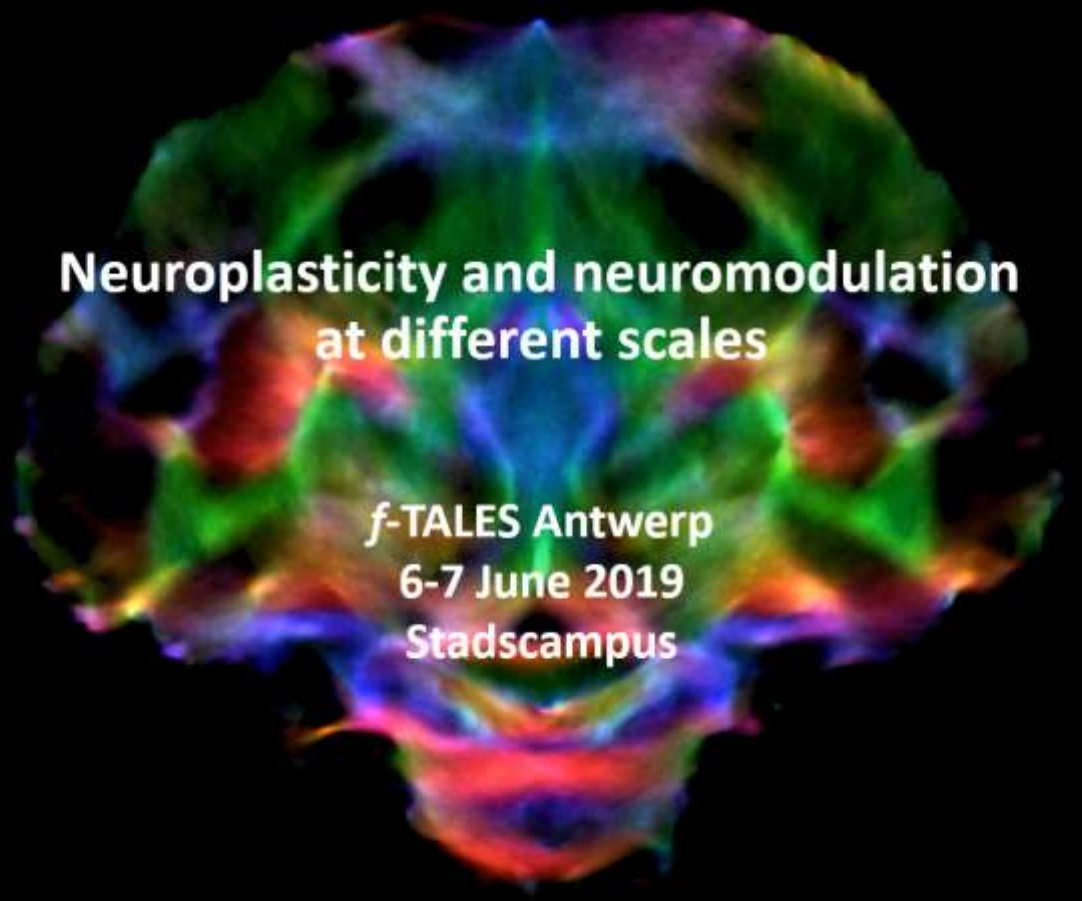

Neuroplasticity and neuromodulation at different scales

f-TALES Antwerp
6-7 June 2019
Stadscampus

zebra finch brain - Bio-Imaging Lab, UA

Abstract Book

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Vlaamse overheid

PROGRAM OVERVIEW – JUNE 6, 2019

Critical periods of enhanced (activity induced) NP to establish brain networks early in life

8:30-9:15 Coffee and registration

9:15-9:30 Welcome & Introduction to day 1: *Annemie Van der Linden*

Session 1 - Chair: Annemie Van der Linden

9:30-10:15 **S01** Critical period mechanisms and neurodevelopmental disorders: lessons from Rett syndrome: *Tommaso Pizzorusso (Florence, Italy)*

10:15-10:40 **S02** Cross-talk between sensory modalities: reprogramming the brain upon vision loss in mice: *Lutgarde Arckens (Leuven)*

10:40-11:00 Coffee break

11:00-11:25 **S03** Neural correlates of song learning accuracy traced by in vivo MRI: *Julie Hamaide (Antwerp)*

11:25-12:10 **S04** From Song to Synapse: Dopamine-dependent reinforcement of song memories and vocal performance: *Richard Mooney (Durham, US)*

12:10-12:40 Poster pitch session *(**even numbered posters**)*

12:40-14:00 Lunch break with posters

Session 2 - Chair: Lutgarde Arckens

14:00-14:15 **T01** High-throughput microscopy exposes a pharmacological window for modifiers of neuronal network connectivity: *Winnok De Vos (Antwerp) – Selected talk from abstracts*

14:15-14:30 **T02** KCNQ2-encephalopathy in a dish: mechanistic insight through 2D and 3D neuronal models: *Nina Dirkx (Antwerp) – Selected talk from abstracts*

14:30-15:15 **S05** Regulating plasticity through instability: *Alain Prochiantz (Paris)*

15:15-15:35 Coffee break

15:35-15:50 **T03** Epigenomic investigation of critical period neuroplasticity in zebrafinch telencephalon reveals major involvement of OTX2/GAD67/GADD45 regulation: *Wim Vanden Berghe (Antwerp) – Selected talk from abstracts*

15:50- 16:05 **T04** The rapid effects of estrogen as neuromodulator in the brain: evidence from humans, rodents and songbirds: *Jasmien Orije (Antwerp) – Selected talk from abstracts*

16:05-16:50 **S06** Functional connectivity and neural dynamics in CP disorder models: *Alessandro Gozzi (Rovereto, Italy)*

Closing discussion

17:00 Conference reception

19:00 Speakers' dinner

POSTERS DAY 1, JUNE 6

P02	G. Majumdar	**Hypothalamic neurogenesis in a migratory bird: An adaptive mechanism**
P04	M. Padmakumar	**Digenic inheritance of rare variants in autism spectrum disorders using platelet studies and genome sequencing**
P06	S. Reynhout	**De novo mutations in specific PP2A subunit encoding genes, including *PPP2CA*, cause a (neuro)developmental disorder**
P08	A. J. Keliris	**DCE-MRI based visualization of brain-fluids-circulation: a window to cerebral distribution of neuromodulatory molecules?**
P10	M. van den Berg	**Functional connectivity in the default mode-like network decreased upon activation of basal forebrain cholinergic neurons in rats**
P12	S. Manzella	**Deciphering biomechanisms and therapeutic response in KCNQ2 encephalopathy using multi-modal characterization of iPSC derived neuronal cultures**
P14	M.-G. Goossens	**Chemogenetic suppression of spontaneous seizures in a rat model for temporal lobe epilepsy**
P16	L. Durieux	**Investigation of the CB1 receptor in cross-modal brain plasticity upon partial vision loss**
P18	S. Valle	**Sexual differentiation of the avian song control system: Insights from a gynandromorphic zebra finch**
P20	M. Hennes	**The astrocyte DREADD toolbox as a mediator of visual cortex plasticity**
P22	J. Timmerman	**Synaptrode: neural interface at the synapse level**
P24	J. Orije	**In vivo online monitoring of testosterone-induced neuroplasticity in a female seasonal songbird**

PROGRAM OVERVIEW – JUNE 7, 2019

Adult (activity induced) NP: learning and neuromodulation

9:00-9:25 Coffee and registration

9:25-9:30 Introduction to day 2: *Georgios A. Keliris*

Session 1 - Chair: Georgios A. Keliris

9:30-10:15 **S07** Concurrent multisite recordings and brain imaging: a study of events related system and synaptic memory consolidation: *Nikos Logothetis (Tübingen, Germany)*

10:15-10:40 **S08** Acquisition of Spatial Search Strategies and Reversal Learning in the Morris Water Maze Depend on Disparate Brain Functional Connectivity in Mice: *Disha Shah (Antwerp/Leuven)*

10:40-11:00 Coffee break

11:00-11:45 **S09** Temporal dynamics of the Locus Coeruleus involvement in learning and memory consolidation: *Oxana Eschenko (Tübingen, Germany)*

11:45-12:10 **S10** Telencephalic neurocircuitry and synaptic plasticity in rodent spatial learning and memory: *Rudi D'Hooge (Leuven)*

12:10-12:40 Poster pitch session *(**odd numbered posters**)*

12:40- 14:00 Lunch break with posters

Session 2 - Chair: Rudi D'Hooge

14:00-14:25 **S11** The role of astrocytes in neuroplasticity: *Ilse Smolders (Brussels)*

14:25-14:40 **T05** The role of neuroplasticity in ECT-induced hippocampal volume changes and cognitive side effects: *Maarten Laroy (Leuven) – Selected talk from abstracts*

14:40-14:55 **T06** In vivo effect of activating optogenetic chloride channel on hippocampal evoked potentials: *Anirudh Acharya (Gent) – Selected talk from abstracts*

14:55-15:10 **T07** Long term chemogenetic suppression of spontaneous seizures in a mouse model for temporal lobe epilepsy: *Jana Desloovere (Gent) – Selected talk from abstracts*

15:10-15:25 **T08** Bottom-up sensory processing can induce negative BOLD responses and reduce functional connectivity in nodes of the default mode-like network in rats: *Rukun Hinz (Antwerp) – Selected talk from abstracts*

15:25-15:45 Coffee break

15:45- 16:30 **S12** Cholinergic modulation of cortical circuits for cognition in rodent and human brain: *Huib Mansvelder (Amsterdam, the Netherlands)*

16:30-16:40 Poster & oral presentation awards

16:40-17:00 Closing remarks & discussion

POSTERS DAY 2, JUNE 7

P01	S. Borrie	**MEK inhibition ameliorates social behaviour deficits in a *Spred1-/-* mouse model for RASopathy disorders**
P03	J. Schröder	**What do stroke survivors actually learn when regaining walking ability? A protocol for a repeated-measurements prospective cohort study**
P05	L. Craeghs	**The effects of childhood cancer treatment on early maternal bonding and adult socio-cognitive functioning in laboratory mice**
P07	D. Vandael	**Stress dependent mental disorders; a molecular and structural basis**
P09	L. Stevens	**Genetic modification of locus coeruleus NE cells for chemogenetic activation remains challenging**
P11	L. Peeters	**Chemogenetic silencing of neurons in mouse cingulate area induces long range neural activity and functional connectivity modulations: a DREADD-fMRI study**
P13	S. Mitra	**Understanding activity dependant amygdalar neural circuitry in appetitive learning**
P15	S. R. Murris	**Electrical microstimulation of the macaque VTA drives subliminal and category specific visual perceptual learning**
P17	A. Ramadan	**Signal discrimination task for characterization of spatial (in)attention in rats**
P19	R. Fanfa Loureiro Chaves	**Unravelling the Relationship Between Brain Connectivity and Gait Outcome in Stroke Survivors**

ABSTRACTS OF INVITED SPEAKERS' PRESENTATIONS (S01-S12)

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S01 Critical period mechanisms and neurodevelopmental disorders: lessons from Rett syndrome

Tommaso Pizzorusso[1]

[1] Dept NEUROFARBA, Univ. of Florence, Florence Italy and Inst Neuroscience CNR, Pisa, Italy

The mouse visual cortex is a classical model for the study of experience-dependent development of the brain and many. Recent work show that visual impairments are also typical in several neurodevelopmental disorders suggesting that impaired molecular regulation during early postnatal life could broadly affect circuit formation. These data prompted us and others to use the visual cortex as a window on atypical development in models of neurodevelopmental disorders. A wealth of studies found that cellular and molecular players regulating critical periods of development such as parvalbumin positive inhibitory interneurons and perineuronal nets are altered in disease models. Moreover, visual assessment has been proposed as a biomarker for several neurodevelopmental disorders including Rett syndrome and related disorders.

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S02 Cross-talk between sensory modalities: reprogramming the brain upon vision loss in mice.

Lutgarde Arckens[1]

[1] Biology, KU Leuven, Leuven Brain Institute , Leuven, Belgium

When a sensory system fails in adult life, as with acquired blindness or deafness, neurons in the sensory deprived brain areas take on new functions becoming strongly responsive to inputs from the spared senses. This form of plasticity, termed cross-modal plasticity, is seen in the adult and is linked to profound behavioral and perceptual changes.

