Schizophrenia risk factor Tcf4 and gene-environment interaction in mice

Badowska D.M.[1], Brzózka M.M.[3], Chowdhury A.[1], Malzahn D.[2], Rossner M.J.[3]
[1]Research Group Gene Expression, Max-Planck-Institute of Experimental Medicine, 37075 Göttingen, Germany. [2]Department of Genetic Epidemiology, University Medical Center, Georg-August University, 37099 Göttingen, Germany. [3]Laboratory of Molecular Neurobiology, Department of Psychiatry, Ludwig-Maximilians-University, 80336 Munich, Germany.

Psychiatric diseases are triggered by the interaction of genetic and environmental risk factors (GxE). To model GxE on the behavioural level in mice, we developed an approach to analysing huge behavioural data sets, which allowed us to compare mice tested in a battery of experiments, in independent cohorts (Badowska et al., 2014). By using multivariate statistics, we merged data measuring similar behaviours into higher-order categories (dimension reduction). This allowed us to create clinically relevant behavioural profiles of mice and visualise them in a single radar chart. We used this approach to study GxE in transgenic mice overexpressing a novel schizophrenia risk gene Tcf4 and subjected them to different, harsh environmental treatments: Isolation rearing, Social defeat or the control condition Enriched environment. These mice displayed schizophrenia-relevant symptoms - deficits in fear memory and behavioural flexibility – only upon Isolation rearing and Social defeat. Enriched environment rescued that phenotype. This result proves GxE in these mice and points at the role of Tcf4 in cognition. Tcf4 overexpressing mice also displayed altered long term plasticity in the hippocampus, increased dendritic spine frequency and up-regulation of several synaptic proteins in the prefrontal cortex. We also tested the behaviour of $Tcf4^{-/+}$ mice, which showed strong cognitive impairment specific to hippocampus-dependent spatial learning. Analysis of Tcf4 expression in these mice revealed down-regulation mainly of the isoforms that are highly expressed in the hippocampus, which is in line with the behavioural phenotype. We conclude that in mice Tcf4 is important predominantly for cognition, which declines upon both overexpression and deficiency of the gene.

Sound feedback removal modulates the perceptuo-motor recalibration after the treadmill walking

Camponogara I.[2], Turchet L.[3], Carner M.[1], Marchioni D.[1], Cesari P.[2]
[1]ENT Dept. Hospital University of Verona, Verona. [2]Neurological and movement sciences department, Univeristy of Verona, Verona. [3]Department of Architecture, Design & Media Technology, Aalborg University, Copenhagen.

The after effect is an unintentional forward drift experienced while attempting to walk in place with eyes closed following few minutes of treadmill walking. Initially this effect was thought as given by the mismatch experienced during the treadmill walking between the visual (no optic flow-no movement) and the proprioceptive (muscles spindles firing-movement) information. Recently it has been shown the persistence of the effect even in absence of vision suggesting that also other information as perceiving the sound of the steps could play a role. To test this hypothesis, a group of six cochlear-implanted patients were recruited, and their forward drift after a treadmill walking with the cochlear system turned off and on measured. Results showed that walking with the system off leads to a 32% shorter drift compared to when walking with the system on. This demonstrates that hearing the steps increases the mismatch among perceptions (visual proprioceptive and auditory) sustaining the importance in perceiving the sounds of actions for actions control.

LTP in the CA1 area of developing hippocampus: molecular pathways controlling synaptic recruitment of GluA4-containing AMPA receptors

Luchkina N.V.[1,2], Coleman S.K.[1], Huupponen J.[1,2], Clarke V.R.J.[1,2,3], Taira T.[1,3], Keinänen K.[2], Lauri S.E.[1,2]
[1]Neuroscience Center, University of Helsinki, Helsinki, Finland. [2]Department of Biosciences, University of Helsinki, Helsinki, Finland. [3]Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland.

Synaptic plasticity, and long-term potentiation (LTP) in particular, plays a critical role in the activity-dependent refinement and fine-tuning of neuronal circuits during development by maintaining and stabilising certain synaptic connections and eliminating others. We show that developmentally restricted expression of AMPA receptor (AMPAR) subunit GluA4 is necessary for protein kinase A (PKA)-dependent LTP at hippocampal CA3-CA1 synapses during the first week of development. Further, the loss of GluA4 expression in parallel with maturation of the circuitry sufficiently explains the developmental switch in LTP signalling requirements from PKA- to Ca2+/calmodulin-dependent protein kinase II

