INTRINSIC ACTIVITY

VOL. 2 (SUPPL. 1)
22 SEPTEMBER 2014

WWW.INTRINSICACTIVITY.ORG
ISSN 2309-8503

PUBLISHED BY APHAR, THE AUSTRIAN PHARMACOLOGICAL SOCIETY

20th Scientific Symposium of the Austrian Pharmacological Society APHAR

Innsbruck, 26–27 September 2014

MEETING ABSTRACTS

Intrinsic Activity is an online, open-access publication medium published by the Austrian Pharmacological Society (APHAR). The Journal welcomes contributions in the fields of Pharmacology, Pharmacotherapy and other fields in biomedicine.

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ISSN: 2309-8503

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Intrinsic Activity, 2014; **2**(Suppl.1)
published online: 22 September 2014
http://www.intrinsicactivity.org

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(available online at http://www.intrinsicactivity.org/2014/2/S1)

Meeting sections:

Austrian Pharmacological Society (APHAR)

A1.1

Decrypting the structure of LeuT$_{Aa}$ employing luminescence resonance energy transfer (LRET)

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.1

Background: Neurotransmitter sodium symporters (NSS) are located in the brain and retrieve neurotransmitters from the synaptic cleft to end synaptic transmission. Solute carrier class 6 proteins (SCLC6) are of great pharmacological importance in terms of their localization and function. The crystal structures obtained from a bacterial homolog, the leucine transporter LeuT$_{Aa}$, in open-to-outward, occluded and open-to-inward conformations are present in frozen state with high resolution. Due to its close kinship with SLC6 proteins, LeuT$_{Aa}$ serves as a paradigm for these transporters.
Methods: In order to address the dynamics of the substrate transport cycle in LeuT$_{Aa}$, we use the lanthanide-based resonance energy transfer (LRET) technique. This method is a spin-off of the fluorescence resonance energy transfer using Förster resonance energy transfer. We employ LRET by introducing the genetically encoded lanthanide-binding tags (LBT) as donor elements. Exogenous cysteine residues labeled with cysteine-specific fluorophores are used as acceptor elements. This technique is an alternative to address the movement of helices with great resolution and has been employed successfully to examine potassium channels.
Results: We screened for the functional LBT mutants using the scintillation proximity assay. The LeuT_A335-LBT-G336 mutant displayed function in terms of its binding activity. Within this background, we generated cysteine mutants. To date, we have successfully measured the intramolecular distances in different LBT_LeuT_Cys mutants. Furthermore, we observed intramolecular distance changes from these purified proteins in detergent micelles. Along these lines we are successfully carrying out LRET measurements of LeuT$_{Aa}$ reconstituted in POPC liposomes, a neutrally charged lipid environment.
Discussion: Our LRET measurements will help us to understand the transport cycle and help to complete the missing steps in the substrate transport cycle of LeuT$_{Aa}$. Currently, we focus on the reconstitution of purified LeuT$_{Aa}$ into liposomes and have our LRET measurements in a reconstituted system that allows us to have a more physiological ionic gradient environment.

A1.2

Pharmacological stimulation of hematopoietic stem cells

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.2

Background: The transplantation of hematopoietic stem cells (HSC) is a standard procedure in the treatment of hematological disorders and applicable to support chemotherapy in cancer. In practice, the clinical outcome is often hampered by severe infections. Hence there is a need for new pharmacological tools which facilitate functional bone marrow reconstitution. In recent years it has been shown that the Gα$_s$ signaling pathway and stimulation of Gα$_s$-coupled EP$_2$ and EP$_4$ prostanoid receptors play an important role in migration and homing of transplanted HSC to the bone marrow niche [1,2]. Here, we test the hypothesis that pretreatment of HCS with treprostinil, a stable prostacyclin (PGI$_2$) analogue, might be a promising therapeutic strategy to stimulate migration of HSC and shorten the time period until the onset of the transplant.
Methods: ***Generation of murine HSC:*** Undifferentiated HSC (Lin$^-$ Sca1$^+$ c-Kit$^+$) were isolated from murine bone marrow, separated by MACS (magnetic-assisted cell sorting) and characterized by fluorescence-activated cell sorting (FACS) [3]. ***Migration assay:*** HSC were pretreated *in vitro* in the absence or presence of 10 µM treprostinil (Trep) in combination with 30 µM forskolin (Fsk). After incubation for either 1 h or 24 h at 37°C in StemSpan™ SFEM

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containing growth factors, 100 µl cells were placed on the top of 2-chamber transwells followed by 4 h incubation at 37 °C with or without 100 ng/ml stromal cell-derived factor 1 (SDF-1) in bottom chambers. Completely migrated cells through a 5 µm filter were counted by a cell counter. ***RT-PCR:*** Total RNA was isolated and reversely transcribed from pretreated and untreated cells according to standard protocol. Prostaglandin receptors (EP_1, EP_2, EP_3, EP_4) and CXCR4 expression were performed using RevertAid First Strand cDNA Synthesis Kit according to standard protocol and adjusted to expression of GAPDH. ***Colony-forming assay (CFA):*** Murine HSC treated with Trep (10 µM) and Fsk (30 µM) or vehicle control were assessed for *in vitro* growth potential with the use of the CFA. The cells were then resuspended in MethoCult containing growth factors required for both colony-forming unit granulo-monocyte (CFU-GM) and colony-forming unit erythrocyte (CFU-E). The number of colonies was scored according to standard criteria. ***Murine and human HSC transplantation:*** Murine HSC cells were pretreated with Trep and Fsk or vehicle control for 1 h at 37 °C. Then, cells were washed, resuspended in PBS and injected into lethally irradiated recipient C57BL/7 mice. One group of recipient mice was also further treated with Trep. This was repeated by using human $CD34^+$ cells derived from umbilical cord blood and applied in to NOD scid gamma mice.

Results: Pretreament of murine and human HCS with treprostinil and forskolin was able to enhance HSC chemotaxis to SDF-1 without affecting cell differentiation. This functional effect is also reflected at the mRNA level. In addition, our data obtained in HSC transplantation indicate an *in vivo* relevance of our findings.

Discussion: To date, no pharmacological strategies are approved for direct stimulation of engraftment and/or migration of HCS. The therapeutic potential and clinical relevance rests in the unmet medical need of patients with either limited HCS cell number availability and/or high risk of infections.

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A1.3

In vivo analysis of cytotoxic effects of alpha-tocopheryl succinate in Ehrlich ascites carcinoma

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Intrinsic Activity, 2014; **2**(Suppl. 1): A1.3

Background: Tumour-specific targeting of distinct molecular pathways involved in the apoptotic process of malignant cell represents a highly beneficial concept for the development of novel anti-cancer therapeutics. Alpha-tocopheryl succinate (α-TOS) has been shown to selectively inhibit cell proliferation and to induce cell death in a variety of transformed cell lines while sparing normal cells. The aim of this study was to evaluate the effects of the administration of α-TOS on the vitality of Ehrlich ascites carcinoma cells (EAC) as well as its influence on the activity of glutathione-dependent antioxidative enzymes in EAC.

Methods: Two days after the transplantation of EAC cells in the abdominal cavity, Swiss mice were intraperitoneally treated with 10 µmol or 20 µmol α-TOS (50 or 100 µl of a 0.2 M solution) each third day, four days in total. Aseptic evacuation of the peritoneal content on the tenth day post-transplantation enabled the collection of EAC, and cell viability and enzyme activity were assessed. All experimental procedures were approved by the Ethical Committee of the University of Novi Sad.

Results: We observed a decreased viability of EAC cells in treated animals, indicated by the leakage of cytosolic lactate dehydrogenase (LDH) into the extracellular compartment, which was significantly increased in the groups treated with 10 µmol ($p = 0.002$) and 20 µmol ($p = 0.01$) α-TOS compared to control. The specific activities of glutathione-dependent antioxidative enzymes showed a dose-dependent decrease in both experimental groups; however, only the decrease in specific activity of glutathione peroxidase (GPX) was statistically significant ($p = 0.003$ for 10 µmol, and $p = 0.0005$ for 20 µmol α-TOS). The concentration of total intracellular proteins was also significantly decreased in treated groups ($p = 0.053$ for 10 µmol, and $p = 0.045$ for 20 µmol α-TOS) compared to control, which is in accordance with results that show a decreased activity of antioxidant enzymes as well as increased leakage of LDH.

Discussion: The results of pro-oxidant activity of α-TOS in EAC cells using the *in vivo* model followed by decreased viability of malignant cells may indicate potential anti-tumour properties of α-TOS, which could be considered as a promising adjuvant agent in developing novel therapeutic strategies.

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, grant no. III41012.

A1.4

Potential role of K^+ currents in the repolarization reserve: the importance of cardiac repolarization reserve in understanding the proarrhythmic side effects of antiarrhythmic drugs

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Intrinsic Activity, 2014; **2**(Suppl. 1): A1.4

Background: Although antiarrhythmic drugs are prescribed to reduce an arrhythmia, they may have the paradoxical effect of actually exacerbating that arrhythmia or causing new or more serious forms. Proarrhythmia is a new or more frequent occurrence of pre-existing arrhythmias, precipitated by antiarrhythmic therapy, which means it is a side effect associated with the administration of some existing antiarrhythmic drugs, as well as drugs for other indications. In other words, it is a tendency of antiarrhythmic drugs to facilitate emergence of new arrhythmias. The CAST and SWORD trials were the first that revealed this dangerous side effect of antiarrhythmic medication. Extensive investigations started then to elucidate the mechanisms which cause proarrhythmia, and in particular special interest was paid to understand the proarrhythmic effect of non-cardiac medication. An important discovery in the investigation of this phenomenon was the introduction of the term of

repolarization reserve by Roden [1]. This term helped to understand how the cardiac repolarization is modified by pharmacological tools.
Methods: This study compared the contribution of the four main potassium currents I_{K1}, I_{to}, I_{Kr} and I_{Ks} currents to cardiac repolarization in mammalian (including human) ventricular preparations by *in vitro* and *in vivo* electrophysiological and molecular biological techniques.
Results: In pathological settings, when repolarization reserve is impaired, the relatively mild block of additional K^+ current can cause marked APD/QT interval prolongation. When this normal repolarization reserve is attenuated the otherwise minimal or moderate potassium current inhibition can result in excessive and potentially proarrhythmic prolongation of the ventricular action potential duration. Congenital ion channel defects, ion channel remodeling due to myocardial infarction, heart failure, diabetes mellitus etc. can lead to impaired repolarization reserve.
Discussion: We should re-evaluate our safety pharmacology concept related to possible QT lengthening effects of drugs to apply tests in preparations where the repolarization reserve is impaired instead of using preparations where this reserve is normal.
Acknowledgements: This research was supported in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 ("National Excellence Program – Elaborating and operating an inland student and researcher personal support system"), a project subsidized by the European Union and co-financed by the European Social Fund, by OTKA (NN-109904 and NK104331) and by the National Research Council Project IDEAS (Programme "Exploratory Research Projects" PN-II-ID-PCE-2012-4-0512).

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A1.5

Role of histone acetylation and dopaminergic signalling in promoting long-term and context-independent fear extinction

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.5

Background: Anxiety disorders are associated with an inability to extinguish learned fear. Current treatments including exposure-based therapies are only partially effective as return of fear is commonly observed. Mirroring anxiety patients, 129S1/SvImJ (S1) mice exhibit profound resistance to induce fear extinction, which is rescued by dietary zinc restriction (ZnR). Here, we show that dietary ZnR can also protect against spontaneous return of fear and fear renewal in a novel context; revealing a treatment to identify key factors leading to sustained and context-independent fear extinction. To elucidate the underlying molecular mechanisms, we assessed gene expression changes following successful ZnR-induced fear extinction. In addition, as acetylation of lysine residues on histone proteins are important steps in promoting gene expression and classical histone deacetylases are Zn-dependent enzymes, we measured lysine acetylation changes following fear extinction in ZnR S1 mice.
Methods: S1 mice were subjected to multi-trial cued fear conditioning/extinction paradigms to measure the ability of ZnR to promote long-term and context-independent protection against the return of fear (spontaneous recovery/fear renewal). Gene expression changes following successful ZnR-induced extinction were quantified 2 h post extinction training using microarrays. Lysine acetylation changes following fear extinction in ZnR S1 mice were measured by fluorescent immunohistochemistry and it was assessed whether the extinction-induced increases in histone acetylation are present in the promoter regions of the differentially regulated genes using chromatin immunoprecipitation.
Results: Gene microarray analysis following successful ZnR-induced fear extinction identified a selective cohort of differentially regulated genes in the amygdala, a brain area known to be involved in extinction. Strikingly, many differentially regulated genes were related to dopaminergic signalling, e.g. dopamine receptor D_1 and D_2 genes, suggesting that the dopaminergic system can be one possible mechanism via which dietary ZnR leads to persistent fear extinction. Indeed, pharmacological enhancement of dopaminergic signalling via L-DOPA following extinction training rescued the impaired extinction consolidation in S1 mice. The results obtained by fluorescent immunohistochemistry and chromatin immunoprecipitation revealed a significant increase in lysine acetylation in the medial prefrontal cortex (mPFC) of extinguishing ZnR S1 mice as compared to non-extinguishing control-fed S1 mice; this was correlated with enhanced histone acetylation in the promoter region of the extinction-regulated dopamine D_1 and D_2 receptor genes in the mPFC of extinguishing ZnR S1 mice.
Discussion: The current data show that ZnR induces extinction of learned fear in a psychopathological animal model and promotes sustained and context-independent fear inhibition, which is an important clinical aim. Changes in gene expression and histone acetylation following successful ZnR-induced fear extinction suggest that the dopaminergic system can be one possible molecular mechanism via which dietary ZnR leads to persistent fear extinction. This is further confirmed as pharmacological enhancement of dopaminergic signalling rescued the extinction consolidation deficits in S1 mice. Collectively, these results suggest that histone deacetylase (HDAC) inhibitors and dopaminergic agents mediate the preservation and context-independency of rescued fear extinction in S1 mice and thus represent promising targets for the development of pharmacological adjuncts for exposure therapy in human anxiety disorders.
Acknowledgements: Supported by the Austrian Science Fund FWF (SFB F4410).

A1.6

The influence of the intestinal environment in drug bioavailability

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.6

Background: The role of the intestinal tract is becoming more profoundly evident in drug metabolism, absorption and overall efficacy. The aim of this review was to summarize the current knowledge regarding presystemic drug metabolism in the gut, and elucidate the role of intestinal microbiota, enterocytes, transporters and various gut enzymes in the fate of orally administered drugs.
Methods: We have analysed original and review articles, published from 2000 to 2014, using the keywords 'gut microflora' and 'drug metabolism'.

Results: The gut luminal fluids, intestinal mucosa and gut microflora contain high concentrations of various enzymes which are involved in metabolism of many drugs. Part of the metabolic capacity seems to be in close relation with the presence of cytochromes. The most common P450 cytochrome subfamily expressed in the mucosa of the small intestine is CYP3A, which represents the major intestinal CYPs. Of particular importance is the synergistic function of P-glycoprotein and CYP3A4 in the small intestine, which limits oral drug bioavailability of a wide variety of compounds. Transporter expression in the intestine affects how much of a drug will reach the systemic circulation after oral administration, thus suggesting that factors which affect their expression and function may change pharmacokinetics, efficacy and safety profiles of drugs. Esterases represent another important group of metabolizing enzymes in the gut. On the other hand, a wide range of phase-II enzymes (UDP-glucuronosyltransferases, sulfotransferases, acetyltransferases, glutathione S-transferases, methyltransferases) are found in the human intestinal mucosa, contributing to the presystemic metabolism of many drugs. Additionally, the intestinal microflora is capable of carrying out a number of metabolic reactions resulting in the production of metabolites required for the physiological activity or conversely in the inactivation or even in the production of toxic products.