Understanding adult cross-modal plasticity is of a broad general clinical relevance particularly for the treatment of neurological injuries and disorders in an aging population. A deep comprehension of the mechanisms is critical whether we seek to help patients compensate or recover from their injury/loss, to control or improve plasticity for therapeutic purposes (e.g. modulating plasticity onset and/or outcomes) or to improve patients' acceptance of neural prostheses.

As a research model for adult plasticity we study the neural activity and circuit changes that occur in the visual cortex of the adult mouse upon monocular enucleation. Monocular enucleation eliminates retinal input from the removed eye and consequently deprives the visual cortex from half its inputs. This results in a massive, transient drop of neural activity in the monocular cortical territory from the removed eye, then followed by an equally profound recovery of activity. A swift potentiation of responses from the spared eye occurs within 3 weeks post injury. A second slower reactivation phase depends on somatosensory inputs.

A long-term goal is to determine the homological and predictive validity of our animal model, the mono-enucleated mouse, for increasingly prevalent human ailments including acquired blindness as well as neurological disorders characterized by abnormal sensory integration i.e. adult synesthesia, autism spectrum disorders, anxiety-related disorders and schizophrenia. This is particularly exciting considering that the model allows the parallel study of two forms of plasticity, unimodal and cross-modal, of which one is only seen in adult animals, and both could be mediated by specific local circuitry and molecular machinery.

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S03 Neural correlates of song learning accuracy traced by in vivo MRI

Julie Hamaide[1], Kristina Lukacova[2], Georgios A. Keliris[1], Marleen Verhoye[1], Annemie Van der Linden[1]

[1] Bio-Imaging Lab, Department of Biomedical Sciences, University of Antwerp, Wilrijk, Belgium.
[2] Centre of Biosciences, Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia.

Human speech and bird song are highly complex and rapid motor behaviors that are learned by imitation and serve to produce complex communication signals vital for social interactions. Both are acquired during a temporally well-defined sensitive period early in life which consists of a sensory learning phase where a perceptual target of speech sounds or a song model are memorized, followed by a sensorimotor learning phase, where human infants or juvenile birds engage in intense vocal practicing and try to match the spectro-temporal characteristics of their own vocalizations to the previously established perceptual targets based on multi-sensory feedback. Owing to the ample similarities at the molecular, functional and behavioral level, songbirds –zebra finches in particular– are currently regarded as the best animal model available to study the neurobiology of vocal learning, i.e. the process underlying human speech, in a laboratory setting.

Even though the brain areas indispensable for speech and song learning are known, the neural circuits important for enhanced or reduced vocal performance –and thus critical for successful communication– remain unclear. We addressed this important question in zebra finches. More specifically, using in vivo Magnetic Resonance Imaging (MRI), we repeatedly mapped the structural architecture of the developing zebra finch brain at seven milestone time-points during and beyond the song learning period, starting as early as 20 days post hatching. In addition to collecting structural MRI data, we recorded the songs of the birds after each imaging session and quantified the learning success by computing the spectral similarity between the tutor song and the song sung by the pupil. We discovered that song imitation accuracy correlates with the structural architecture of four distinct brain areas, none of which pertain to the traditionally studied song control system. Furthermore, the structural properties of the secondary auditory region NCM of the left hemisphere, are capable of predicting future song copying accuracy, already at the earliest stages of the song learning process, before vocal practicing. We conjecture that our results not only identify new networks engaged in vocal learning accuracy but also underscore the importance of early life inputs and role models in future learning success and in actively sculpting the structural properties of the brain.

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S04 From Song to Synapse: Dopamine-dependent reinforcement of song memories and vocal performance

Richard Mooney

Department of Neurobiology, Duke University School of Medicine. Durham, NC 27710 USA

Vocalizations are an essential medium for social recognition in most vertebrates. Whereas many types of vocalizations are innate, humans and a few other animal groups culturally transmit their species typical vocalizations. How the brain enables the learning of complex, culturally transmitted behaviors such as speech remains largely unknown. The presentation will cover fundamental discoveries concerning how the brain enables the learning and production of birdsong, a behavior with many parallels to human speech. Similar to speech learning, birdsong learning requires that the pupil first memorize and then imitate the song of a tutor. Experiments in songbirds have begun to identify the synaptic mechanisms that underlie the pupil's ability to memorize and imitate the tutor song. Indeed, in vivo multiphoton imaging has revealed that hearing a tutor song rapidly changes the size and strength of synapses in the pupil's brain. Gain and loss of function methods, including genetic perturbations of neural activity in freely singing birds, have established the synaptic mechanisms that are both necessary and sufficient for forming an auditory memory of the tutor song and converting this memory into a vocal pattern. Furthermore, high-resolution electrophysiological recordings in freely singing wild songbirds has helped to provide the first evidence of auditory-vocal mirror neurons, which are theorized to facilitate human speech, and has linked their auditory activity to the categorical perception of different vocal dialects. Finally, neuromodulators such as dopamine have been shown to play an important role not only in song learning but also in modulating song behavior as a function of the bird's social context, pointing to ways in which social cues gain access to vocal machinery to facilitate social signaling, courtship, and affiliation.

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S05 Regulating plasticity through instability

A. Prochiantz[1], J. Apulei[1], J. Gilabert[1], A. A. Di Nardo[1].

[1] Center for Interdisciplinary Research in Biology, College de France, Paris, France

Accumulation of non-cell autonomous Otx2 homeoprotein in postnatal mouse cortex is implicated in both the onset and closure of critical period plasticity. A genetic point mutation in the glycosaminoglycan recognition motif of Otx2 results in Otx2 mis-localization, which leads to dynamic turnover of selected perineuronal net components and broadly delays maturation of pivotal parvalbumin interneurons in primary sensory and prefrontal cortices. Consequently, critical periods are delayed across modalities, suggesting a potential global role for Otx2 function in establishing mental health. This hypothesis is supported by recent studies suggesting that some psychiatric diseases may be associated with dysregulated critical periods of plasticity in regions of the cortex that affect mood and cognition. In this presentation, on the basis of experimental data gathered in our laboratory and in the recent literature, a possible role of OTX2 in the regulation of mood and cognition will be discussed.

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S06 Functional connectivity and neural dynamics in CP disorder models

Alessandro Gozzi

Functional Neuroimaging Laboratory, Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia, Rovereto (TN), Italy

Prominent and yet highly heterogeneous abnormalities in interregional connectivity characterize CP disorders, such as autism. However, several fundamental questions as to the neural basis and significance of this phenomenon remain unaddressed. For one, what drives functional disconnectivity in autism and CP disorders? Does etiopathological variability account for heterogeneous connectivity in autism? And can we link autism-associated genetic alterations to specific patterns of functional disconnectivity?

To address these questions, we have developed fMRI-based methods for connectivity mapping in the laboratory mouse, where specific autism-related etiologies can be isolated and investigated with high precision. We observed significant mouse to human correspondences in network organization, and conserved patterns of disconnectivity in rodents and humans harboring autism-associated 16p11.2 chromosome microdeletion, or mTOR-related pruning deficits. By extending this approach to multiple autism risk genes, we then showed that different genetic etiologies lead to a spectrum of heterogeneous, yet classifiable cross-mutational connectivity fingerprints, that are associated with disrupted network dynamics. Our results establish a mechanistic link between neuro-genetic abnormalities, specific patterns of disconnectivity and their heterogeneous expression across clinical populations.

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S07 Concurrent multisite recordings and brain imaging: a study of events related to system and synaptic memory consolidation

Nikos K. Logothetis

Physiology of Cognitive Processes, Max-Planck-Institute for Biological Cybernetics, Tuebingen, Germany

Experimental work in animals and humans suggests that various short-lasting patterns of neural activity, including single- or multiple-cycle oscillatory episodes, may reflect state changes of self-organizing large-scale networks. Such state-marking events, including K complexes, spindles, hippocampal sharp wave ripples (SPW-R), and ponto-geniculo-occipital (PGO) waves, are in fact thought to regulate cognitive capacities, such as learning, memory encoding and consolidation, as well as memory-guided decision making. Although the neural events themselves have long been studied in great detail with neurophysiological methods, the actual brain-states related to them remain elusive, primarily due to a dearth of methodologies permitting concurrent recordings in various structures and mapping of whole-brain activity. In an effort to map and study cooperative patterns of a large number of brain structures associated with intrinsic events. such patterns, we recently developed so-called neural event triggered fMRI (NET-fMRI) and used it to understand the dynamics of the networks related to SPW-R and PGO events, both considered to be critical for the sequential states of system and synaptic memory consolidation during sleep. In my talk I'll present novel results demonstrating the importance of multidisciplinary and multimodal approaches.

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S08 Acquisition of Spatial Search Strategies and Reversal Learning in the Morris Water Maze Depend on Disparate Brain Functional Connectivity in Mice

Disha Shah[1], Marleen Verhoye[2], Annemie Van der Linden[2], Rudi D'Hooge[3]

[1] Laboratory for the Research of Neurodegenerative Diseases, Department of Neuroscience, VIB center for Brain and Disease Research, KU Leuven, O&N4 Herestraat 49 Box 602, 3000 Leuven, Belgium.
[2] Bio-Imaging Lab, Department of Biomedical Sciences, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium.
[3] Laboratory of Biological Psychology, Department of Psychology, KU Leuven, Tiensestraat 102, 3000 Leuven, Belgium.

Learning has been proposed to coincide with changes in connections between brain regions. In the present study, we used resting-state fMRI (rsfMRI) to map brain-wide functional connectivity (FC) in mice that were trained in the hidden-platform version of the Morris water maze. C57BL6 mice were investigated in a small animal MRI scanner following 2, 10 or 15 days of acquisition learning, or 5 days of reversal learning. Spatial learning coincided with progressive and changing FC between telencephalic regions that have been implemented in spatial learning (such as hippocampus, cingulate, visual and motor cortex). Search strategy assessment demonstrated that the use of cognitively advanced spatial strategies correlated positively with extensive telencephalic connectivity, whereas non-spatial strategies correlated negatively with connectivity. FC patterns were different and more extensive after reversal learning compared to after extended acquisition learning, which could explain why reversal learning has been shown to be more sensitive to subtle functional defects.

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S09 Temporal dynamics of the Locus Coeruleus involvement in learning and memory consolidation

Oxana Eschenko

Physiology of Cognitive Processes, Max-Planck-Institute for Biological Cybernetics, Tuebingen, Germany

Learning experience induces brain activity changes leading to stabilization of newly encoded information within neuronal networks. Learning-induced processes occur at the molecular, cellular and systems levels and have complex temporal dynamics. According to a broadly accepted view, synaptic plasticity, neuronal replay, and cross-regional interactions are the key processes underlying memory consolidation. Both, cellular- and systems-level consolidation dependent upon the input from neuromodulatory centers; the noradrenergic brainstem nucleus Locus Coeruleus (LC) is being one of them. The optimal level of noradrenergic neurotransmission is essential during information encoding, but the LC activity it also critical 'off-line', when experience-activated neuronal ensembles replay and protein-dependent synaptic modifications occur. Noradrenaline (NE) release from diffuse terminals of LC neurons creates a window of heightened synaptic plasticity promoting both synaptic strengthening and weakening. Earlier studies revealed the role of LC-NE system for the late phase of memory consolidation (*1-3*); yet, the temporal dynamics of the LC involvement in systems consolidation remains largely unexplored. The hippocampal ripples and thalamo-cortical spindles are considered to mediate 'off-line' hippocampal-cortical communication underlying systems consolidation. Sustained increase of LC tonic firing suppresses generation of both oscillatory events. We hypothesized that phasic, but not tonic NE release promotes plasticity within reactivated neuronal assemblies. Conversely, experimentally induced LC bursts at times of ripples caused spatial memory deficit (*4*). Furthermore, the LC activity is transiently elevated prior cortical Up-states and also during sleep spindles (*5*), but it is reduced around ripples with the most pronounced LC suppression occurring at times of ripple/spindle coupling. Our new results suggest that reduced NE neurotransmission may be necessary for generation of a brain state that is favorable for 'off-line' inter-regional information transfer. The temporal windows of heightened LC activity and the functional significance of LC activation for 'off-line' information processing remain yet to be discovered.

