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POSTER

Effect of time-of-day on the efficiency of a short-term personalized Intermittent Work Exercise Program (IWEP) on endurance parameters and blood pressure among older adults

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Regularphysical activity offers primary and secondary prevention of several chronic diseases such as cardio-respiratoryamongolder adults. Recent research suggests that manipulating the timing of training and the circadian-rhythm can produce significant benefits on the physical performance. The purpose of this study is to investigate the effect of time-of-day on the efficiency of the IWEP on endurance parameters and blood pressure among seniors.

One-hundredsedentarysubjects over sixty years (mean age: 64 ± 3 years) free from cardiac and pulmonary diseasewere enrolled in this study.Participantswere dividedinto two groups: Morning training group (MG: n=50)and Evening training Group (EG: n=50). The IWEP consisted of a 36-min cycling workout twice a week over a 9-week period.The firstventilatory threshold(VT_1), heart rate (HR) at pre-training VT_1, totalworkload, systolic (SBP) and diastolic bloodpressure (DBP) were measured beforeand after training program.

The IWEP has decreased the SBPin both groups (MG:-4,7%and EG:-4,5%, all $p<0.05$) without significant change in DBP. Morning training was more effective than evening training on VT_1 (MG: 33,8% *vs.*EG: 24,8%; $p<0.05$), and on totalworkload (MG: 28,1% *vs.*EG: 21,3%; $p<0.05$), whereas no significant change was observed between groups in HR at pre-training VT_1.

Overall,the IWEP program showed a significant improvement in systolic blood pressure values for older adults.The time-of-day showed that MG is more efficient in the improvement of endurance parameters than EG. In this study we opted to use a short-term study of 9-week, a long-term study should better separate effects of time-of-day training amonghealthy seniors.

Active and living thicknanofibrous implant based on growth factor and stem cells for regenerativenanomedecine

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New-generation implants focus on robust, durable, and rapid tissue regeneration to shortenrecovery times and decreaserisks of postoperative complications for patients. Herein, wedescribe a new-generationthicknanofibrous implant functionalizedwith active containers of growthfactors and stem cells for regenerativenanomedicine.

A thickelectrospun poly(e-caprolactone) nanofibrous implant (from 700 mm to 1 cm thick) wasfunctionalizedwithchitosan and bonemorphogeneticprotein BMP-7 as growth factor using layer-by-layer technology, producingfishscale-likechitosan/BMP-7 nanoreservoirs. This extracellular matrix-mimickingscaffoldenabled*in vitro*colonization and boneregeneration by humanprimaryosteoblasts, as shown by expression of osteocalcin, osteopontin, and bonesialoprotein (BSPII), 21 daysafterseeding. *In vivo* implantation in mouse calvariadefectsshowedsignificantly more newlymineralizedextracellular matrix in the functionalized implant compared to a barescaffoldafter 30 days' implantation, as shown by histological scanning electronmicroscopy/energy dispersive X-ray microscopystudy and calcein injection. We have as wellbifunctionalizedour BMP-7 therapeutic implant by addinghumanmesenchymal stem cells (hMSCs). The activity of this BMP-7-functionalized implant wasagainfurtherenhanced by the addition of hMSCs to the implant (living materials), *in vivo*, as demonstrated by the analysis of new bone formation and calcification after 30 days' implantation in micewithcalvariadefects.

Therefore, implants functionalizedwith BMP-7 nanocontainersassociatedwithhMSCscanact as an accelerator of *in vivo*bonemineralization and regeneration.

How cellular plasticity is promoted and controlled in an organism?

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We are interested in the mechanisms allowing a cell to change its identity. For this, we use a natural transdifferentiation event where a rectal cell converts into a moto-neuron, in *C. elegans*. Work in the lab has shown that this cell type conversion involves first the erasure of the rectal identity, before re-differentiation into a moto-neuron. However this transient de-differentiation is not coupled with a reversal to a multi- or pluri-potent state. Further work has shown that a nuclear complex is necessary for the de-differentiation step, and that its members are conserved pluripotency factors. Pluripotency is one important cellular property of early embryonic cells in mammals that allows these cells to give rise to a variety of differentiated cells types, and ultimately to a functional organism. How is pluripotency established and maintained has been the subject of intensive work in the last decades, but is still not fully understood. Landmark experiments by Yamanaka show that 4 transcription factors, *oct3/4, sox-2, c-myc* and *klf4,* are sufficient to turn specialised cells into pluripotent cells, called iPS cells. In addition, sox2 and oct4 are at the center of the so-called pluripotency network, including sall4 or mta1, which is necessary to maintain pluripotency. We have found that these same 4 factors are necessary to promote de-differentiation in vivo, while not establishing pluripotency.