Discussion: This review [1] highlights the influence of the intestinal microflora, intestinal transporters and enzymes on the fate of drugs in the host. The combined activities of drug transporters and metabolic enzymes present in luminal fluids and in the intestinal mucosa have shown to significantly influence pharmacokinetic profile of a range of drugs. However, despite broad investigations, this complex system has not yet been fully clarified. Deepening our knowledge in this area, we will be in a better position to predict the behavior of drug in the organism and to optimize the therapy for patients.

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, grant no. III41012.

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A1.7

Repeated water avoidance stress causes resilience against colitis-induced behavioural changes in mice

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.7

Background: Inflammatory bowel disease (IBD) is associated with an increased risk for several psychiatric disorders including generalized anxiety and major depression. The aim of this work was to investigate whether dextran sulfate sodium (DSS)-induced colitis is a valid model to study the gut–brain axis in IBD. We investigated behavioural, hormonal, and neuropeptide changes in the DSS colitis model alone and in combination with water avoidance stress (WAS).

Methods: Four groups of male C57BL/6N mice were studied: control mice, mice treated with DSS (2% in drinking water), mice exposed to WAS for 1 hour daily, and mice treated with DSS+WAS. After 1 week of treatment, emotional behaviour was assessed with the open field (OF) test, the social interaction test (SIT) and the tail suspension test (TST). Colonic myeloperoxidase (MPO) was used to evaluate colitis severity. In a separate set of animals, plasma corticosterone was measured by ELISA, and hippocampal and hypothalamic expression of glucocorticoid receptors (GR), mineralocorticoid receptors (MR), corticotropin-releasing factor (CRF), neuropeptide Y (NPY), brain-derived neurotrophic factor (BDNF), cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) were measured by RT-PCR. Statistical analysis was done with two-way ANOVA, one factor being DSS, and the other factor being WAS.

Results: DSS treatment caused a significant increase in colonic MPO ($p < 0.001$); this increase was unaffected by WAS. In the OF test, DSS treatment had an anxiogenic effect as it reduced the time spent in the central zone ($p < 0.01$) and the number of central zone visits ($p < 0.001$). Social interaction in the SIT was attenuated by DSS treatment ($p < 0.05$). These behavioural effects of DSS treatment were absent in mice subjected to DSS+WAS treatment; WAS alone had no effect on the behaviour in the OF test and SIT. The immobility time in the TST remained unchanged by the DSS and WAS treatments. The plasma levels of corticosterone were significantly elevated in the presence of both WAS and DSS ($p < 0.01$). In the hippocampus, DSS significantly reduced the relative expression of BDNF ($p < 0.001$), NPY mRNA ($p < 0.01$) and MR ($p < 0.001$) whereas WAS significantly decreased the relative expression of CRF mRNA ($p < 0.05$). Hippocampal GR and COX-1 were not affected by DSS or WAS. In the hypothalamus, the relative expression of NPY mRNA was significantly elevated in the presence of both WAS and DSS ($p < 0.001$). DSS-induced colitis increased the relative expression of hypothalamic but not hippocampal COX-2 mRNA ($p < 0.01$). The expression of GR, MR, COX-1, CRF and BDNF mRNA in the hypothalamus was not significantly affected by DSS or WAS.

Discussion: DSS-induced colitis caused behavioural and neuropeptide changes comparable to those observed in chronic stress models. Repeated WAS caused resilience to the behavioural changes associated with DSS-induced colitis although colitis was not changed. The WAS-evoked resilience to the behavioural effects of colitis may be related to increased circulating corticosterone and increased hypothalamic NPY gene expression.

Acknowledgements: Supported by the Austrian Science Fund FWF (grant P23097-B18).

A1.8

Retinal morphology of a $Ca_V1.4$ mouse model for congenital stationary night blindness type 2

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.8

Background: $Ca_V1.4$ L-type calcium channels, encoded by the *CACNA1F* gene, are most abundantly expressed in retinal photoreceptor synapses and are important for synaptic transmission between photoreceptor and second order neurons. Mutations in the *CACNA1F* gene are linked to the congenital stationary night blindness type 2 (CSNB2) in humans. The gain-of-function mutation $Ca_V1.4_{I745T}$ was discovered in a New Zealand family with severe clinical pictures of CSNB2. Functional analyses in a heterologous expression system showed a strong leftward shift in the $Ca_V1.4$ activation curve. This finding indicates a reduction in the photoreceptors' dynamic range. Electroretinograms of mutant mice

carrying the $Ca_V1.4_{I745T}$ mutation (IT mice) revealed a decrease in rod and a loss of cone responses upon light stimuli. This finding correlates well with ERGs from human patients. Here, we focus on the morphological consequences of this mutation in the IT mouse model.

Methods: ***Immunohistochemical analyses:*** Eyes from 10 to 11 weeks old wild-type (wt, C57BL/6N) and IT mice were collected, fixed with 4% paraformaldehyde for 20 min and 2 hours, cryoprotected via graded sucrose steps (10% and 20%, or only 30%) and embedded in 1:1 mixtures of OCT and sucrose (20% or 30%, respectively). Sixteen-µm-thick sections were produced for staining. For the washing steps, 1×PBS, 0.1% Triton X-100, 0.05% sodium azide was used. The first antibody was incubated overnight at 4°C and the second antibody was incubated for 1 hour at room temperature. Samples were imaged with a fluorescence microscope (Zeiss, Axiovert 200M). Three mice per mouse model were analyzed. ***Quantitative RT-PCR:*** This was performed from whole retinas, brain and muscle from 8-week-old wt and IT mice.

Results: Immunohistological analyses of IT retinas showed a reduction in thickness of the mutant retina. In qRT-PCR experiments a reduction in expression of $Ca_V1.4$, β2, and α2–δ4 subunits was evident in IT mice. Both results are likely to be explained by a loss of mainly rod photoreceptors. However, because cone function also was affected, we used cone-specific markers to screen for aberrations in the cone morphology. We observed a shortening of cone outer segments. Moreover, in some cones sprouting was detectable. Second order neurons as such, rod and cone bipolar cells, as well as horizontal cells formed elongated ectopic dendrites to establish contact with photoreceptor synapses. Synaptic morphology resembled immature synapses, a finding that supports the role of $Ca_V1.4$ for synapse development and maturation.

Discussion: Overall, the $Ca_V1.4_{I745T}$ gain-of-function mutation leads to a disrupted integrity of the outer plexiform layer in mice. We further suggest that the IT mouse model can help to improve our understanding of the underlying mechanism and pathophysiology of the CSNB2 dysfunction also in humans.

Acknowledgements: This study was funded by the Austrian Science Fund FWF (P22526, SFB F44020 and F44060) and the Medical University of Vienna.

A1.9

Cytosolic heat shock proteins as key players in serotonin transporter folding

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.9

Background: Solute carrier 6 (SLC6) proteins exit the endoplasmic reticulum (ER) compartments by recruiting the coat protein complex II (COPII) machinery. The COPII component SEC24 is responsible for the interaction with specific ER export motifs located on the C-terminal domains of cargo proteins. We have previously shown that ER export of the serotonin transporter (SERT) relies exclusively on SEC24C [1] and that mutations in the C-terminal SEC24 binding motif of SERT impair folding of the transporter [2]. We here propose a model where SCL6 transporters first associate with heat shock proteins (HSPs) until they adopt their final stable conformation, which allows release of HSPs and renders the ER export motif accessible to SEC24C.

Methods: We examined the role of HSPs in SERT folding by measuring specific [^{3}H]5-HT uptake, [^{3}H]imipramine binding, GST pull-down assays, immunoblotting and FRET microscopy.

Results: We verify that HSP isoforms 70 and 90β are involved in SERT folding, based on the following evidence: (i) SERT interacts with HSP70 only in the ER compartments but not at the plasma membrane, (ii) the presence of an HSP binding motif on the SERT C-terminus, (iii) co-immunoprecipitation of HSP70-1A and HSP90β with SERT, (iv) siRNA-induced knock-down or inhibition of HSP70 and HSP90β affects the affinity of pharmacochaperones (e.g. noribogaine), reflective of the folding state of SERT mutants, (v) HSP70 shows a dramatically higher association with a SERT mutant stalled in an intermediate stage of folding and (vi) these effects could be recapitulated in JAR cells endogenously expressing SERT.

Discussion: Our results shed new light onto protein folding mechanisms of SERT and other SLC6 family members. Inhibition or depletion of HSPs promotes cell surface expression of folding-deficient transporter mutants. ER resident luminal chaperones (e.g. calnexin) are required for protein folding. We here identify cytosolic HSP chaperones as additional key players in the protein folding trajectory of SERT. Depleting HSPs and/or treating ER-retained SERT variants with pharmacological chaperones can rescue their expression and activity at the cell surface. An association between HSP70 activity and response to antidepressant treatment has already been reported in the literature [3]. Our findings may have therapeutic potential, particularly in the treatment of neuro-psychiatric disorders resulting from defective folding of monoamine transporter proteins.

Acknowledgements: This research was financially supported by a programme grant SFB35.

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A1.10

$Ca_V1.3$ L-type calcium channel contribution to retinal activity

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.10

Background: The retina is built up by different neuronal cell layers, roughly consisting of an input region, the photoreceptors, at which light stimuli are transduced to neuronal code and an output region represented by the ganglion cells. Bipolar, horizontal and amacrine cells further integrate signals from the photoreceptor, process and finally forward them to ganglion cells. Communication between these cell layers is mainly mediated by chemical synapses at which upon calcium influx neurotransmitter is released. L-type calcium channels, among those $Ca_V1.4$ and $Ca_V1.3$ channels, play a key role in mediating persistent calcium influx required for vesicle fusion. The functional importance of $Ca_V1.4$ channels is evident from the fact that mutations in $Ca_V1.4$ α1 subunits impair signal transmission between photoreceptor cells and second-order neurons. Reports

about the expression pattern of $Ca_V1.3$ channels in the retina are heterogeneous, but there are indications that $Ca_V1.3$ is expressed in a certain type of mouse amacrine cells and in salamander ganglion cells. However, the role of $Ca_V1.3$ channels in the retina and their contribution to retinal activity is mainly unknown.

Methods: To elucidate whether $Ca_V1.3$ channels contribute to retinal activity we performed multi-electrode analysis of whole-mount retinas of wild-type (WT) and $Ca_V1.3$-deficient ($Ca_V1.3$-KO) mice. Retinas were dark-adapted for 30 min before and after eye dissection. Ganglion cell activity elicited by light–dark stimuli (full-field flashes or white-noise jitter) was recorded at 37 °C during continuous superfusion with carbogen-equilibrated Ames buffer. ***Retina stimulation:*** full-field flashes: 0.5 s 14 mW/m^2 light pulses with 1.5 s 20 µW/m^2 dark interval, 300 repetitions; white-noise jitter: 32×32 checkerboard flicker based on a zero mean Gaussian white noise with a standard deviation of 0.3, pixel size corresponds to 75.6 µm of retina, 12,000 frames and 6 repetitions. Spike sorting was conducted using custom-made scripts.

Results: Spike-triggered averages of the ganglion cell responses evoked by white-noise jitter stimulation of $Ca_V1.3$-deficient mice retinas revealed increased time-to-peak response latencies (latency [ms]: WT: ON: 65 ± 1, OFF: 74 ± 3; $Ca_V1.3$-KO: ON: 92 ± 5 OFF: 97 ± 3). Receptive field size seemed to be unaffected (size [µm]: WT: ON: 123 ± 4, OFF: 141 ± 8; $Ca_V1.3$-KO: ON: 118 ± 12, OFF: 142 ± 12). Twelve or 17 electrodes of 2 mice each, WT and $Ca_V1.3$-KO, respectively, were analysed. Data: mean ± SEM.

Discussion: From our experiments we suggest that $Ca_V1.3$ calcium channels can indeed contribute to retinal activity. Though, further approaches like pharmacological silencing of ON and OFF retinal signaling pathways and/or combined intracellular recordings as well as immuncytochemical analyses will be required to further elucidate the role of retinal $Ca_V1.3$ channels.

Acknowledgements: Funding: FWF (P22526 to A.K.), SFB F44 (02 to J.S. and A.K., 06 to G.J.O.) and the Medical University Vienna.

A1.11

Signaling mechanisms of 5-HT$_2$ receptors in primary sensory neurons

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Intrinsic Activity, 2014; **2**(Suppl. 1): A1.11

Background: Serotonin (5-HT) is an inflammatory mediator and involved in pain sensation. Ionotropic 5-HT$_3$ receptors of dorsal root ganglion (DRG) neurons are thought to mediate this effect. Here, the contribution of metabotropic 5-HT receptors and their functional interactions with K_V7 and TRPV1 channels was investigated.

Methods: Using the perforated patch clamp technique on DRG neurons of newborn rats in primary cell culture, effects of 5-HT receptor ligands on membrane potential and various currents were investigated.

Results: 5-HT increased the excitability of DRG neurons and caused depolarizations. This effect was not altered by the 5-HT$_3$ receptor antagonist tropisetron, but reduced by the 5-HT$_2$ receptor antagonist ritanserin. The 5-HT$_2$ receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine ((±)-DOI) also raised the excitability of DRG neurons. Currents through K_V7 channels of DRG neurons were not inhibited by 5-HT, but reduced by (±)-DOI in a concentration-dependent manner by up to 32.6 ± 6.9% (n = 20). Furthermore, K_V7 channels of DRG neurons were inhibited in the presence of ritanserin by up to 14.5 ± 3.8% (n = 9), and the effects of (±)-DOI and ritanserin were additive. Currents through channels formed by $K_V7.2/K_V7.3$ heteromers expressed in tsA201 cells (which do not express 5-HT$_2$ receptors) were also significantly attenuated by (±)-DOI (up to 17.1 ± 5.6%) and ritanserin (up to 24.6 ± 7.8%) (n = 13), but were not altered by another 5-HT$_2$ receptor antagonist (ketanserin) nor by 5-HT (n = 11). In tsA201 cells coexpressing 5-HT$_2$ receptors and $K_V7.2/K_V7.3$ heteromers, 5-HT also failed to suppress currents (n = 14), whereas coexpressed muscarinic M_1 and bradykinin B_2 receptors mediated an inhibition. Recombinant 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, nevertheless, mediated increases in intracellular Ca^{2+}. In DRG neurons, 5-HT$_2$ receptor activation enhanced currents through TRPV1 channels.

Discussion: These results indicate that the 5-HT$_2$ receptor ligands (±)-DOI and ritanserin can interact with neuronal K_V7 channels independently of 5-HT$_2$ receptors. In DRG neurons, activation of 5-HT$_2$ receptors mediates enhanced excitability, an effect that involves sensitization of TRPV1 channels.