1. P. Roullet, S. Sara, *Neural Plast* **6**, 63-68 (1998).
2. S. Tronel, M. G. Feenstra, S. J. Sara, *Learn Memory* **11**, 453-458 (2004).
3. O. Eschenko, S. J. Sara, *Cereb Cortex* **18**, 2596-2603 (2008).
4. Y. Novitskaya, S. J. Sara, N. K. Logothetis, O. Eschenko, *Learning & Memory* **23**, 238-248 (2016).
5. O. Eschenko, C. Magri, S. Panzeri, S. J. Sara, *Cerebral Cortex* **22**, 426-435 (2012).

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S10 Telencephalic neurocircuitry and synaptic plasticity in rodent spatial learning and memory

Rudi D'Hooge

Biological Psychology, KU Leuven, Leuven, Belgium

Human episodic memory is the cornerstone of human neurocognition that is affected by cerebral aging, neurodegeneration and psychopathology. Morris-type and radial-arm maze tasks assess spatial learning and episodic memory ability in laboratory rodents, and are the most widely used, valid and reliable tests to model higher cognition in these animals. Hippocampus, dorsal striatum and medial prefrontal cortex are central hubs in the telencephalic neurocircuitry, which includes parallel neurocircuits that control different aspects of spatial learning and memory ability. Prefrontal-hippocampal connectivity, the major associative system in the rodent brain, is critical for navigation in physical space. Functional connections between prefrontal cortex and dorsal striatum are probably more important for motivational or goal-directed aspects of spatial learning. Different forms of synaptic plasticity have been found in hippocampus, dorsal striatum and medial prefrontal cortex. They have been suggested to underlie the involvement of telencephalic neurocircuitry in spatial learning and memory, and to play a role in the pathophysiology of two brain pathologies with episodic memory impairments as core symptoms (Alzheimer's disease and schizophrenia). We therefore suggest that elucidation of the functions of rodent telencephalic neurocircuitry will contribute to translatable preclinical research on these most devastating brain disorders.

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S11 The role of astrocytes in neuroplasticity

Ilse Smolders[1], Yana Van Den Herrewegen[1], Ann Van Eeckhaut[1], Zuner A Bortolotto[2], Dimitri De Bundel[1]

[1] Department of Pharmaceutical and Pharmacological Sciences, Research group Experimental Pharmacology, Center for Neurosciences, Vrije Universiteit Brussel, Brussels, Belgium

[2] Centre for Synaptic Plasticity, University of Bristol, Bristol, United Kingdom

It's exciting times for deciphering the role of astrocytes in brain functions. Indeed, cell type-specific strategies such as chemogenetics allow us to finetune neuronal actions and behaviour by precisely and selectively modulating astrocytes activity. Chemogenetic modulation is based on virus-driven expression of genetically modified receptors, the so-called Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), in a specific cell type of interest, and selective activation of these DREADDs by an otherwise biologically inert ligand, the designer drug. We studied chemogenetic manipulation of astrocytes in acute coronal hippocampal slices prepared from C57Bl6/J mice previously injected with viral vectors driving DREADD-hM3Dq expression in astrocytes or a control viral vector. The designer drug CNO enhanced calcium activity in astrocytes of Gq-transfected slices but not in control slices. Subsequently, we performed extracellular field recordings in CA1 area upon Schaffer collateral stimulation and found significant increases in fEPSP amplitude in Gq-DREADD transfected but not control slices. These data are in line with the data published by Adamsky et al (2018) who showed that astrocytic hippocampal activation is sufficient to generate NMDA receptor-dependent synaptic potentiation. These authors additionally demonstrated that chemogenetic astrocyte activation also improved memory function and cognitive performance beyond what could be achieved by activating nerve cells. We are now characterizing the in vivo effects of astrocyte modulation on spatial learning and memory in an animal model of epilepsy with established cognitive dysfunction (Van Den Herrewegen et al, 2019). Chemogenetic Gq-mediated astrocyte activation might pave the way to appoint astrocyte modulation as a new treatment strategy for improving cognitive function and to rescue cognitive dysfunction.

- Adamsky A, Kol A, Kreisel T, Doron A, Ozeri-Engelhard N, Melcer T, et al. Astrocytic Activation Generates De Novo Neuronal Potentiation and Memory Enhancement. Cell. 2018;174(1):59–71.
- Van Den Herrewegen Y, Denewet L, Buckinx A, Albertini G, Van Eeckhaut A, Smolders I, et al. The Barnes Maze Task Reveals Specific Impairment of Spatial Learning Strategy in the Intrahippocampal Kainic Acid Model for Temporal Lobe Epilepsy. Neurochem Res. 2019;44(3):600–608.

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S12 Cholinergic modulation of cortical circuits for cognition in rodent and human brain

Huib Mansvelder

Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam

Acetylcholine (ACh) signaling shapes neuronal circuit development and underlies specific aspects of cognitive functions and behaviors, including attention, learning, memory and motivation. During behavior, activation of acetylcholine receptors by ACh alters the activation state of neurons, and neuronal circuits most likely process information differently with elevated levels of ACh. In several brain regions, ACh has been shown to alter synaptic strength as well. By changing the rules for synaptic plasticity, ACh can have prolonged effects on and rearrange connectivity between neurons that outlasts its presence. From recent discoveries in the mouse, rat, monkey and human brain, a picture emerges in which the basal forebrain (BF) cholinergic system targets the neocortex with much more spatial and temporal detail than previously considered. Fast cholinergic synapses acting on a millisecond time scale are abundant in the mammalian cerebral cortex, and provide BF cholinergic neurons with the possibility to rapidly alter information flow in cortical microcircuits. Our recent findings reveal novel mechanisms of how cholinergic projections from the BF affect synaptic strength in several brain areas of the rodent brain, with behavioral consequences. I will discuss how these findings translate to human brain circuitries.

ABSTRACTS OF ORAL PRESENTATIONS
(T01-T08)

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T01 High-throughput microscopy exposes a pharmacological window for modifiers of neuronal network connectivity

Marlies Verschuuren[1], Peter Verstraelen[1], Gerardo García-Díaz Barriga[1], Ines Cilissen[1,4], Emma Coninx[2,3], Mieke Verslegers[3], Peter Larsen[4], Rony Nuydens[4], Winnok H. De Vos[1]

[1]Laboratory of Cell Biology & Histology, University of Antwerp, Department of Veterinary Sciences, Antwerp, Belgium
[2]Animal Physiology and Neurobiology, KU Leuven, Department of Biology, Antwerp, Belgium
[3]Radiobiology Unit, Institute of Environment, Health and Safety, Belgian Nuclear Research Centre, Mol, Belgium
[4]Janssen Research & Development, a division of Janssen Pharmaceutica NV, Beerse, Belgium

Virtually all neurodegenerative disorders share a cellular phenotype of dysregulated synaptic plasticity. Thus, exposing pathways that modulate this process may hold promise for therapeutic lead discovery. Capitalizing on the throughput of modern automated microscopy and the rich information content returned by dedicated image analyses, we have established a pipeline that queries both morphological and functional correlates of connectivity in primary neuronal culture. Using this pipeline, we unveiled a connectivity signature that was specific to the cell culture type (brain region) and age (days *in vitro*). With a focused compound screen, we found that inhibition of dual leucine zipper kinase activity increased neuronal connectivity in unperturbed cultures and also exerted neuroprotective effects in cultures grown under sub-optimal (aged or anti-oxidant depleted) or challenged (hTau.P301L overexpressing) growth conditions. Our results illustrate that analyzing microscopy images with deep coverage enables sensitive interrogation of neuronal connectivity and allows exposing a dose and time window for pharmacological interventions. In doing so, we revealed a broad-spectrum neuroprotective effect of DLK inhibition. *In extensu*, the approach holds promise for identifying druggable nodes that modulate neuronal plasticity in the context of neurodegenerative conditions.

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T02 KCNQ2-ENCEPHALOPATHY IN A DISH: MECHANISTIC INSIGHT THROUGH 2D AND 3D NEURONAL MODELS

Nina Dirkx[1,2], Bob Asselbergh[3], Peter Verstraelen[4], Tine Deconinck[1,2], Bavo Heeman[3], Winnok De Vos[4], Peter De Jonghe[1,2,6], Michele Guigliano[2,5], Sarah Weckhuysen[1,2,6]

[1] Neurogenetics Group, University of Antwerp, Antwerp, Belgium
[2] Institute Born-Bunge, University of Antwerp, Antwerp, Belgium
[3] Center for Molecular Neurology, VIB and University of Antwerp, Antwerp Belgium
[4] Laboratory of Cell Biology and Histology, University of Antwerp, Antwerp, Belgium
[5] Theoretical Neurobiology & Neuroengineering lab, University of Antwerp, Antwerp, Belgium
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Heterozygous pathogenic variants in the gene KCNQ2 are associated with both severe KCNQ2-encephalopathy (KCNQ2-E), characterised by neonatal seizures and developmental delay, and a self-limiting epilepsy syndrome called benign Familial Neonatal Epilepsy (KCNQ2-BFNE), where development is completely normal. Pathogenic variants leading to KCNQ2-E have a dominant negative (DN) or gain-of-function (GOF) effect, whereas haploinsufficiency results in KCNQ2-BFNE. KCNQ2 encodes for a subunit of the M-channel, which generates a current that is very important for regulation of the resting membrane potential and control of neuronal excitability. While the role of KCNQ2 in epilepsy is straightforward, as dysfunction of the M-channel affects neuronal excitability, its role in neurodevelopment is less well understood. Currently used therapies for KCNQ2-E consists of symptomatic treatment, mainly directed at the (often difficult to treat) seizure activity. So far, no therapies influencing the developmental outcome of KCNQ2-E exist. To develop such a therapy, it is of utmost importance to increase our understanding of the mechanism underlying the neurodevelopmental delay observed in these patients.

To understand how KCNQ2 affects neuronal development, we are generating 2D and 3D neuronal cultures (brain organoids) derived from human induced pluripotent stem cells (hiPSC). So far, we developed cell lines from 1 GOF and 3 DN KCNQ2-E variants, as well as 2 KNCQ2-BFNE variants, and 2 control individuals. By studying 2D neuronal co-cultures of excitatory and inhibitory neurons, generated via a fast overexpression protocol, we aim to develop a read-out system for both the epileptic and the neurodevelopmental features of KCNQ2-E, and to develop a platform that can be used for future drug screening. The brain organoids cultures are used to study the effect of the mutations in a more heterogeneous and complex neuronal network and to identify the affected cell types. Preliminary staining data reveal structural differences (i.e., abnormal morphology and expanded ventricular lumen) in KCNQ2-GOF vs. WT patient-derived brain organoids, 28 days post differentiation.

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T03 Epigenomic investigation of critical period neuroplasticity in zebrafinch telencephalon reveals major involvement of OTX2/GAD67/GADD45 regulation

Jolien Diddens[1], Louis Coussement[2], Carolina Frankl-Vilches[3], Gaurav Majumdar[6], Sandra Steyaert[2], Sita ter Haar[4], Jeroen Galle[2], Ellen De Meester[5], Sarah De Keulenaer[5], Wim Van Criekinge[2], Charlotte Cornil[4], Jacques Balthazart[4], Annemie Van Der Linden[6], Tim De Meyer[2], Wim Vanden Berghe[1*]

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Epigenetic changes in DNA methylation (5mC) are thought to regulate closure of critical period plasticity during song learning in zebrafinch. RNA transcriptome and DNA methylome analysis in male and female zebrafinch telencephalon identified extensive changes in pathways related to neurogenesis, axonal guidance, neuronal migration, critical period synaptic plasticity and postsynaptic spine maturation involved in memory and sensory learning processes. Of particular interest, neurodevelopmental changes could be observed in parvalbumin, GAD67 and OTX2 expression, which are crucially involved in the timing of critical period. Furthermore, analysis of sexually dimorphic gene expression further identified differential expression of GADD45G, involved in DNA repair, active DNA demethylation and temporal dynamics of learning-induced immediate early gene (IEG) expression. In this context, OTX2 induced GADD45b/g expression was found to modulate juvenile mice neuronal plasticity through changes in MeCP2 foci within parvalbumin interneurons and in methylation states of several plasticity gene promoters to trigger critical period plasticity yet suppress adult plasticity. At the epigenetic level, a large number of chromatin, DNA/RNA methylation writer-reader-eraser proteins reveal age and sex dependent gene expression changes during neurodevelopment. Using reduced representation bisulfite sequencing (RRBS), we identified cumulative neurodevelopmental age dependent CpG DNA methylation differences, driving gene expression changes. Along the same line, immunofluorescence and ISH experiments show age dependent accumulation of 5mC levels in the song control system, with differentiation dependent decline of DNMT expression levels. Remarkably, the majority of sexually dimorphic genes resulted from an incomplete dosage compensation of Z chromosome genes, rather than

DNA methylation differences. Analysis of upstream regulators reveals important combinatorial gene expression control involving multiple transcription factor (p53, E2F1) and nuclear hormone receptors (ESR1, ESR2, PGR, NR3C1, THRB, PPAR) which might contribute to sex and age specific neurodevelopmental trajectories.

