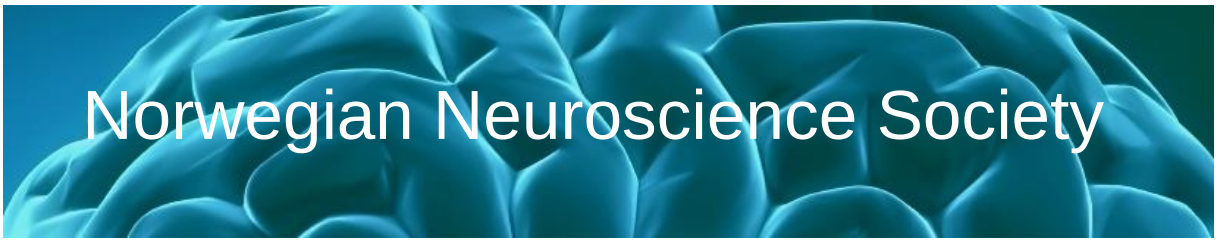

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NNS is an interest organization that represents all neuroscience related disciplines and practices in Norway. All neuroscientists who are working towards a PhD and above are welcome to become a member.

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- assist in the training of neuroscience researchers and other relevant professionals
- promote the interests of neuroscientists vis-à-vis policy makers, funding agencies and media
- spread knowledge from neuroscience research to the general population
- represent the Norwegian neuroscience community at the Federation of European Neuroscience Societies (FENS) and other relevant regional and international fora

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Questions? Please contact the NNS secretariat: hanne.lehn@ntnu.no

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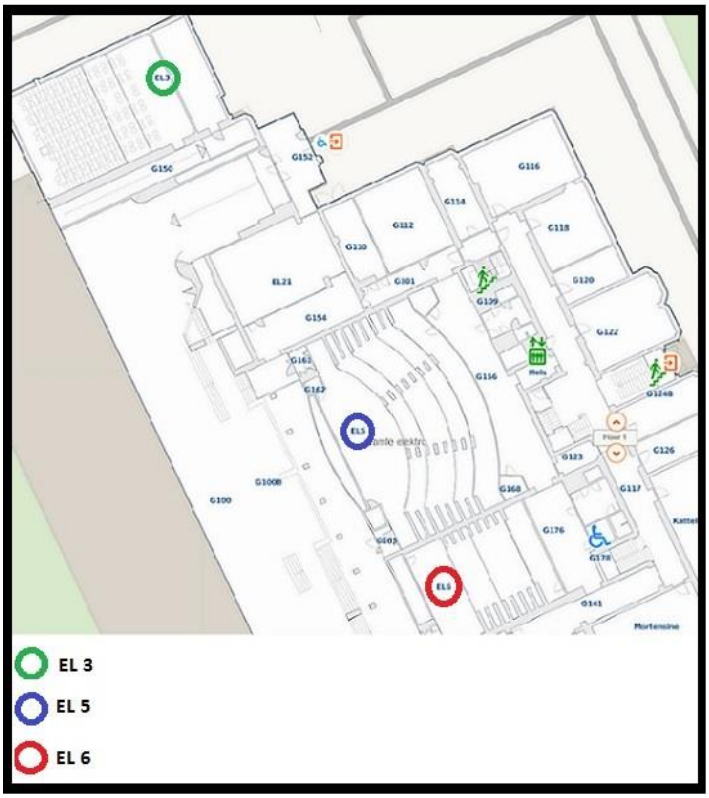
MAP

Estimated walking distances in minutes:

1. Scandic Lerkendal <--> Elektrobygget	11min	950m
2. Elektrobygget <--> Banksalen	18min	1600m
3. Banksalen <--> Café To Tårn	6min	550m
4. Café To Tårn <--> Scandic Lerkendal	25min	1900m