A1.12

The role of hydrogen sulfide in autonomic nervous system

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Intrinsic Activity, 2014; **2**(Suppl. 1): A1.12

Background: Hydrogen sulfide (H_2S) is a toxic gas also produced in mammalian tissues where it can exert various functions. H_2S has also been shown to act as endogenous neuromodulator. A recent study showed that H_2S is endogenously generated and released in sympathetic ganglia and potentiates ganglionic transmission [1].

Methods: Experiments were performed on primary cultures of rat superior cervical ganglion (SCG) or on transfected tsA cells. Neurotransmitter release was determined by measuring the outflow of radioactivity from cultures labelled with [^{3}H]noradrenaline. Electrophysiological recordings were performed by using the perforated patch-clamp technique.

Results: In radiotracer release experiments, tritium overflow triggered by either electrical fields or by depolarizing K^+ concentrations was reduced by 0.1 to 1 mM of the H_2S donor NaHS in a concentration-dependent manner. In contrast, overflow triggered by activation of nicotinic acetylcholine receptors (by 100 µM dimethylphenylpiperazinium; DMPP) was enhanced in a concentration-dependent manner. This latter effect was paralleled by an enhancement of DMPP-evoked currents by NaHS in SCG neurons. The inhibitory effect, in contrast, is rather related to an inhibition of voltage-gated Ca^{2+} currents by NaHS. In addition, we found NaHS to transiently enhance spontaneous transmitter release and to reduce currents through K_V7 channels, whether endogenously expressed in SCG neurons or heterologously expressed in tsA cells.

Discussion: These results show that H_2S regulates various functions of ganglionic neurons, the most prominent effect being a potentiation of nicotinic acetylcholine receptors.

Acknowledgements: M.D.R. is member of the doctoral programme “Cell Communication in Health and Disease” (CCHD; co-financed by the Austrian Science Fund FWF and the Medical Univesity of Vienna; W1205).

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A1.13

Transmembrane proteins of *Fasciola hepatica:* identification and characterization of new putative drug targets

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.13

Background: *Fasciola hepatica*, a parasitic flatworm (Platyhelminthes: Digenea), is the cause of one of the most important diseases affecting animal health all over the world, causing the so-called liver fluke disease (fascioliasis). As vaccinations against this parasite are not available yet, anthelmintic drugs, like triclabendazole (TCBZ), are the treatment of choice. During the last decades more and more flukes resistant to these drugs have been found. Molecular mechanisms underlying TCBZ resistance could be mediated by various mechanisms: (i) accelerated metabolism of drugs, (ii) mutations in target proteins which eliminate drug sensitivity, (iii) accelerated drug efflux via transmembrane transporters of the family of ABC transporters. The last scenario is very common in nature and is also suspected to have major role in drug resistance against TCBZ in *F. hepatica*.
Methods: Using adult flukes obtained from Northern Ireland and Lower Austria, next-generation sequencing (NGS) has been performed to identify the mutation that confers drug resistance between TCBZ-resistant and susceptible flukes. In parallel, we also performed RACE (rapid amplification of cDNA ends) to generate a full length transporter of *F. hepatica* which has not been cloned before.
Results: We did preliminary work to see the difference between TCBZ-resistant and -susceptible flukes, if there is at all. A bioinformatics part is the next step that we will take into consideration. We also have managed to go further for the sequences of transporters of *F. hepatica*; however, we are not at that step where we can go for functional assays yet.
Discussion: Using the findings of the all-experimental methods, this project proposes to identify genes of *F. hepatica* that play a crucial role in the life cycle and in the development of TCBZ drug resistance, to characterize the encoded proteins and to screen for ligands which may lead to the design of new anthelmintic therapeutic strategies.
Acknowledgements: Supported by AIT (1.G8.00016.0.0).

A1.14

Amphetamines and SERT: what's the N-tail got to do with it?

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Background: We have previously reported that deletion of the first 64 amino acids of the serotonin transporter (SERT) N-tail region, leads to complete abolition of amphetamine-induced efflux [1]. We have also identified a highly conserved threonine (Thr 81), residing at the juxtamembrane region of the N-tail, as another key site for amphetamine-induced reverse transport by SERT [1]. In this study, we further elucidate how the N-tail domain promotes outward substrate transport by monoamine transporters.
Methods: A series of truncated variants or alanine mutants were created along the N-tail domain of hSERT by PCR and site-directed mutagenesis (Quikchange kit, Stratagene). To examine whether the mutated versions of SERT affected the delivery of SERT proteins to the plasma membrane, we examined all (YFP-tagged) mutations by confocal laser scanning microscopy. We used tryptic digestion to assess conformational changes in the N-tail induced by a variety of buffer (ion replacement) conditions in the absence or presence of amphetamine, imipramine or ibogaine. An extensive pharmacological characterisation of the mutants was carried out using radioligand uptake, release and binding assays.
Results: None of the N-tail mutants investigated in the present study impaired the trafficking of SERT to the cell surface. However, detrimental effects were observed in amphetamine-induced substrate release by several mutants, particularly those located in the mid fragment of the N-tail. These defects in outward transport, however, were not due to impaired serotonin uptake kinetics (i.e. no significant changes in the K_m and V_{max} values compared to the wild-type SERT). Moreover, all mutants displayed normal affinity for amphetamines and ibogaine, measured from inhibition of imipramine binding. In outward-facing conformations of SERT, the N-tail was found to be protected from cleavage by trypsin. Remarkably, amphetamines were able to shield the transporter from becoming digested even in the inward-facing conformational states.
Discussion: The N-tail domain plays an indispensable part in asserting amphetamine action on monoamine transporters. Our current data provide new evidence in support of the recently proposed mechanistic model [1], where the N-tail acts as a lever activating the second moiety of the transporter oligomer, allowing substrate release to take place.
Acknowledgements: This project was supported by SFB35.

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A1.15

Resveratrol and its derivative piceatannol modulate mitochondrial Ca^{2+} uptake via inhibition of ATP synthase

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.15

Background: Mitochondria, the cellular power plants, supply the cell with ATP and are crucially involved in cell differentiation, cell cycle and intracellular signalling. Because they are essential for cell survival, their role in human diseases and aging processes are under intensive investigation. The transfer of Ca^{2+} into the matrix of mitochondria is an important signalling process that substantially contributes to multiple cellular functions. For instance, the Ca^{2+} concentration within mitochondria ($[Ca^{2+}]_{mito}$) controls the metabolic activity of this organelle and can also provoke cell death pathways.
Methods: To investigate the role and possible mechanism(s) of cell death pathways triggered by mitochondrial Ca^{2+}, we used resveratrol and its derivative, piceatannol, which are known to cause apoptosis in certain cancer cells. Using genetically encoded, mitochondria- or endoplasmic reticulum (ER)-targeted, fluorescent Ca^{2+} probes like FRET-based 4mtD3cpv as well as the fluorescent dye Fura-2AM, we investigated how these compounds affect Ca^{2+} homeostasis in HeLa cells. Moreover, we studied the effect of these compounds on cell viability by MTT assay.
Results: Our results indicate that chemical compounds such as resveratrol, piceatannol and *N,N'*-dicyclohexylcarbodiimide (DCCD), which specifically bind to the F_1 subunit of the ATP synthase, boost

an IP_3-generated mitochondrial Ca^{2+} uptake via inhibition of SERCA. Inhibition of SERCA caused a slower refilling of ER Ca^{2+} stores, a higher Ca^{2+} concentration in the gap between mitochondria and ER and finally an increased mitochondrial Ca^{2+} uptake. Moreover, extensive mitochondrial Ca^{2+} uptake resulted in mitochondrial Ca^{2+} overload, opening of the mitochondrial permeability transition pore (mPTP) and ultimately cell death.

Discussion: The anti-cancer properties of resveratrol and its derivative piceatannol are due to modulation of mitochondrial Ca^{2+} uptake via inhibition of mitochondrial ATP synthase, a multi-protein complex of the inner mitochondrial membrane. Since inhibition of ATP synthase leads to apoptosis in glycolytic cells, ATP synthase is a promising target for anti-cancer drugs.

Acknowledgements: C.T.M.'s work is funded by the Austrian Science Fund FWF (DK Metabolic and Cardiovascular Disease) at the Medical University of Graz.

A1.16

Age-dependent sensitivity to glucocorticoids in the developing mouse basolateral nucleus of the amygdala

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.16

Background: Experiences of severe trauma during childhood are thought to be risk factors for developing mental disorders, such as anxiety and mood disorders, later in life. Correspondingly, exposure of rodents to early-life stress has been shown to affect neuronal circuitry and emotional behavior in adulthood, indicating a significant impact of stress on brain development. One current hypothesis proposes that the developing central nervous system is more sensitive to environmental influences, such as stress, than the adult. To test this hypothesis, we compared long-lasting effects of systemic corticosterone (CORT) administrations in two distinct early developmental periods.

Methods: We performed whole-cell patch clamp recordings of brain slices of C57BL/6J mice, contextual fear conditioning and extinction behavioral testing, as well as quantitative real-time PCR experiments.

Results: Mice exposed to early-neonatal CORT treatment on postnatal days (PD) 2–4 exhibited strongly enhanced excitability of neurons of the basolateral nucleus of the amygdala (BLA) in early adolescence and displayed impaired extinction of contextually conditioned fear memory, a type of behavior in which the BLA plays an important role. Furthermore, gene-expression of NMDA receptor subunits as well as calcium-activated K^+ channels was reduced in the amygdala. In contrast, exposure to the same CORT concentrations in a late-neonatal period (PD17–19) did not significantly affect BLA electrophysiology or extinction learning in adolescence.

Discussion: Glucocorticoid exposure in early-neonatal life appears to affect the development of passive membrane properties of amygdala neurons and lead to increased amygdala excitability in adolescence. Our findings indicate that the treatment with glucocorticoids in early postnatal days could be a suitable model to study the mechanisms that link early-life stress and psychiatric disorders. Furthermore, our results emphasize the importance of a stress-free environment, especially in early developmental periods, as these periods appear to be highly sensitive to stress hormones. We therefore conclude that the early-neonatal period presents a window of opportunity for CORT to induce long-lasting alterations in amygdala electrophysiology and related behavior.

Acknowledgements: This work was supported by the following grants to M.S.: KAKENHI (grant number 23500474) and Intramural Research Grant for Neurological and Psychiatric Disorders of the National Center of Neurology and Psychiatry (grant number 24-2 & 25-1).

A1.17

Investigating the pharmacology of L-type Ca^{2+} channels using stable cell lines and brain activity patterns

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.17

Background: L-type Ca^{2+} channels (LTCCs) respond to membrane depolarization by opening their conductive pore and thereby allow Ca^{2+} ions to enter the cell. $Ca_V1.3$, one of the two main brain LTCC isoforms, has been recently implicated in the pathophysiology of Parkinson's disease. LTCCs are particular sensitive towards organic Ca^{2+} channel blockers (CCBs), such as the dihydropyridine isradipine, which mostly act in a state-dependent way. Thereby, parameters such as the shape and frequency of the depolarizing stimuli can strongly influence drug responsiveness. Since usually cardiac myocyte-like long and infrequent square pulses are used to study the pharmacology of CCBs, IC_{50} values for CCB-mediated inhibition during neuronal activity patterns are unknown. Therefore, we generated stable cell lines of $Ca_V1.3$ channel constructs capable of reproducibly quantifying the pharmacological activity of CCBs under physiological recording conditions (2 mM Ca^{2+}) using neuronal activity stimuli.

Methods: Inducible cell lines stably expressing human $Ca_V1.3$ long and short splice variant ($hCa_V1.3_L$, $hCa_V1.3_S$) were generated using the Flp-In™ T-REx™ system (together with β3 and α2–δ1) and the expression of full-length α1 subunits biochemically confirmed using western blot analysis. Biophysical and pharmacological channel properties were measured by the patch-clamp whole-cell technique and compared with the same constructs transiently transfected in tsA-201 cells using the Ca^{2+} phosphate precipitation method.

Results: Stable cell lines expressing $hCa_V1.3_L$ and $hCa_V1.3_S$ exhibit regularly large current amplitudes (500–2000 pA) and similar biophysical properties compared to transiently transfected constructs. However, a ~8 mV left-shift of the Ca^{2+} (I_{Ca}) current–voltage relationship towards more hyperpolarized voltages as well as faster I_{Ca} inactivation were observed for both constructs in the stable cell lines. We show that the high expression level allow reliable recordings of $Ca_V1.3$ currents even under stimulation conditions (such as action-potential-like command voltages) that induce substantial steady-state inactivation of I_{Ca}. At present, we are also generating cell lines stably expressing $rbCa_V1.2_S$ and $Ca_V1.3$ channels containing *CACNA1D* mutations causing human diseases.

Discussion: In conclusion, our data demonstrate that inducible cell lines stably expressing LTCCs are a feasible approach for extensive drug screening under physiological recording conditions (2 mM Ca^{2+}) where large and consistent currents are required.

Acknowledgements: This work was supported by the Austrian Science Fund FWF (F44020, W11).

A1.18

Pyrimidine-2,4,6-triones are a new class of voltage-gated L-type Ca^{2+} channel activators

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Intrinsic Activity, 2014; **2**(Suppl. 1): A1.18

Background: The motor symptoms of Parkinson's disease, a non-curable neurodegenerative disorder, reflect the specific loss of dopaminergic substantia nigra pars compacta neurons. The cell death of these pacemaker neurons seems to be attributable to $Ca_V1.3$-mediated dendritic Ca^{2+} transients that add to an elevated level of mitochondrial oxidative stress. $Ca_V1.2$ and $Ca_V1.3$, the two main L-type Ca^{2+} channel (LTCC) isoforms found in the brain, cannot be efficiently discriminated in a pharmacological manner. Since $Ca_V1.2$ is also highly expressed in the cardiovascular system, a $Ca_V1.3$-selective blocker could constitute a promising neuro-protective therapy without blood-pressure lowering side effects.
Methods: Using the patch-clamp technique, we investigated the pharmacological modulation by Cp8 (a pyrimidine-2,4,6-trione derivative, recently reported as the first highly selective $Ca_V1.3$ blocker [1]) of I_{Ba} (10 or 15 mM) or I_{Ca} (15 mM) through $Ca_V1.3$ (rat or human long splice variant, $rCa_V1.3_L$, $hCa_V1.3_L$) and $Ca_V1.2$ (rabbit long or short C-terminus, $rbCa_V1.2_L$, $rbCa_V1.2_S$) α_1 subunits expressed together with β3 and α2–δ1 subunits in tsA-201 cells or using mouse chromaffin cells (MCCs; 2 mM Ca^{2+}).
Results: Unexpectedly, a change in gating kinetics of I_{Ba} and I_{Ca} through different $Ca_V1.2$ and $Ca_V1.3$ channel constructs, closely resembling the activity of known LTCC activators such as FPL 64176, was observed using 50 µM of Cp8. This modulation was characterized by a slowing of activation and inactivation as well as a profound enhancement of tail currents. However, in a minority of cells using Ba^{2+} as charge carrier no change in gating kinetics but a weak and non-selective inhibition of both channel isoforms could be observed. Furthermore, the activating properties of Cp8 could be confirmed on native LTCCs in mouse chromaffin cells (MCCs; 2 mM Ca^{2+}) where non-L-type currents were spared. Additionally, 50 µM Cp8 also increased the spontaneous firing frequency of MCCs and the total Ca^{2+} load during action potentials.
Discussion: Apart from a weak inhibition of both, $Ca_V1.2$ and $Ca_V1.3$, in a minority of cells using Ba^{2+} as charge carrier, neither a potent nor a $Ca_V1.3$-selective (reported IC_{50} 24.3 ± 0.7 µM [1]) inhibition by Cp8 was observed. Moreover, Cp8 induced an LTCC activator-like change in current gating kinetics in all Ca^{2+} and the majority of Ba^{2+} recordings, therefore suggesting that pyrimidine-2,4,6-triones can act as a new class of Ca^{2+} channel activators.
Acknowledgements: This work was supported by the Austrian Science Fund FWF (F44020, W11).

