8th European Molecular and Cellular Cognition Society Meeting

A FENS Satellite Meeting

Abstracts

8th EMCCS-FENS Satellite Meeting

Date: July 6th, 2018
The meeting will start at 9:00 and end at 19:00, including a poster session.

Venue: CharitéCrossOver (CCO) Auditorium, Charité Campus

Address: Virchoweg 6, 10117 Berlin, Germany

Organizing committee:
Angel Barco (Universidad Miguel Hernández de Elche, Instituto de Neurociencias, Spain)
Andre Fischer (Deutsches Zentrum für Neurodegenerative Erkrankungen e.V., Germany)
Kobi Rosenblum (University of Haifa, Israel)

Administrative organization:
Shunit Ben Ari (University of Haifa, Israel)
Alina Elkobi (University of Haifa, Israel)
Ulrike Kramer (Deutsches Zentrum für Neurodegenerative Erkrankungen e.V., Germany)

Program

9:00-9:10 Welcome: Kobi Rosenblum (President of EMCCS, University of Haifa, Israel)

Session1: Neuronal plasticity and brain circuits

Chair: Steven A. Kushner (Erasmus University Medical Center, the Netherlands)

09:10-09:35
Raffaella Tonini (Instituto Italiano di Technologia, Genova, Italy). Serotonergic mechanisms of plasticity at striatal circuits

09:35-10:00
Marianne Fyhn (University of Oslo, Norway). Removal of perineuronal nets impairs remote memory recall

10:00-10:25
Yiota Poirazi (Insitute of Molecular Biology & Biotechnology, Greece). Learning and remembering with dendrites: insights from computational models

10:25-10:50 Break

Session 2: Synaptic plasticity in intellectual disabilities and neurodegenerative diseases

Chair: Claudia Bagni (University of Lausanne, Switzerland)

10:50-11:15
Frédéric Saudou (University Grenoble Alpes, France). Huntington's disease: from axonal transport to neurotrophin signaling and circuits defects

11:15-11:40
Ted Abel (University of Iowa, USA). Male-specific deficits in reward learning in mouse models of autism

11:40-12:15 General Assembly of EMCCS

12:15-14:30 Lunch and poster session

Session 3: Molecular and cellular mechanisms of learning and memory

Chair: Riccardo Brambilla (Cardiff University, United Kingdom)

14:30-14:45
Adonis Yiannakas (University of Haifa). Distinct roles for insular cortex GABAergic interneuron subtypes during the acquisition and retrieval of conditioned taste aversion learning

14:45-15:10
Oliver Stork (Otto-von-Guericke University Magdeburg). Local hippocampal circuits controlling the strength of context fear memory

15:10-15:35
Bong-Kiun Kaang (Seoul National University, South Korea) Inter-regional connectivity among engram neurons after memory formation

15:35-15:50
Cemil Kerimoglu (Deutsches Zentrum für Neurodegenerative Erkrankungen e.V., Germany). RNA-dependent intergenerational inheritance of enhanced cognitive ability after environmental enrichment

15:50-16:05
Adar Adamsky (The Hebrew University of Jerusalem, Israel). Astrocytic inhibition in CA1 impairs remote memory acquisition by disrupting CA1 to ACC communication)

16:05-16:30 Break

Session 4: Molecular and cellular mechanisms of stress and emotional processing

Chair: Jocelyne Caboche (Université Pierre et Marie Curie, France)

16:30-16:55

Emmanuel Valjent (Centre National de la Recherche Scientifique, France) Dopamine signaling in the extended amygdala regulates defensive behaviors

16:55-17:20

Giovanni Marsicano (Institut National de la Santé et de la Recherche Médicale, France). Title is to be announced

17:20-17:45

Asya Rolls (Rappaport Medical School, Technion, Israel Institute of Technology). The neuro-immune axis: using the brain to control immunity

17:45-18:00

Nancy Gallus (University of Alabama at Birmingham). Enhancer RNAs are necessary and sufficient for activity-dependent neuronal gene transcription

18:00-18:15 Concluding remarks: Kobi Rosenblum and Andre Fischer

Morphometric characterisation of the *substantia nigra, pars compacta* and identification of morpho-electrical subtypes among its dopaminergic neurons

Olivier Hagens*, Henry Markram

Laboratory of Neural Microcircuitry, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

* Corresponding author: olivier.hagens@epfl.ch

Apart from their prominent role in motor control, dopamine (DA)-releasing cells in the *substantia nigra, pars compacta* (SNc) are also involved in the coordination and integration of several cognitive abilities such as visuospatial processing, and in the regulation of attention. In this study, we provide a comprehensive characterisation of DA cells in the murine P14 SNc. First, we use lightsheet microscopy to image cleared SNc tissue expressing the fluorescent tdTomato protein under the control of the *DA transporter* (DAT) promotor. We also infused the clarified tissue with an α-tyrosine hydroxylase (TH) antibody. TH is the rate-limiting enzyme in the synthesis of DA. Morphometric analyses established the extent of the SNc, the number of SNc DA cells and their density. The almost perfect DAT/TH co-expression demonstrates that fluorescing cells are indeed *bona fide* DA neurons. Next, we performed whole-cell patch clamp on >275 such identified dopaminergic cells in acute midbrain slices; 102 of these neurons were 3D-reconstructed, 221 were fully electrically characterised. Clustering analyses on the >30 morphologic and >100 electrophysiological features extracted from each cell uncovered nine novel morpho-electrical subtypes. This detailed description of the SNc and its subtypes of dopaminergic neurons may help in accelerating medical investigations in diseases linked to an imbalance of the DA system, such as Parkinson's disease. It also holds the potential to tease apart the specific involvement of DA in behaviours such as motor planning and sensorimotor integration.

Insights into the effects of cued-fear extinction within the reconsolidation window on cell signalling and behaviour.

Emma N Cahill[1]*, Melissa A Wood[1], Barry J Everitt[1], Amy L Milton[1]

[1] *University of Cambridge, Dept. of Psychology, Cambridge CB2 3EB, United Kingdom.*

*Corresponding author: enc22@cam.ac.uk

Aims: Extinction of a cued-fear memory within the reconsolidation window has received interest for its therapeutic potential to prevent fear reacquisition by reconsolidation interference. However, considerable debate remains regarding the mechanisms controlling the retrieval-extinction effect. The aim of these studies was to investigate the role of prediction error and destabilisation in the behavioural effect and analyse how the procedure recruits signalling pathways converging onto calcineurin (PP2B) and pERK in brain regions implicated in fear extinction. **Methods**: Here, the dependency of this effect on prediction error or memory destabilisation was examined in adult male rats using pavlovian cued-fear conditioning. The requirement for prediction error was assessed by using a reinforced or non-reinforced memory retrieval trial before extinction, compared to a no-retrieval, extinction only control. The requirement for memory destabilisation, and thus reconsolidation, for the retrieval-extinction effect was subsequently examined using region-specific pharmacology to block receptor subtypes required for memory destabilisation. The signalling pathways recruited were analysed by quantitative Western blot. **Results**: Both the reinforced (no prediction error) and non-reinforced retrieval sessions led to a decrease in fear reacquisition, suggesting that engagement of prediction error does not influence the occurrence of retrieval-extinction. Intra-basolateral amygdala antagonism of dopamine D1-receptors or glutamate GluN2B-containing NMDARs did not prevent the reacquisition of fear associated with the retrieval-extinction procedure. **Conclusions**: Together, these data suggest that retrieval-extinction does not always require memory destabilisation, since behavioural or pharmacological interventions that prevent destabilisation did not disrupt any capacity to attenuate fear.

Modulation of mesolimbic dopamine transmission by brain lipid composition: consequences on reward processing.

Fabien Ducrocq[1,2,*], Roman Walle[1,2,] Asma Oummadi[1,2], Clementine Bosch-Bouju[1,2], Xavier Fioramonti[1,2], Suzanne van der Veldt[1,2], Sophie Layé[1,2], David Ma[3], Véronique De Smedt-Peyrusse[1,2] and Pierre Trifilieff[1,2]

[1]*INRA, Nutrition et Neurobiologie intégrée, UMR 1286, 33076 Bordeaux, France*
[2]*University of Bordeaux, Nutrition et Neurobiologie intégrée, UMR 1286, 33076 Bordeaux, France*
[3]*Department of Human Health and Nutritional Sciences, University of Guelph, 50 Stone Rd. E., Guelph, ON, Canada N1G2W1*
* fabien.ducrocq@inra.fr

Various, though distinct psychiatric disorders, such as Schizophrenia, bipolar disorder or major depression are associated with a dysfunction of the reward system linked to an alteration of dopamine transmission. Furthermore, these pathologies are also accompanied by changes in brain lipid composition and in particular a decrease in the content of docosahexaenoic acid (DHA), the main n-3 polyunsaturated fatty acid (PUFA) in the brain. However, despite that n-3 PUFA supplementation seems to improve or prevent some psychiatric symptoms, the implication of brain lipid composition in the etiology of psychiatric endophenotypes has been overlooked. Using operant conditioning tasks in mice, we show that developmental n-3 PUFA deficiency leads to motivational deficit that is not reversed by postnatal n-3 PUFA supplementation. Moreover, n-3 PUFA deficiency leads to alterations in electrophysiological properties of medium spiny neurons (MSNs) in the nucleus accumbens (NAc) that could account for the behavioral deficits. Indeed, D1-MSNs displayed a decrease in neuronal excitability in parallel with an increase of inhibitory input onto these neurons that are reversed by the D2R agonist quinpirole, suggesting an increase of the inhibitory input of D2-MSNs on D1-MSNs within the NAc. Accordingly, using a transgenic approach that allows the expression of the fatty acid desaturase FAT1 in a cre-dependent manner, we show that rescuing appropriate PUFA levels in D2R-expressing neurons selectively, is sufficient to reverse both the motivational deficits and alterations in electrophysiological properties of MSNs induced by n-3 PUFA deficiency. We demonstrate that alteration of PUFAs in discrete neuronal population can alter neuronal function and associated behavior.