We are now closely investigating how these factors function during the initial phases of transdifferentiation. Indeed Arnaud Ahier has shown that *ceh-6/oct4* and *sox-2* can be required at successive steps of transdifferentiation, prior to the initiation. To understand whether and what intrinsic properties of CEH-6/OCT4 and SOX-2 could underlie their ability to promote de-differentiation but not pluripotency in the worm, as opposed to their mammalian counterparts, Arnaud has examined CEH-6 and SOX-2 interaction surfaces in *C. elegans*. We are further trying to understand which domains of CEH-6/OCT4 and SOX-2 are important for the transdifferentiation process as well as mapping the interaction pattern of ceh-6 and sox-2 with respect to their transcriptional activity, and will report on our latest findings at the meeting.

Role of BTLA in Systemic Lupus Erythematosus

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The balance between co-stimulatory and co-inhibitory receptors determines the fate of immune responses. Co-inhibitory receptors limit T-cell activation and may direct the immune response toward tolerance, and thus play an important role for the prevention of autoimmune diseases such as systemic lupus erythematosus (SLE). B and T lymphocyte attenuator (BTLA) is an inhibitory receptor of the CD28 family expressed on $CD4^+$ T cells and B cells. The ligation of BTLA with its ligand HVEM (Herpesvirus-entry mediator) attenuates T-cell activation, leading to decreased cell proliferation, cytokine production and cell cycle progression. Consistent with an inhibitory role for BTLA in T cells, BTLA deficient mice exhibit enhanced specific antibody responses and sensitivity to EAE. Moreover, the lupus disease was exacerbated in lupus mice deficient for BTLA suggesting a protective role of BTLA in lupus. These recent observations lead us to investigate the involvement of this newly described co-inhibitory receptor in human SLE. Indeed, altered expression and/or functionality of BTLA could result in defective regulation of the immune response leading to the emergence of autoimmunity.

Our preliminary experiments revealed higher BTLA expression on lupus Tregs, whereas the expression of BTLA is lowered on lupus $CD27^-$ memory B cells compared to controls. Moreover, we observed an altered capacity of BTLA to inhibit $CD4^+$ T cell activation in SLE patients. As BTLA expression does not seem to be impaired in lupus $CD4^+$ T cells, the next step is to analyze whether BTLA is efficiently recruited at the TCR synapse following stimulation. Finally, we plan to investigate the impact of BTLA on B cell functions in lupus patients. Our preliminary results are promising and suggest that the BTLA pathway could be altered in lupus B and T cells. The final objective is to define whether BTLA could be considered as a new interesting and potent target for the development of a therapeutic strategy in lupus.

Orthographic processing and lexical access in patients with schizophrenia: Analysis of the N170 component

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Reading has been proposed among the cognitive functions affected in schizophrenia. Evidence comes from standardized tests of passage (GORT, WJTA) or single-word reading (WRAT), for which patients exhibit lower scores than comparison subjects (Martinez et al., 2013; Revheim et al., 2006, 2014). As skillful readers are characterized by the speed and effortlessness with which they recognize written words, the degree of automaticity of word recognition processes seems to be the best indicator of reading level. The present study aimed to investigate the automatization of word recognition processes in patients with schizophrenia. We focused on the first step of word processing, i.e., the orthographic processing by exploring the coding of sublexical representations and the access to lexical representations. The orthographic sublexical coding was explored by manipulating mean bigram frequency in words (dependencies among letters in word-forms) while the lexical access was explored by manipulating lexical frequency. A group of patients with schizophrenia and a control group carried out a lexical decision task (i.e., is the presented stimulus a word or not?). Reaction times and the early N170 neuronal evoked response were registered to measure the degree of automatization of word recognition processes.

Behavioral results indicated a significant interaction between bigram frequency and lexical frequency. An inhibitory effect of bigram frequency appeared with high frequency words whereas a facilitatory effect of bigram frequency appeared with low frequency words. No effect interacted with the group. Electrophysiological results indicated a significant effect of bigram frequency and a significant effect of lexical frequency on N170 amplitudes. Larger N170 amplitudes were observed for low frequency than for high frequency words; larger N170 amplitudes were observed for low bigram frequency than for high bigram frequency words. Most importantly, no effect interacted with the group.