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A1.19

Anti-inflammatory and antinociceptive effects of a new peripherally acting µ opioid receptor agonist in experimental models of colitis in mice

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Background: Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the gastrointestinal (GI) tract, characterized by intestinal inflammation and mucosal damage. To date, the prevailing means of treating IBD have been unable to offer satisfactory long-term solutions. The µ opioid peptide (MOP) receptor, the main target for effective pain relief, is widely distributed in the central and peripheral nervous systems, as well as in peripheral non-neuronal tissues. In the periphery, the expression of MOP receptors in the GI tract has been reported in neuronal (myenteric and submucosal plexus), muscular and immune cells. This localization of MOP receptors is closely related to the role of MOP agonists in the GI tract, where they act as antinociceptive and immunomodulatory agents and inhibit GI motility. Several lines of evidence showed that the MOP receptor is involved in the pathophysiology of inflammatory GI disorders, directing toward the potential clinical relevance of targeting MOP receptors for the treatment of IBD. In this study, we describe the anti-inflammatory and antinociceptive actions of 6β-tryptophan-substituted 14-*O*-methyloxymorphone (HS1333), a novel peripherally acting MOP agonist, in mouse models of IBD.
Methods: Radioligand binding assays were performed using rodent brain membranes to determine opioid receptor affinity and selectivity. [^{35}S]GTPγS functional assays with Chinese hamster ovary cells expressing human opioid receptors were used to assess potency and opioid agonism. *In vitro* inhibitory effect on nuclear factor-κB (NF-κB) activation was assessed on THP-1 Blue cells by QUANTI-Blue assay. Anti-inflammatory effects were determined in mice using trinitrobenzene sulfonic acid (TNBS, intracolonic application)- and dextran sodium sulphate (DSS, orally)-induced colitis. Anti-nociceptive activities were assessed using acetic acid-induced abdominal stretching and mustard oil-induced acute colitis in mice.
Results: *In vitro* binding studies showed that HS1333 displays high MOP binding affinity, and potent and full agonist activities toward the MOP receptor. It significantly inhibited the NF-κB activation in THP-1 Blue cells upon stimulation with tumor necrosis factor-α (TNF-α) or lipopolysaccharide (LPS). After subcutaneous (s.c.) administration, the MOP receptor agonist HS1333 significantly attenuated TNBS- and DSS-induced colitis by showing preventive and therapeutic intestinal anti-inflammatory effects. Dose-dependent and significant inhibition of pain behavior was produced by s.c. HS1333 in two mouse models of abdominal pain induced by intraperitoneal acetic acid or intracolonic mustard oil.
Discussion: The present findings establish the efficacy of HS1333, having combined anti-inflammatory and antinociceptive properties, in experimental models of colitis, and the potential of peripheral MOP agonists as valuable therapeutics for the treatment of IBD.
Acknowledgements: Supported by the Austrian Science Fund FWF (P21350), the Tyrolean Research Fund (UNI-0404/949) and S&T Cooperation Fund Austria-Poland 2013-2014 (PL13/2013).

A1.20

Sewarine, an indole alkaloid from *Rhazya stricta* and a κ opioid receptor antagonist, induces apoptosis via caspase activation in various cancer cell lines, and inhibits NF-κB activation

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.20

Background: Cancer is a global health problem, and nowadays effective cancer therapy still remains one of the major medical challenges. Recent statistics show that in 2011 37,100 people were newly diagnosed with cancer in Austria, and cancer was the second reason of mortality in the USA in 2012. The different treatment strategies available (i.e. chemotherapy, immunotherapy, radiation and surgery) are largely associated with lack of efficacy, development of resistance and a plethora of side effects. Several lines of evidence indicate that the present cancer therapies exert their antitumor effect primarily by triggering apoptosis in cancer cells. Currently, cancer research has been continuously engaged in the identification of novel, more efficacious and safer anticancer drugs. Medicinal plants are tremendous sources of new drug candidates and are gaining increasing momentum for cancer therapy. Extracts of *Rhazya stricta*, a common medicinal plant used in traditional oriental medicine, are used to treat cancer, inflammatory conditions and chronic rheumatism. Scientific studies support the pharmacological activities (i.e. antitumor, anti-inflammatory and antioxidant) of extracts of *R. stricta* attributed to the presence of indole alkaloids. However, there are no reports describing the anticancer or other actions of any of these alkaloids. Recently, we have identified a phenolic indole alkaloid from *R. stricta*, sewarine, as the first naturally derived κ opioid peptide (KOP) antagonist. The present study was undertaken to investigate the potential anticancer and anti-inflammatory properties of sewarine *in vitro*, and mechanisms thereof.

Methods: Apoptosis induction was detected in different cancer cell lines by propidium iodide FACS analyses. Regulation of the pro- and anti-apoptotic proteins was determined by immunoblot. The effect on nuclear factor-κB (NF-κB) activation was assessed in human monocytic (THP-1 Blue) cell supernatants using QUANTI-Blue assay.

Results: Sewarine was found to induce apoptosis in a variety of human cancer cell lines, including leukemia (CEM, MOLT-3 and Jurkat), neuroblastoma (SH-SY5Y) and breast cancer cells (MCF-7), in a time- and concentration-dependent manner. Co-treatment with the pan-caspase inhibitor (zVAD-FMK) significantly reduced the level of sewarine-induced apoptosis, indicating that the activation of caspases is essential for the pro-apoptotic effects of sewarine. Sewarine activated pro-apoptotic BH3-only proteins (Noxa, Bim and Puma) and repressed the expression of anti-apoptotic proteins, Bcl-2 and survivin. The immunosuppressive effect of sewarine was assessed by determining its potential to inhibit NF-κB, a well-established marker for immune and inflammatory responses. A concentration-dependent inhibition of NF-κB activation was produced by sewarine in THP-1 Blue cells upon stimulation with tumor necrosis factor-α (TNF-α) or lipopolysaccharide (LPS).

Discussion: Sewarine, an indole alkaloid from *R. stricta* with KOP antagonistic properties, has potent pro-apoptotic effects via activation of caspase pathways and by modulating pro-apoptotic and anti-apoptotic proteins, together with an inhibitory effect of NF-κB activation. Our present findings provide the first evidence and a rational for the recognized anticancer effects described for the alkaloid extracts of *R. stricta*.

A1.21

Diphenethylamine derivatives, a novel class of κ opioid receptor ligands: molecular modeling, synthesis and pharmacological activities

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.21

Background: In recent years, the κ opioid peptide (KOP) receptor has received great attention as a prominent drug discovery target toward treatment of pain, depression, and drug addiction. There is considerable evidence that selective KOP agonists produce antinociception in animal models, while they do not cause physical dependence or respiratory depression, therefore being greatly attractive as potential analgesics. On the other hand, KOP antagonists and partial agonists have prospective value as antidepressants, anxiolytics and anti-addiction drugs [1]. The breakthrough in the opioid field made in 2012 in resolving the 3D structure of the human KOP receptor is nowadays providing significant details of the ligand binding pathways into this receptor at the molecular level, and ultimately gives essential insights for the design of ligands with new pharmacological properties targeting the KOP receptor. Recently, we have described the discovery of a new molecular scaffold for KOP ligands within the class of diphenethylamines [2]. The *N*-cyclopropylmethyl-substituted analogue was a selective KOP partial agonist, while the *N*-cyclobutylmethyl-substituted derivative (HS665) was identified as a novel highly selective KOP agonist with potent antinociceptive activity. With the currently available crystal structure of the human KOP receptor, the present study was undertaken to investigate the structural features that promote binding of these diphenethylamines to the KOP receptor via docking and molecular dynamics simulations.

Methods: The structural features that promote binding of the diphenethylamines to the human KOP receptor was investigated using docking calculations and molecular dynamics simulations. Radioligand and functional binding assays were performed with newly designed ligands in Chinese hamster ovary (CHO) cells expressing the human opioid receptors.

Results: *In silico* investigations revealed that the hydrogen bond formed by the phenolic hydroxyl group of HS665 with His291 is essential for KOP affinity and agonist activity. The hydrophobic pocket formed by the residues Val108, Ile316, and Tyr320 appears to be important for hosting the *N*-substituent, indicating that a chain of six carbon atoms is the critical length for agonist interaction. Biological studies with the radiolabeled form of HS665 confirmed its high affinity and KOP receptor specificity, making this molecule a valuable tool in probing KOP receptor pharmacology.

Discussion: The combination of molecular modeling and Pharmacological outcomes aided in the design of novel interesting KOP ligands from the series of diphenethylamines that was supported by structure–activity relationship studies. The current investigations provide further understanding of the binding mode of diphenethyl-

amine derivatives as a novel class of KOP ligands, and may be instrumental to the development of new KOP receptor therapeutics.

Acknowledgements: Supported by the National Institute on Drug Abuse (USA).

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A1.22

The C-terminus of SERT provides distinct motifs for ER export and protein folding

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.22

Background: The serotonin transporter (SERT) belongs to the class of monoamine neurotransmitter transporters. The protein is responsible for the reuptake of serotonin, released into the synaptic cleft, back into the intracellular compartment. Two fundamental requirements for fulfilling this function are: correct protein folding and vesicular export, both on the level of the endoplasmic reticulum (ER). In our recent works, we describe distinct protein motifs on the SERT C-terminus playing a major role in supporting folding and export [1,2,3]. We identified a motif for specific binding of SERT to the COPII subunit SEC24C; a hydrophobic motif for binding HSP70; an amphipathic helix harbouring biochemical properties which support protein folding. In conclusion, we are convinced that the C-terminus of SERT is a key regulator for designating the whole protein as correctly or incorrectly folded.
Methods: Mutations were introduced into the C-terminus of SERT and the effect of these mutations on protein folding, protein–protein interactions and ER export were examined using: substrate binding, GST pulldown, co-immunoprecipitation, Förster resonance energy transfer (FRET), confocal microscopy, serotonin uptake.
Results: Three C-terminal motifs determining protein folding and ER export are described in this work: (i) $RIIK^{607–610}$ defines specific binding to the COPII subunit SEC24C; (ii) $RLIIT^{596–600}$ binds the heat shock protein 70 (HSP70); (iii) an amphipathic helix which supports correct folding.
Discussion: A profound knowledge about the folding and export of monoamine neurotransmitter transporters is of great scientific and medical importance. Especially in the light of mental disorders like depression, bipolar disorder, attention deficit hyperactivity disorder (ADHD), or neurodegenerative diseases like Parkinson's disease. In the future, it will be especially interesting to investigate ways of driving the appropriate transporters to their correct conformation and thereby guiding them to the ER export machinery. In particular, our hope is to achieve this goal by pharmacochaperoning monoamine transporters.
Acknowledgements: This work was supported by the Austrian Science Fund FWF, SFB35 and Cell communication in Health and Disease (CCHD).

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A1.23

The effects of the neurosteroid dehydroepiandrosterone on rat behavior in the forced swim test

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.23

Background: Neurosteroid dehydroepiandrosterone (DHEA) has been associated with various functions in the central nervous system, including modulation of memory and behavior. It has been suggested that the effects of DHEA are mediated through several neurotransmitter systems; however its mechanisms of action are not fully understood. This study aimed to investigate the behavioral profile of DHEA in the forced swim test (FST), and also its impact on locomotor activity.
Methods: FST was performed in a glass cylinder, 45 cm high, 20 cm diameter, filled with water up to a height of 30 cm. On the first day, male Wistar rats were forced to swim for 15 min. Rats were re-exposed to the FST for a single 5 min session, after the acute and chronic challenge with vehicle or DHEA. The measurement of locomotor activity was performed in a clear Plexiglas box (40×25×35 cm) for 30 min without any habituation period. In the experiments, the animals received DHEA (2, 10, and 50 mg/kg) or vehicle. Afterwards, the capability of bicuculline (0.5, 1, and 2 mg/kg) to antagonize effects of DHEA was checked. Throughout the study, drugs were given intraperitoneally, 30 min before testing. The data were assessed by one-way ANOVA. If the ANOVA was significant, each treatment condition was compared with control by Dunnett's test ($\alpha = 0.05$). Where appropriate, the influence of the antagonist bicuculline on the effect of DHEA was assessed.
Results: In FST, ANOVA indicated statistically significant effects of DHEA. Dunnett's analysis showed that DHEA significantly decreased the duration of immobility at the dose of 10 mg/kg, exerting acute, but also chronic antidepressant-like effects. These effects were antagonized by bicuculline (2 mg/kg), a specific antagonist of the $GABA_A$ receptor. However, DHEA did not induce significant differences in time of struggling behavior. ANOVA did not show a significant effect of treatment on locomotor activity.
Discussion: These data experimentally support the findings that under certain circumstances, DHEA might have triggered antide-pressant-like effects in rats. Furthermore, these effects were not confounded by change in motor function. Bicuculline abolished the action of DHEA, confirming partially GABA-ergic mediation of the effect. However the molecular and neuronal substrates linking the actions of DHEA to specific $GABA_A$ receptors remain to be further elucidated and linked to human neuropsychiatric disorders.

Acknowledgements: This work was supported in part by the Ministry of Education, Science and Technological Development, Republic of Serbia (grant no. 175076).

A1.24

What is the somatodendritic serotonin transporter good for?

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Background: Neurotransmitter transporters belonging to the solute carrier 6 gene family are endowed with C-termini that vary greatly in sequence. However, the C-termini of these neurotransmitter transporters harbor a conserved RI/RL motif. The RI motif has been shown to interact with the endoplasmic reticulum (ER) export machinery, most notably the COPII component SEC24, of which four isoforms exist, SEC24A–D [1,2]. Mutation of conserved RI/RL to AA results in impaired ER export. It remains interesting to understand if the conserved R is sufficient enough for the recruitment of SEC24 isoforms. The aim of the current research is to understand if the conserved R607 of the human serotonin transporter (SERT) is sufficient enough for recruitment of SEC24C.
Methods: siRNA were used to knockdown SEC24 isoforms A–D in HEK 293 cells. The SERT mutant was characterized using uptake and binding assays.
Results: The SERT R607A mutant does not recruit SEC24C for ER export. K_m and K_d values were similar for SERT and the SERT mutant.
Discussion: The SERT R607A mutant eventually reaches the plasma membrane in a SEC24C-independent manner. The SERT R607A mutant does not show folding defects.