Validation in rodent models of a novel approach to treat mood disorders based on ERK signalling stimulation

Ilaria Morella[1], Simon Brooks[2], Riccardo Brambilla[1]

[1] *Neuroscience and Mental Health Research Institute, Cardiff University, Cardiff, UK*
[2] *School of Biosciences, Cardiff University, Cardiff, UK*

Depression and anxiety are the most prevalent disorders of the nervous system affecting hundreds of millions people worldwide. Compelling evidence has demonstrated that several components of the Ras-ERK cascade are dysregulated in the brains of suicide patients, resulting in a general decrease in ERK signalling. Although these data support the potential role of ERK cascade as a new therapeutic target, to date no drugs are available to selectively potentiate the ERK pathway. We have recently developed a novel compound that potentiates ERK signalling in the brain and has remarkable cognitive enhancing properties. In this work, we demonstrate that a single systemic injection is sufficient to activate in the brain ERK 1/2 and MSK-1 from 1 hour up to 6 hours after injection in wild-type mice. Interestingly, this compound can positively affect motivation during progressive ratio schedule and increase sucrose consumption in naïve mice following a single systemic injection. Furthermore, this compound significantly decreases anxiety in naïve mice in the light/dark box test and in the elevated plus maze. In conclusion, our proprietary compound represents an interesting new candidate to treat mood disorders.

Neurofascin knock down in the basolateral amygdala mediates resilience of memory and plasticity in the dorsal dentate gyrus under stress

Rinki Saha[1], Martin Kriebel[2], Hansjürgen Volkmer[2], Gal Richter-Levin[1,3,4], Anne Albrecht[1,4,*]

[1] *Sagol Department of Neurobiology, University of Haifa, Haifa, Israel*
[2] *NMI Natural and Medical Sciences Institute, University of Tübingen, Tübingen, Germany*
[3] *Department of Psychology, University of Haifa, Haifa, Israel*
[4] *The Institute for the Study of Affective Neuroscience, University of Haifa, Haifa, Israel*
* Corresponding author: anne1.albrecht@ovgu.de

Activation of the amygdala is one of the hallmarks of acute stress reactions and a central element of the negative impact of stress on hippocampus-dependent memory and cognition. Stress-induced psychopathologies, such as posttraumatic stress disorder, exhibit a sustained hyperactivity of the amygdala, triggered at least in part by deficits in GABAergic inhibition that lead to shifts in amygdalo-hippocampal interaction. Here, we have utilized lentiviral knock down of neurofascin to reduce GABAergic inhibition specifically at the axon initial segment (AIS) of principal neurons within the basolateral amygdala (BLA) of rats. Metaplastic effects of such a BLA modulation on hippocampal synaptic function were assessed using BLA priming prior to the induction of long-term potentiation (LTP) on dentate gyrus synapses in anaesthetized rats in vivo. The knock down of neurofascin in the BLA prevented a priming-induced impairment on LTP maintenance in the dentate gyrus. At the behavioral level, a similar effect was observable, with neurofascin knock down preventing the detrimental impact of acute traumatic stress on hippocampus-dependent spatial memory retrieval in a water maze task. These findings suggest that reducing GABAergic inhibition specifically at the AIS synapses of the BLA alters amygdalo-hippocampal interactions such that it attenuates the adverse impact of acute stress exposure on cognition-related hippocampal functions.

Mesopontine inputs to VTA dopamine neurons regulate depressive-like behaviors

Sebastian P. Fernandez [1, 2], Loïc Broussot [1, 2], Fabio Marti [4, 5], Xavier Mouska [1, 2], Mariano Soiza-Reilly [3, 4], Hélène Marie [1, 2], Philippe Faure [4, 5], Jacques Barik [1, 2*].

[1] *Université Côte d'Azur, Nice, France.*
[2] *Institut de Pharmacologie Moléculaire & Cellulaire, CNRS UMR7275, Valbonne, France.*
[3] *Institut du Fer à Moulin, INSERM, UMRS-839, Paris, France.*
[4] *Université Pierre et Marie Curie, Paris, France.*
[5] *Neurosciences Paris Seine, INSERM U 1130, CNRS UMR 8246, Paris, France.*
* Corresponding author: barik@ipmc.cnrs.fr

Depression is a major human blight with more than 350 million people affected worldwide. Yet, curative treatments are lacking due to our still very limited understanding of its pathophysiology. Long-lasting periods of stress as well as traumatic stressful experiences trigger molecular and cellular maladaptations that affect emotional and social behaviors. Hence, stress is a primary environmental factor that is acknowledged as a key element in the developmental root of a large part of mental disorders including depression and thus a leading cause of disability worldwide. Dysregulation of dopamine (DA) neurons from the ventral tegmental area (VTA) has been causally linked to the appearance of social withdrawal and anhedonia, two classical manifestations of depression. However, little is known about the relevant inputs that regulate the changes in activity of VTA DA neurons that are responsible for the appearance of these behavioral maladaptations. Here, we exposed mice to chronic social defeat stress (CSD), a preclinical paradigm of depression. We combined ex-vivo patch recordings in genetically-tagged neuronal cell types to dissect the impact of social stress on excitatory inputs to the VTA. To causally link these cellular changes to the appearance of social withdrawal and anhedonia we used chemogenetic approaches. Our data indicate that mesopontine excitatory inputs to the VTA are essential to promote depressive-like behaviors. This work will favor the development of circuit-based interventions to alleviate symptoms of depression.

Altered social predispositions in chicks exposed to valproate during embryonic development

Paola Sgadò [1,*], Elena Lorenzi[1], Alessandra Pross[1], Orsola Rosa Salva[1], Elisabetta Versace[1,2], Samantha Pedrana[1], Chiara Spiazzi[1], Nicolò Taricco[1] and Giorgio Vallortigara[1]

[1] *Animal Cognition and Neuroscience Laboratory, Center for Mind/Brain Sciences, University of Trento, Piazza della Manifattura 1, 38068 Rovereto (TN), Italy.*
[2] *Queen Mary University of London, Department of Biological and Experimental Psychology- School of Biological and Chemical Sciences, London, United Kingdom.*
* Corresponding author: paola.sgado@unitn.it

Predispositions to attend to social stimuli influence the expression of social behavior in newborns of several vertebrate species, including human infants and chicks of the domestic fowl. A remarkable difference has been recently reported in early social predispositions of human newborns at high vs. low familial risk of Autism Spectrum Disorder (ASD). Little is known however, about the neurobiological bases of social predispositions, both in ASD and in typically developing newborns. To uncover the molecular and neurobiological bases of these early biomarkers of social behaviors and their role on ASD-relevant phenotypes, we developed a new model of ASD-like social impairments in chicks, based on the injection of valproic acid (VPA) during embryonic development. VPA is an anticonvulsant and mood stabilizer, exposure to VPA during pregnancy have been shown to increased risk of developing ASD. Animal studies using prenatal exposure to VPA have also been carried out to model the core signs of ASD. To assess innate social predispositions, newly-hatched, visually-naïve chicks were exposed to stimuli that contained animacy cues, either stationary (face-like configurations) or dynamic (speed-changes). We also assessed filial imprinting to evaluate alteration in learning mechanisms associated to social predispositions. Our results show a dramatic effect of VPA on the chicks' social predispositions, without altering the experience-dependent mechanisms associated with filial imprinting. The data indicate a specific effect of VPA on development of these early social orienting mechanisms, opening new perspectives to investigate the molecular and neurobiological mechanisms relevant for development of early ASD symptoms.

Aberrant hippocampal transmission and cognitive behavior in mice with stargazin mutation linked to intellectual disability

Gladys Caldeira[1], Susana R Louros[1], Ângela Inácio[1], Tiago Rondão[1], Nuno Beltrão[1,2], Marina V Rodrigues[1], Joana Guedes[1], João Peça[1], Ana Luísa Carvalho[1,2,*]

[1]*CNC-Center for Neuroscience and Cell Biology, University of Coimbra, 3004-504 Coimbra, Portugal*
[2]*Department of Life Sciences, University of Coimbra, 3004-504 Coimbra, Portugal*
* Corresponding author: alc@cnc.uc.pt

Mutations in genes encoding proteins with a synaptic role often contribute to neurodevelopmental disorders such as intellectual disability, characterized by impaired intellectual and adaptive functioning. One such gene is CACNG2, encoding the synaptic protein stargazin, a transmembrane AMPA receptor regulatory proteins (TARP) which regulates synaptic AMPA receptor trafficking and AMPA receptor channel gating, and is required for Hebbian and homeostatic forms of synaptic plasticity. We tested whether stargazin is implicated in the pathogenesis of neurodevelopmental disorders. We found that the intellectual disability-linked V143L variant of stargazin presents increased cell surface mobility, and disrupts synaptic AMPA receptor traffic and homeostatic upscaling of AMPA receptors. We characterized mutant mice harboring the V143L stargazin mutation, and found reduced dendritic complexity and decreased frequency of AMPAR-miniature EPSCs in hippocampal CA1 pyramidal neurons in these mice. Furthermore, behavioral analysis showed that mice with the V143L stargazin mutation manifest impaired object displacement recognition and contextual fear memory, as well as aberrant behavior in marble burying, nest building and social interactions. Our findings demonstrate that mutations in stargazin contribute to altered hippocampal transmission and abnormal cognitive behavior and suggest a role for stargazin in the pathophysiology of cognitive disorders.