In summary, our study characterized the neurodevelopmental dynamics of epigenetic control mechanisms of pattern age and sex dependent gene expression in the developing zebra finch brain and song control system, which may contribute in wiring of neuronal sensory circuits and modulation of synaptic plasticity during the critical period.

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T04 The rapid effects of estrogen as neuromodulator in the brain: evidence from humans, rodents and songbirds

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Estrogen is mostly known for its role in female reproduction. However, recent research has extended beyond the gonadal role of estrogens and explored their complex actions within the brain. Estrogen receptors are expressed in brain regions other than the hypothalamus, like hippocampus, amygdala and auditory cortex. Furthermore, aromatase, the enzyme for estrogen synthesis, is highly expressed in these brain regions critical for memory encoding and consolidation, a feature that is conserved across species [1]. This renders these cognitive regions susceptible for hormonal changes and could explain sex differences in cognitive behaviors, like spatial orientation and verbal fluency [2]. Most of these long lasting effects of estrogens on cognition are the result of genomic and epigenetic modulation of gene transcription during development.

Next to these long lasting effects of estrogen on neural function, recent research established more rapid effects of estrogen on learning and memory consolidation. This has mostly been studied in rodents, where estrogen treatment prior or directly after training can enhance memory in various spatial navigation and object recognition tasks. This is associated with increased dendritic spine density within the hippocampus. Interestingly, estrogen treatment >2 hours past training does not improve memory consolidation, indicating that there is a time sensitive critical period in which estrogens are effective in enhancing memory. These kind of rapid effects rely on non-genomic mechanisms, where presynaptic aromatase rapidly produces neuro-estrogens, which act as a neuromodulator, priming neurons by structurally and functionally changing their dendritic spines. Depending on whether this neural priming is followed by activity, these dendritic spines are stabilized and lead to activity-dependent neuroplasticity[3]. Similar non-genomic mechanism could play a role in songbirds, where estrogens may enhance vocal learning and auditory memory consolidation in NCM (the songbird homologue of the auditory association cortex) [4].

In humans, a similar critical period is suggested for the effectiveness of estrogen treatment in menopausal women with cognitive decline, where only women who started treatment during or soon after menopause showed beneficial effects on verbal and working memory [2].

During this talk we highlight the role of estrogens in cognitive behaviors and uncover the underlying mechanisms by which estrogens can modulate memory and neuroplasticity, using evidence derived from rodents, songbirds and humans.

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T05 The role of neuroplasticity in ECT-induced hippocampal volume changes and cognitive side effects

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Background and objective: Electroconvulsive therapy (ECT) induced hippocampal volume change (HVC), which is assumed to be related to the process of neuroplasticity, is a robust phenomenon seen in patients treated with ECT. The similar time course and treatment related characteristics of ECT-induced HVC and ECT-related cognitive effects suggests a relation, that is to date, understudied. This study investigates whether HVC following ECT predicts the change in memory performance six months after the end of the ECT treatment.

Methods: Hippocampal volume (HV) was measured via high-resolution 3D T1-weighted images in 88 patients with late life depression, within 1 week before and after ECT. Memory performance was assessed before and six months after ECT. Multiple linear regression was used to examine whether change in memory performance could be predicted based on ECT-induced changes in HV.

Results: Larger right HVC predicts less pronounced improvement on the VAT (visual memory) in the whole sample. For the 8-Word Test (verbal memory) and the Category Fluency Test (semantic memory), the effect is only present in patients who switched from right unilateral to bitemporal stimulation after six ECT sessions. HVC in the left hemisphere was not significantly related to change in memory performance.

Conclusion: A larger change in right HV during ECT is associated with an adverse improvement in memory performance up to six months post ECT. These findings suggest a possible role for neuroplasticity in the appearance of both the ECT-induced HVC and ECT-related cognitive effects, contributing to the 'transient ECT-induced neuroplasticity hypothesis' of cognitive effects in ECT (see Figure).

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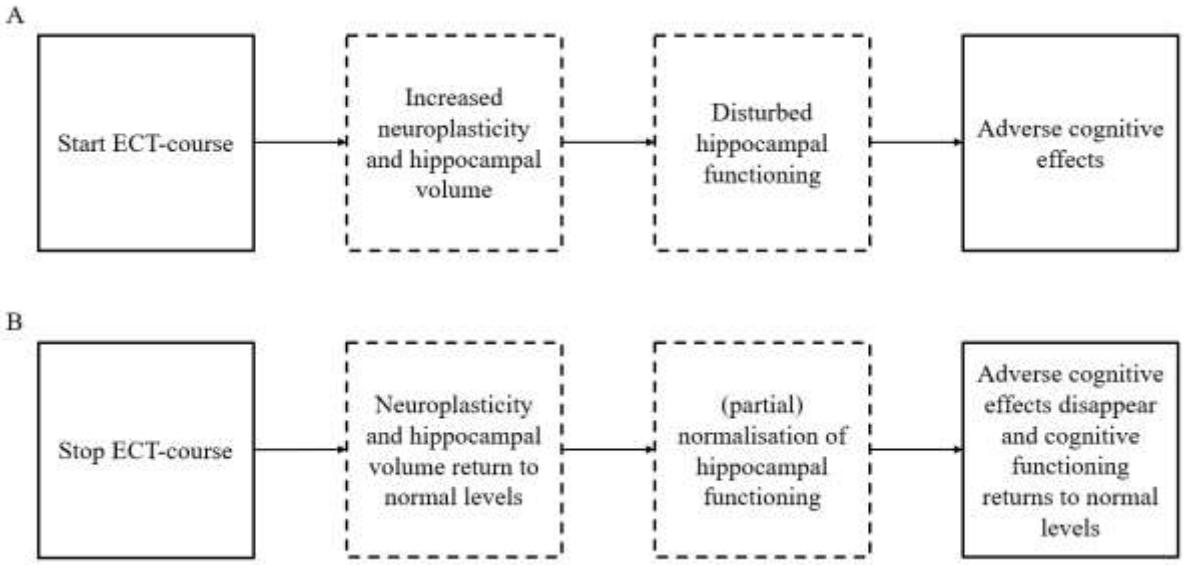

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T06 In vivo effect of activating optogenetic chloride channel on hippocampal evoked potentials

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Background and aims:

Electrical stimulation of axon collaterals of CA3 pyramidal cells of hippocampus (Schaffer collaterals) gives evoked potentials (EPs) in CA1 region. These EPs represent synaptic input (i.e. field Excitatory Postsynaptic Potential) and neuronal output (population spike) of a population of CA1 neurons. In this study, we tested microbial blue-light activated chloride channel, GtACR2, to suppress hippocampal CA1 EPs *in vivo* in isoflurane anaesthetized rats.

Methods:

Delivery of viral vector (AAV2/7-CaMKIIα-hGtACR2-mCherry), and implantation of stimulation electrode and optrode (optic-fibre + recording electrode) (three weeks later) in CA1 subfield of rat hippocampus was done through stereotaxic procedures. A biphasic charge-balanced square-wave pulses (200 µs pulse width) of a fixed intensity was then used to obtain EPs in isoflurane anaesthetized rats. The averaged ratio of EPs parameters—fEPSP amplitude, fEPSP slope, and population spike (PS) amplitude was compared and calculated for when GtACR2 was activated to when not activated (GtACR2 On/ GtACR2 Off: Mean ± SEM) for each animal.

Results:

To determine the effect of activating GtACR2 on EPs (dose-response relations), three light power densities (LPDs) out of the optic fiber tip, i.e., 32 mW/mm^2, 159 mW/mm^2, and 318 mW/mm^2, and for each of the LPDs three pulse durations (5 ms, 10 ms and 20 ms) were tested by delivering a pulse of light 1 ms after the electrical stimulation. All of of the LPDs showed a decrease in amplitudes of fEPSP and population spike (PS) and fEPSP slope, and LPD of 159 mW/mm^2 (10 ms) could decrease PS amplitude in all of the animals (GtAR2 On/GtACR2 Off: 0.21 ± 0.06) (n = 10).

Conclusion:

Inhibition of transient evoked neuronal-population responses invivo was possible in the given experimental conditions.

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T07 Long term chemogenetic suppression of spontaneous seizures in a mouse model for temporal lobe epilepsy

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AIM More than one third of patients with epilepsy continue to have seizures despite treatment with anti-epileptic drugs. In this study we evaluate whether selective silencing of excitatory hippocampal neurons using the inhibitory Designer Receptor Exclusively Activated by Designer Drugs (DREADD) hM4Di leads to suppression of spontaneous hippocampal seizures in the intrahippocampal kainic acid (IHKA) mouse model for TLE.

METHODS Mice (n=60) were injected with kainic acid in the right hippocampus (200ng/50nl, AP -2mm ML +1.5mm DV -1.8mm relative to bregma). Three weeks later, animals were injected in the KA-lesioned hippocampus with 500nl adeno-associated-viral vector carrying genes encoding hM4Di-mCherry fusion protein (2.7E+13 GC/ml, DREADD group, n = 43) or mCherry only (2.1E+13 GC/ml, non-DREADD group, n=13) under transcriptional control of CamKIIalpha promotor, specific for excitatory neurons. Subsequently a bipolar recording electrode was implanted at the injection site. Mice with most frequent hippocampal seizures were selected for further treatment. DREADD animals were treated with three subclinical single doses of clozapine (0.01, 0.03 and 0.1mg/kg ; n=8, 9 and 10) and with repeated doses (every 8 hours for 3 days , n=5, 4 and 5). Non-DREADD animals (n=4) received a single 0.1mg/kg clozapine injection. The fraction of time an animal spent in seizures (FTS) was used as a measure for epileptic activity.

RESULTS Administration of 0.1mg/kg clozapine resulted in a strong inhibition of epileptic activity only in DREADD-expressing animals (baseline (BL) FTS values of 16 ± 2 % and 21 ± 4 %, post treatment FTS values of 6 ± 1 % and 20 ± 2 % in DREADD and non-DREADD animals respectively). Both groups were statistically different from each other for at least 18 hours after

treatment. Acute administration of 0.1mg/kg lead to a stronger seizure suppression in comparison to 0.03 and 0.01mg/kg injection (BL FTS values of 15 ± 2 % and 18 ± 3 % reduced to 10 ± 1 % and 11 ± 1 % post treatment with 0.03 and 0.01mg/kg respectively). Similarly, sustained treatment for three consecutive days resulted into a dose-dependent suppression of epileptic activity (0.1mg/kg resulted in a more potent suppression than 0.03 and 0.01mg/kg; 0.03 mg/kg resulted in a more potent suppression than 0.01mg/kg, BL FTS values of 15 ± 2 %, 21 ± 2 % and 25 ± 2% reduced to 3 ± 2%, 6 ± 2% and 11 ± 2% during chronic treatment with 0.1, 0.03 and 0.01mg/kg respectively).

CONCLUSION This study shows the potency of chemogenetics to robustly and chronically suppress spontaneous epileptic seizures. Moreover, this indicates that excitatory hippocampal neurons are essential in the seizure generation process in the IHKA mouse model. Additionally we show that different subclinical doses of clozapine can be used to activate DREADDs and that 0.1mg/kg clozapine is the most potent dose to suppress spontaneous seizures. This could lead to epilepsy therapy where a systemically administered drug very selectively modulates specific neurons of the seizure network, resulting in a very potent seizure suppression.

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T08 Bottom-up sensory processing can induce negative BOLD responses and reduce functional connectivity in nodes of the default mode-like network in rats.