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A1.25

Pharmacological properties of rosemary essential oil in experimental animals

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Intrinsic Activity, 2014; **2**(Suppl. 1): A1.25

Background: Rosemary essential oil (REO) is classified as a traditional herbal medicine but additional studies are needed to complete the knowledge of its pharmacological effects. The aim of this research was to examine the influence of rosemary essential oil on pharmacological effects of diazepam and pentobarbital in experimental animals, and to examine antioxidant activity of essential oil and its hepatoprotective potential.
Methods: Pharmacodynamic experiments included pentobarbital-induced sleeping time test, while interaction with diazepam was examined by the rotarod test. Antioxidant activity of the REO was evaluated *in vitro* by the DPPH test and by determining phenolic content using the Folin–Ciocalteu reagent. The hepatoprotective effect of REO was evaluated *in vivo* in CCl_4-induced liver injury of albino Wistar rats.
Results: Seven-days pretreatment with REO significantly reduced pentobarbital-induced sleeping time, compared to the control group ($p < 0.05$), while single-dose pretreatment with REO in the dose of 20 mg/kg significantly prolonged sleeping time compared to controls ($p < 0.05$). Both doses, applied repeatedly, caused significantly longer retention of mice on the rotarod compared to controls ($p < 0.05$). The investigated essential oil exerted antioxidant activity, the IC_{50} value was 77.6 µl/ml. All biochemical parameters referring to oxidative stress induced by CCl_4 were also significantly reversed by oral administration of REO.
Discussion: Rosemary essential oil affected pharmacological properties of diazepam and pentobarbital. Beside its radical-scavenging activity it also mediates a hepatoprotective effect through activation of physiological defense mechanisms.
Acknowledgements: This research was supported by the Provincial Secretariat for Science and Technological Development, Autonomous Province of Vojvodina (project no. 114-451-2458/2011) and by the Ministry of Science, Republic of Serbia (project no. 41012).

A1.26

Investigating the molecular mechanisms underlying the differential subcellular targeting of the metabotropic glutamate receptor 1 in the cerebellar cortex

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Intrinsic Activity, 2014; **2**(Suppl. 1): A1.26

Background: Type 1 metabotropic glutamate ($mGlu_1$) receptors play a pivotal role in different forms of synaptic plasticity in the cerebellar cortex, e.g. long-term depression at excitatory synaptic inputs to Purkinje cells (PCs) and rebound potentiation at inhibitory postsynaptic potentials. These various forms of plasticity might depend at least in part on subsynaptic arrangement of the receptor that can be regulated by protein–protein interactions. To elucidate the molecular mechanisms implicated in the differential subcellular targeting and the physiological functions of $mGlu_1$ receptors in the cerebellar cortex, we have searched for novel interaction partners of these receptors. To this aim, we have selected an unbiased proteomic approach, namely co-immunoprecipitation (Co-IP) followed by liquid chromatography–tandem mass spectrometry (LC-MS/MS).
Methods: Co-IP of $mGlu_{1(\alpha)}$ receptor was performed from C57BL/6N mouse cerebellar homogenates using highly specific polyclonal antibodies. Confirmation of the specificity was obtained using tissue obtained from *Grm1* knockout (KO) mice. High-speed membrane fractions were solubilized in buffer containing 1% non-ionic and 0.1% ionic detergents. Co-IP was carried out using affinity-purified guinea-pig or rabbit polyclonal antibodies directed against the carboxy-terminal tail of the $mGlu_{1(\alpha)}$ receptor and protein A conjugated to magnetic beads. Eluted proteins were then resolved on SDS-PAGE and analyzed by quantitative mass spectrometry.
Results: LC-MS/MS analysis of the Co-IP eluates identified a number of well-known direct and indirect interactors such as Homer proteins, TRP channels and GluD2 receptors. A novel putative interaction partner, namely KCTD12, was identified in all LC-MS/MS analyses performed on wild-type eluates, but not on *Grm1*-KO ones. KCTD12 is already known as a $GABA_B$ receptor auxiliary subunit, which is involved in the desensitization of $GABA_B$ receptor

responses, and shows a distinct expression pattern in PCs. To investigate whether the KCTD family of proteins are common interaction partners of group 1 mGlu receptors, we are currently performing Co-IP for $mGlu_5$ receptors from mice hippocampal homogenates. In order to elucidate the mechanisms that regulate the coupling between $mGlu_1$ receptors and KCTD12, we have generated a recombinant mammalian cell line stably expressing KCTD12. Heterologous co-expression of different $mGlu_1$ receptor mutants is ongoing to investigate the type and site of interaction besides the role that KCTD12 might play on $mGlu_1$ receptor trafficking and function.

Discussion: Our findings suggest a novel $mGlu_1$ receptor interaction partner, namely KCTD12, in the mouse cerebellum. Further investigations will elucidate the role that KCTD12 might play on $mGlu_1$ receptor localization and function in the cerebellar cortex.

Acknowledgements: This project is part of the SPIN Doctorate program funded by the Austrian Science Fund FWF (project W012060-10 to F.F.).

A1.27

Activation of kappa opioid receptors reduces seizure activity in a mouse model of temporal lobe epilepsy

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.27

Background: Neuropsychiatric disorders are one of the main challenges of medicine with epilepsies representing some of the most frequent. Temporal lobe epilepsy (TLE) is the most common type of epilepsies and is often accompanied by marked neuronal degeneration. It was shown that the deletion of prodynorphin in mice and low expression in humans is associated with increased epilepsy vulnerability. Dynorphin targets opioid receptors and in particular the kappa opioid receptor (KOPr). KOPr are located in strategically ideal places to control hippocampal excitability also in chronic TLE. The aim of this study was to investigate the potential of KOPr agonists as antiepileptic drugs and compare it to new generation of anti-epileptic drugs (AEDs) in a mouse model of drug-resistant TLE.

Methods: Fifteen C57BL6/N male mice were injected unilaterally with saline ($n = 5$) or kainic acid (KA; 1 nmol in 50 nl saline; $n = 10$) into dorsal hippocampus. While saline injected animals did not show any signs of EEG or histopathological alterations, KA caused acute and delayed behavioral, pathological and EEG effects. Four-channel EEG traces were recorded from ipsi- and controlateral hippocampi and motorcortices applying depth- and surface electrodes from freely moving mice, respectively. The KOPr-specific agonist U-50488H, saline or one the new AEDs oxcarbazepine, lamotrigine and levetiracetam were applied i.p., while the biased KOPr partial agonist 6'-GNTI was delivered i.c.v. through a guide canula. Number and duration of EEG seizures were automatically evaluated (Sciworks software) for the 45 min preceding and following the injections. Another group of animals (20 male mice C57BL6/N) was tested in the conditioned place avoidance (CPA) paradigm for U-50488H and 6'-GNTI. For the acquisition of CPA, the conditioning procedure comprised a pre-test session on day one, four consecutive training days (two training session per day, a total of four training sessions each for drug or saline) and a post-test on day six.

Results: Spike trains, sharp waves and paroxysmal discharges in the ipsilateral hippocampus were observed starting from about 14 days after KA injection. Prolonged EEG seizures occurred frequently after about 4 weeks. Such paroxysmal discharges were accompanied by behavioural arrest and stereotypic behaviour like head nodding. Application of either U-50488H or 6'-GNTI decreases EEG abnormalities caused by KA in a dose-dependent manner. All the AEDs showed significant effects, at least at the highest dose administered. As expected, the CPA experiments revealed that the animals conditioned to U-50488H developed avoidance for the compartment paired with this drug. On the other hand the biased KOPr agonist 6'-GNTI did not produce any avoidance.

Discussion: Our data demonstrate the anticonvulsant action of KOPr agonists in the chronic phase of epilepsy, comparable to the effect of 2.5 mg/kg diazepam. Furthermore, we demonstrate that the biased KOPr partial agonist 6'-GNTI does not induce place avoidance in the CPA paradigm. The absence of KOPr-induced dysphoria is probably due to the fact that 6'-GNTI does not recruit the β-arrestin pathway.

Acknowledgements: This work was supported by the Austrian Science Fund FWF (W1206-BO5).

A1.28

Histone deacetylase inhibitors rescue impaired fear extinction in a persistent and context-independent manner

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.28

Background: A novel strategy in the treatment of anxiety and fear-related disorders is to augment extinction-based therapy by boosting gene transcription via administration of histone deacetylase inhibitors (HDACi) during extinction consolidation (a time point where new gene synthesis is required to initiate the formation of fear-inhibitory memories). However, information regarding the persistency and context-independency of this approach in pathological models of impaired fear extinction remains largely unknown. We have previously shown that MS-275 (HDAC1-, HDAC2- and HDAC3-isoform inhibitor) applied during the critical extinction consolidation period can rescue impaired extinction consolidation/retrieval in 'weak' conditioned 129S1/SvImJ mice. Here, we assessed whether MS-275 can promote persistent and context-independent fear inhibition and also to identify potential extinction-relevant molecular mechanisms invoked.

Methods: S1 mice were subjected to multi-trial cued fear conditioning/extinction paradigms to assess the ability of pharmaceutical ligands, including the HDACi MS-275 and the dopamine precursor L-DOPA, to promote long-term and context-independent protection against the return of fear (spontaneous recovery/fear renewal). Histone acetylation changes following fear extinction were measured using fluorescent immunohistochemistry and extinction-induced increases in histone acetylation in the promoter regions of dopaminergic receptor genes were quantified using chromatin immunoprecipitation.

Results: Here, we replicated the finding that MS-275 can rescue impaired fear extinction in S1 mice, and now show that MS-275 can promote a persistent (reduced fear during a long-term memory test) and context-independent (reduced fear in a novel context) fear-inhibitory memory. Rescue of impaired fear extinction was associated with higher histone acetylation in MS-275 treated mice in the infralimbic cortex, a brain region where long-term memories are primarily stored. Moreover, enhanced histone acetylation was observed in the promoter region of the dopamine D_1 receptor. The

finding that increasing dopaminergic signalling, via L-DOPA, rescued impaired fear extinction consolidation in S1 mice provided functional proof-of-principal that dopaminergic signalling is an important signalling pathway promoted by enhanced histone acetylation.

Discussion: These data reveal that HDACi rescued impaired fear extinction in extinction-impaired S1 mice in a persistent and context-independent manner, which is of high clinical relevance. Moreover, these data reveal that this rescue of impaired fear extinction was associated with enhanced expression of a neuroplasticity-related gene within a key fear-extinction-relevant brain region, revealing a potential molecular mechanism via which HDACi may rescue aberrant fear. This was further underscored by the finding that pharmaceutical enhancement of dopaminergic signalling was able to rescue extinction deficits in S1 mice. Collectively, these results identify that HDACi's display promising potential as pharmacological adjuncts in exposure-based therapy to rescue impaired fear extinction in a persistent manner.

Acknowledgements: Funded by the Austrian Science Fund FWF (SFB F4410).

A1.29

Analyzing the role of diffusion for A_{2A} receptor function in hippocampal neurons

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.29

Background: The adenosine A_{2A} receptor is a classical G protein-coupled receptor and has a very long carboxyl terminus that lacks the canonical palmitoylation site. In addition, the A_{2A} receptor differs in the mode of G protein activation. Coupling to its cognate protein G_s occurs via restricted collision coupling and is contingent on the presence of cholesterol. We explored the contribution of the hydrophobic core and of the extended C-terminus by examining diffusion of quantum-dot-labeled receptor variants in dissociated hippocampal neurons.

Methods: We used the technique of "single particle tracking", which has a high spatial and temporal resolution to study receptor diffusion on the cell membrane. We labeled A_{2A} receptors expressed on hippocampal neurons with quantum dots, video-recorded the movement of the fluorescent dots and used computer-assisted detection and diffusional analysis of the obtained trajectories. Finally, we utilized hidden Markov models to further analyze the data.

Results: The analysis of all trajectories revealed two diffusion states of the A_{2A} receptor. Agonist activation reduced the transition between the two states and promoted the accumulation in the state of slow mobility. However, this agonist-induced redistribution was abolished with a truncated A_{2A} receptor, which lacks the last 101 residues, and agonist-induced decrease in diffusivity was substantially reduced. We identified a fragment comprising the SH3 and the guanylate kinase (GUK) domain of synapse-associated protein 102 (SAP102) as a candidate that bound to the A_{2A} receptor C-terminus. The over-expression of SAP102 precluded the access of the A_{2A} receptor to the compartment with reduced mobility. In contrast, an A_{2A} receptor with mutated C-terminus (with ^{383}DVELL^{387} replaced by ^{383}RVRAA^{387}) was insensitive to the action of SAP102.

Discussion: Our observations demonstrate that agonist-induced changes in the hydrophobic core cannot fully explain the change in mobility of the A_{2A} receptor. The extended carboxyl terminus interacts with scaffolding molecules such as SAP102, which has an additional regulatory influence on A_{2A} receptor confinement in neurons.

Acknowledgements: This work was supported by grants P21002 and PIRG-GA-2008-230970 from the Austrian Science Fund FWF and the European Union Seventh Framework Program (FP7/2007–2013), respectively; I.G. was supported by a Doc-fForte stipend of the Austrian Academy of Sciences.

A1.30

The anticonvulsant retigabine is a subtype selective modulator of $GABA_A$ receptors

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.30

Background: Retigabine is a novel antiepileptic drug whose purported mechanism of action is the opening of K_V7 potassium channels. Nevertheless, at concentrations of 10 µM or above, this drug has also been reported to enhance currents through $GABA_A$ receptors. We sought to investigate this latter effect of retigabine in more detail.

Methods: For $GABA_A$ receptor current measurements, we used the perforated-patch method on cultured primary hippocampal neurons or tsA201 cells expressing various $GABA_A$ receptor subunit combinations. For K_V7 currents, $K_V7.2/7.3$ heteromers were expressed in tsA201 cells. Retigabine effects on seizure-like activity were investigated in hippocampal neurons in current-clamp mode under low Mg^{2+} conditions and/or in presence of the K_V7 blocker XE 991.

Results: In primary cultures of hippocampal neurons, retigabine reduced seizure-like activity triggered by low Mg^{2+} in a concentration-dependent manner with half-maximal inhibition at about 1 µM. This inhibitory effect was not altered when K_V7 channels were blocked with XE 991. Currents in hippocampal neurons evoked by increasing concentrations of GABA were not affected by 10 µM retigabine. However, when phasic GABAergic inhibition was prevented due to the presence of penicillin, retigabine did enhance GABA-induced currents. When tested in tsA201 cells expressing various combinations of $GABA_A$ receptor subunits, 10 µM retigabine enhanced currents through α1β2δ, α4β3δ, and α6β2δ receptors, but left currents through α1β2γ2S, α4β3γ2S, α6β2γ2S receptors unaltered. With αβ receptors, retigabine diminished currents through α1β2 and α4β3, but increased currents through α6β2 receptors. Modulation of α4β2δ and α4β3δ receptors by retigabine was the same. The enhancement of currents through α1β2δ receptors by retigabine was concentration-dependent and became significant at 1 µM.

Discussion: These results demonstrate that retigabine is a subtype-selective modulator of $GABA_A$ receptors with preference for extrasynaptic δ-containing receptors. This property apparently contributes to its antiepileptic efficacy.

Acknowledgements: This study was supported by the Austrian Science Fund FWF (project numbers W1205, P23658 and P23670), and the CCHD doctoral programme funded by FWF and the Medical University of Vienna.