Behavioral and electrophysiological data showed that patients with schizophrenia, as comparison subjects, were sensitive to orthographic sublexical and lexical information in the course of lexical access. First, patients showed an unimpaired perceptual coding of orthographic properties within the first 200 ms of word perception, suggesting preserved abilities to encode letter position in letter strings and orthographic regularities throughout exposure to print. Second, the results indicated that the activation of lexical representations occurs at an early stage of word processing, suggesting preserved abilities to rapidly map the visual features of a word to its mental representation.

Detection of errors and conflict during a long sustained attention task in patients with schizophrenia.

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The main objective of this study is to better understand, in a time on task perspective, cognitive control impairments in schizophrenia, manifested in altered neural signatures during a long sustained attention task. Patients with schizophrenia and healthy controls (matched for age, gender and level of education) participate in this study. The task used is the sustained attention to response task (SART) for 30 minutes. Behavioral and ERP measures are recorded. For their analysis, the task is divided into two 15-minute periods. Our preliminary results obtained with 2 groups of 16 subjects each, reveal that schizophrenic patients are globally slower but able to maintain a stable level of performance over the course of the task. More importantly they reveal error- and conflict-monitoring disturbances in schizophrenic patients: patients exhibit an attenuated ERN and N200. This finding might be interpreted in terms of similar cognitive and neural mechanisms underlying the two ERP components.

Identifying novel viral RNA binding proteins in Drosophila

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Viruses are obligate intracellular parasites, which can infect all types of cells. Activation of antiviral pathways largely relies on the sensing of viral nucleic acids (NA), which often harbor specific marks that are recognized by sensor molecules. In mammals, a number of pattern recognition receptors and effectors molecules recognize viral NAs (e.g. members of the RLR, TLR and NLR families, IFITs). In flies, the only known sensor for viral RNAs to date is Dicer-2, a key component of the RNAi pathway. Our aim is to find novel RNA sensors in flies. Candidate proteins are identified by affinity purification-mass spectrometry (AP-MS) following binding to various RNA species. Each candidate protein is then be characterized and functionally validated in Drosophila (using a cell line and *in vivo*). For this, we establish knock-out (KO) mutants in the Drosophila S2 cell line using the CRISPR/Cas9 technology. As proof of principle, we generated KO cells lines for Dicer-2 and AGO2, which are two main components of the antiviral pathway already known. The same approach will be applied to the candidate genes identified in the AP-MS screen. After establishment of the KO lines, the cells are infected with several viruses and viral replication is monitored. Using this approach, we identified one molecule, which we named polyI:C binding protein (PIC), that is required for replication of the Picorna-like virus CrPV (Cricket Paralysis Virus). Experiments are in progress to elucidate the precise function of the PIC protein.

Contribution of different immunoglobulin subtypes to the protection of HIV patients

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It is now widely accepted thatantibodies (Abs) directed against HIV-1 (Human Immunodeficiency Virus type 1) play an essential role in the protection against infection with this virus. Thus, current vaccination strategies strive to induce HIV-specific Abs, several types of which were shown to beof interest. Among them, neutralizing Ab (NAbs) are able to protect macaques against experimental infection but are difficult to induce by vaccination. The RV144 Phase III trial demonstrated a 31% reduction of infection in the absence of NAbs,and this decrease of infection correlated with the induction of anti-gp120 immunoglobulin (Ig) G3Abs in patients. Furthermore, a study of HIC patients (HIV Controllers having an undetectable viral load without therapy) showed that anti-gp41 IgG2scorrelate with the lack of disease progression. These studies suggest that the Fc fragment (crystallizable) and the heavy chain of the Ab, determining its isotype, play a crucial role in protection.

The goal of this study is to characterize the isotypes and functional responses of Abs induced in sera of 37 HIC patients, 14 LTNP patients (Long-Term Non-progressors, with asteady rate of CD4 T lymphocytes) and 21 "progressor" patients (progressing to disease). We hypothesize that LTNP and HIC patients haveinduced a particularly favorable Ab response to control the disease progression or the viral replication. Therefore we have analyzed the distribution of the Ab response in the different cohorts. IgG isotypes have been purified and analyzed for their functional activities.