(CaMKII)-dependent. At immature synapses, activation of PKA leads to a robust potentiation of AMPA receptor function via the upregulation of GluA4. In neonatal GluA4 deficient mice, GluA1 is upregulated as an apparent compensatory mechanism and LTP depends on CaMKII, similar to LTP at mature synapses. Further, lentiviral expression of GluA4 in CA1 neurons confers a PKA-dependent synaptic potentiation and LTP regardless of the developmental stage. Thus, GluA4 defines the signalling requirements for LTP. Moreover, we demonstrate that PKA-dependent insertion of GluA4 is critical for silent synapse activation and strengthening of AMPAR-mediated transmission at immature synapses during network development. In the absence of GluA4, strengthening of AMPAR-mediated transmission is significantly delayed and the number of silent synapses is decreased. By introducing GST-fusion proteins corresponding to the intracellular part of GluA4 (with different mutations) into neurons during electrophysiological recordings, we show that PKA-induced insertion of GluA4 requires a previously unidentified molecular mechanism involving an interaction between the membrane proximal region and the extreme C-terminal sequence of the GluA4 C-terminal domain. Therefore, GluA4/PKA-mediated activation of silent or initially weak synapses is a critical mechanism facilitating the functional maturation of glutamatergic circuitry during the critical period of experience-dependent fine-tuning.

Sequential Development of Glutamatergic and GABAergic Synapses on Principal Neurons in the Rat Neocortex

Naskar S.[1], Balzani E.[1], Tucci V.[1], Cancedda L.[1]

[1]Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, via Morego, 30, 16163 Genova, Italy

A large number of morphofunctional studies have revealed that GABAergic synapses develop before Glutamatergic synapses in different areas of the brain and in different species, ranging from reptiles to humans. Nevertheless, only a handful of studies on the GABA-Glutamate sequence of synaptogenesis exists in the developing neocortex, and they provide contrasting results. To provide detailed functional evidences of the GABA-Glutamate sequence of synaptogenesis in the developing rodent neocortex, we performed electrophysiology, morphology and behavior experiments. We found that Glutamatergic miniature postsynaptic currents (mPSCs) appear earlier than GABAergic mPSCs in pyramidal neurons in Layer 2/3 of the neocortex. Notably, frequencies of both Glutamatergic and GABAergic mPSCs show an abrupt increase at postnatal day 9 (P9). This is possibly modulated by Serotonin because inhibition of serotonin reuptake with Selective Serotonin Reuptake Inhibitors (SSRIs) accelerates the

development of Glutamatergic and GABAergic synapses. Conversely, we found that, the frequencies of both Glutamatergic and GABAergic mPSCs increases gradually In Layer 5. Moreover, both Glutamatergic and GABAergic synaptogenesis in Layer 5 precedes that in Layer 2/3. In order to investigate whether the abrupt increase in the frequencies of Glutamatergic and GABAergic mPSCs at P9 correlates with the development of motor and sensory behaviors, we are currently performing behavioral experiments in rat pups aged between P2 and P10.

Astroglial type-1 cannabinoid receptors (CB1) are required for memory formation

Oliveira da Cruz J.F.[*1,2,3], Robin L.[*1,2], Langlais V.C.[*1,2], Busquets-Garcia A.[1,2], Soria-Gomez E.[1,2], Drago F.[3], Panatier A.[1,2], Georges F.[1], Metna-Laurent M.[1,2], Oliet S.[*1,2], Marsicano S.[*1,2]

[1]University of Bordeaux, France. [2]INSERM U862 NeuroCentre Magendie, France. [3]University of Catania, Italy. *contributed equally.

Cannabinoids and endocannabinoids modulate learning and memory through the type-1 cannabinoid receptor (CB1). Brain astrocytes have been shown to participate in memory processes through many different mechanisms, including the release of gliotransmitters at the so-called “tripartite synapse”. Interestingly, similarly to neurons, astrocytes express functional CB1 receptors capable of modulating the effects of exogenously administered cannabinoid agonists on hippocampal synaptic plasticity and working memory. However, the endogenous physiological role of astroglial CB1 receptors in specific memory processes is not known. Here we show that astrocyte CB1 receptors are necessary for the formation of object recognition memory and the induction of NMDA-dependent long-term potentiation (LTP), via the astroglial modulation of the occupancy of the N-methyl-D-Aspartate receptor (NMDAR) co-agonist binding site. The conditional genetic deletion of CB1 receptors from mouse brain astrocytes (GFAP-CB1-KO mice) impairs *in vivo* hippocampal LTP induction and object recognition memory. Notably, the administration of D-serine, a gliotransmitter acting as co-agonist NMDAR, is able to restore the formation of recognition memories and the induction of LTP in mutant mice. Finally, GFAP-CB1-KO mice exhibit strongly reduced occupancy of the co-agonist binding site of NMDARs. Thus, endogenous astroglial CB1 receptors are necessary for the formation of object recognition memory through the modulation of synaptic neuron-glial interactions.

Social Microbes & Neurodevelopment – Absence of Microbiota during Early Life Increases Activity-Related Transcriptional Pathways in the Amygdala.

Stilling R.M.[1,2], Ryan M.J.[1,2], Hoban A.E.[1,2], Shanahan F.[1], Clarke G.[1,2], Claesson M.J.[1,2], Dinan T.G.[1,2], Cryan J.F.[1,2]

[1]Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland. [2]Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland.