A1.31

Region-specific differences in the activity of mitochondrial complexes I and II in the mouse brain

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.31

Background: Mitochondrial dysfunction appears to be a common factor in neurodegenerative diseases. However, such diseases differ markedly in the nervous tissue affected.
Methods: To test potential differences in mitochondrial respiratory capacity of different brain tissues under physiological or pathological conditions, we established a protocol for the analysis of oxidative phosphorylation (OXPHOS) of small amounts of defined brain tissues of mice. This protocol enables us to measure independently the activities of complex I, complex II and complex IV (CI–IV, respectively), as well as the overall OXPHOS and electron-transfer system (ETS) capacity in a single run from as a little as 2 mg tissue applying the OROBOROS high-resolution respirometry system. The reproducibility within one experiment (2 parallels from the same tissue sample) as well as between experiments is very high.
Results: We observed significantly higher activities of CI in the motorcortex and of CII in the striatum when comparing motorcortex, striatum, hippocampus and brainstem obtained from healthy adult, male C57BL6/J mice. No differences were found for the ETS capacity itself and CIV activity (both, for oxygen consumption per mass and if normalized to the ETS capacity). Motorcortex and hippocampus differed in their OXPHOS capacity if normalized to the ETS capacity. We are currently performing additional experiments on healthy adult, female C57BL6/J mice to determine if the observed patterns of OXPHOS are sex-specific.
Discussion: The established protocol allows detailed analysis of mitochondrial function from small amounts of specific tissues. It thus enables comparison of different brain tissues implicated in neurodegenerative diseases of the healthy mouse and in disease models, leaving enough material for further studies on the tissues.

A1.32

Cellular and subcellular localization of metabotropic glutamate (mGlu) receptor 4 in the rodent amygdala

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.32

Background: Group III metabotropic glutamate (mGlu) receptors, namely $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$, are located presynaptically at the active zone where they regulate neurotransmitter release through G protein-coupling and second messenger pathways. In recent years, great interest was raised about these receptors as potential targets for new anxiolytic drugs. For example, the novel $mGlu_4$ receptor positive allosteric modulator VU 0155041 was shown to reduce anxiety-like behavior in wild-type male mice. Recent studies on mice with gene-targeted deletion of $mGlu_4$ have also suggested a functional role for this receptor in the acquisition and formation of fear memories. Since amygdala plays a pivotal role in fear learning, we have investigated the cellular and subcellular localization of $mGlu_4$ receptors in this limbic region.
Methods: Cellular distribution of the $mGlu_4$ receptor was carried out using both conventional epifluorescence as well as confocal microscopy on mouse (C57BL/6) brain slices. Specficity of immunostaining was controlled in tissue of $mGlu_4$ receptor null mice. Subcellular localization for the $mGlu_4$ receptor was performed by means of the pre-embedding immunoelectron microscopy on both rat and mouse amygdala slices. To quantitavely investigate $mGlu_4$ receptor immunoreactivity we are taking advantage of stochastic optical reconstruction microscopy (STORM). This super-resolution technique utilizes sequential activation and time-resolved localization of photoswitchable fluorophores to create high-resolution images.
Results: Our preliminary immunohistochemical results in mouse brain slices showed an intense punctate immunoreactivity decorating mostly large aspiny dendrites surrounding the intercalated cell clusters (ITC) between the basolateral complex and the central nucleus. Additional pre-embedding immunoelectron microscopy confirmed the exclusive presynaptic location of $mGlu_4$ receptors, forming both symmetric and asymmetric synapses with dendritic shafts and spines. Of particular interest was the observation that terminals containing $mGlu_4$ receptors made symmetric synapses, most likely inhibitory, on spine necks, suggesting an input-specific regulation.
Discussion: The highly discrete distribution in the amygdala of $mGlu_4$ receptors was similar to that observed for other group III mGlu receptors such as $mGlu_7$ and $mGlu_8$. In an earlier study, we have shown that these latter receptors have a complex anatomical segregation in different neuronal pathways of the fear circuit being present on multiple extrinsic and intrinsic inputs and having large $mGlu_{1(a)}$-positive cells surrounding the intercalated cell masses (ITCs) as one of the primary cell targets. Although additional investigation is needed, we hypothesize that $mGlu_4$-labelled terminals might target a different cell class as compared to those containg others mGlu group III receptors. Our goal is also to characterize the origin of $mGlu_4$-positive inputs and to investigate the co-existence with other group III mGlu receptors.
Acknowledgements: This project is part of the SFB F44 and funded by the Land Tirol (Austria).

A1.33

Connectivity of CR^+ (VIP^+) interneurons in the basolateral amygdala

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.33

Background: The amygdala is known to be involved in simple forms of emotional learning, such as fear conditioning and fear extinction. Interneurons (INs) of the basolateral amygdaloid complex (BLA) represent a highly heterogeneous group of cells that tightly regulate principal cell (P cell) excitability. The unambiguous identification of functionally distinct classes of BLA INs requires the precise definition of their dendritic and axonal pattern, firing activity and presynaptic afferents, together with their neurochemical features. The majority of BLA INs contacts glutamatergic P cells at different plasma membrane specializations. On the other hand, some INs preferentially target other types of INs (interneuron-selective interneurons, ISIs). Among them, calretinin (CR^+) INs, which also express vasoactive intestinal polypeptide (VIP), were found to contact calbindin (CB^+) INs and to establish a reciprocal connection in the rat BLA. In hippocampus and cortex, VIP^+ INs contact dendrite-targeting INs, which in turn lead to a disinhibition of P cells. Such disinhibitory mechanisms may be important in the modulation

of amygdala-related behaviors. BLA INs are believed to receive mainly glutamatergic inputs from cortex, thalamus and local P cells in different arrangements. However, little is known about the VIP^+ (CR^+) pre-synaptic partners. One way to investigate their functional role is to characterize the microcircuits in which they are embedded. Our work focuses on the identification of first order pre-synaptic inputs as well as post-synaptic targets of this specific class of INs in order to elucidate their role in fear and extinction behaviors.

Methods: We will take advantage of the mono-trans-synaptic tracing approach using the mutant rabies virus EnvA-ΔG, in order to retrogradely identify first-order afferents. The mutated virus lacks the gene encoding its envelope glycoprotein (RG) necessary for further viral spread and is pseudotyped with the avian sarcoma leucosis envelope glycoprotein (EnvA) so that only the cells that express the EnvA receptor TVA (not constitutively expressed in mammalian cells), can be infected. To reconstitute the infectious RV it is necessary to express the TVA receptor, along with the native RG, in a specific cell population. Through the use of a Cre-dependent TVA mouse line, crossed with a CR-Cre or VIP-Cre mouse line, the TVA receptor is expressed only in CR^+ (VIP^+) cells. The stereotactic injection in BLA of an adeno-associated viral vector (AAV) that expresses also in a Cre-dependent fashion the native RG along with the pseudotyped rabies virus coupled with a fluorescent reporter molecule, allows the mono-trans-synaptic spreading of the rabies infection only from TVA-CR^+ (VIP^+) cells.

Results: We injected CR-Cre:TVA mice with a 1:1 mixture of a Cre-dependent AAV carrying the RG and an EnvA-ΔG-RFP, therefore allowing the spreading from CR^+ cells. Preliminary immunofluorescence analysis on injected brains revealed direct presynaptic partners in several forebrain areas including: piriform, enthorinal and temporal cortex, zona incerta, and ventral hippocampus (stratum oriens and stratum pyramidale), along with local presynaptic partners in BLA. Interestingly, the shape and position of hippocampal cells would suggest an interneuronal origin. So far, we have investigated several interneuronal markers (somatostatin, calretinin, and calbindin), but none appeared to coexist with the RV.

Discussion: Relating structure to function is a central goal of modern neuroscience. Our preliminary experiments point in the direction of a broad but organized distribution of presynaptic partners to CR^+ INs in BLA. Further analysis is needed to elucidate the distinctives of these afferent inputs.

Acknowledgements: This project is part of the SPIN Doctorate program funded by the Austrian Science Fund FWF (project W012060-10 to F.F.).

A1.34

Cardiac ion channel profile of the antiepileptic drug retigabine

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.34

Background: Retigabine was recently approved as a novel antiepileptic drug for the adjunctive treatment of partial onset seizures. At low-micromolar concentrations, the drug acts as an opener of neural K_V7 potassium channels ($K_V7.2$–7.5; *KCNQ2–5*), whereby K_V7 channels expressed in the heart ($K_V7.1$; *KCNQ1*) are not affected. Nevertheless, a slight but significant prolongation of the QT interval in the electrocardiogram has been demonstrated in a cardiac safety study in healthy subjects treated with retigabine (1,200 mg/day). In addition, cardiac arrhythmias were observed in a few subjects after retigabine application. This suggests that the drug may affect cardiac ion channels in therapeutic concentrations (free C_{max}, 1–5 µM), and may thereby represent a cardiac arrhythmia risk.

Methods: Ionic currents and action potentials were studied by the whole-cell patch-clamp technique. Cardiac ion channels were expressed in tSA201 cells and ventricular cardiomyocytes were isolated from Langendorff perfused guinea-pig hearts.

Results: In the present study, we therefore tested the effects of retigabine on human cardiac voltage-gated ion channels with major importance for electrical impulse propagation in the heart. These were $hK_V11.1$ potassium, $hNa_V1.5$ sodium, and $hCa_V1.2$ calcium channels, which, when functionally modulated by drugs, can account for undesirable cardiac adverse events. We found that retigabine significantly inhibits currents through $hK_V11.1$, $hNa_V1.5$, as well as $hCa_V1.2$ channels in concentrations ≥10 µM. In addition, retigabine at concentrations ≥10 µM shortened the action potential in guinea pig ventricular cardiomyocytes.

Discussion: We conclude that inhibition of cardiac ion channels and changes in action-potential duration occur at retigabine concentrations ≥10 µM, concentrations which are probably higher than those reached in the plasma of patients after “normal” drug application. The drug may therefore be relatively safe with respect to cardiac arrhythmia generation. However, in the case of intoxications due to retigabine overdoses, co-medication or other “second hit” factors, the drug’s inhibitory effects on channels may become relevant.

Acknowledgements: This work was supported by the Austrian Science Fund FWF (P19352 and P23060 to K.H.).

A1.35

Suggested role of MKP-1 in regulation of the inflammatory response in a human intestinal epithelia cell (Caco-2) model

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.35

Background: Gram-negative bacteria are enhanced in inflamed intestine, leading to an increase of LPS in the lumen that may cause intestinal inflammation, such as necrotizing enterocolitis. Mitogen-activated protein kinase phosphatase 1 (MKP-1), a non-receptor, dual-specificity phosphoprotein phosphatase (DUSP1), controls MAP kinases that are involved in regulation of various physiological and pathophysiological processes where inflammatory events occur. The aim of our study was to demonstrate the role of MKP-1 in an inflammatory intestinal epithelial cell model.

Methods: Differentiated Caco-2 cells were treated with 0, 50, 100, 250, 500 and 1000 ng/ml of LPS for 4 h, 6 h and 24 h incubation time. The amount of cytotoxicity was determined using the LDH assay. The cell transepithelial electrical resistance (TER) was measured using impedance (*Z*) measurement. The membrane permeability was tested by selective transport of small fluorescein thiocarbamoyl (FITC)-dextran (3–5 kDa). The protein expression of MKP-1, and as control glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was tested by western blot analysis. IL-8 production was quantified by means of a commercial ELISA.

Results: LPS dose-dependently increased very slightly the amount of cytotoxicity after 4 h or 6 h, but the cytotoxicity increased significantly after 24 h. The protein expression of MKP-1 was down-regulated and the release of IL-8 increased after 4 h incubation time (Fig. 1). Furthermore, LPS at concentrations above 500 ng/ml significantly increased the membrane permeability of small FITC-dextran molecules, although the membrane integrity (TER value) was relatively unchanged.

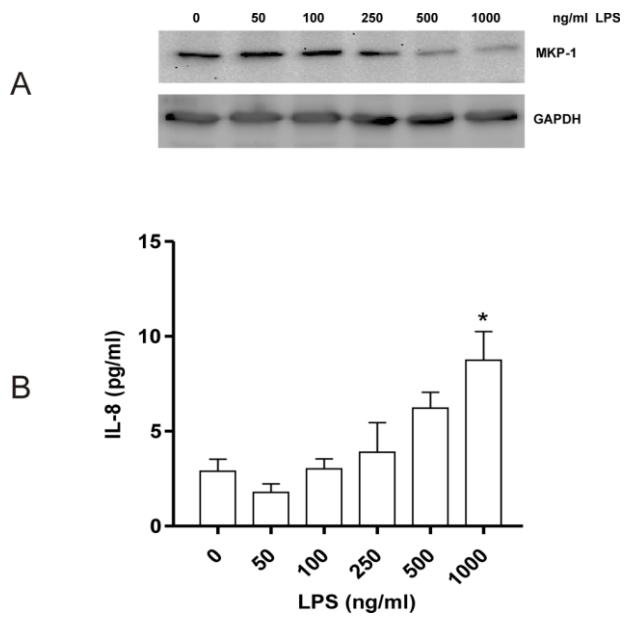

Figure 1: Dose-dependency of LPS on (**A**) the MKP-1 and GAPDH protein expression, and (**B**) IL-8 release. Differentiated Caco-2 cells were treated with LPS (0, 50, 100, 250, 500, 1000 ng/ml) for 4 hours. IL-8 production in the cell culture supernatant was determined using ELISA assay and the protein expression of MKP-1 and GPADH were determined by means of western blot analysis. *$p < 0.05$ vs. 0 ng/ml LPS; means ± SEM, $n = 3–4$.

Discussion: MKP-1 may be involved in the regulation of inflammation in response to LPS in human intestinal epithelia cells.
Acknowledgements: This project has been funded by the State of Upper Austria.

A1.36

Successful rescue of impaired fear extinction leads to dynamic regulation of microRNAs in the amygdala

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.36

Background: Current treatments to overcome excessive fear in anxiety disorders, including exposure-based therapy, are far from being optimal. A basic principle of exposure-based therapy is the formation of long-lasting fear-inhibitory memories, which depends on *de novo* gene transcription of plasticity-related genes, amongst others. Recent evidence suggests that gene transcription after a learning event is influenced by non-protein-coding RNAs (ncRNAs) including microRNAs (miRNAs), for example, miR-128b which has been demonstrated to be involved in the formation of fear-inhibitory memory. However the role played by miRNAs promoting enduring fear-inhibitory memories in individuals with extinction deficits remains to be determined.
Methods: Mimicking anxiety patients, 129S1/SvImJ (S1) mice exhibit profound resistance to induce fear extinction. We have shown that persistent context-independent extinction in S1 mice can be induced using dietary zinc restriction. Utilizing dietary zinc restriction as a tool, we can elucidate the underlying mechanisms leading to the successful rescue of impaired fear extinction. To examine to role of miRNAs in this process we used microarray technology and subsequent RT-PCR to assess the regulation of miRNAs in the amygdala of S1 mice 2 h after the successful rescue of impaired fear extinction. Anatomical localization of candidate miRNAs was demonstrated by fluorescent *in situ* hybridization.
Results: Microarray analysis and consecutive RT-PCR confirmation revealed an altered regulation in a select set of miRNAs, including an increase in miR-144 expression in the amygdala of S1 mice. Genes regulated by miR-144 remain to be elucidated; however we show regulation of the predicted target retinoic acid receptor beta (RXRβ), a plasticity-related gene, in S1 mice during rescue of impaired extinction. Experiments establishing the link between miR-144 and fear-inhibitory memories are ongoing. To examine the anatomical localization of candidate miRNAs in a neuronal subtype-specific manner we have set up and validated a fluorescent *in situ* hybridization. Initial results on candidate miR-144 demonstrate a highly selective expression restricted to the central amygdala.
Discussion: In conclusion, rescuing impaired fear extinction in a persistent and context-independent manner is associated with dynamic regulation of the expression of select miRNAs, which can interact with protein-coding synaptic plasticity genes. One candidate, miR-144, exhibits a highly selective expression restricted to the central amygdala, and we have confirmed the downregulation of RXRβ, a predicted target of miR-144. Understanding the role of miRNAs in the rescue of impaired fear extinction may shed light on novel therapeutic targets to treating these debilitating disorders.
Acknowledgements: Supported by the the Austrian Science Fund FWF (SFB F44, Signal Processing In Neurons (SPIN)).