RNA-dependent intergenerational inheritance of enhanced cognitive ability after environmental enrichment

Eva Benito[1,*], Cemil Kerimoglu[1,2,*], Binu Ramachandran[3], Qihui Zhou[4], Tonatiuh Pena[1,5] Vincenzo Capece[1,5], Gaurav Jain[1,5], Susanne Burkhardt[1], Roman Stilling[1], Rezaul Islam[1], Dieter Edbauer[4], Camin Dean[3] and André Fischer[1,2]

1 *Department for Epigenetics and Systems Medicine in Neurodegenerative Diseases, German Center for Neurodegenerative Diseases (DZNE) Göttingen, von Siebold Str. 3A, 37075 Göttingen, Germany*
2 *Department of Psychiatry and Psychotherapy, University Medical Center Göttingen, Griesbachstr. 5, 37077 Göttingen, Germany*
3 *Trans-synaptic Signaling Group, European Neuroscience Institute, Grisebachstr. 5, 37077 Goettingen, Germany.*
4 *German Center for Neurodegenerative Diseases (DZNE), Munich, Schillerstr. 44, 80336 Munich, Germany*
5 *Bioinformatics Unit, German Center for Neurodegenerative Diseases (DZNE) Göttingen, von Siebold Str. 3A, 37075 Göttingen, Germany*
Corresponding author: andre.fischer@dzne.de * equal contribution

Aims: Recent studies evoke the idea that acquired information can be transmitted to subsequent generations via non-genetic mechanisms. Physical exercise in combination with cognitive training (environmental enrichment) is known to improve memory function and lower the risk for cognitive diseases. Whether environmental enrichment in fathers prior to conception would also transmit a cognitive benefit to the offspring has not yet been investigated. **Methods:** Adult (3 month-old) male mice were housed either in standard home cages (HC) or exposed to environmental enrichment (EE). Memory function and hippocampal long term potentiation were assessed in F0, F1 and F2 generations. Moreover, fertilized oocytes were injected with either (i) sperm RNA of home-caged mice, (ii) sperm RNA of mice exposed to environmental enrichment or (iii) sperm RNA of mice exposed to environmental enrichment combined with microRNA212/132 inhibitors. The offspring were tested for memory function and long term potentiation at adult age. **Results:** Exposure of adult male mice to environmental enrichment transmits the acquired cognitive advantage and enhanced synaptic plasticity to the offspring. We furthermore show that this effect is mediated via sperm RNA and can partly be explained by microRNA212/132. **Conclusions:** Our findings have broad implications not only for reproductive medicine but also for healthcare and lifestyle of humans in general. For example, extrapolating to humans, our study suggests that fathers should exercise prior to conceiving in order to pass cognitive benefits on to their children.

Astrocytes shape habitual behavior via regulation of glutamate transporter EAAT2

Arjen J. Boender [1], Raffaella Tonini [1*]

[1] *Neuromodulation of Cortical and Subcortical Circuits Laboratory, Neuroscience and Brain Technologies Department, Italian Institute of Technology, Genova, Italy*

* Corresponding author: raffaella.tonini@iit.it

Habit learning automates newly acquired actions. and is an adaptive behavior that depends on glutamatergic signaling in the dorsolateral striatum (DLS). Glutamate clearance is shaped by astrocytes, which affects synaptic functions and neuronal network activity, but the impact of this cellular process on the behavioral level remains elusive. Disturbances in astrocytic glutamate transporter EAAT2 have been related to neurological dysfunctions, but it is unknown how environmental cues modulate EAAT2 expression to support the acquisition of novel behaviors. In this study, we investigate the relationship between experience-dependent plasticity of EAAT2 expression levels and habit learning. We find that habit formation is associated with the upregulation of EAAT2 in the DLS. Reducing EAAT2 protein levels by chemogenetic activation of astrocytic Gq-signaling or by *in vivo* transient knockdown of EAAT2 in the DLS interferes with habitual control of behavior. Astrocytes are emerging as critical regulators of higher brain functions, and by demonstrating behavioral-relevant plasticity of astrocytic glutamate transporters, we provide novel mechanistic insights on their involvement in cognitive processes.

Distinct roles for insular cortex GABAergic interneuron subtypes during the acquisition and retrieval of conditioned taste aversion learning

Adonis Yiannakas [1], Kobi Rosenblum [1, 2]

[1] *Sagol Dept. of Neurobiology University of Haifa, Haifa, Israel*
[2] *Center for Gene Manipulation in the Brain, University of Haifa, Haifa, Israel*
* Corresponding author: email [kobir@psy.haifa.ac.il

The insular cortex (IC) is required for acquisition and retention of taste memories, and it is known to subserve taste learning paradigms, including conditioned taste aversion (CTA), where an association is formed between a novel taste and malaise. CTA involves GABAergic neurotransmission and induction of ERK MAPK signaling at the IC. Aims: Here, we aimed to elucidate how activity within GABAergic interneuron subtypes of the IC contributes to the different phases of associative learning, and how this relates to IC ERK phosphorylation. Methods: In our studies, adult glutamate decarboxylase 2 (GAD2)-Cre, parvalbumin (PV)-Cre and vasoactive intestinal peptide (VIP)-Cre male mice were stereotaxically injected with Cre-dependent AAV8 at the IC, driving local expression of fluorescent mCherry and the inhibitory chemogenetic receptors (hMD4Gi, n=6), or a control vector (n=6), to the targeted interneuron subtypes. Following recovery and training, mice were administered clozapine N-oxide (CNO; 0.5mg/kg) prior to acquisition or retrieval of CTA. Animals were behaviorally assessed and sacrificed 20min following CTA retrieval. Injection sites and ERK phosphorylation were examined using fluorescent immunohistochemistry. Results: Consistent with previous pharmacological studies implicating GABAergic neurotransmission at the IC in CTA learning, chemogenetic inhibition of IC interneurons disrupted CTA behavior. Interestingly, chemogenetic inhibition of PV and VIP interneurons differentially modulated aversion to the conditioned tastants, depending on the timing of the aforementioned interventions. Conclusions: Our results suggest activation of VIP and PV IC interneurons to regulate the dominance and valence of the taste memories, at least in part through their influence on IC ERK phosphorylation during retrieval.

Adult hippocampal MeCP2 preserves the genomic responsiveness to learning required for long-term memory formation

Kubra Gulmez Karaca[1], David V.C. Brito[1], Benjamin Zeuch[1] and Ana M.M. Oliveira[1],*

[1] *Department of Neurobiology, Interdisciplinary Centre for Neurosciences (IZN), University of Heidelberg, Heidelberg, Germany*
* Corresponding author: oliveira@nbio.uni-heidelberg.de

MeCP2 is required both during postnatal neurodevelopment and throughout the adult life for brain function. Although it is well accepted that MeCP2 in the maturing nervous system is critical for establishing normal development, the functions of MeCP2 during adulthood are poorly understood. Particularly, the requirement of hippocampal MeCP2 for cognitive abilities in the adult is not studied. To characterize the role of MeCP2 in adult neuronal function and cognition, we used a temporal and region-specific disruption of MeCP2 expression in the hippocampus of adult male mice. We found that MeCP2 is required for long-term memory formation and that it controls the learning-induced transcriptional response of hippocampal neurons required for memory consolidation. Furthermore, we uncovered MeCP2 functions in the adult hippocampus that may underlie cognitive integrity. We showed that MeCP2 maintains the developmentally established chromatin configuration and epigenetic landscape of CA1 neurons throughout the adulthood, and that it regulates the expression of neuronal and immune-related genes in the adult hippocampus. Overall, our findings identify MeCP2 as a maintenance factor in the adult hippocampus that preserves signal responsiveness of the genome and allows for integrity of cognitive functions. This study provides new insight into how MeCP2 maintains adult brain functions, but also into the mechanisms underlying the cognitive impairments observed in RTT patients and highlights the understudied role of DNA methylation interpretation in adult cognitive processes.

Differential distribution of molecularly defined GABAergic interneurons along the rostrocaudal axis of the mouse perirhinal cortex

Maximiliano José Nigro[1*], Menno P. Witter[1]

[1] *Kavli Institute for Systems Neuroscience, Centre for Neural Computation, Egil and Pauline Braathen and Fred Kavli Centre for Cortical Microcircuits, NTNU Norwegian University of Science and Technology Trondheim, Norway*
[*]Corresponding author: maximiliano.j.nigro@ntnu.no

Aim: the perirhinal cortex, together with the postrhinal and entorhinal cortex, are part of the parahippocampal region of the medial temporal lobe. The perirhinal cortex receives sensory information from different uni- and multisensory cortical areas and relays it to the lateral entorhinal cortex. As such, the perirhinal cortex plays a pivotal role in multisensory integration, object recognition, and memory formation. Propagation of neocortical information along this perirhinal-entorhinal path is subject to a strong inhibitory control (Martina et al., 2001; Kajiwara et al., 2003). Here we quantified the distribution of molecularly defined GABAergic populations in the perirhinal cortex. Methods: we used immunofluorescence to describe the differential distribution of parvalbumin (PV), somatostatin (SST), and vasoactive intestinal peptide (VIP) expressing interneurons (INs) along the rostrocaudal axis of the perirhinal cortex of the GAD67-GFP mouse. These markers label non-overlapping populations of GABAergic interneurons that participate in specific inhibitory circuit motifs. Results: we found that PV-INs are differentially distributed along the rostrocaudal axis of the perirhinal cortex, with higher levels rostrally, whereas these neurons account for a low percent of GABAergic neurons at mid-caudal parts of the perirhinal cortex. The distribution of SST and VIP-INs appears homogeneous along the rostrocaudal axis of the perirhinal cortex. Conclusions: our results suggest that different inhibitory circuits might contribute to the cortico-hippocampal communication along the rostrocaudal axis of the perirhinal cortex. Ongoing anatomical and electrophysiological studies will reveal the contribution of specific interneuron types to the circuits of the perirhinal cortex.