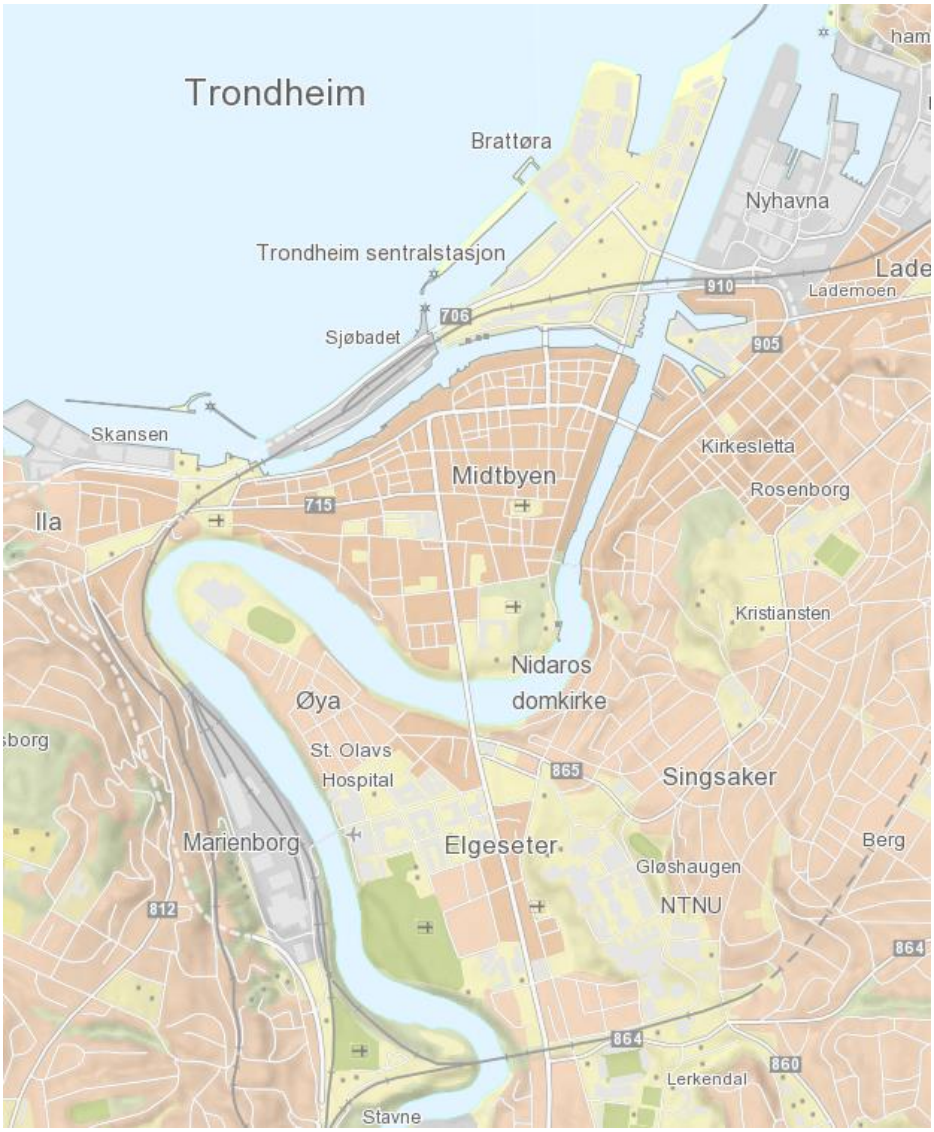


PHOTO CREDIT: TRONDHEIM KOMMUNE

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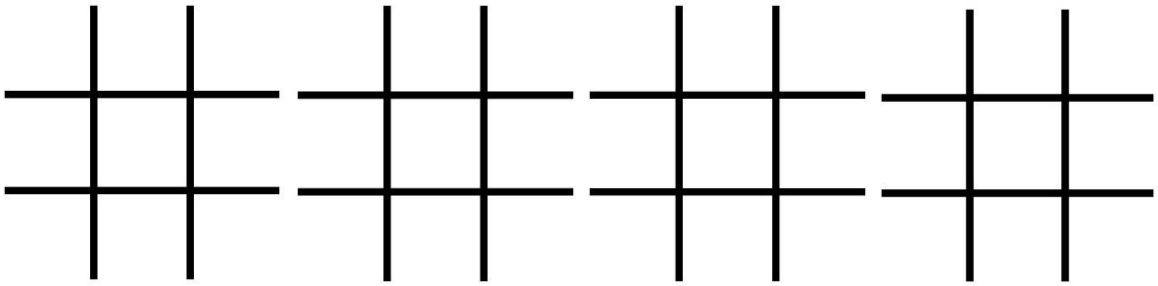
THANK YOU!

Thank you for attending and contributing at our conference. We highly appreciate your participation and presence – we hope you have enjoyed meeting people with similar interest and that the atmosphere have suited you well. A special thanks to exhibitors for sponsoring and to Scandinacian Physiologial Society for financial support.

We hope to meet you again at the next Nordic Neuroscience meeting!

TIC TAC TOE



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REMEMBER ALL?

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