A1.37

Effects of two different surface coatings of superparamagnetic iron oxide nanoparticles on membrane properties in a human intestinal barrier model

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Intrinsic Activity, 2014; **2**(Suppl. 1):A1.37

Background: Superparamagnetic iron oxide nanoparticles (SPION) have a promising potential for use in various approaches such as magnetic drug targeting, magnetic resonance imaging, and tissue regeneration. Despite multiple promising benefits of SPION, these particles must be comprehensively toxicologically and pharmacologically characterized before clinical use; this is necessary also in the view of an oral exposure scenario. Here, we compare the biological effects of lauric acid (LA)-stabilized SPION ($SPION^{LA}$) and $SPION^{LA}$ with an additional corona formed by bovine serum albumin ($SPION^{LA\text{-}BSA}$) on an intestinal barrier model *in vitro*. Both particle species were intensively physico-chemically characterized previously.
Methods: To establish an intestinal barrier model, Caco-2 cells seeded onto inserts were differentiated for 21 days. After that, the Caco-2 barrier model was treated apically with $SPION^{LA}$ and $SPION^{LA\text{-}BSA}$ for 3 h, 6 h, and 24 h. The amount of reactive oxygen species (ROS) and cytotoxicity were determined using Rd123 and LDH assay. The cell transepithelial electrical resistance (TER) was determined using impedance (Z) measurement. The membrane permeability was tested by selective transport of small fluorescein thiocarbamoyl (FITC)-dextran (3–5 kDa). The amount of SPION on the apical and basolateral side in the cell culture supernatant was measured photometrically at 370 nm and in the inside of cells by means of Berlin blue / hematoxylin staining.

Results: Both SPION types dose-dependently increased very slightly the amount of reactive oxygen species (ROS) and the cytotoxicity in a time-dependent manner. The concentration of $SPION^{LA}$ and $SPION^{LA\text{-}BSA}$ was increased dose-dependently only on the apical side and in the inside of cells, but not on the basolateral side. Only, $SPION^{LA}$ at a concentration above 100 µg/ml decreased the membrane integrity (TER value). However, the permeability of small FITC-dextran molecules was unchanged after treatment with both types of SPION. These results were in accordance with determination of SPION concentrations on the apical and basolateral side which were measured photometrically.

Discussion: Altogether, $SPION^{LA\text{-}BSA}$ was more biocompatible regarding its effect on barrier properties than $SPION^{LA}$. The understanding how different types of SPION affect the intestinal barrier can help to find the appropriate SPION type and dose for therapeutic applications.

Acknowledgements: The project was funded by the State of Upper Austria, the Bavarian Ministry for Environment and Consumer Protection (74-U8793-2012/7-35), and the Cluster of Excellence Engineering of Advanced Materials (EAM).

A1.38

Comparative application of common virtual screening tools for the identification of novel µ opioid receptor agonists and antagonists

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Background: The µ opioid peptide (MOP) receptor, a G protein-coupled receptor (GPCR), is one of the oldest targeted opioid receptors for the treatment of pain and other human disorders including ileus, alcohol and drug addiction. Several opioid drugs, i.e. MOP agonists and antagonists, are available for clinical use or are valuable research probes. However, there is still limited information on the molecular mechanisms underlying the different biological effects of various ligands interacting with the MOP receptor. In 2012, the crystal structure of this receptor was elucidated in complex with the irreversible MOP antagonist β-funaltrexamine [1], and could provide first insights into potential binding modes. Within this study, commonly used virtual screening tools, including pharmacophore and shape-based modeling, and docking, were applied to identify novel MOP ligands, and to investigate whether these methods could discriminate between agonists and antagonists.

Methods: Multiple agonist and antagonist models were generated with the programs LigandScout 3.1 and ROCS 3.0.0. A docking workflow using GOLD 5.2 was established. The Maybridge database was screened with all methods and the resulting hits were ranked according to their respective Fit value. Top-ranked agonist and antagonist hits from all methods were tested in a radioligand binding assay at the human MOP receptor.

Results: The evaluation of the theoretical validation illustrated the differences in the suitability of the various *in silico* methods represented by pharmacophore and shape-based modeling, and docking. Particularly, docking appeared as an interesting approach since different interaction patterns of agonists and antagonists observed in this validation run suggested a possible mode of action. In the course of the prospective virtual screening, eighteen structurally novel and diverse molecules were selected for the biological evaluation and three of them showed a weak interaction with the human MOP receptor.

Discussion: The results obtained in this study suggest that some virtual screening tools might be better suitable than others. This may account for the investigation of the MOP receptor in particular, but might also apply for G protein-coupled receptors in general. Therefore, the method of choice for conducting GPCR-related projects should be carefully selected.

Acknowledgements: Supported by Tyrolean Research Fund, Verein zur Förderung der wissenschaftlichen Ausbildung und Tätigkeit von Südtirolern an der Landesuniversität Innsbruck, Openeye, the University of Innsbruck (Young Talents Grant) and the Austrian Science Fund FWF (TRP19-B18 and TRP16-B18).

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A1.39

Changes in the expression of histone deacetylase 1–11 mRNAs in the hippocampus in two mouse models of temporal lobe epilepsy

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Background: Histone modifications involve deacetylation of histone proteins by HDAC families of enzymes contributing to transcriptional silencing of gene expression. There are four different classes, comprising 11 HDAC isoforms. We investigated changes in the expression of HDAC mRNAs in an animal model of temporal lobe epilepsy (TLE).

Methods: C57BL/6N mice were injected unilaterally with kainic acid (KA; 0.350 nmol/70 nl) into the dorsal hippocampus (CA1). EEGs were continuously recorded subdurally for 4 weeks and revealed an initial status epilepticus, followed by about 2 severe spontaneous seizures per day. In the injected hippocampus, losses in CA1 and CA3 pyramidal cells were observed after 24 h and granule cell dispersion after 7 days. Expression levels of HDAC 1–11 mRNAs were investigated by *in situ* hybridization 2, 4, 6, 12, 24 and 48 h, and 7, 14 and 28 days after KA.

Results: In the dentate gyrus, HDAC 1, 2, 4, 7 and 11 mRNAs were significantly decreased 4 h after KA ipsi- and contralateral to the injection relating to increased seizure activity during the initial status epilepticus and presumably contributing to increased expression of different genes. In contrast, HDAC5 mRNA levels were significantly increased 4 and 12 h after KA presumably resulting in silencing of certain genes. HDAC3 mRNA levels were transiently increased (contralaterally) and those of HDAC4 mRNA decreased after 24 to 48 h. Most interestingly, 7 to 28 days after KA injection we observed also a pronounced increase in HDAC9 mRNA expression in granule cells of the injected hippocampus correlating with the concomitantly developing granule cell dispersion.

Discussion: Decreases in HDAC 1, 2, 4, 7 and 11 during the status epilepticus may initiate augmented gene expression of various genes and may induce epileptogenesis. Specific upregulation of HDAC 5 and 9 in granule cells may be related to granule cell dispersion.

Acknowledgements: Supported by the European Science Foundation (Eurocores Programme; EuroEPIGENOMICS) and the Austrian Science Fund FWF.

A1.40

The role of neurokinin-B-expressing neurons in the basolateral amygdala in fear conditioning and extinction

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.40

Background: Anxiety disorders constitute a major burden for the society and are characterized by pathological expression of anxiety and fear. Recent evidence points towards an important role of neuropeptides in modulating fear and anxiety. Among the tachykinin neuropeptide family, substance P is promoting anxiety-related behavior, whereas the role of neurokinin B (NKB), acting predominantly on neurokinin NK_3 receptors, is not clear yet. Interestingly, NKB is highly expressed in the amygdala, a brain area central for emotional processing. Thus, we wanted to investigate the role of NKB and NKB-expressing neurons in the amygdala in fear behavior.
Methods: To test the specific role of NKB-expressing neurons in the basolateral amygdala (BLA), double-immunolabeling of NKB or their precursor preprotachykinin B (PPTB) with specific interneuron markers were performed. In addition, Tac2-Cre mice were locally injected into the BLA with a FLEXed rAAV vector expressing tetanus light chain (TeLC) for specific and local permanent silencing of NKB neurons, followed by Pavlovian fear conditioning two weeks later. Pavlovian fear conditioning was used as a simple form of associative learning by pairing a tone with a mild electric foot shock. Fear extinction was performed the following day by repetitive exposure to the tone without foot shock.
Results: NKB or PPTB are localized in calretinin-expressing interneurons but not in parvalbumin-, somatostatin- or calbindin-expressing interneurons in the BLA. The inhibition of NKB-expressing neurons in the BLA did not affect fear acquisition but facilitated fear extinction. This change was also observed in extinction recall, demonstrating a persistent effect of inhibiting NKB neurons.
Discussion: Our data indicate that NKB-expressing neurons in the BLA are regulating fear extinction within BLA. Further studies will clarify the exact mechanism and the contribution of NKB itself in this process.
Acknowledgements: Austrian Science Fund (P22830, P25851), MUI Start (ST2013042001) and CAPES Foundation, Ministry of Education, Brazil (BEX2439-13-1 to G.L.).

A1.41

Modulation of survival circuits in extinction of conditioned fear

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.41

Background: Emotions control evolutionary conserved behaviour that is central to survival in a natural environment. Although neurobiological substrates of emotionally controlled circuitries are increasingly evident, their mutual influences are not.
Methods: Here we employed Pavlovian fear conditioning and *ex vivo* slice electrophysiology to investigate interactions between hunger and fear, two life-sustaining survival circuits.
Results: In mice, fasting before fear acquisition specifically impaired long-term fear memory, while fasting before fear extinction facilitated extinction learning. Accordingly, genetic deletion of a feeding-relevant gene that reduces appetite, the Y_4 receptor gene, completely impaired fear extinction, a phenomenon that was rescued by fasting. Facilitated feed-forward inhibition between the basolateral and central amygdala, a synaptic correlate of fear extinction involving the medial intercalated cells, was absent in Y_4 knockout mice. Fasting before extinction learning, however, re-established facilitated feed-forward inhibition in these mice.
Discussion: Hence, consolidation of fear and extinction memories is differentially controlled by hunger.
Acknowledgements: Supported by the Austrian Science Fund FWF (P22830) and Tiroler Wissenschaftsfonds TWF.

A1.42

Presynaptic NPY Y_2 receptors reduce GABAergic neurotransmission within the central extended amygdala

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Intrinsic Activity, 2014; **2**(Suppl.1):A1.42

Background: Neuropeptide Y (NPY) is an evolutionarily conserved neuropeptide that is widely expressed throughout the mammalian brain and modulates the function of brain circuits involved in feeding, learning and memory, and survival behaviors. NPY and NPY receptors are expressed throughout the central extended amygdala and pharmacological interventions of this network have demonstrated that NPY signaling has an important role in influencing affective behaviors including fear and anxiety. Thus, modulating NPY signaling within the central extended amygdala may have therapeutic potential in the treatment of anxiety disorders. We have found that the NPY Y_2 receptor is highly expressed in a projection between the medial subdivision of the central amygdala (CeM) and bed nucleus of the stria terminalis (BNST). Thus, we investigated how the Y_2 receptor modulates neurotransmission in this projection.
Methods: Spontaneous and evoked GABAergic neurotransmission was measured by performing whole-cell patch clamp recordings of neurons of the CeM and BNST in the presence and absence of the Y_2 agonist, $PYY_{3–36}$.
Results: In wild-type mice, selective activation of the Y_2 receptor using the Y_2 receptor agonist $PYY_{3–36}$ reduced the frequency but not amplitude of GABA-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) in neurons of both the CeM and BNST. Whole-cell recordings of paired-pulse-evoked inhibitory postsynaptic currents in the BNST and CeM indicated that $PYY_{3–36}$ reduces the presynaptic release probability of GABA. We next explored how NPY signaling modulates GABAergic neurotransmission within the central extended amygdala by performing electrophysiological recordings in brain slices from NPY knockout and NPY Y_2 receptor knockout mice. Interestingly, the basal frequency of spontaneous inhibitory postsynaptic currents in the CeM was elevated in both NPY knockout and NPY Y_2 receptor knockout mice, however this was not observed in the BNST.
Discussion: Taken together, these results indicate that the NPY Y_2 receptor modulates GABAergic neurotransmission within the central extended amygdala and mice lacking key components of the NPY system exhibit alterations in basal inhibitory neurotransmission.
Acknowledgements: Supported by the Austrian Science Fund FWF (P25851, P22830) and MUI Start (ST20130420001).

A1.43

Development of epilepsy after spatially restricted viral-vector-mediated silencing of parvalbumin-expressing GABAergic interneurons in the mouse

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Intrinsic Activity, 2014; **2**(Suppl. 1): A1.43

Background: Parvalbumin (PV)-expressing GABAergic interneurons in the CA1 sector of the hippocampus and in the subiculum comprise basket cells and axo-axonic cells targeting the pyramidal cell soma and the axon initial segments, respectively. They potently control the firing frequency and timing of pyramidal cells. In patients with temporal lobe epilepsy (TLE) and in animal models of TLE degeneration of PV-expressing interneurons in the hippocampal formation has been observed by us and others. The goal of this study was to test whether impaired function of PV-GABA interneurons in the hippocampal/parahippocampal region of mice may be causative for development of epilepsy. We specifically silenced PV-expressing interneurons in subregions of the hippocampus and investigated the effect on development of spontaneous seizures.
Methods: Locally restricted stereotaxic injections of an AAV vector containing the GFP-tagged tetanus toxin light chain (TeLC) reading frame (inverted in a flip-excision (FLEX) cassette) were performed in mice expressing Cre-recombinase under the PV promoter. TeLC-mediated cleavage of vesicle-associated membrane protein 2 (VAMP2) stops vesicle fusion thereby inhibiting GABA release from PV-expressing interneurons. Respective viral vectors containing GFP alone were used for control injections. Mice were also implanted with transmitters for telemetric EEG recording and continuous video and EEG recordings were carried out for 30 (or up to 50) days after virus injection.
Results: Double-fluorescence immunohistochemistry confirmed strong expression of TeLC in PV-expressing interneurons at the injection site. About 40% of mice developed at least 2 spontaneous recurrent seizures within 5–30 days. The mean number of seizures was 2.2 ± 0.33 per week. Each seizure was preceded by several minutes of low-frequency population spikes. These occurred mostly in clusters followed by seizure-free intervals of 4–6 days. Animals developing no spontaneous seizures revealed a decreased seizure threshold upon application of pentylenetetrazole.
Discussion: The data underline the crucial role of PV-expressing GABAergic interneurons for the control of principal cell firing in the hippocampal/parahippocampal region. We show that even a spatially restricted loss of function of these interneurons (without neuronal damage) can lead to the development of spontaneous recurrent seizures.
Acknowledgements: Supported by the Austrian Science Fund FWF (P26680).