Forebrain-specific Setd1b histone methyltransferase deletion is associated with behavioral deficits and unique transcriptomic signature in mice

Alexandra Michurina [1], Sakib M. Sadman [1], Cemil Kerimoglu [1], Andrea Kranz [2] Francis A. Stewart [2], Andre Fischer [1*]

[1] *German Center for Neurodegenerative Diseases, Epigenetics and Systems Medicine in Neurodegenerative Diseases, Göttingen 37075, Germany*
[2] *Biotechnology Center, Dresden University of Technology, Dresden, 01069, Germany*
* Corresponding author: Andre.Fischer@dzne.de

Cumulative evidence of an emerging 12q24.3 microdeletion syndrome in a number of studies has been associated with a causative *Setd1b* gene, a component of the SET1 histone methyltransferase complex mediating H3K4-trimethylation, yet its function in the brain *in vivo* is remaining unclear. In this study we accessed gene expression profiles of conditional knock-out mice (cKO) with selective ablation of *Setd1b* methyltransferase in the excitatory neurons of the forebrain. Whole tissue RNA-seq revealed massive transcriptional changes in two hippocampal sub-regions, cornu ammonis (CA) and dentate gyrus, including pathways involved in memory formation, behavioral fear response, dopaminergic and serotoninergic systems and synaptic organization proteins. Differentially expressed genes (DEG) did not have significant overlap with genes, differentially expressed upon similar models of *Mll1* and *Mll2* cKO, indicating non-redundant functions of SET-family members for regulating the transcription levels via trimethylation. In the ongoing experiments we are comparing occupancy of H3K4me3 mark with DEG, combining ChIP-seq and RNA-seq from sorted neuronal cells of the CA hippocampal region. In the behavioral tests *Setd1b* cKO mice showed decreased anxiety in elevated-plus maze, displayed a completely impaired nesting behavior and exhibited severe hippocampal-dependent spatial memory deficits in the Morris water maze. An almost two-fold higher immobility time in the forced swimming test indicated possible depressive symptoms in the cKOs. In the absence of proper genotype-phenotype correlation of human 12q24.3 microdeletion, our study for the first time highlights *Setd1b* as an essential substrate for the neuronal plasticity, behavior and gene expression programs in the central nervous system.

Activation of Dopamine receptor D1/MEK/mTOR pathway induces protein synthesis by eEF2K inactivation and eEF2 dephosphorylation

Iliana Barrera[1] Orit David[1], Kobi Rosenblum[1,2]

[1]*Sagol Department of Neurobiology, University of Haifa, Haifa, Israel*
[2]*Center for Gene Manipulation in the Brain, University of Haifa, Haifa, Israel*
* Corresponding author *iliana.barrera34@gmail.com

Dynamic regulation of mRNA translation is a key mechanism for modulating memory and synaptic plasticity consolidation. Dopamine affects behavior, cellular and molecular functions including protein synthesis. However, the mechanism underlying this process is poorly understood. Aim: Here, we tested the hypothesis that dopamine via the D1 receptor modulates the elongation phase of mRNA translation in neurons by the regulation of the eukaryotic elongation factor 2 kinase (eEF2K). Methods: We used mouse-derived primary cortical culture and a novel technique to measure protein synthesis by providing cells with tRNA labeled as FRET pares and exploiting the proximity in at translating ribosome. Results: We show that activation of dopamine D1 but not D2 receptor leads to inactivation of eEF2K and dephosphorylation of the eukaryotic elongation factor 2 (eEF2) at Thr^{56}, indicating enhanced elongation in a time-dependent manner. D1 receptor activation through N-Methly-D-asparate receptor (NMDAR) stimulation activates the MEK/mTOR pathway, inducing *de-novo* protein synthesis in in dendrites, and to a lesser extent, in the cell soma of mouse cortical neurons. This increase is dependent on the dephosphorylation of eEF2, since the inhibition of the MEK/mTOR pathway by U0126 compound, a MEK inhibitor, did not have an effect on *de-novo* protein synthesis in cortical neurons from eEF2K-KO mice treated with D1 receptor agonist, SKF38393. Conclusions: These results establish the role of D1/MEK/mTOR/eEF2K signaling cascade in regulating local protein translation in cortical neurons, which sheds light on the complexly of dopaminergic function in neurons.

Enhancer RNAs are necessary and sufficient for activity-dependent neuronal gene transcription

Nancy V. N. Gallus*, Rhiana C. Simon, Aaron J. Salisbury, Kendra D. Bunner, Jasmin S. Revanna, Katherine E. Savell, Faraz A. Sultan, & Jeremy J. Day

Department of Neurobiology and Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, Birmingham, AL 35294, USA
* Corresponding author: ngallus@uab.edu

Distal enhancer elements in DNA enable chromatin reorganization and facilitate gene expression programs to regulate cell fate and function. A significant fraction of regulatory DNA regions, like enhancer sites, are subject to bidirectional, RNA polymerase II-dependent transcription that results in non-coding enhancer RNAs. While evidence suggests that eRNAs are involved in the regulation of gene expression through interactions with transcription factors and epigenetic modifiers, their potential role in neuronal function and development remains unclear. Here, we used cortical neuronal cultures to investigate the regulation and localization of specific eRNAs arising from enhancers surrounding Fos (also known as c-Fos), an immediate early gene that codes for a transcription factor implicated in neuronal plasticity and cognitive processes. We show that eRNA transcription from Fos enhancers is dynamically modulated by various forms of neuronal activity, requires RNA polymerase II, and precedes induction of Fos mRNA. To investigate the localization of eRNAs at the single-neuron level, we employed single-molecule RNA FISH with multiplexed probes to separately identify mRNA and eRNA transcripts. Anti-sense based knockdown of Fos eRNAs selectively reduced Fos mRNA. Targeted stimulation of eRNA synthesis from Fos enhancers using CRISPR-dCas9 fusion proteins increased Fos mRNA expression, with limited cross-talk between enhancers. Similarly, CRISPR-targeted delivery of eRNA to a Fos enhancer elevated mRNA induction following neuronal depolarization. Finally, we show that knockdown of a single Fos eRNA is sufficient to alter neuronal physiology in vitro using a Multi Electrode Array system. Overall, these findings indicate that eRNAs directly modulate gene expression and neuronal function.

Role of Ndr2, a serine/threonine kinase, on the mossy fiber development and its possible contribution to the Pallister-Killian Syndrome.

Deniz A. Madencioglu[1, *], Gürsel Çalışkan[1], Kati Rehberg[1], Jorge R. Bergado-Acosta[1], Pingan Yuanxiang[2], Anne Kummer[1], Iris Müller[1], Yunus E. Demiray[1], Michael R. Kreutz[2], Oliver Stork[1]

[1]*Department of Genetics and Molecular Neurobiology, Institute of Biology, Otto-von-Guericke-University, Magdeburg, Germany*
[2]*Research Group Neuroplasticity, Leibniz Institute for Neurobiology, Magdeburg, Germany*
* Corresponding author: deniz.madencioglu@ovgu.de

The Ndr (nuclear Dbf2-related) family members are highly conserved serine/threonine protein kinases, which function in many cellular processes extending from cytokinesis to neurite outgrowth. In humans, the NDR2 gene is located on chromosome 12p. Tetrasomy of 12p results in a rare genetic disorder called Pallister-Killian mosaic syndrome, which is characterized by multiple congenital anomalies including mental retardation. To investigate the putative consequences of the overexpression of Ndr2, we generated a conditional transgenic mouse model overexpressing Ndr2 in postmigratory forebrain neurons. In the hippocampus, these animals show approximately doubled expression levels compared to endogenous Ndr2 and localization of the transgene to both dendrites and axons. Profound expression was observed in the hippocampal mossy fibers (MF), which lead to a reduction of terminals in the ventral hippocampus and reduced excitability at the MF-CA3 synapse. Consequently, the generation of intrinsic sharp wave ripples and carbachol-induced gamma oscillations were reduced in the ventral CA3. This disruption of network activity was associated with behavioral impairments as transgenic mice displayed increased exploratory behavior in the open field test and impaired hippocampus-dependent learning in an active avoidance paradigm. Our results suggest that increased expression of Ndr2 impairs the hippocampal circuit function and thus may contribute to the mental retardation phenotype of the Pallister-Killian syndrome.

Supported by the German Research Foundation.

Studying the role of Cajal-Retzius cells in the maturation of the hippocampal network

Giulia Quattrocolo [1] *, Edvard I. Moser [1]

[1] *Kavli Institute for Systems Neuroscience – Centre for Neural Computation, NTNU, Trondheim, Norway*
* Corresponding author: giulia.quattrocolo@ntnu.no

Aims: Cajal-Retzius (CR) cells are critical orchestrators of brain development and play a pivotal role in the formation of cortical areas. While cortical CR cells die during the first week of postnatal development, CR cells are still observed in the hippocampus for several months after birth. During these months, hippocampal-parahippocampal circuits are reaching maturity. What is the function of hippocampal CR cells during postnatal development? Methods: To address this question we decided to ablate CR cells from early time points of postnatal development and determine how their ablation affects maturation of the hippocampal network and hippocampal-dependent behaviors. To achieve the ablation, we inject a Cre-dependent virus expressing diphtheria toxin fragment A (DTA) into the hippocampus of P0 pups of the Pde1c-cre transgenic mouse line. This line is highly selective for CR cells, in the hippocampus, therefore CR cells are the only neurons affected by virally-induced DTA expression. Results and Conclusions: We here show that the toxin successfully ablates hippocampal CR cells, reducing their number to around 50% by the second postnatal week. We are currently testing the effect of CR cells ablation by looking at markers of neuronal development and network maturation. By using in vitro and in vivo physiology, we will investigate alterations in synaptic connectivity in the hippocampal circuit and in the maturation of the spatial memory system. Preliminary data suggest that the levels of hippocampal BDNF are increased when the number of CR cells is reduced, pointing to possible alterations in plasticity and synaptic strength.