Rukun Hinz[1], Lore M. Peeters[1], Disha Shah[1], Stephan Missault[1], Michaël Belloy[1], Verdi Vanreusel[1], Meriam Malekzadeh[1], Marleen Verhoye[1], Annemie Van der Linden[1], Georgios A. Keliris[1]

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Introduction: The brain's default mode network (DMN) is active during rest and deactivates as well as decreases its functional connectivity (FC) during externally oriented (top-down) attention demanding tasks. Moreover, the switching from active to inactive state during tasks has been shown to influence task performance. However, top-down attention tasks cannot always easily be employed to investigate the function of DMN e.g. in preclinical research using anesthetized animals. To this end, we investigated whether and how bottom-up visual processing affects DMN in anesthetized rats.

Method: Male long Evans rats (N=12) 4 months of age were scanned on a 9.4T BioSpec Bruker MRI scanner. Animals were anesthetized using an S.C. bolus injection of medetomidine followed by a continuous infusion. The fMRI scans were acquired using a GE-EPI sequence. For each subject, resting state fMRI scans were acquired during (a) complete rest (RSB) and (b) continuous random visual stimulation (CVS). Last, we performed a visual block-design fMRI scan. The CVS consisted of a train of continuous short pulses of light with a random duration and random inter-light intervals (both between 50-250ms). The visual fMRI scan was acquired using white light flickering at 4Hz and 15 ON/OFF blocks of 10s and 40s respectively.

Results/Discussion: Visual stimulation during the block-design fMRI demonstrated expected activations of visual processing regions, i.e. lateral geniculate nucleus, superior colliculus (SC), and visual cortex (VC). Interestingly, we also observed deactivation of DMN regions i.e. Temporal association cortex (TeA) and retrosplenial cortex (RS). Comparison of FC between RSB and CVS showed that continuous random visual stimulation significantly decreased functional connectivity between regions of the DMN i.e. cingulate cortex (Cg)-RS, Cg-parietal association cortex (PtA), RS-TeA, RS-PtA and TeA-PtA as well as between DMN and visual ROIs i.e. Cg-VC, RS-VC, TeA-VC and PtA-VC.

Conclusion: This study demonstrates that visual stimulation in anesthetized rats results in an activation of visual areas and deactivation of nodes of the DMN in rats. In addition, we observed that bottom-up visual processing induced decreased functional connectivity within DMN and between DMN and visual regions. Results provide a functional demonstration of DMN in rats and suggest that bottom-up visual stimulation can influence DMN activity and connectivity in a similar way as top-down attention tasks in humans.

ABSTRACTS OF POSTER PRESENTATIONS (P01-P24)

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P01 MEK inhibition ameliorates social behaviour deficits in a *Spred1-/-* mouse model for RASopathy disorders

Sarah Borrie[1], Ellen Plasschaert[1], Ype Elgersma[2], Steven Kushner[2], Alexa Horner[3], Maksym Kopanitsa[3], Akihiko Yoshimura[4], Eric Legius[1], Hilde Brems[1]

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RASopathies are neuro-cardio-facio-cutaneous disorders stemming from mutations in the Ras-MAPK pathway. Legius syndrome is a rare RASopathy disorder caused by mutations in the *SPRED1* gene. SPRED1 protein negatively regulates activation of Ras by its interaction with neurofibromin, a Ras-GAP protein. Incidentally, the phenotype of Legius syndrome is very similar to that of Neurofibromatosis type 1, another RASopathy caused by mutations in the *NF1* gene that encodes neurofibromin. Common to both disorders are cognitive deficits and increased incidence of autism spectrum disorder (ASD). ASD is defined by core symptoms of impaired social behaviour and communication, and restricted interests and repetitive behaviours. Mouse models for RASopathies exhibit cognitive deficits consistent with human phenotypes, however it is not known whether they also recapitulate ASD-like symptoms.

Here we examined autism-linked behaviours in *Spred1-/-* mice as a model for Legius syndrome, and probed the mechanisms underlying the behavioural phenotypes. *Spred1-/-* mice have deficits in social dominance in the automated tube test and monitoring ultrasonic vocalisations revealed impairments in social communication in *Spred1-/-* mice. Behaviour assays measuring the response to novelty showed that *Spred1-/-* mice exhibit reduced nesting behaviour, marble burying and investigation of novel objects, suggesting lack of or restricted interest. Additionally, associative learning in an touchscreen operant conditioning protocol was impaired in *Spred1-/-* mice. Targeting the Ras-MAPK pathway by treating adult mice with the specific MEK inhibitor PD325901 could reverse the deficits in social dominance and novelty investigation in *Spred1-/-* mice, but could not rescue the cognitive impairments. This indicates the specificity of dysregulation of RAS-MAPK pathway activity in mediating ASD-like deficits in *Spred1-/-* mice. In conclusion, these results suggest the importance of correct regulation of Ras-MAPK signalling for the maintenance of social behaviours.

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P02 Hypothalamic neurogenesis in a migratory bird: An adaptive mechanism

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Reversible changes in body mass are typical of latitudinal avian migrants. This is important as they need energy reserves to fly thousands of miles. Long photoperiod has been shown to induce both body mass increase and migratory restlessness (representing migration). It is not yet known if both phenomenon which are induced by photoperiodic changes share common parallel regulatory pathways. In contrast resident bird species shows no difference in their body mass pattern over the seasons. The neuro-molecular mechanism underlying the body weight increase in mammals has recently been studied in depth revealing a role of hypothalamic neurogenesis. We hypothesize that a similar mechanism is adopted by the migratory redheaded buntings (*Emberiza bruniceps*) to regulate their body weight. We further compared the effects with non-migratory resident Indian weaverbird (*Ploceus philippinus*) using it as a natural 'mutant' for photoperiod induced body weight increase. Birds in 4 groups were exposed for 30 days to a short photoperiod (10L:14D) with a regular or high calorie diet, and a long photoperiod (14L:10D) with a regular or high calorie diet. We recorded before and after the experiment, the body temperature, food intake, fat score, body mass, testis size, and continuously monitored daily activity pattern. As a physiological measure, blood glucose and triglycerides levels were measured. Hypothalamic expression of DCX and NPY was measured as neurobiological correlates. We found a significant effect of food on photoperiodic induction of migratory phenotype in buntings. High calorie diet significantly lowered the activity levels in buntings under short days. Interestingly, there was a shift in food preference in buntings, not weaverbirds. The migratory birds demonstrated high plasticity in dorsomedial hypothalamus (DMH) in response to both food and photoperiod. DMH showed both high number of new neurons/μm^2 and their arborization complexity in buntings, as compared to the weaverbirds. The expression of NPY was also found associated with both the photoperiod and food types in buntings. We for the first time show that there is a neurogenic niche in mediobasal hypothalamus (MBH) of birds too, and propose hypothalamic neurogenesis/plasticity acts as an adaptive mechanism involved in the development of migratory phenotype which is an important life history state in migratory birds.

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P03 What do stroke survivors actually learn when regaining walking ability? A protocol for a repeated-measurements prospective cohort study.

Jonas Schröder[1,2], Wim Saeys[1,2,3], Laetitia Yperzeele[4], Gert Kwakkel[5] and Steven Truijen[1,2]

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Objective

During a stroke, ischemic damage to brain tissue causes a loss of neurological functions, e.g. generating coordinated movements (i.e., paresis) required to walk. During the first 3 months post-stroke, typically large improvements in paresis and walking ability are observed. However, previous studies suggest that these improvements are mainly driven by learning to shift control towards the non-paretic body side (i.e., using compensation strategies) rather than by the restoration of inter-limb coordination.

Objective

Stroke survivors from the first days onwards are assessed repetitively to identify the neurological and behavioral changes responsible for improvements in walking.

Methods

In total, 24 participants with leg paresis (i.e., NIHSS item 6 >0) and limited walking ability (i.e., FAC ≤3) will be included within the first 14 days after a first-ever hemispheric stroke. Longitudinal changes in selected outcomes will be investigated at 3, 5, 8, 12 and >24 weeks post-stroke. Leg paresis (i.e., Fugl-Meyer Assessment and Motricity Index) as well as standing (i.e., posturography) and walking ability (i.e., walking speed) will be assessed. In addition, kinetic and electromyographic analyses during upright activity will be performed as soon as participants are able to stand unsupported. For this purpose, the center-of-pressure displacements and muscle activation patterns will be calculated to estimate the contribution of paretic and non-paretic side to the control of balance and locomotion.

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Discussion

In the present study, we will investigate behavioral restitution and compensation strategies for improvements in walking during the first 6 months post-stroke (see Fig). A better understanding of what patients actually learn when regaining walking ability is required to give evidence-based guidelines for clinicians in order to choose for restorative or adaptive treatment strategies.

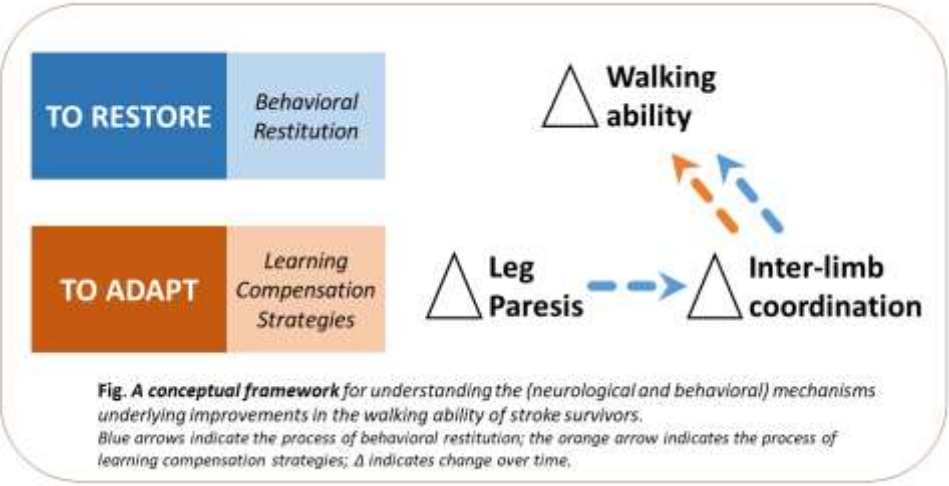

Fig. *A conceptual framework* *for understanding the (neurological and behavioral) mechanisms underlying improvements in the walking ability of stroke survivors. Blue arrows indicate the process of behavioral restitution; the orange arrow indicates the process of learning compensation strategies; Δ indicates change over time.*

Acknowledgement:

Jonas Schröder is a SB PhD-fellow (application:1S64819N) at the FWO, Research Foundation Flanders, Belgium.

Conflict of interest:

The authors declare that there is no conflict of interest regarding the described research activity.

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P04 Digenic inheritance of rare variants in autism spectrum disorders using platelet studies and genome sequencing

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Autism spectrum disorder (ASD) is a group of early-onset heterogenous neurodevelopmental disorders characterised by deficits in social communication and interaction and abnormal repetitive behaviour. It has high heritability and the disease aetiology involves complex gene-environment interactions. Comorbidities and endophenotypes of ASD have proved to be valuable to understand its clinical and pathophysiological characteristics further. Blood platelets are mainly known to prevent bleeding, though they also modulate processes in cancer, inflammation, and neurological disorders. They have the potential of being a peripheral biomarker and cell model for autism since they share common biological and molecular characteristics with neurons. In particular, platelet dense granules contain neurotransmitters like serotonin, and molecular players controlling their formation and function are similarly regulated in platelets and neurons. The major platelet receptor αIIbβ3 has recently been studied in ASD as a regulator of serotonin transport. Though many studies revealed an association between platelet markers and ASD, there is still a big gap in linking all these markers and explaining the “platelet phenotype” in autism.

Whole genome sequencing (WGS) was performed on patients diagnosed with idiopathic ASD and mild platelet secretion defects as endophenotype. High impact variants in potential candidate genes identified followed an interesting digenic inheritance pattern in the more than two independent families. Functional genetic studies using splice vectors, state-of-art cell lines, RNA-seq on neural precursors formed from patient derived induced pluripotent stem (iPS) cells, and isogenic mock patients created by CRISPR-Cas9 system were performed to further characterise the identified candidate genes in relation to neuronal and platelet function. Nervous system development, endosomal biogenesis and neurotransmitter transport are few of the top disrupted pathways in this cohort of ASD patients with platelet defects. Therefore, here we leverage the potential of platelets as a peripheral biomarker to understand ASD pathophysiology and try to explore the pathways involved in the observed “platelet phenotype” in ASD.