APHAR Section of Clinical Pharmacology

A2.1

Biliary amphotericin B pharmacokinetics and pharmacodynamics

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Background: Fungal cholangitis is a life-threatening condition affecting mainly immune-compromised persons, patients with choledocholithiasis, cancer, bile duct strictures, primary sclerosing cholangitis and liver transplant recipients. Because of its broad fungicidal activity, amphotericin B (AMB), particularly less toxic lipid-formulated AMB, is a therapeutic option. Therefore, biliary penetration of AMB was determined in four patients treated with AMB lipid formulations. Activity of AMB in bile at therapeutically achievable concentrations was assessed by *in vitro* simulation in order to detect an eventual ambience effect.
Methods: Two patients received liposomal AMB, two patients AMB colloidal dispersion. AMB concentration–time profiles in bile and plasma were determined in three liver transplant recipients. Bile was collected via T-tube or bile duct drainage. In addition, one sample was obtained by endoscopy from a fourth patient. The samples were purified by solid phase extraction. AMB was extracted with dimethyl sulfoxide and methanol and quantified by high-pressure liquid chromatography (HPLC). *In vitro* simulation was performed with isolates of *Candida albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei* incubated with AMB at concentrations of 0.025, 0.05, 0.10, 1.00 and 5.00 mg/l, respectively, dissolved in porcine bile, RPMI medium and RPMI medium at pH 7.8. Inocula of 10,000 cells of each *Candida* strain were incubated for 0, 7, 12, 24 and 48 hours, seeded by a spiral platter and manually counted.
Results: Biliary AMB concentrations (maximum 1.28 mg/l) were lower and displayed a slower rise and decline in comparison with plasma levels. The highest penetration ratio as expressed by the ratio between the area under the time–total-AMB-concentration curve in bile and plasma over the sampling period ($AUC_{0-n\ bile}$ / $AUC_{0-n\ TO\ plasma}$) amounted to 0.14. *C. albicans* and *C. tropicalis* presented a proliferation in bile similar to that in RPMI media whereas colony forming units (CFU) of *C. krusei* and *C. glabrata* remained constant over 48 hours. A biliary AMB concentration of 1 mg/l or less was not effective against the four *Candida* strains. Even the highest AMB concentration (5.00 mg/l) which exceeds biliary concentrations measured in our patients did not cause a relevant decline in CFU. Thus, AMB activity in porcine bile was lower than in culture medium.

Discussion: In the majority of bile samples, AMB concentrations were similar to or even markedly below the *in vitro* MIC values reported for relevant pathogens. *In vitro* simulation revealed a decreased antifungal activity of AMB in bile in comparison with RPMI suggesting an ambiance effect of bile on AMB pharmacodynamics. Based on these data, a reliable response of fungal cholangitis to treatment with AMB lipid formulations cannot be anticipated.

Acknowledgements: We thank Chiesi, Pfizer and MSD for financial support

Ecucation and Training in Pharmacology

A3.1

European Certified Pharmacologists (EuCP): standards for postgraduate professional training in pharmacology established by EPHAR, the Federation of European Pharmacological Societies

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Background: In many countries world-wide, pharmacologists have raised concerns that pharmacology as a discipline is under threats of disappearing. Departments of pharmacology have been abolished or merged with other units to form larger entities and in many cases not even the former laboratories have retained their distinction as pharmacological units. The new European Certified Pharmacologist (EuCP) scheme of EPHAR, the Federation of European Pharmacological Societies, is intended to provide a distinctly visible documentation which certifies that bearers excel in standards of education, skills, experience and professional standing in pharmacology.

Methods: Draft guidelines were prepared by a dedicated subgroup of EPHAR. In a further two-day workshop, delegates from twenty-one out of the twenty-seven EPHAR member societies discussed and prepared the final guidelines document (www.ephar.org/eucp).

Results: ***Certification procedures:*** National EuCP programs will be validated by an international EuCP committee. The national EuCP programs can be based on pre-existing certifications or diplomas or can be set up anew. Diploma programs of an external body (e.g. Medical Chambers or similar) must be validated by the society and checked whether they fulfil all EuCP criteria. Once the national EuCP system is accredited by the EuCP committee, the national societies of pharmacology will be responsible for the evaluation of all documents supplied by individual applicants and upon approval will forward the names of the applicants to the EuCP committee which will issue the EuCP certification (with a period of validity of five years). All certified EuCPs shall re-affirm their certification credentials on a five-year basis by supplying documentation of the continued professional practice and continuing professional development. ***Certification requirements:*** General requirements include an academic degree (MD, PhD, MSc or equivalent), documentation of training (both theoretical and practical), at least 5 years of pharmacological experience, current professional engagement in pharmacology, active membership in the national society of pharmacology, and a minimum number of professional contributions documented by publications, reports, expert opinions or similar. Training requirements as defined by the EuCP guidelines include fields of theoretical knowledge, practical awareness and practical skills; given the large diversity of the field of pharmacology, these are grouped into compulsory and elective items. ***Current status:*** As of June 2014, fifteen of the 27 member societies, representing 5,200 of a total of some 10,000 individual members, have already officially approved and adopted the EuCP certification scheme. The project has also already gained recognition by LifeTrain (Wolzt *et al.:* this meeting, [1]), a subproject of the European Union's Innovative Medicines Initiative (IMI).

Discussion: Besides providing common, high standards for professional excellence in pharmacology, the EuCP project shall also provide incentives for European pharmacological societies to develop and set up appropriate training courses for potential EuCP candidates and to implement or provide opportunities for continuing professional development.

Acknowledgements: EPHAR thanks N. Başaran of EUROTOX for helpful discussions; the generous support of the Austrian, Italian and Spanish Pharmacological Societies is gratefully acknowledged.

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A3.2

LifeTrain: towards a European framework for continuing professional development in biomedical sciences

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Intrinsic Activity, 2014; **2**(Suppl. 1):A3.2

Background: The medicines research and development process has recently undergone considerable change and will continue to change: biomedical professionals are now expected to be much more agile than previously, moving and collaborating between disciplines, sectors and geographical locations. This necessitates that they continually develop and maintain the required competencies to work most effectively. By its multi-disciplinary nature, the discipline of pharmacology is crucial and can serve as a role model in the whole value chain of medicines research and development. The Innovative Medicines Initiative (IMI) is Europe's largest public–private partnership which aims at improving the research environment in all sciences involved in medicines research (www.imi.europa.eu). The IMI Education and Training projects have developed LifeTrain, an emerging pan-European framework for continuing professional development (CPD) in the biomedical sciences [1].

Methods: LifeTrain's approach (www.lifetrain.eu) focuses on working collaboratively to develop competency profiles for the different roles required in medicines research and development. It was developed with four major stakeholder groups: professional/scientific bodies, course providers, employers and individual professionals. The LifeTrain agreed principles also promote the IMI quality standards for continuing professional development [2]. LifeTrain is supported by the on-course® resource, a comprehendsive pan-European course catalogue that serves course seekers to navigate the 'jungle' of post-graduate eduation and training opportunities offered in Europe (www.on-course.eu) [3]. LifeTrain is coordinated by the EMTRAIN project (www.emtrain.eu), on behalf of all the IMI-funded Education and Training projects.

Results: The LifeTrain framework is comprised of four sets of agreed principles; one for each of the four stakeholders groups, and has a growing list of signatories who have agreed to the principles of the framework and to work towards their implementation. Responsibility for learning lies with the individual professional, but is supported by employers, professional/scientific bodies and course providers working together to provide an appropriate environment for learning. Within the LifeTrain framework, several stakeholder groups, including the IMI projects Eu2P (www.eu2p.eu), PharmaTrain (www.pharmatrain.eu) and SafeSciMET (www.safescimet.eu) as well as professional bodies like EPHAR (www.ephar.org) are currently developing certification processes to recognise that bearers excel in standards of education, skills, experience and professional standing in their respective disciplines [4]. Inspired by the LifeTrain principles, EPHAR has recently launched their new European Certified Pharmacologist (EuCP) scheme (www.ephar.org/eucp) [5].

Discussion: With an increasing number of IMI LifeTrain signatories the current approaches to CPD in the biomedical sciences will dramatically change. Individual professionals will be guided by clearly defined competency profiles. They will develop and maintain their personal competency portfolios recognised by both professional/scientific bodies and employers. This stimulates mutual recognition of competencies to facilitate mobility: across disciplines; between academia, industry and regulatory authorities; and across geographical boundaries.

Acknowledgements: EMTRAIN LifeTrain Core Group, IMI Education and Training Projects, LifeTrain signatories (to be found on www.lifetrain.eu).

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A3.3

on-course®: a portal for in-service training and career development for biomedical scientists

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Intrinsic Activity, 2014; **2**(Suppl. 1):A3.3

Background: The Innovative Medicines Initiative (IMI; www.imi.europa.eu), Europe's largest public–private funding partnership for biomedical research, was established to speed up the development of better medicines for patients in Europe. IMI education and training projects were funded specifically to address the skills and competency requirements needed by the biomedical sector in Europe. IMI supports the European Medicines Research Training Network (EMTRAIN; www.emtrain.eu) as a collaborative education and training project with 12 academic and 15 industrial partners. One of EMTRAIN's actions has been to build the interactive online database on-course® (www.on-course.eu) to catalogue all EU postgraduate biomedical courses (continuing professional development (CPD), master and PhD) [1] to overcome the difficulty in accessing the right training programmes of the right quality. Finding a course containing high-quality content at the right price, time, location and in particular with the appropriate recognition by employers and/or professional/scientific bodies has been largely left to luck in the past.

Methods: The on-course® portal enables scientists to navigate the 'jungle' of postgraduate courses, address education and training gaps/quality and enhance interaction between course seekers and course providers. The on-course® portal has been designed to allow course seekers to apply tailored search strategies. It has been populated through a manual website search of over 1,000 course providers in over 30 EU Member and Associated States.

Results: As of July 2014, on-course® contains information (including details for costs, credits, location, language of tuition, detailed course description, breakdown of modules (for master and PhD courses)) on 3,020 master courses, 973 PhD and 2,285 CPD courses. The IMI education and training topics (SafeSciMet, EU2P, PharmaTrain and EMTRAIN) have also developed a set of quality standards for CPD courses which are displayed on the course details page [2]. Uniquely, the catalogue not only helps course seekers find an appropriate course, but also provides data for research on cross-sectional and longitudinal trends in post-graduate course provision in Europe. Thus far, on-course® has published on course fee trends and academic rankings for master courses [3] and is currently preparing a detailed overview of the distribution of English-taught master courses. on-course® is an integral part of an EMTRAIN-led work stream called LifeTrain that is helping to build a common European framework for mutual recognition of CPD in biomedical sciences [4]. In addition we have developed a database of learning methodologies to support learners.

Discussion: on-course® continues to grow by approximately 200 new courses being entered per month. Towards the end of September 2014, on-course® will be re-launched, using a new content management system. It will appear with a refined interface, improved functionalities and new features and services. It will switch to the XCRI data format (eXchanging Course Related Information) which is used to share information about courses between education institutions and course database compilers (http://www.xcri.co.uk/).

Acknowledgements: EMTRAIN Consortium, on-course® Development Group, IMI Education and Training Projects.

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A3.4

on-course®: a research tool for analysing cross-sectional and longitudinal trends in post-graduate course provision in Europe

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Intrinsic Activity, 2014; **2**(Suppl. 1): A3.4

Background: The Innovative Medicines Initiative (IMI; www.imi.europa.eu) supports the European Medicines Research Training Network (EMTRAIN; www.emtrain.eu) as a collaborative education and training project with 12 academic and 15 industrial partners. One of EMTRAIN's deliverables is the online database on-course® (www.on-course.eu), which compiles information about European postgraduate biomedical courses (continuing professional development (CPD), master and PhD) [1] to overcome the difficulty in finding and accessing training programmes. The amount and the quality of the information contained in the on-course® platform provides both an effective search tool for course seekers, and a unique resource for research and analyses of cross-sectional and longitudinal trends in post-graduate course provision in Europe. So far, two key questions have been investigated: (1) does course provider reputation as assessed by university ranking correlate with course fees for master trainings? and (2) what is the impact of using English-taught medium in biomedical master courses in Europe?
Methods: (1) For the question of correlation between fees and university ranking, the master course data and fees were reviewed from the on-course® catalogue and the correlation between course fees and university ranking was analysed. The on-course® platform contained—at the time of the analysis (August 2013)—2,360 courses leading to a master's degree, of which 1,951 courses were predominantly set in a teaching environment (taught courses) and 409 were predominantly set in a research environment (research courses). After weighting the merits of the various ranking systems we chose Webometrics Ranking (http://www.webometrics.info/en). This system provided the most comprehensive list (over 21,000) of ranked higher education institutions in the world and was the only ranking system that could be applied to every institute captured on on-course® [2]. Our analysis looked at courses from 370 universities that Webometrics ranked in Europe between position 1 and position 2,712. (2) A more recent investigation (July 2014) of language trends in 2,370 taught master's courses looked at: (i) The distribution and ratio of English-taught courses in EU member states, (ii) the influence of English-taught medium on student and staff mobility, and (iii) the influence of English-taught medium on course fees and university ranking.
Results: For question 1 (course fees vs. university ranking), we observed a negative and significant ($p > 0.001$) correlation between research master's course fees and university ranking for EU student fees and non-EU student fees where the top-ranking universities correlated with higher fees. We observed a similar significant trend for taught master's fees for non-EU student fees. However, our results showed no significant correlation between university ranking and taught master's course fees offered to EU students, suggesting that EU students are paying the same for biomedical courses regardless of the quality of the university [2]. For question 2 (English-taught course), we found that the number of English-taught master courses increased by 26% between the end of 2011 and June 2013 in European countries where English is not the primary language. There are several reasons that may be responsible for this growth that include greater scope for inter-country development/delivery of joint curricula, increased competitiveness of the course on the international market, improved employability for the student and an increase in domestic demand for such courses We observed a wide variation in the number and ratio to non-English-taught courses across Europe. We also found that the use of English medium is positively correlated with mobility, an increase in course fees and better ranking. Details of these results are being prepared for a publication.
Discussion: The amount and quality of the information gathered in on-course® has proven to be a unique resource for research on trends and gaps in the biomedical education and training sector. Further investigations of on-course® data will follow. The results will inform the European biomedical education and training community (course providers, employers, professional/scientific bodies and individual professionals/scientists) about trends and gaps. These results will be shared primarily through the on-course® and the LifeTrain (www.lifetrain.eu; [3]) platforms.
Acknowledgements: EMTRAIN Consortium, EMTRAIN Gap Team, on-course® Development Group, EMTRAIN LifeTrain Core Group

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