MicroRNA-186-5p controls GluA2 surface expression and synaptic scaling in hippocampal neurons

Mariline Silva[1,2,3], Beatriz Rodrigues[1,2,3], Joana Fernandes[1], Sandra Santos[1], Laura Carreto[4], Manuel Santos[4], Paulo Pinheiro[1], Ana Luísa Carvalho[1,5,*]

[1] *Synapse Biology Group, CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal*
[2] *Doctoral Program in Experimental Biology and Biomedicine, University of Coimbra, Coimbra, Portugal*
[3] *Institute for Interdisciplinary Research, University of Coimbra (IIIUC), Coimbra, Portugal*
[4] *Department of Medical Sciences and Institute of Biomedicine - iBiMED, University of Aveiro, Aveiro, Portugal*
[5] *Department of Life Sciences, University of Coimbra, Coimbra, Portugal*
* Corresponding author: alc@cnc.uc.pt

INTRODUCTION: Homeostatic synaptic scaling is a compensatory negative-feedback response to fluctuations in synaptic strength induced by developmental or learning-related processes, in order to maintain neuronal firing within a physiological range. Although several components of the synaptic scaling apparatus have been characterized, the intrinsic regulatory mechanisms promoting scaling remain largely unknown. MicroRNAs, pivotal posttranscriptional regulators, have been reported to control distinct mechanisms at different stages of synaptic scaling. However, few miRNAs have been functionally studied and their role in synaptic scaling mechanisms is still vastly undervalued. AIMS: Clarify the intrinsic mechanisms underlying synaptic scaling. METHODS: We assessed the effects of chronic blockade of AMPA and NMDA receptors in primary cultures of rat hippocampal neurons, using immunofluorescence and electrophysiological studies. We then evaluated the effects of chronic synaptic activity blockade in the neuronal transcriptome (using Gene Expression MicroArrays) and microRNA profile (using a 16-microRNA screening panel). Finally, we manipulated the levels of miR-186-5p, by either expressing the precursor form of miR-186 or by expressing inhibitors of miR-186-5p. RESULTS: Here, we report that chronic blockade of glutamate receptors of the AMPA and NMDA types induces changes in the neuronal transcriptome and miRNA profile, leading to synaptic upscaling. We found that activity regulation of miR-186-5p is required for synaptic upscaling and that miR-186-5p is a regulator of GluA2 expression, implicating this miRNA in the regulation of synaptic scaling mechanisms. CONCLUSIONS: Our findings elucidate a novel activity-dependent miRNA-mediated mechanism for regulation of AMPA receptor expression.

A systems approach identifying microRNAs as marker of cognitive decline

Md. Rezaul Islam[1], T. Berulava[1], M. Navarro[2], V. Elerdashvili[1], TP. Centeno[1], G. Jain[1], E. Benito[1], S. Burckhardt[1], P. Rao[2], V. Capece[1], M. Boroomandi[1], B. Malchow[3], P. Falkai[3], F. Sananbenesi[4] A. Fischer[1,2]

[1] *German Center for Neurodegenerative Diseases, Epigenetics and Systems Medicine, Goettingen, GERMANY*
[2] *Research Group for Genome Dynamics in Brain Diseases, Griesbachstr. 5, 37077 Göttingen, GERMANY*
[3] *Department of Psychiatry and Psychotherapy, Medical Center of the University of Munich, Nußbaumstr. 7, 80336 München, GERMANY*
[4] *University Medical Center Goettingen, Dept. For Psychiatry and Psychotherapy, Goettingen, GERMANY*

Aging is the major risk factor for cognitive decline and dementia. However, there is a substantial inter-individual variability amongst individuals and while some develop age-associated memory impairment other undergo “healthy aging” accompanied by preserved cognitive function. Suitable biomarker to reliably predict cognitive decline are still missing. In this study, we employ a longitudinal approach to test the hypothesis that circulating microRNA levels can inform about the cognitive status. We investigated two cohorts of mice from 12 to 16.5 month of age. One group was housed in home cages, while the other was subjected to spatial reference memory testing every 1.5 months. Blood was collected at 4 time points (12, 13.5, 15 and 16.5 months) and small RNA sequencing was performed. We observed that learning abilities of mice significantly declined between 13.5 and 16.5 months of age. Comparative and weighted co-expression analysis of sequencing data revealed microRNA signatures linked to cognitive performance. Of these signatures, we decided to further study microRNA-181a-5p that was highly correlated to age-associated memory decline in blood and various brain tissues. Of note circulating miR-181a levels also correlated with memory function in humans. Moreover, mice injected with miR-181a mimic displayed memory impairment and inhibition of miR-181a could reinstate memory in aged mice. In summary, our data provides evidence that the analysis of circulating miRNAs can inform about cognitive status.

Disrupted AMPA receptor function and homeostatic synaptic plasticity upon genetic- or antibody-mediated loss of autism-associated CASPR2

Dominique Fernandes [1, 2, 3], Sandra Santos [1], Ester Coutinho [4,#], Jessica L. Whitt [3,#], Tiago Rondão [1,5], M. Isabel Leite [4], Camilla Buckley [4], Hey-Kyoung Lee [3], Ana Luísa Carvalho [1,5,*]

[1] *Synapse Biology Group, CNC – Centre for Neuroscience and Cell Biology, University of Coimbra, Portugal;*
[2] *PDBEB, Doctoral Programme in Experimental Biology and Biomedicine, CNC & Institute for Interdisciplinary Research, University of Coimbra (IIIUC), Portugal;*
[3] *The Zanvyl Krieger Mind/Brain Institute & The Solomon Snyder Department of Neurosciences, Johns Hopkins University, USA;*
[4] *Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, University of Oxford, UK;*
[5] *Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Portugal.*
[#]These authors contributed equally to the work

* Corresponding author: Ana Luísa Carvalho – alc@cnc.uc.pt

Aims: Mutations in the *CNTNAP2* gene have been recurrently implicated in neuropsychiatric disorders such as autism, schizophrenia and intellectual disability, and autoantibodies targeting the *CNTNAP2*-encoded protein CASPR2 have also been found in association with autoimmune encephalitis. However, the pathogenesis ensuing from perturbations in CASPR2 function is still unclear. Here, we aimed to investigate the pathogenic mechanisms associated to CASPR2 loss-of-function.
Methods: Using either a genetic silencing strategy, or autoimmune encephalitis patient-derived CASPR2-antibodies, we aimed at perturbing Caspr2 function in rat primary cortical neurons and in the mouse primary visual cortex *in vivo*. Perturbations in glutamatergic function and synaptic plasticity were assessed through immunofluorescence and electrophysiological techniques following prolonged neuronal activity inhibition or sensory deprivation. Results: We show that Caspr2 is expressed in cortical excitatory synapses, and that it interacts with the GluA1 subunit of AMPA receptors. Silencing Caspr2 *in vitro* decreases the basal synaptic content of GluA1-containing AMPARs in cortical neurons, and hinders homeostatic synaptic scaling of AMPARs following prolonged neuronal inactivity. Caspr2 is further required for experience-dependent plasticity *in vivo*, since its loss in the mouse visual cortex prevents the scaling of AMPAR-mediated currents following paradigms of chronic visual deprivation. Finally, we observe that patient-derived CASPR2-antibodies alter dendritic levels of Caspr2 and synaptic GluA1-AMPAR content in cortical neurons, and perturb excitatory transmission in the visual cortex. Conclusions: Our results indicate that genetic- and antibody-mediated loss of Caspr2 impair AMPAR function and excitatory synaptic transmission in the cortex, and reveal a requirement for Caspr2 in the regulation of homeostatic and experience-dependent synaptic plasticity. Overall, these findings suggest that disruption of such mechanisms may underlie the pathogenesis of CASPR2-related disorders.

Social place cells in the bat hippocampus

David Omer, Shir R. Maimon, Liora Las, Nachum Ulanovsky

Department of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel

Social animals have to know the spatial positions of conspecifics. However, it is unknown how the position of others is represented in the brain. We designed a spatial observational-learning task, where an observer-bat mimicked a demonstrator-bat, while we recorded hippocampal dorsal-CA1 neurons from the observer-bat. A neuronal subpopulation represented the position of the other bat, in allocentric coordinates. About half of these 'social place-cells' represented also the observer's own position, i.e. were place-cells. The representation of the demonstrator-bat did not reflect self-movement or trajectory-planning by the observer. Some neurons represented also the position of inanimate moving objects; however, their representation differed from the representation of the demonstrator-bat. This suggests a role for hippocampal CA1 neurons in social-spatial cognition.