We found a variable induction of IgG isotypes: only a smallnumber ofpatients in each groupinduce anti-HIV IgG2s and IgG3s. Remarkably, the anti-HIV IgG2scorrelated to the total amount of IgG2s in sera of LTNP patients but not of "progressors", suggesting a different regulation of this particular IgG isotype in patients controlling infection. Analysis of the inhibitory functions of purified IgG2s and IgG3sshowed that these two isotypesdisplayneutralizing activities. In addition, we observed an Fc-dependent inhibitory activity in thepurified IgG3 fraction.

These results show that polyclonal sera contain a wide variety of anti-HIV Abisotypes with various inhibitory functions. An in-depth characterization of the IgG isotypes induced in HIC patients and their inhibitory activities willguide the design of new immunogens that are able to induce functionally relevant Abs by vaccination.

Loss of chronic morphine-induced hyperalgesia in mu opioid receptor knockout female and male mice

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Opiates are potent analgesics but their clinical use is limited by adverse events including analgesic tolerance and opioid induced hyperalgesia (OIH). Here, we examined the involvement of mu opioid receptors in OIH by comparing chronic morphine effect in wild-type (WT) mice and mu opioid receptor knockout (KO) mice.

Two protocols of repeated morphine administration were compared, consisting of 6 days 20 mg/kg/d intraperitoneal (ip) injections, and 4 days 60 mg/kg/d ip injections, followed by sensitivity thresholds scoring. Both protocols produced analgesic tolerance and OIH in female and male WT animals. However, no OIH was detected in mu receptor KO mice. These behavioural results have been observed in several tests exploring mechanical, heat and cold pain modalities.

Also, the morphine metabolite morphine-3-glucuronide (M3G) previously shown to mediate OIH, did produce hyperalgesia in WT but not in mu receptor KO animals. In a ligand activated GTPγS binding assay, G protein activation triggered by M3G was reversed by the selective mu receptor antagonist ([H-D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2] (CTOP) in brain membrane preparations from WT mice, and was absent when using brain membranes from KO mice.

Altogether, our findings on WT and mu opioid receptor KO mice indicate that mu receptor is required for hyperalgesia induced by both chronic morphine and M3G acute administration.

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Specific interaction between HIV-1 precursor Pr55^{Gag} and genomic RNA : the importance of Pr55^{Gag} p6 domain.

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Our group is interested in the molecular mechanisms leading to the specific selection of the human immunodeficiency virus type 1 (HIV-1) genomic RNA (gRNA) from cellular RNAs and other spliced viral RNA pools. This crucial step for viral replication is finely regulated by the specific interaction between the viral precursor Pr55^{Gag} and highly structured domains in the 5' untranslated region (5'UTR) of gRNA. This region includes the Psi element (*Packaging signal*) which contains the SL1 stem-loop allowing the viral RNA dimerisation through a loop-loop interaction due to a palindromic sequence in its apical loop. Up to now a major difficulty has been to express and purify functional full-length Pr55^{Gag}, and thus most *in vitro* studies have used a truncated protein missing Pr55^{Gag} C-terminal p6 domain (GagΔp6). Recently our group obtained a functional full length Pr55^{Gag}. According to our dynamic light scattering (DLS) data, Pr55^{Gag} is mostly present in solution as a trimer, and the analysis of the stoechiometry of Pr55^{Gag}-RNA complexes showed that two trimers of proteins bind to the gRNA. Based on Pr55^{Gag} intrinsic fluorescence signal conferred by several Trp residues, we analyzed Pr55^{Gag} binding to a large set of viral RNA fragments corresponding to different regions and mutants of the 5'UTR of gRNA. We showed that gRNA dimerization plays an important role for Pr55^{Gag}-gRNA interaction. Additionally, SL1 lateral loop was identified as the Pr55^{Gag} highest affinity binding site within the 5'UTR region of gRNA, allowing the discrimination of gRNA from spliced viral RNAs. We next investigated whether Pr55^{Gag} p6 domain could impact Pr55^{Gag} binding specificity. Our DLS data show that unlike the full length protein, GagΔp6 is mostly present in solution as a dimer and approximately two dimers of proteins bind to the viral RNA. Importantly, by analyzing GagΔp6 binding to the same RNA sets as was done for full length Pr55^{Gag} by steady-state fluorescence spectroscopy, we were able to compare precisely the affinity of those two proteins for the different viral RNAs. Our results clearly indicate a different binding mode for the GagΔp6 compared to the wild-type protein and show an important role of the p6 domain in the specificity of the Pr55^{Gag}-gRNA interaction *in vitro*. Moreover, currently ongoing DLS titration experiments would complete this analysis of Pr55^{Gag} p6 domain in order to establish its role regarding Pr55^{Gag}-RNA interaction also during the viral assembly step.