The tight association of the human body with trillions of colonizing microbes is the result of a long evolutionary history. Only recently we started to understand how this symbiosis also affects brain function and behaviour. Now, increasing evidence implicates host-microbe interactions at all levels within the body including the brain and in neurodevelopmental and stress-related disorders such as autism and anxiety. Germ-free (GF) mice, devoid of any microbiota throughout development, are a well-established tool to study the effects of absence microbes on host physiology. Recent findings confirm that GF animals demonstrate altered anxiety-related and impaired social behaviour. As such, our lab could recently show that GF mice demonstrate lack of pro-social behaviour. However, the underlying mechanisms and pathways involved are only insufficiently understood. To elucidate the molecular underpinnings of microbe-brain interaction, we therefore exploited unbiased, genome-wide transcriptional profiling to determine gene expression in the amygdala of GF mice and GF mice, colonised at weaning. Using RNA-sequencing we found significant changes at the level of differential gene expression, exon usage and RNA-editing. Most surprisingly, we noticed upregulation of several immediate early response genes such as Fos, Fosb, Egr2 or Nr4a1 in association with increased CREB signalling in GF mice under baseline conditions. In a subsequent behavioural stimulation experiment, we further identified these genes as key response genes to social interaction exposure in the amygdala.

In conclusion, our data suggest altered baseline neuronal activity in the amygdala of germ-free animals, which is established during early life and may have implications for understanding development and treatment of neurodevelopmental disorders such as autism.

The age and usage of the synaptic vesicle determine its ability to release

Truckenbrodt S.[1,2,3], Viplav A.[1,4,5], Denker A.[1,6], Fornasiero E.F.[1,3], Rizzoli S.O.[1,3]
[1]European Neuroscience Institute, Göttingen, Germany. [2]IMPRS for Molecular Biology, Göttingen, Germany. [3]University of Göttingen Medical Centre, Department for Neuro- and Sensory Physiology, Göttingen, Germany. [4]Master Molecular Biology Programme, University of Vienna, Austria. [5]Cells in Motion Cluster of Excellence at the University of Münster, Germany. [6]Hubrecht Institute, Utrecht, The Netherlands.

Organelles change with age; alterations in protein composition and protein damage can unpredictably alter their behaviour. Old organelles are thus degraded via damage response mechanisms. While most cellular processes will tolerate the continued use of ageing organelles until degradation, we identified a mechanism that measures the operational age of synaptic vesicles, anticipates damage, and removes them from active neurotransmission long before recognition by classical damage response mechanisms. We found that newly synthesised synaptic vesicles are preferentially employed in neurotransmitter release. They recycle only ~270 times in hippocampal cultures, over 12-24 hours, but are not degraded for another 1-2 days after functional inactivation. Increasing their release frequency accelerates inactivation accordingly. This is achieved through a "molecular timer" built into the recycling process itself: during release, the plasma membrane SNARE SNAP25 contaminates synaptic vesicles. There, SNAP25 blocks CSP (2-3 copies per vesicle), which in young vesicles promotes fusion via a trans-complex with SNAP25 on the cell membrane. But once CSP is blocked in futile cis-complexes with SNAP25 on the ageing vesicle itself, this interaction cannot take place anymore and release is no longer facilitated – the vesicle is inactivated.

Presynaptic protein synthesis is required for endocannabinoid-mediated long-term depression of inhibition

Younts T.J.[1,2], Klein M.E. [1], Monday H.R.[1], Castillo P.E.[1]
[1]Albert Einstein College of Medicine. [2]University College London.

Protein synthesis in neurons sculpts and maintains synapses during activity-dependent long-term plasticity such as long-term potentiation (LTP) and depression (LTD). It is generally accepted that translation in postsynaptic compartments supports local and persistent changes in synaptic function. However, it is still unclear whether presynaptic protein synthesis is necessary for long-term plasticity in the mature mammalian brain. In the hippocampus, endocannabinoid (eCB)-mediated long-term depression of

inhibition (iLTD) is expressed presynaptically at a subset of inhibitory interneuron synapses. We examined in acute hippocampal rat slices the role of presynaptic protein synthesis in iLTD by combining paired electrophysiological recordings with single cell manipulations that selectively block protein synthesis. Interfering with translation initiation or ribosome-mediated peptide elongation revealed that rapid protein synthesis is required for iLTD. Mechanistically, presynaptic type-1 cannabinoid receptors (CB1Rs) engage a signaling pathway involving protein kinase A (PKA), the mammalian target of rapamycin (mTOR), and the eukaryotic initiation factors eIF4F and eIF2α. iLTD was also independent of somatic transcription and microtubule-based trafficking. Protein synthesis was specific to long-term plasticity because basal synaptic transmission and short-term plasticity remained intact when translation was blocked. Our results indicate that protein synthesis required for iLTD occurs in presynaptic compartments and strongly suggest that presynaptic CB1Rs signal to the translation machinery to persistently and locally modify inhibitory neurotransmitter release in the mammalian brain.