Dopamine D1 receptors produce a switch-like PKA responses in the nucleus of striatal neurons

Pierre Vincent [1, 2*], Liliana Castro [1, 2], Anu Nair [3, 4, 5], Jeanette Kotaleski [3, 6], Cédric Yapo [1, 2]

[1] *Sorbonne Université, CNRS, Biological Adaptation and Ageing, F-75005 Paris, France.*
[2] *Member of the Bio-Psy Labex.*
[3] *Science for Life Laboratory, School of Computer Science and Communication, KTH Royal Institute of Technology, Stockholm, 10044, Sweden*
[4] *National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India*
[5] *Manipal University, Manipal, India*
[6] *Department of Neuroscience, Karolinska Institutet, Solna, 17177, Sweden** Corresponding author: pierre.vincent@upmc.fr

Dopamine release by midbrain dopaminergic neurons in both prefrontal cortex and striatum is critically involved in action selection and reward-mediated learning. D1 dopamine receptors transduce the dopamine signal into an increase in intracellular cAMP, and PKA activation, eventually leading to the induction of immediate early genes. The striatum had already been shown to be exquisitely sensitive to brief dopamine stimulations at the level of cFos expression (Castro et al, J Physiol 2013), but why the PKA signal was so effective in the nucleus of striatal neurons remained unclear. Here, we combined live-cell imaging of PKA-dependent phosphorylation in mouse brain slices with computational modeling to investigate how transient dopamine signals are translated into nuclear PKA activity. We compared data from cortical pyramidal neurons and striatal medium spiny neurons. We observed that the nuclear PKA signal in striatal neurons featured an ultrasensitive responsiveness, associated with fast, all or none responses, which is not consistent with the commonly accepted theory of a slow and passive diffusion of catalytic PKA in the nucleus. Our numerical model suggests that a positive feed-forward mechanism, inhibiting nuclear phosphatase activity, could be responsible for this non-linear pattern of nuclear PKA response. DARPP-32, a protein highly enriched in this brain structure, may be responsible for this particular feature of signal integration observed in the striatum, an hypothesis that will be tested in future work. These observations provide a biological substrate to the well recognized function of the striatum in learning: the non-linear switch-like integration allows for a better detection of the transient dopamine signals that are associated with a reward.

Activity-dependent Chromatin Architecture Remodeling

Ana Martín González [1], Agnieska Czechowska [1], Elzbieta Januszewicz [1], Kuba Robert Filipkowski [2], Grzegorz Wilczynski [1], Adriana Magalska [1*]

[1] *Laboratory of Molecular and Systemic Morphology, Nencki Institute of Experimental Biology, Warsaw, Poland)*
[2] *Laboratory of Behaviour and Metabolism Research, Mossakowski Medical Research Centre, Warsaw, Poland)*
* Corresponding author: a.magalska@nencki.gov.pl

Spatial chromatin organization is crucial for transcriptional regulation and is particularly important in neurons, as these cells severely change their transcriptome upon external stimuli. In our study, we used different paradigms of neuronal stimulation to induce activity-dependent changes in rat neurons. We stimulated in vitro cultured hippocampal neurons with KCl or a mixture of picrotoxin, rolipram and forskoline. In vivo neural activity was induced in hippocampal and amygdala neurons by acute treatment with kainite or fear conditioning, respectively. Our recent studies showed that, as a result of chemically induced long-term potentiation (cLTP), the chromatin starts to condense to small foci visible as bright spots in DNA staining, which later form bigger areas of condensed chromatin and an apparently condensed nuclear rim. Such a phenomenon occurs also in the hippocampal neurons of rats stimulated with kainite, and in amygdala neurons (both pyramidal and interneurons) of rats subjected to fear conditioning, a more physiological paradigm. This activity-dependent chromatin reorganization in response to LTP -which has never been described before- is fast and takes place before the expression of IEGs, being therefore independent of transcription. The epigenetic modifications of the chromatin can have long-lasting effects on neuronal function and thereby represent a still largely unexplored molecular substrate for neuronal plasticity.
Project supported by SonataBis5 National Science Centre grant nr 2015/18/E/NZ3/00730 and Marie Sklodowska-Curie grant agreement nr 665735.

Sex-specific regulation of fear memory and chronic stress responsivity by targeted epigenetic editing of Cdk5

AS Sase [1], SI Lombroso [1], BA Santhumayor [1], RR Wood [1], RL Neve [3], EA Heller [1,2*]

[1]*Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, USA*
[2]*The Penn Epigenetics Institute, University of Pennsylvania, Philadelphia, USA*
[3]*Gene Delivery Technology Core, Massachusetts General Hospital, Boston, USA** Corresponding author: *
Corresponding author: eheller@pennmedicine.upenn.edu

Sex-differences in the expression and prevalence of mood disorders, such as post-traumatic stress disorder (PTSD) and major depressive disorder (MDD), have led to a growing interest in the sex-specific molecular and epigenetic mechanisms underlying these diseases. Cyclin dependent kinase 5 (Cdk5) is known to underlie both fear memory and chronic stress susceptibility in male mice. Given our recent finding that targeted histone acetylation of Cdk5 regulates social stress in male mice, we hypothesized that such a mechanism may be functionally relevant in female mice as well. We therefore aimed to (1) examine Cdk5 regulation in both male and female mice, and (2) apply epigenetic editing to decipher the direct causal relevance of acetylation of Cdk5 to PTSD and MDD preclinical paradigms. We applied contextual fear conditioning (FC) to model PTSD, and chronic unpredictable stress (CUS) to model MDD. We found male-specific activation of Cdk5 expression in both CA1 and NAc, that correlated with male-specific histone acetylation at the Cdk5 promoter. We then examined regulation of fear memory retrieval or pro-depressive phenotypes following targeted epigenetic editing of Cdk5. CUS or FC were paired with viral expression of zinc-finger proteins targeting histone acetylation to the Cdk5 promoter in either hippocampus (CA1) or nucleus accumbens (NAc), respectively. Targeted histone acetylation of Cdk5 promoter attenuated stress responsivity and fear memory retrieval in female, but not male mice. Taken together we have uncovered a female-specific role of Cdk5 acetylation and expression in attenuating long-term fear memory and susceptibility to chronic unpredictable stress.

The epigenetic factor CBP is required for the differentiation and function of medial ganglionic eminence-derived interneurons

Alejandro Medrano-Fernández[1], Jose M. Delgado-Garcia[2], Raudel Sánchez-Campusano[2], Román Olivares[1], Agnès Gruart[2], Angel Barco[1*]

[1]*Instituto de Neurociencias (Universidad Miguel Hernández - Consejo Superior de Investigaciones Científicas). Av. Santiago Ramón y Cajal s/n. Sant Joan d'Alacant. 03550. Alicante, Spain.*
[2]*Division of Neurosciences, Pablo de Olavide University, Seville-41013, Spain.*
* Corresponding author: abarco@umh.es

The development of inhibitory circuits depends on the action of a network of transcription factors and epigenetic regulators that are critical for interneuron specification and differentiation. Although the identity of many of these transcription factors is well established, much less is known about the specific contribution of the chromatin-modifying enzymes that sculpt the interneuron epigenome. Here, we generated a mouse model in which the lysine acetyltransferase CBP is specifically removed from neural progenitors at the median ganglionic eminence (MGE), the structure where the most abundant types of cortical interneurons are born. Ablation of CBP interfered with the development of MGE-derived interneurons, causing a reduction in the number of functionally mature interneurons in the adult forebrain. Genetic fate mapping experiments not only demonstrated that CBP ablation impacts on different interneuron classes, but also unveiled a compensatory increment of non-MGE-derived interneurons that cushions the excitatory-inhibitory imbalance. Consistent with having a reduced number of interneurons, CBP-deficient mice exhibited a high incidence of spontaneous epileptic seizures, and alterations in brain rhythms and enhanced low gamma activity during status epilepticus. These perturbations led to abnormal behavior including hyperlocomotion, increased anxiety and cognitive impairments. Overall, our study demonstrates that CBP is essential for interneuron development and the proper functioning of inhibitory circuitry *in vivo*.

ADAR1 dual function as RNA editing and potential DNA binding enzyme in the activity-dependent regulation of adaptive behaviour in the mouse

Paul Marshall [1*], Qiongyi Zhao[1], Xiang Li [1], Wei Wei [1] Esmi Zajaczkowski[1] Laura Leighton[1] Timothy Bredy[1]

[1] *University of Queensland (Queensland Brain Institute, Brisbane, and Australia)*
* Corresponding author: marshall.r.paul@gmail.com

RNA editing has been correlatively linked to behaviour in organisms ranging from flies to humans, with the highest levels of the enzymes mediating this process found in primates, humans, and in some instance in a brain-specific manner. Mechanistically, this occurs through the deamination of specific base nucleotides, including adenosine in the case of the adenosine deaminase (ADAR). However, not all variants of ADAR appear to operate solely on RNA. For example, ADAR1 can also bind to DNA but the functional relevance of this capacity in the brain remains relatively unexplored. In order to begin to assess the potential RNA-independent function of ADAR1 in neurons, and in the context of behavioural adaptation, ADAR1 ChIP-seq was performed on activated primary cortical neurons (PCN's) in vitro, and on cortical neurons derived from adult mice subjected to behavioural training. We have discovered that ADAR1 binds a number of targets on DNA in an activity-dependent manner. In addition, we have found that lentiviral-mediated knockdown of ADAR1 in the infralimbic cortex leads to an enhancement of fear-related memory. It remains to be seen to what extent this effect on memory is mediated primarily by RNA editing or by ADAR1's capacity to bind to DNA.