Identification of genes causing congenital myopathies

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Congenital myopathies are genetic disorders characterized by distinctive morphological abnormalities in skeletal muscle fibers. They define a class of severe muscle diseases with a strong impact on patient survival and quality of life. The main subclasses include nemaline myopathy (with protein aggregates or rod), cores myopathy (central core or multi-minicore, well demarcated areas devoid of mitochondria) and centronuclear myopathy (centralization of nuclei). A large number of genes has already been described for causing congenital myopathies. However, about half of the patients do not have a genetic diagnosis supporting the implication of a large number of yet unidentified genes.

Massively parallel sequencing offers an unbiased and integrated approach to accelerate the identification and characterization of the genetic basis of congenital myopathies. Myocapture is a consortium of research teams, clinicians and sequencing platform working together to characterize the clinical, histological and genetic data of patients. The strategy was to sequence 1000 exomes of patients and their family, previously excluded for known myopathy-causing gene. For exome data analysis, efficient bio-informatic pipelines have been developed in house and shown to be very powerful to identify the mutations responsible for the disease.

Within this project, we studied a non-consanguineous Franco-Lebanese family with three affected children suffering from severe neonatal hypotonia, swallowing troubles and weak limb reflexes. Structural abnormalities on biopsy were not specific of any classical congenital myopathy. We sequenced the exome of the six family members, the two parents and their four children and filtered the variants according to a recessive mode of inheritance. All affected members carried two variants in SCN4A, a sodium channel highly expressed in muscle, compatible with a compound heterozygous segregation. A missense mutation in a well conserved amino-acid was transmitted by the father and a mutation affecting an essential splice donor site was transmitted by the mother. The mutations were well covered and confirmed by Sanger sequencing.

This example of integrated approach helped to expand the phenotype of diseases associated with mutations in SCN4A, previously described in other diseases such as congenital myotonia and potassium-related periodic paralysis. We identified SCN4A as a gene causing a new type of myopathy characterized by a clinical improvement over time and an overlap between classical congenital myopathy and dystrophy on muscle biopsy. Thus, this integrated clinic-molecular approach refines the classification of myopathies.

The identification, validation and characterization of novel implicated genes in congenital myopathies such as SCN4A will allow the development of novel diagnosis protocols to improve genetic counseling, including eventual prenatal or pre-implantation diagnosis. Moreover, the identification of novel genes is an important step for the discoveryof new therapeutic targets.

Double compartmented and hybrid implant outfitted with well-organized 3D stem cells for osteochondral regenerative nanomedicine

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Articular cartilage repair remains challenging, because most clinical failures are due to the lack of subchondral bone regeneration. We report an innovative approach improving cartilage repair by regenerating a robust subchondral bone, supporting articular cartilage.

We developed a compartmented living implant containing triple-3D structure: stem cells as microtissues for embryonic endochondral development mimic, nanofibrous collagen to enhance mineralization for subchondral bone and alginate hydrogel for cartilage regeneration.

This system mimics the natural gradient of the osteochondral unit, using only one kind of stem cell, targeting their ability to express specific bone or cartilage proteins. Mineralization gradient of articular cartilage and the natural 'glue' between subchondral bone and cartilage were reproduced *in vitro.*

Effects of acute and chronic light exposure on hedonic eating behavior: molecular and neurobiological mechanisms

Supervisors: Jorge Mendoza and Andries Kalsbeek

Light is the principal synchronizer for the mammalian circadian clock in the suprachiasmatic nucleus (SCN). Intrinsically photosensitive retinal ganglion cells (ipRGCs), expressing the photopigment melanopsin, respond directly to light and send this information to different brain regions, including the SCN, which may modulate diverse behaviors, such as locomotion, mood and feeding.