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P05 The effects of childhood cancer treatment on early maternal bonding and adult socio-cognitive functioning in laboratory mice

Livine Craeghs[1], Zsuzsanna Callaerts-Vegh[1], Rudi D'Hooge[1]

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Objective: Early (adverse) life events can affect cognition and social behavior in adults. Childhood cancer is such an adverse life event and 2 factors, i.e. chemotherapy and the nature of parental care could affect behavior later in life. Chemotherapeutics have long-lasting behavioral effects (chemo-brain), especially when applied during development. However, stable and positive parental interactions are stress-reducing and might increase a child's resilience to adverse events. In this project we investigate 1) the severity of developmental chemotherapy-induced socio-cognitive defects during pre-weaning and adult life, and 2) how chemotherapy affects the relationship between a mother and pup.

Methods: We used a mouse model (C57BL/6), since mice are social mammals and mother-pup relationships are crucial for the survival and thriving of pups. Pups were injected with methotrexate (MTX) or vehicle (i.c.v., bilaterally) at one week of age. We assessed changes in behavior in pups and the mothers. When offspring reached adulthood, their behavior was assessed in an extensive testing battery spanning exploratory, emotional, cognitive and social behavior.

Results: Mothers, whose pups were exposed to MTX, spent significantly more time nursing their pups and less time off-nest when monitored 24h after injection. The number of ultrasonic vocalizations by the pups after maternal separation did not change when injected with MTX. However, the pups' preference towards their mother was altered 2 weeks after MTX exposure, especially in females. During adulthood, no differences were found in levels of exploration, anxiety, depression-like behavior, fear memory, spatial-dependent learning and memory, reversal learning and working memory. However, we observed robust changes in social behavior, e.g. alterations in dominance, sociability and a trend towards impaired social memory.

Conclusion: Our first results indicate changes in the pups' as well as the mother's behavior after MTX injections. Also during adulthood, mice show impaired social behavior when exposed to MTX at early age.

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P06 De novo mutations in specific PP2A subunit encoding genes, including *PPP2CA*, cause a (neuro)developmental disorder

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Type 2A protein phosphatases (PP2As) are highly expressed in the brain and regulate neuronal signaling by catalyzing phospho-Ser/Thr dephosphorylations of diverse substrates. PP2A holoenzymes comprise catalytic C-, scaffolding A-, and regulatory B-type subunits, which determine substrate specificity and physiological function. Interestingly, *de novo* mutations in genes encoding subunits Aα and B56δ, and less frequently, B56β and B56γ, have recently been implicated in intellectual disability/developmental delay (ID/DD).

Here, we report on 16 individuals with a *de novo* mutation in *PPP2CA*, encoding the catalytic Cα subunit. These patients were phenotypically characterized by mild to severe ID/DD, behavioral problems, variable types of epilepsy, hypotonia and brain abnormalities. In contrast to ID-related variants in the A- and B-subunit, which are all missense mutations, the mutational spectrum in *PPP2CA* is more diverse, comprising a partial gene deletion, a frameshift, three nonsense mutations, a single amino acid duplication, one recurrent and eight non-recurrent missense mutations. More strikingly, functional studies showed complete PP2A dysfunction in four individuals with seemingly milder ID, providing the first evidence that *PPP2CA* could be a haploinsufficient gene in humans. Next to this, whereas mutations in *PPP2R5D* (B56δ) and *PPP2R1A* (Aα) rather suggest a dominant-negative effect on B56δ-regulated PP2A complexes, Cα variants induce a wide range of PP2A deficiencies, affecting holoenzyme assembly, post-translational modifications and/or activity, but overall, mainly affecting B56(δ) holoenzyme functionality. Hence, *de novo* mutations in *PPP2CA*, *PPP2R1A*, and *PPP2R5D* constitute a spectrum of overlapping ID syndromes, characterized by mild to severe phenotypes and functional convergence by severe PP2A dysfunction, which we suggest, collectively, to call “PP2A-related (neuro)developmental disorders.”

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P07 Stress dependent mental disorders; a molecular and structural basis

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The stress response is the lifesaving mechanism. It helps an organism to react and to adapt to a stressor. However, prolonged or excessive stress is a major causative factor in multiple mental diseases such as major depressive disorder, anxiety disorders, and dementia. Little is known how acute stress affects neuronal circuitry at a structural level. We focus on the effects of the corticotropine releasing factor (CRF) system in acute stress. CRF is one of the major peptides in the stress response. Besides its hormonal actions in the blood, CRF is endogenously expressed in distinct regions of the brain, related to the stress response like the hippocampus. In brain, CRF acts as a neuromodulator and can contribute to the functional and structural properties of synapses. We found that acute application of physiological doses of CRF induced the formation of the dendritic spines *ex-vivo*. In addition, functional electrophysiological studies show an increase in calcium-dependent paired-pulse facilitation together with an increase of the presynaptic vesicular pools.

In chronic stages, CRF activates the G-protein coupled receptor named CRF-R1, and it leads to a reduction of the spine formation and complexity of dendritic tree. Beside CRF-R1, CRF can bind with a lower affinity to another receptor, CRF-R2. To understand the role of these receptors in acute stress, we use shRNA-mediated protein knock down. Spine density quantification shows a reduction of the spine numbers in CA1 due to CRF-R2 knockdown. In parallel, we asked whether acute CRF and CRF-Rs signaling might affect spine induction via the cyclin-dependent kinase-5 (Cdk5) pathway. Blocking of Cdk5 pathway, by its inhibitor roscovitine, together with CRF acute application failed to increase spine density. To conclude, CRF has a beneficial neuromodulator effect in acute stages on morphological and functional synapse properties.

To date, the molecular signaling downstream of the binding of CRF neuropeptides to their receptors is not well understood. Further research to unravel these mechanisms and to elucidate the signaling pathways in stress-related morphological and molecular alterations will contribute to the better understanding of stress-induced brain changes.

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P08 DCE-MRI based visualization of brain-fluids-circulation: a window to cerebral distribution of neuromodulatory molecules?

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Introduction. Proper CNS function critically depends on maintaining its homeostasis that requires an efficient solutes clearance and distribution of essential molecules. Recently a glial-dependent brain fluids transport route was discovered, so called glymphatic system (GS). This system uses the perivascular network of the brain for cerebrospinal fluid (CSF) transport, and the exchange with interstitial fluids (ISF), facilitated by AQP4 present at astrocytes end-feet. The malfunction of the GS has been suggested to contribute to abnormal protein accumulation in Alzheimer disease (AD). Further, the changes in CSF secretion and choroid plexus has been reported to occur in AD and upon ageing. In our research we investigate how and if the efficacy of brain-fluid-circulation (BFC) via CSF-ISF exchange influences the function of neurons by means of the distribution of molecules (e.g. neuromodulators, gliosignaling molecules, OTX2) via the extracellular space (ECS) under healthy and pathological conditions. To this end, we are using dynamic contrast enhanced MRI (DCE-MRI) for macroscale visualization of BFC in conjunction with microscale evaluation.

Methods. DCE-MRI was implemented and used for the whole-brain visualization of BFC in vivo following the labelling of CSF with a gadolinium (Gd)-based T1-contrast agents, Gd-DOTA. Data were acquired on 9.4 T in wild-type rats (Wistar Han, Fischer F344) and TgF344-AD rats by repeated acquisition of 3D T1-weighted MR images (3D FLASH) every 4 min. The acquisition was started before (baseline), and continued during and after injection of contrast agent into the CSF, and until the signal returns back to the baseline intensity levels indicating the washout of the contrast agent (TA~4-6h). The analysis of the time-dependent signal intensity changes (i.e. kinetic time courses (TACs) of signal intensity changes, cluster analysis) was performed to extract the information about the regional efficacy of BFC. Brain samples were collected for a complementary ex-vivo analysis at microscale.

Results & conclusions. We could demonstrate that DCE-MR is a valuable method for mapping kinetics and spatial distribution of the CSF-ISF exchange including: anatomical influx nodes, the distribution and retention of imaging tracers in the regions of interest and the clearance routes across the entire brain. Furthermore, we could detect the regional changes in CSF-ISF exchange under pathophysiological conditions and link those to underlying molecular fingerprints and functional alterations.

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P09 Genetic modification of locus coeruleus NE cells for chemogenetic activation remains challenging

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Aim: The Locus Coeruleus-Noradrenaline (LC-NE) system plays an important role in vagus nerve stimulation (VNS) induced effects on brain excitability and epileptic seizure control. Chemogenetic approaches to selectively modulate LC may allow dedicated investigation of the role of the LC-NE pathway in the regulation of brain excitability. This study investigated the feasibility to express the excitatory hM3Dq DREADD (Designer Receptor Exclusively Activated by Designer Drugs) in LC neurons and perform unit recording of genetically modified LC neurons.

Methods: Thirty-seven male rats were injected with 10nl of adeno-associated viral vector AAV2/7-PRSx8-hM3Dq-mCherry (n=20) or AAV2/7-PRSx8-eGFP (n=17) in the LC. Three weeks later LC unit recordings were performed in anesthetized rats to investigate the effects of clozapine (0.01 and 0.1 mg/kg, s.c.) as DREADD ligand to activate the modified LC neurons. After characterization of LC-NE neuron by a phasic burst inhibition after a foot pinch, baseline was recorded followed by subsequent administration of 0.01 mg/kg and 0.1 mg/kg clozapine. A decreased firing rate after clonidine (0.04mg/kg, s.c.) injection was used to confirm LC identity of the recorded neuron. Determination of hM3Dq-mcherry expression levels was performed using immunofluorescence staining.

Results: Successful unit recordings were performed in 12 animals and a total of 12 neurons were recorded (n=5 control, n=7 DREADD). There was no difference in baseline LC firing rate between DREADD (2.67 ± 0.26Hz) and control LC neurons (1.88 ± 0.61Hz; P=0.21). Clozapine (0.01 mg/kg) had no effect on the mean firing rate of recorded LC neurons whereas an increased firing rate was observed after the highest dose (0.1 mg/kg) irrespective whether they came from DREADD or control animals (P=0.006). Co-labeling of LC neurons and mCherry-tag showed that 21 ± 2.3% LC neurons are expressing the hM3Dq receptor. Aspecific expression of hM3Dq-mCherry was also observed in non LC neurons (26 ± 4.1%).

Conclusions: LC unit recording is feasible following manipulations for DREADD induction. In the current experimental set-up we were not able to draw conclusions on clozapine-activation dosages due to a low efficiency and selectivity of LC neuron transduction.

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P10 Functional connectivity in the default mode-like network decreased upon activation of basal forebrain cholinergic neurons in rats

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The default mode network (DMN) consists of several brain regions which are activated during states of rest or quiet wakefulness. This network has received a lot of attention since its function is still poorly understood and alterations are observed in several neurological disorders, such as schizophrenia and Alzheimer's Disease. An important characteristic of the DMN is the suppression of activity and decrease in functional connectivity during attention demanding tasks. Recent research suggests that the basal forebrain regulates the activity of the DMN. Therefore, we hypothesized that activation of basal forebrain cholinergic neurons, which are strongly involved in attention, will decrease the FC of the DMN in rats.

In this experiment, cholinergic neurons in the basal forebrain of rats were transfected with hM3Dq receptors, which are excitatory designer receptors exclusively activated by designer drugs (DREADDS). These DREADDs increase neuronal activity upon administration of the inert ligand Clozapine-N-Oxide (CNO). Chat-cre Long Evans rats (n=28) received a unilateral stereotactic injection of a viral construct containing the DREADD gene (n=16) or a sham virus (n=12) in the nucleus basalis of Meynert. Magnetic resonance imaging (MRI) was performed 2 months after the transfection. A 10-minute baseline scan was acquired, after which CNO was administered and phMRI scans were acquired for 35 minutes.

In the DREADD expressing animals, we observed a significant decrease in FC within different nodes of the DMN upon activation of the basal forebrain cholinergic neurons. These changes in FC were not observed in sham animals indicating that CNO did not activate endogenous receptors and the effects seen in the DREADD animals were due to specific activation of the basal forebrain cholinergic neurons.

Hence, we can conclude that activation of the cholinergic neurons in the right basal forebrain, significantly decreases FC in different nodes of the DMN. These results imply a direct role of the basal forebrain cholinergic system in the modulation of the activity of the DMN. Since the basal forebrain cholinergic system is an important modulator of attention, these results indicate that when the attention system is activated, FC within the DMN decreases. Deeper understanding of the modulatory role of acetylcholine in DMN regulation might yield valuable insights into the function of the DMN. Furthermore, investigating the DMN in rats could provide valuable insights into mechanisms which drive the alterations of DMN activity in neurological disorders.