Functional remodeling of glutamatergic inputs to Locus Coeruleus neurons during the adolescence to adulthood transition

Andrea Locarno[1], Ieva Misevičiūtė[1], Francesco Papaleo[1], Giovanni Marsicano[2-3], Beat Lutz[4], François Georges[5-6], Raffaella Tonini[1, *]

[1] *Fondazione Istituto Italiano di Tecnologia, Genova, Italy*
[2] *INSERM, NeuroCentre Magendie, Bordeaux, France*
[3] *Université de Bordeaux, Bordeaux, France*
[4] *Institute for Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Mainz, Germany*
[5] *Institut des Maladies Neurodégénératives, Bordeaux, France*
[6] *CNRS, Institut des Maladies Neurodégénératives, Bordeaux, France*
* Corresponding author: raffaella.tonini@iit.it

The adolescent and adult brain responds differently to environmental stressors. These stimuli are encoded at the level of the medial prefrontal cortex (mPFC) and amygdala (Amyg) circuits, suggesting that unbalanced activity between mPFC- and Amyg- may contribute to the higher emotional reactivity and risk-taking behavior observed during adolescence. The mPFC and Amyg send excitatory afferents to the Locus Coeruleus (LC) nucleus, the major source of norepinephrine for the entire forebrain. This neuromodulatory nucleus has been associated with attention, behavioral flexibility, arousal, emotional learning, and stress response. Whether glutamatergic inputs to LC neurons can undergo experience-dependent synaptic plasticity and whether this plasticity differs between adolescent and adults remains to be established. To address these questions, we investigated spike-timing dependent plasticity at excitatory LC synapses. We found that glutamatergic inputs to LC neurons express a Hebbian form of t-LTD in adult mice (P45-P60), which requires the activation of CB1 receptor. The induction rules of STDP were inverted in young mice (P23-P28), indicating a post-natal functional remodeling of LC glutamatergic synapses. Young animals LTP relies upon CB1 receptor and Corticotropin Releasing Factor 1 (CRF1) receptor activation. The involvement of CRF1R suggests a role of Amyg circuit in this form of plasticity. Our results point to divergent synaptic substrates for some of the different adrenergic-mediated behavioral responses between adolescent and adult subjects.

Astrocytic inhibition in CA1 impairs remote memory acquisition by disrupting CA1 to ACC communication

Adar Adamsky[1]*, Adi Kol[1], Tirzah Kreisel[1], Maya Groysman[2], Michael London[1,3], Inbal Goshen[1]

[1] *Edmond and Lily Safra Center for Brain Sciences (ELSC), The Hebrew University of Jerusalem, Jerusalem, Israel.*
[2] *ELSC Vector Core Facility, The Hebrew University of Jerusalem, Jerusalem, Israel.*
[3] *Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel.*
* Corresponding author: adar.adamsky@gmail.com

Astrocytes were recently shown to monitor and directly modulate neuronal activity in their domain, and are therefore necessary for synaptic plasticity, the infrastructure for memory formation and stabilization. However, only few studies have directly investigated the role of astrocytes in cognitive functions in behaving animals. Here, we employ a chemogenetic tool to specifically inhibit astrocytic activity, in conjunction with behavior and histology, and examine their role in memory formation and retrieval. The synthetic Gi-Coupled receptor hM4Di was selectively expressed in hippocampal CA1 astrocytes, allowing specific disruption of their activity by the designer drug CNO. Gi-pathway recruitment in hippocampal astrocytes by CNO application during memory acquisition resulted in intact recent recall one day after learning, but severely impaired remote recall one month later. Furthermore, this hippocampal astrocytic inhibition during acquisition resulted in decreased neuronal cFos levels during remote recall in both CA1 and the anterior cingulate cortex (ACC), a region known to be involved in remote memory. These findings suggest that the effects of CA1 astrocytic inhibition are not restricted to this brain region. Indeed, inhibiting CA1 astrocytes during acquisition did not significantly affect local activity in this region, but it did inhibit the recruitment of the ACC, where no manipulation took place, during memory acquisition. Finally, similar to CA1 astrocytic inhibition, ACC astrocytic inhibition during memory acquisition also specifically impaired remote recall. These results suggest that Gi-pathway recruitment in CA1 astrocytes might affect long-distance communication between hippocampal and frontal memory ensembles, consequently affecting remote memory.

Mimicking age-associated Gadd45γ decline results in memory impairments in young mice

David VC Brito[1], Janina Kupke[1], Kübra Gülmez Karaca[1], Benjamin Zeuch[1] and Ana MM Oliveira[*1]

[1] *Department of Neurobiology, Interdisciplinary Centre for Neurosciences (IZN), University of Heidelberg, INF 364, 69120 Heidelberg, Germany.*
* Corresponding author: oliveira@nbio.uni-heidelberg.de

With an increasingly aged population age-associated cognitive decline is a major health and socio-economic burden. Understanding the mechanisms underlying progressive cognitive loss is required to develop future therapies. The underlying causes of age-associated cognitive decline are largely unknown. We found that Gadd45γ expression is decreased during basal conditions and upon spatial learning in the hippocampus of aged mice, compared to young adult mice. This decrease is selective, as other family members are expressed at comparable levels in young adult and aged mice. We hypothesized that if Gadd45γ would be involved in age-associated cognitive decline, then reducing its expression in the hippocampus of young mice should promote memory deficits. Indeed, we found that knocking down Gadd45γ in the hippocampus of young adult mice, generates age-like memory deficits in long-term and short-term memories. Abolishing the expression of other family members did not produce the same phenotype. Hence, our findings show a selective role for Gadd45γ in memory formation, which may underlie memory deficits observed during aging. Next, we identified signaling pathways regulated by Gadd45γ that may mediate its role in plasticity-related mechanisms. Particularly, decreasing the expression of Gadd45γ disrupted MAPK and downstream AP-1 signaling. Indeed, we found that transcription of AP-1 target genes was decreased in conditions of Gadd45γ knockdown. These alterations were not due to altered calcium influx, which suggests Gadd45γ may act directly at the MAPK signaling cascade. This data suggests that age-associated decrease in Gadd45γ expression may be involved in cognitive deficits possibly by abnormal MAPK activation and downstream AP-1 signaling and gene expression required for memory.

Huntington's disease striatal super-enhancer signature

Rafael Alcala Vida[1,], Jonathan Seguin[1], Anne Molitor[2], Caroline Lotz[1], Aurélie Bombardier[1], Stéphanie Le Gras[3], Céline Keime[3], Jean-Christophe Cassel[1], Anne-Laurence Boutillier[1], Thomas Sexton[2], Karine Merienne[1]

[1] *Team epigenetics and dynamics of memory systems, Laboratory of cognitive and adaptive neurosciences (LNCA), UMR 7364, CNRS / Strasbourg university, Strasbourg, France*
[2] *Department of functional genomics and cancer, Institute of Genetics and Molecular and Cellular Biology (IGBMC), UMR 7104, CNRS / INSERM / Strasbourg University, Strasbourg, France*
[3] *High throughput sequencing platform, Institute of Genetics and Molecular and Cellular Biology (IGBMC), UMR 7104, CNRS / INSERM / Strasbourg University, Strasbourg, France*
* Corresponding author: karine.merienne@unistra.fr

Huntington's disease (HD) is a progressive neurodegenerative disease, affecting primarily the striatum. Transcriptional dysregulation is believed to contribute to HD. However, the underlying mechanism is unclear. Using ChIPseq and RNAseq on the striatum of HD R6/1 transgenic mice, we found that down-regulated genes are enriched in striatal identity genes, controlled by a super-enhancer. H3K27ac, enhancer transcription and recruitment of RNA polymerase II (RNAPII) were selectively reduced at R6/1 striatal super-enhancers, indicating that altered super-enhancer activity underlies down-regulation of striatal identity genes in HD. Our 4Cseq data using R6/1 striatum further suggest that disruption of chromatin 3D architecture contributes to altered expression of striatal identity genes regulated by a super-enhancer. To investigate functional consequences of epigenetic alterations in HD, R6/1 mice were trained to learn striatum-dependent cognitive task. In contrast to wild-type (WT) animals, R6/1 mice were impaired in this task. ChIPseq data generated using the striatum of "trained" and "home cage" mice showed an increase of H3K27ac and RNAPII at genes implicated in synaptic plasticity and regulated by a super-enhancer, in trained vs home cage WT animals. However, this "plasticity" signature was absent in trained R6/1 mice, suggesting that aberrant RNAPII dynamics and inadequate histone acetylation at these genes preclude synaptic plasticity and contribute to R6/1 behavioural deficits. Finally, we generated ChIPseq data using the striatum of HD patients and knockin mice. HD striatal "super-enhancer" signature was conserved across models and our analyses further revealed that it establishes early, at presymptomatic stage.

ASD-like social behavior deficits in mouse models for RASopathy disorders

Sarah C. Borrie[1 *], Ellen Plasschaert[1], Ype Elgersma[2], Steven Kushner[2], Eric Legius[1], Hilde Brems[1]

[1] *Department of Human Genetics, KU Leuven, Leuven, Belgium.*
[2] *Erasmus Medical Center, Rotterdam, The Netherlands.*
* Corresponding author: sarah.borrie@kuleuven.be

Neurofibromatosis type 1 and Legius syndrome are two related RASopathy disorders, stemming from mutations in the RAS–MAPK pathway, in the *NF1* and *SPRED1* genes respectively. NF1 is a RAS-GAP protein, negatively regulating activation of RAS, and SPRED1 interacts with NF1. Common to both disorders are neurological problems including cognitive deficits and increased incidence of autism spectrum disorder (ASD). Mouse models for these disorders, *Nf1+/-* and *Spred1-/-* mice, exhibit cognitive deficits consistent with human phenotypes, but it is not known whether they also recapitulate the ASD-like symptoms, and what the molecular mechanisms underlying such phenotypes are. Here we examined social behaviours in *Spred1-/-* and *Nf1+/-* mice, to ask if social deficits are observed in these mouse models, and whether any observed deficits depend upon RAS-MAPK signalling. *Spred1-/-* mice displayed abnormal social behaviour in the automated tube test compared to wildtype controls, and impairments in nesting behaviour. Studies in *Nf1+/-* mice also demonstrated similar social deficits in the tube test. Social deficits in *Spred1-/-* could be reversed in adult mice by inhibiting the RAS-MAPK pathway with MEK inhibitors. Fluorescent in situ hybridisation revealed that similar to *Nf1*, *Spred1* mRNA is expressed in both inhibitory and excitatory neurons, indicating that further investigation of the relative contribution of these cell types to social behaviour phenotypes in this model is warranted. These findings suggest that RAS–MAPK pathway overactivation underlies altered social behaviour in a mouse model for RASopathy-linked ASD.