In addition to homeostatic- also hedonic mechanisms are thought to be a major determinant for food intake, particularly for palatable foods. For the homeostatic pathway, nuclei from the mediobasal hypothalamus, such as the arcuate nuclei and the lateral hypothalamus, play an important role, on the other hand corticolimbic structures are more implicated in the reward aspect of feeding. Interestingly, some of these structures receive projections from the retinal ipRGCs cells. Hence, light in some way might regulate feeding behavior and metabolism.

Therefore, the aim of this project is to study the acute and chronic effects of light (at different times of the day, different intensities and wavelengths) on regular and palatable food intake and metabolism in mice, and to reveal the possible neurobiological mechanism implicated.

miR-135a-5p-mediated downregulation of protein tyrosine phosphatase delta (PTPRD) is a candidate driver of HCV-associated hepatocarcinogenesis

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Background and Aims: Hepatitis C Virus (HCV) infection is a main cause of hepatocellular carcinoma (HCC). Even though novel antivirals can efficiently eradicate HCV infection, HCC risk remains elevated after viral clearance. HCV contributes to HCC development by perturbing signaling circuitry, and these signaling events are tightly regulated by protein phosphatases. However, the impact of HCV on phosphatases is largely unknown. Thus we aimed to identify phosphatases that are potentially relevant for HCC development and that respond to HCV infection *in vivo*.

Methods: Using RT-qPCR and FISH we screened and validated protein phosphatase and microRNA (miRNA) expression in liver biopsies of HCV patients and in primary human hepatocytes. Phosphatase expression in paired liver biopsies of HCC patients was correlated to patient survival and tumor recurrence.

Results: We show that tumor suppressor PTPRD is consistently downregulated upon HCV infection *in vivo*. Moreover, we demonstrate that PTPRD expression is impaired in tumor lesions of paired liver biopsies and that high levels of PTPRD in adjacent liver tissue of HCC patients correlate with survival and reduced tumor recurrence after surgical resection. We identified miR-135a-5p as a mechanistic regulator of hepatic PTPRD expression in HCV patients.

Conclusions: Our results demonstrate impaired PTPRD levels in infected hepatocytes and HCCs potentially represent a hallmark of liver disease progression. PTPRD is a tumor suppressor and phosphatase of STAT3, a HCV co-factor. HCV may maintain STAT3 activity via miR-135a-5p-mediated downregulation of its negative regulator PTPRD, leaving the liver more prone to malignant transformation as a side-effect.

Disclosure of Interest: None Declared

The role of the sympathetic hyperactivity in the development and modulation of glucose metabolism

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Introduction: The causal role of sympathetic nervous system in the development of metabolic disorders has not been clearly defined. The aim of the study was to determine if a chronic increase of the sympathetic nervous activity could, by itself, trigger the development of metabolic disorders. For that purpose, we used a transgenic mouse model, in which the gene encoding for the reuptake norepinephrine transporter (NET) has been deleted; these animals display increased urinary catecholamine excretion. **Methods:**Thecarbohydrate metabolism status was characterizedat ages of 10, 20, 25 weeksin heterozygous NET knockout mice (+/-) and wild-type mice (+/+). From the age of 25 weeks old, +/- and +/+ mice received normal water or a 30% w/v fructose in drinking water during a 15 week-long periodto induce metabolic disorders. Metabolic parameters (plasma triglycerides and total cholesterol, total fat volume,intraperitoneal glucose tolerance test, IPGTT, insulin tolerance test, ITT, insulin rates) were measured after a 5h fasting period before and at the end of the diet period. **Results:**+/- mice displayed clear glucose intolerance already at 10 weeks of age (area under the curve (%) = 24859 vs 18016, p=0.004, respectively) and just before starting the fructose diet (AUC (%) = 24524 *vs* 20573, p=0.005 for +/- and +/+ mice respectively at 25 weeks of age).Moreover sensibility to insulin seemed to be diminished in +/- mice compared to +/+ mice (insulin plasma concentration = 1.12 vs 0.86 µg/L, and HOMA-IR = 11.09 vs 8.36, respectively), which is associated with an increase of urinary noradrenaline level (2598 vs 1521 nmol/L respectively, p=0.02). These animals were also much more sensitive to high fructose, since a 20% increase in the AUC of the IPGTT was obtained at the end of the high fructose diet, compared to the 2% increase in +/+ mice. This disorder wasassociated with an increase of the insulin resistance (HOMA-IR = 11.13 vs 18.11, p=0.01, and insulin plasma concentration = 1.06 vs 1.84µg/L, p=0.01 before and after fructose diet respectively). No differences were observed in lipid metabolism and in total body fat proportion between the groups.**Conclusion:** Our data suggest that constitutive chronicsympathetic hyperactivitycan induceby itself a carbohydrate metabolismdisorder and amplify the susceptibility to diet-induced metabolic dysfunction. Moreover, the involved mechanism seemed to be independent fromtotal adipose tissue and from lipid metabolism.