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P11 Chemogenetic silencing of neurons in mouse cingulate area induces long range neural activity and functional connectivity modulations: a DREADD-fMRI study

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Introduction. The anterior cingulate area (ACA) is an integral part of the prefrontal cortex and has been implicated in several cognitive functions. Anatomical and functional imaging studies identified the ACA as a hub region that is highly interconnected with numerous brain regions and involved in multiple functional networks. In this study, we combined inhibitory kappa opioid-receptor DREADDs (KORD) in the mouse ACA and non-invasive functional MRI in order to link inhibition of the ACA to whole brain network activity and integrity.

Methods. During a stereotactic surgery, mice received either AAV-CaMKII-HA-KORD-IRES-mCitrine (n=12) or a control virus AAV-CaMKIIa-EGFP (n=9) in the right ACA. Imaging procedures started at least three weeks after viral injections and were performed on a 9.4 T MRI system (Bruker Biospec). For all scans mice were anesthetized with a s.c. bolus of 0.05 mg/kg medetomidine, followed by a s.c. infusion of 0.1 mg/kg/h medetomidine and 0.3% isoflurane. Two separate pharmacological fMRI (phMRI) acquisitions were performed in different scanning sessions using two concentrations of SalB (low: 3 mg/kg, high: 6mg/kg). PhMRI scans (Two-shot GE-EPI: TE= 20 ms, TR = 15000 ms, matrix = 64x64, FOV = (20x20) mm^2, 16 slices) were acquired starting 10 min before to 50 min after s.c. SalB administration (duration 1h). Resting state fMRI scans (GE-EPI: TE = 16 ms, TR = 2000 ms, matrix = 128x64, FOV = (20x20) mm^2, 16 slices) of KORD expressing mice were acquired 10 min after s.c. administration of SalB (3mg/kg) or a control compound, DMSO.

Results. The phMRI scans revealed that inhibition of the neurons in the right ACA induced significantly altered BOLD signals in the ACA as well as in connected brain regions. Both BOLD signal decreases and increases were observed in connected brain regions. Comparisons of the two doses of SalB did not show any significant results. Further, the results of the rsfMRI scans revealed functional connectivity decreases in the connected brain regions.

Conclusion. This study combines the use of KORD with in vivo MRI to assess large scale network activity and functional connectivity in response to inhibition of a specific brain region. We showed that KORD-induced inhibition of the right ACA could mediate BOLD signal decreases and increases in connected brain regions throughout both hemispheres. Furthermore, these connected brain regions also showed decreases functional connectivity

measures. To conclude, this study identifies the DREADD technology as a valuable tool that can be used in combination with non-invasive imaging methods.

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P12 Deciphering biomechanisms and therapeutic response in KCNQ2 encephalopathy using multi-modal characterization of iPSC derived neuronal cultures

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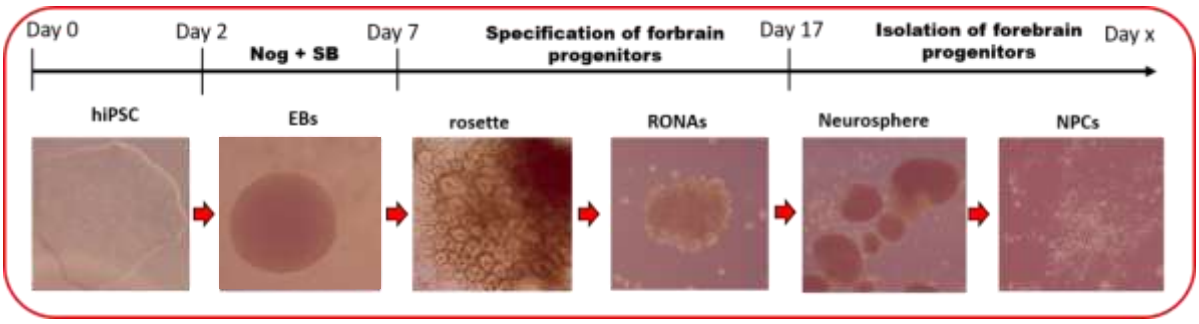
Developmental and epileptic encephalopathies (DEE) are severe disorders characterized by epilepsy and developmental delay. KCNQ2-encephalopathy (KCNQ2-E) is the most frequent neonatal DEE, caused by de novo dominant negative mutations in the potassium channel subunit gene KCNQ2. Interestingly, inherited loss of function mutations in KCNQ2 lead to neonatal seizures with normal development (Benign Familial Neonatal Epilepsy, KCNQ2-B). The presence of developmental delay thus seems to be related to a more severe defect of the neuronal potassium current. However, how this leads to the neurodevelopmental problems of KCNQ2-E is not yet understood. Animal models are so far not available. This lack of mechanistic understanding is reflected by the lack of treatments that influence development, and all available treatments for KCNQ2-E indeed purely target seizures. In this project, we will generate neuronal cultures derived from human induced pluripotent stem cells from KCNQ2-E and KCNQ2-B patients, and model these disorders within their neuronal context. We will perform electrophysiology, high content microscopy, and transcriptomics of different cell types in our cultures, generating a readout for both the epileptic and neurodevelopmental aspects. We will next study the potential impact of targeted treatment with a potassium channel opener on the different readout modalities, and define therapeutic pathways using compound screening.



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P13 Understanding activity dependant amygdalar neural circuitry in appetitive learning

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Amygdala is the centre for emotive responses in the brain. It has been been implicated in fear learning and conditioning, aversive responses as well as appetitive learning and responses - the later being comparatively less studied. Fragmented information is available pertaining to the role of each amygdalar sub-regions and interaction of its neuronal subgroups with the afferent and efferent connections to different brain regions in appetitive learning. This needs to be investigated to have a better understanding of the neural networks pertaining to positive valence. We performed recombination based viral labelling in mice for tracing direct projections from basolateral and central amygdala to nucleus accumbens, and from sweet cortex to basolateral amygdala. We also did viral vector based activity dependant labelling in different amygdalar subregions to track their projections and changes in spine properties in mice subjected to appetitive learning. We combined these to standard immuno-histochemistry against neuronal markers of different subtypes of amygdalar neurons and post-synaptic markers. The afore-mentioned techniques helped us to trace the efferent projections from baso-lateral amygdala to the central amygdala and nucleus accumbens and investigate their changes and interactions with different neuronal populations upon appetitive training.

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P14 Chemogenetic suppression of spontaneous seizures in a rat model for temporal lobe epilepsy

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The hippocampus is believed to play a crucial role in seizure generation in temporal lobe epilepsy (TLE), a common form of medication-resistant epilepsy. This preclinical study evaluated chemogenetics as a potential therapy for TLE. Using this approach, excitatory neurons of the epileptic hippocampus were selectively inhibited through ligand-based activation of an inhibitory Designer Receptor Exclusively Activated by Designer Drugs (DREADD).

The intraperitoneal kainic acid rat model for TLE was used. Animals (n=6) were injected in right hippocampus with adeno-associated viral vector carrying CamKIIα-hM4Di-mCherry. Two weeks after injection, rats were bilaterally implanted with depth electrodes in the dentate gyrus and CA1 region of both hippocampi. Seizure frequency before and after activating DREADDs with subclinical doses of clozapine was determined using continuous video-EEG recordings.

First, EEG was monitored during a baseline period of six days. Next, single injections of different clozapine doses (0.01, 0.1 or 1 mg/kg bodyweight/24h, s.c.) and vehicle were compared. For each dose, EEG was monitored during three days of treatment. Finally, one dose was selected to evaluate an improved dosing scheme in a randomized-blind trial. EEG was monitored during a baseline period of one days, followed by one day of treatment with clozapine (0.1mg/kg bodyweight/6h) or vehicle. In all experiments, seizure frequency during baseline recordings was used to normalize the data.

Clozapine-induced activation of DREADDs had a dose-dependent seizure suppressing effect. Clozapine doses of 0.01, 0.1 and 1 mg/kg resulted in a clear lag in average cumulative seizure frequency of about 2, 5 and 8 hours respectively. Repeated clozapine administration resulted in a strong suppression of epileptic seizures in all animals tested. During treatment, the average daily seizure frequency was reduced with 86%±7% (SEM).

Clozapine-mediated activation of hM4Di DREADDs in excitatory hippocampal neurons temporary suppresses spontaneous seizures in a rat model for TLE in a dose-dependent way. Repeated clozapine administration results in a sustained suppression of epileptic seizures.

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P15 Electrical microstimulation of the macaque VTA drives subliminal and category specific visual perceptual learning

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Visual Perceptual Learning (VPL) refers to the phenomenon in which performance on a discrimination or detection task in the visual domain improves after repeated practice. Characteristic for VPL is that it can be sensitive to very specific aspects of visual features and is able to induce long lasting changes in behaviour and neural processes. Interestingly, under some conditions, VPL does not require awareness of the subject. It is hypothesized that VPL depends at least in part on plasticity of the adult brain and the visual cortex in particular. The neuromodulator dopamine (DA) is associated with a wide range of adaptive behaviours and DA is tightly linked to our ability to learn. Furthermore, electrical microstimulation of the DA-rich ventral tegmental area (VTA-EM) in rodents can induce cortical plasticity.

Here, we set out to investigate changes in behaviour and brain plasticity (through fMRI) after pairing subliminal complex stimulus categories of faces and bodies with VTA-EM (100 Hz, 200 ms). Two male rhesus monkeys (*Macaca mulatta*), with chronic electrodes targeting the VTA, were trained to discriminate the profile (left/right-ward oriented) of images of faces and bodies in the periphery of their right or left visual field (RVF/LVF). In the next phase, we paired one image category (faces or bodies) at subliminal signal-to-noise-ratio (SNR) levels with VTA-EM while the animals were performing a difficult orthogonal colour discrimination task. Prior to this phase, we had tested that the SNR levels and stimulus durations chosen did not interact with colour task performance, to ensure that the stimuli (faces and bodies) were subliminal. Finally, we compared profile discrimination performance before and after the VTA-EM-category pairing sessions at different SNR levels. Profile discrimination performance for the (previously) stimulated category improved significantly, but rather surprisingly, at the higher SNR levels. This improvement in performance was stronger when animals had to discriminate the profile of the previously paired stimulus category in the LVF, contralateral to the hemisphere with the VTA electrodes. In addition, subtle differences in fMRI responses to the VTA-EM paired category indicate the involvement of several subcortical areas such as the hippocampus, claustrum/putamen and cerebellum. In conclusion, Pavlovian pairing with VTA-EM can significantly alter perception of complex subliminal stimuli. Remarkably, this is accompanied by changes in representations of these stimuli, mainly in subcortical areas.

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P16 Investigation of the CB1 receptor in cross-modal brain plasticity upon partial vision loss

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When sensory inputs become reduced or lost, the brain can undergo neuroplasticity events in order to reorganize the deprived cortex to support new function. Monocular enucleation (ME) is a validated model to study visual cortex reorganization in relation to one-eyed vision. The partially deprived binocular visual cortex will become reactivated by open-eye potentiation though inputs coming from the remaining eye. The deprived monocular visual cortex will however become responsive to **non-visual inputs,** the cortical neurons will start to respond to whisker stimulation, a typical example of **cross-modal brain plasticity**. Interestingly, cortical reactivation induced by this cross-modal plasticity phase appears to be age-dependent. Previous research revealed that adolescent mice (P45) do not show this plasticity phenomenon while adult mice (P120) do, and that an altered state of the **GABAergic system** plays a key role in this age dependency. **CB1R** is the main endocannabinoid (ECB) receptor of the central nervous system and as a neuronal and astroglial receptor is capable of regulating GABAergic synaptic transmission. Moreover, this receptor shows a similar age-dependent cortical expression pattern and is already known to be an important mediator of synaptic plasticity. With this project, we aim to decipher the **involvement of the CB1R in ME-induced cross-modal plasticity as well as its contribution to age-related differences in neuroplasticity in general.** Therefore, we will characterize CB1R expression in the ME model and attempt to identify causality between ECB signalling and cross-modal brain plasticity by using a pharmacological approach. In addition, we will interrogate the link between cell-specific adaptations to the GABAergic system, ECBs and cross-modal plasticity by *ex vivo* slice electrophysiology and decipher the specific contribution of the astroglial CB1R to cross-modal plasticity via a cell type specific gene manipulation strategy.