Isoform-selective inhibitors reveal a double dissociation between the functions of PKMζ and PKCι/λ during LTP and long-term memory maintenance in wild-type and PKMζ-null mice

Panayiotis Tsokas[1, 2*], Changchi Hsieh[1], Mini P Sajan[4], Mildred Acevedo-Duncan[5], Robert V. Farese[4], Andre A. Fenton[6], Todd C. Sacktor[1, 2, 3]

[1] *SUNY Downstate Medical Center, Physiology and Pharmacology, The Robert F. Furchgott Center for Neural and Behavioral Science, Brooklyn NY, USA*
[2] *SUNY Downstate Medical Center, Anesthesiology, Brooklyn NY, USA*
[3] *SUNY Downstate Medical Center, Neurology, Brooklyn NY, USA*
[4] *University of South Florida, James A Haley Veterans Medical Center, Department of Internal Medicine, Tampa FL, USA*
[5] *University of South Florida, Department of Chemistry, Tampa FL, USA*
[6] *New York University, Center for Neural Science, New York NY, USA*
* Corresponding author: tsokasp@gmail.com

Protein kinase Mζ (PKMζ) is a persistently active, brain-specific atypical PKC isoform thought to be essential for maintaining the protein synthesis-dependent phase of long-term potentiation (late-LTP) and long-term memory (LTM). Because the catalytic sites of atypical PKCs are very similar, first generation PKMζ-inhibitors such as ZIP also inhibit the other atypical PKC, PKCι/λ, which compensates for PKMζ in PKMζ-null mice (Tsokas et al., 2016). Recent studies with a selective inhibitor of PKCι/λ revealed that it reverses late-LTP and LTM maintenance in PKMζ-null mice, but not wild-type mice. To address whether the persistent action of PKMζ is required for maintaining LTP in wild-type mice, we identified a novel, small molecule negative allosteric modulator of PKMζ (NSA), which inhibits PKCζ autophosphorylation with an IC50 of ~100 nM without effect on PKCι/λ autophosphorylation up to 100 μM. NSA (10 μM) reverses late-LTP maintenance without affecting baseline synaptic transmission when applied to slices from wild-type mice 3 h post-tetanization. Importantly, NSA does not reverse late-LTP in PKMζ-null mice that lack the drug's target protein. Similarly, bilateral intra-hippocampal injection of NSA (5 nmol/hippocampus) 1 day post-training eliminates spatial LTM in wild-type mice, but not PKMζ-null mice. Combined with the previous results, these findings reveal a double dissociation between the effects of PKMζ and PKCι/λ inhibition on LTP and LTM in wild-type and PKMζ-null mice and demonstrate that PKMζ maintains physiologically normal late-LTP and spatial LTM.

ARHGAP8 Regulates Excitatory Synapses

Jeannette Schmidt [1,2,3], Inês B Santos [1,4], Joana S Ferreira [1,5], Renato Socodato [6,7], João B Relvas [6,7], Paulo Pinheiro [1] , Ana Luisa Carvalho [1,4 *]

[1] *Synapse Biology Group, CNC – Center for Neurosciences and Cell Biology, University of Coimbra, Coimbra, Portugal*
[2] *PDBEB, Doctoral Programme in Experimental Biology and Biomedicine, CNC, University of Coimbra, Coimbra, Portugal*
[3] *Institute for Interdisciplinary Research, University of Coimbra (IIIU), Coimbra, Portugal*
[4] *Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal*
[5] *Development and Adaptation of Neuronal Circuits Group, IINS - Interdisciplinary Institute for Neuroscience, University of Bordeaux, Bordeaux, France*
[6] *Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Portugal*
[7] *Instituto de Biologia Molecular e Celular (IBMC), University of Porto, Portugal*
* Corresponding author: alc@cnc.uc.pt

During development and other processes that include changes to cell architecture, such as synaptic plasticity, neurons need to undergo extensive reorganization of their actin scaffolding. Synaptic plasticity is associated to mechanisms such as dynamic changes in spine size and number and the ability of excitatory glutamate-activated synapses to alter their strength in response to changes in activity patterns. Intraneuronal actin dynamics are orchestrated by members of the Rho small GTPase subfamily. Acting as binary switches, they

cycle between active GTP-bound and inactive GDP-bound states, into which they are pushed by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs), respectively. A variety of these regulators have by now been shown to play critical roles in cognitive deficits and intellectual disability. Using quantitative mass spectrometry, we identified ARHGAP8, a RhoGAP, as a novel component in neurons with mouse neuronal cells lacking the developmentally regulated NMDA-type glutamate receptor subunit GluN2B presenting a complete loss of the protein in their postsynaptic densities (PSDs). Additionally, dendritic spines of these cells display enhanced RhoA activity patterns in fluorescence resonance energy transfer (FRET) assays. Further characterization of ARHGAP8 revealed a brain-wide expression pattern with several hotspots, as well as partial localization to active synapses that is dependent on GluN2B. Artificially modifying ARHGAP8 levels in isolated neurons lead to drastic changes in synaptic levels of AMPA receptors and in synaptic strength. Overall our data hints at an important regulatory role for ARHGAP8 in neurons and more specifically excitatory synapses.

New mouse object-in-place recognition task for in vivo two-photon calcium imaging in the Mobile Home Cage

Olga Ivashkina [1, 2, *], Ksenia Toropova [1, 2], Anna Gruzdeva [1, 2], Ekaterina Kulikova [3], Konstantin Anokhin [1, 2, 4]

[1] *Neuroscience Department, NRC "Kurchatov Institute", Moscow Russia*
[2] *Center for neuroscience and cognitive sciences, Lomonosov Moscow State University, Moscow, Russia*
[3] *Moscow Institute of Physics and Technology, Moscow, Russia*
[4] *PK Anokhin Institute of Normal Physiology, Moscow, Russia*
* Corresponding author: oivashkina@gmail.com

The goal of our study was to investigate neuronal specializations in the retrosplenial cortex and hippocampus to a new environment and objects in it. To this end, we used in vivo two-photon calcium imaging in mice during object-in-place recognition task. As two-photon imaging requires head fixation of animals, we used the Mobile Home Cage (MHC, Neurotar Ltd) setup – an air-lifted mobile cage, where a head-fixed animal can move around and explore the environment. First, we developed the object-in-place recognition task in the MHC on C57 Bl/6 mice with implanted headposts (Neurotar Ltd). Mice were habituated to the MHC conditions during 14 days with session time increasing from 10 to 50 min. The object-in-place task consisted of 7 sessions. During sessions mice explored empty MHC, MHC with new proximal cues, MHC with two novel objects, MHC with one of familiar objects displaced (place recognition task), and MHC with a familiar and a novel object (object recognition task). We showed that mice actively explored MHC with cues and objects. In the place recognition task, mice spent more time exploring and sniffing the familiar object in the new location, demonstrating place recognition memory. In the object recognition task, animals preferentially explored and sniffed the novel object, demonstrating object recognition memory. After we successfully developed the MHC-based object-in-place task, we performed two-photon calcium imaging using GCaMP6 in the retrosplenial cortex during task sessions. Supported by RSCF 14-15-00685 and RFBR 17-04-02054

In vivo two-photon calcium imaging of engram cortical neurons TRAPed during sound fear conditioning in mice

Anna Gruzdeva[1, 2, *], Olga Ivashkina[1, 2], Konstantin Anokhin[1, 2, 3]

[1] *NRC "Kurchatov Institute", Moscow, Russian Federation*
[2] *Center for Neural and Cognitive Sciences, Lomonosov Moscow University, Moscow, Russian Federation*
[3] *P.K. Anokhin Institute of Normal Physiology, Moscow, Russian Federation*
* Corresponding author: annadronova@mail.ru

Neuronal ensembles or engrams are thought to encode memories. Although in vivo calcium imaging is an emerging technique to analyze activity of the same neurons during different behaviors, specific calcium responses of engram neurons across different memory states such as systems consolidation, recall or extinction remains unexplored. *In this study we describe employment of novel Fos-Cre-GCaMP transgenic*

mouse strain for investigation of long-term changes in engram neurons activity. We used targeted recombination in active populations *(TRAP) strategy to introduce calcium sensor into the engram neurons of conditioned fear in the mouse brain. The tamoxifen was injected one day before the training, that the neurons that expressed c-fos during fear conditioning also started to express the* GCaMP3 *calcium sensor. The total number of GCaMP3 positive neurons reached maximum on the fourth day after the tamoxifen injection and remain stable for at least two months. On the fifth day we started* two-photon calcium imaging of TRAPed neurons in the mouse parietal cortex *during* presentation of the conditioned sound (CS). *T*hree types of neuron were described: neurons that responded without CS, neurons that responded during CS and neurons that did not respond. Also, we reveal that usage of *Fos-Cre-GCaMP mice allows to* record calcium dynamic in dendritic spines of TRAPed neurons. Thus in vivo neuronal calcium imaging in the brain of conditioned Fos-Cre-GCaMP mice allows to investigate various forms of activity in the specific TRAPed neurons to address question about allocation, stability and dynamics of memory engram.

Supported by RSCF grant #14-15-00685