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P17 Signal discrimination task for characterization of spatial (in)attention in rats

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Introduction: Spatial inattention (neglect) is a clinical syndrome characterized by failure to allocate attention to stimuli from a delimited sector of space. It is a frequent sequel of stroke inflicting - most commonly – right frontal and/or parietal lobes. Extinction is an attenuated form of the syndrome where awareness of a stimulus manifesting in the contralesional hemispace is obstructed by another salient stimulus simultaneously appearing in the ipsilesional perceptual hemifield. Theoretical accounts of this phenomenon have pointed to an extreme case of potential interhemispheric competition where the intact hemisphere “wins” and continuously suppresses the contralesional one. Spatial inattention has also been reliably replicated in rodents. Notably, the diagnosis of inattention in humans, and characterization of the lesion-induced deficits in animals rely largely on qualitative methods. Rodents are becoming a favorite in studies of perception owing to the feasibility of genetic modification of specific neuronal subpopulations. The exploitation of the full potential of molecular techniques in rodents, however, is contingent upon the development of rigorous behavioral tasks to discern the outcome of neuronal manipulations. We have developed a behavioral task to parametrically gauge spatial (in)attention in rats. The task design is straightforward, readily learnt by the rats, and can be employed in various research contexts.

Objective: Adapting double simultaneous stimulation (DSS) test of extinction to rats and employ it as a measure of spatial attention with and without neuronal manipulations.

Methodology: Automated operant conditioning chamber controlled and monitored by python-based utilities (pyControl). The system comprises custom made operant conditioning chambers containing 3 contiguous nose pokes. The nose pokes are equipped with infrared photodiodes and interior LED. Two LEDs are installed above the two lateral ports. Two pipes project through holes right below the port openings to supply water reward. The behavioral chamber is placed inside a light and sound attenuating box containing an infrared-sensitive webcam for real-time video tracking. Subjects (L.E rats) are trained to discriminate between two simultaneously presented visual stimuli and to make a nose poke at the brighter side.

Results: Psychometric curves representing the probability of a subject to perceive a lateralized stimulus brighter relative to a competing stimulus on the other side. Parametrically manipulating the intensities on both sides enabled the identification of a subject-specific perceptual threshold. The curves demonstrated performance increases over time and reached relative

stability during the last few days. Each subject's baseline perceptual threshold was estimated. In future experiments this will be monitored after attentional manipulations.

Conclusion: We have developed a promising behavioral task to index the attention deficits in rats. The task will be used as tool to monitor behavioral changes in a larger scheme of investigating neuronal network disturbances underlying spatial inattention and evaluating the effect of cholinergic neuromodulation on restoring functional connectivity and promoting recovery.

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P18 Sexual differentiation of the avian song control system: Insights from a gynandromorphic zebra finch

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Numerous sex differences in brain structure and function exist, and such differences have traditionally been thought to result from differences in early exposure to gonadal hormones. Recent evidence suggests, however, that the sexual differentiation process of the brain is more complex, involving interactions between hormones, sex chromosomes and the environment. Naturally occurring lateral gynandromorphs, in which one side of the animal is genetically female and the other male, provide a unique opportunity to investigate the relative role of genetic sex and gonadal hormones. In species of songbirds in which the males sing a courtship song, the song control system is sexually differentiated such that males have a larger forebrain neural song circuit. In the first characterization of a gynandromorphic zebra finch, researchers found that the genetically male (right) half of the brain had a more masculinized song system, with larger nuclei compared to the female (left) half. These differences existed despite both sides being exposed to identical gonadal hormone levels, providing evidence that the sexual differentiation of the song control system is partially controlled by genetic, rather than strictly hormonal, factors.

We discovered another lateral gynandromorphic zebra finch and further characterized aspects of the song control system that have been demonstrated to be sexually differentiated. Our gynandromorph exhibited the reverse phenotype (right: female, left: male) to the one previously studied and possessed atrophied gonads. This bird produced an unusual song, often “stuttering” or repeating the same syllable. The telencephalon volume on the male side was larger (23%) than on the female side. Similar to the previous gynandromorph, the size of the song control nuclei, most notably HVC, was larger (190%) on the male (left) half of the brain, but lateral differences in the size of the other nuclei were less pronounced. Perineuronal net (PNN) expression, which is associated with neuroplasticity of the song system and is higher in male finches, was similar on both sides of the gynandromorph brain. Aromatase expression was also not lateralized but was low across multiple brain regions when compared to control males. Taken together, these results suggest that genetic factors play a role in overall brain architecture (telencephalon and song system growth) but that hormonal and/or additional factors may be important in some specific sexual differentiation processes. Work is currently ongoing to determine the genetic sex of cells on each side of the brain and to quantify lateralization in neurogenesis.

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P19 Unravelling the Relationship Between Brain Connectivity and Gait Outcome in Stroke Survivors

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Introduction: Stroke incidence is increasing and, consequently, so are the number of motor impaired patients. Reliable biomarkers for gait outcome prediction and early onset rehabilitation are believed to greatly improve the results of rehabilitation for stroke survivors. However, the current literature lacks evidence on such biomarkers as well as on the effect of early gait training on the recovery of brain connectivity. **Objectives:** This project has three objectives targeted by two work packages. Firstly, to characterize functional and structural brain connectivity related to motor cortex of stroke survivors under conventional treatment in the early phase and over 6 months. Secondly, to use changes in functional and structural brain connectivity as biomarkers for gait recovery at 6 months post stroke. Lastly, to characterize changes in functional and structural brain connectivity influenced by early and intensive gait training. **Methods:** Brain structural connectivity will be assessed by Diffusion Tensor Imaging (DTI) and functional connectivity by Resting State Functional Magnetic Resonance Imaging (RS-fMRI). Gait function will be assessed by kinetics and kinematics measures as well as electromyography. Balance will be analysed by two force plates measuring centre of pressure displacement. Biomechanical data will be combined with clinical data from the Motricity Index, Fugl-Meyer Motor Assessment, and Functional Ambulatory Category. **Expected Outcomes:** The results will open the possibility of choosing rehabilitation strategies in an individualized way and improving subject selection for clinical research.

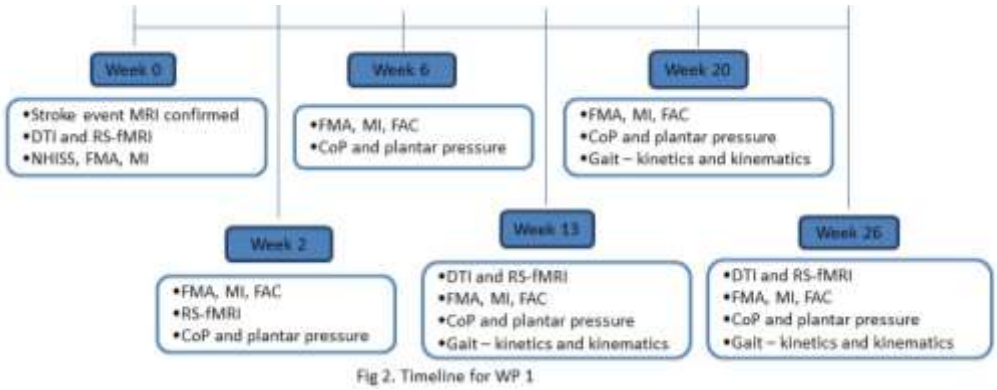

Fig 2. Timeline for WP 1

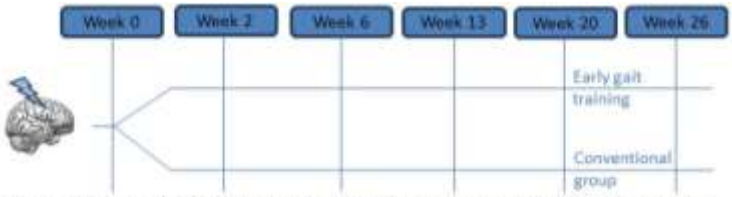

Fig 3. Timeline for WP 2. Patients will be randomized into two groups. Early gait training will happen within the first 6 weeks while the conventional group will receive the same amount of therapy .

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P20 The astrocyte DREADD toolbox as a mediator of visual cortex plasticity

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P22 Synaptrode: neural interface at the synapse level

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Understanding information processing systems requires analysis down to the level of physical implementation. In neural networks this corresponds to the structure-functional relationships of the constitutive neural subpopulations [1]. Structural development of neural networks is regulated in part by a diverse set of synaptogenic proteins, which are expressed on neural cell membranes and induce synapse formation only with distinct complementary partners [2]. With the synaptrode project, we aim to create electrode surfaces covered with synaptogenic proteins. Immobilized synaptogenic proteins have been shown to selectively induce synapse-like connections in vitro [3]. The physical proximity of the formed synapse-like connection, and the specificity embedded in the molecular code of the synaptogenic proteins would allow membrane depolarization measurements of neural subpopulations targeted by synaptogenic protein.

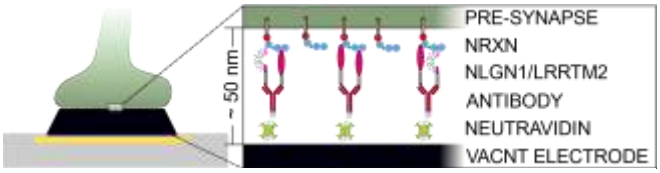

Figure1. Schematic representation

The approach involves 3D vertically aligned carbon nanotube (VACNT) electrodes, functionalized with synaptogenic proteins. Biotin linkers are grafted to the VACNT electrodes by EDC/NHS coupling after a brief O2 plasma activation step. The VACNT electrodes are incubated with NeutrAvidin protein which in turn captures biotin-tailed anti-Fc antibodies. In a final incubation step the antibodies immobilize the synaptogenic proteins on the electrode surface. The combined biochemical and topological-mechanical cues of the protein-functionalized electrodes result in a close (<50 nm) physical connection between the electrode and the target neurons (Figure 1) which will allow stable and specific membrane depolarization recording.

To date, we have successfully fabricated VACNT electrodes and have immobilized NeutrAvidin on the CNT surface via a biotin linker. Currently we are performing the first in vitro proof-of-concept cell culture experiments and designing custom microelectrode arrays (MEAs) for further experiments.

Acknowledgment

The authors would especially like to thank Dr. M. De Volder from the University of Cambridge (UK) for assisting with the growth of the VACNTs.

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P24 In vivo online monitoring of testosterone-induced neuroplasticity in a female seasonal songbird

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One of the best models to study natural occurring adult neuroplasticity is the seasonal neuroplasticity of the song control system (SCS) in seasonal songbirds, which is largely driven by the photoperiod-induced increase in testosterone. Until now, studies unravelling the relationship between testosterone, song performance and neuroplasticity of the SCS used invasive techniques, which did not allow to obtain insights into the dynamic changes over time in each subject. To overcome this, the current study includes in vivo Magnetic Resonance Imaging to study the effect of testosterone on neuroplasticity and song behavior longitudinally in thirteen female photosensitive starlings (Sturnus vulgaris) which received a subcutaneous testosterone implant for 3 weeks. Birds were repeatedly investigated using in vivo diffusion tensor imaging (DTI) while their songs were recorded and plasma testosterone levels determined at 6 different time points before, during and after removal of the testosterone implants.

Changes in plasma testosterone levels correlated with a rapid increase in song rate and the volume of the nucleus robustus arcopallialis (RA) and with fast changes in several DTI parameters in Area X. However, these effects were completely reversible after implant removal. In addition, the testosterone-induced gradual development of a masculinized song repertoire displayed a positive correlation with Fractional Anisotropy (FA) values (a DTI metric sensitive to white matter and axonal changes) of the HVC-RA connection and of part of the Lamina Mesopallialis, which contains the HVC-Area X and LMAN-RA connections. These results confirm many of the previously reported testosterone-induced effects, but identify for the first time a more complete picture of the temporal profile of these changes. The study reveals a distinction between fast (days) and slower (weeks) effects of testosterone on song behavior and neuroplasticity, how these effects of testosterone correlate at the intra-individual level and exposes the important contribution of both the song motor-and anterior forebrain pathway in the seasonal recapitulation of singing performance.