Brain clocks: the circadian timing of the lateral habenula

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Behavioral and physiological functions of organisms are periodic. Cycles near of 24h are termed circadian (*circa*: close to; *dien*: day) rhythms. These rhythms are the result of a molecular clock composed by positive and negative loops of the expression of clock genes in different tissues. The master pacemaker, which coordinates and synchronizes other body clocks to the environment, is localized in the brain specifically in the hypothalamic suprachiasmatic nucleus (SCN)[a]. The lateral habenula (LHb), a small brain structure emerging as key nucleus in dopamine and serotonin control, show circadian clock properties: firing rate and clock genes expression oscillate in a 24h range, LHb can be affected by photic stimulation, and have connections with the SCN clock[b]. However, little is known about the clock machinery of LHb. Hence, the aim of this study is to decipher whether the habenula oscillates in a self-sustained manner or is SCN dependent. We use transgenic mice expressing a luciferase reporter (PER2::LUCIFERASE) to investigate the endogenous circadian rhythms of clock protein PER2 in the habenula explants of animals carrying a bilateral SCN lesion.

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[a] Albrecht, 2012

[b] Salaberry and Mendoza, 2016

Dynamic aspects of translation initiation and its control in *Staphylococcus aureus*

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Bacterial cells must constantly sense their environments to ensure rapid and appropriate responses to unfavorable conditions. Regulation of translation contributes to the rapid adjustment of bacterial physiology in response to environmental changes and stress stimuli. Many of these regulations take place at the translation initiation phase, the rate-limiting step of protein synthesis. Even if the translation initiation process is most likely very similar in bacteria, low GC content Gram-positive bacteria, as *Staphylococcus aureus*, seem to differ on the mechanism by which structured mRNAs are recognized and adapted on the ribosome. One of the peculiarities of these Gram-positive bacterial ribosomes is the presence of a ribosomal protein S1, which is shorter and has a different domain organization. Extensive *in vivo* and *in vitro* studies have demonstrated the implication of S1 in the translation of structured mRNAs in the Gram-negative bacteria*Escherichia coli*, but nothing is known about the S1 functions in *S. aureus*.

My project is focused on the study of translation initiation in *S. aureus,* an opportunistic pathogen and a serious threat for human health.More specifically, the functions of protein S1 in RNA metabolism will be studied. Analysis of the ribosome content by mass spectrometrysuggested that S1 is not strongly associated to the ribosome. Preliminary data show that the purified ribosomes do not bind to mRNAs carrying structural elements sequestering the Shine and Dalgarno sequence. We wonder whether the shorter protein S1 in *S. aureus* contribute to the translation of structured mRNAs outside from the ribosome. In a first attempt, co-immunoprecipitation (co-IP) assays were used to characterize the targets of S1. Proteomic analysis and preliminary RNA-seq data have been performed to characterize the proteins and RNAs, respectively, which were co-immunoprecipitated with S1. Among the co-IP RNAs, we surprisingly identified three small non-coding RNAs, many tRNAs and some specific mRNAs encoding virulence factors. I am presently validating these interactions and will analyze the functional consequences of this novel RNA-protein (RNP) complex on *S. aureus*physiology and pathogenesis.

Lateral Habenula: involvement in working memory

The lateral habenula (LHb) is a structure involved in decision making based on the rewarding and aversive valences of incoming stimuli, mainly through its connection with the basal ganglia and the dopaminergic system. Very few is known about its involvement in memory processes. Here we investigated the potential role of the LHb in working memory (WM) given its key anatomical position between the prefrontal cortex and the dopaminergic system, which are key actors of WM processing.
Sixteen food-restricted male Long-Evans rats were trained on a delayed non-matching to position paradigm in 9-hole operant chambers. Two pairs were used (holes 1 and 7; holes 2 and 8). Rats received 1 session per day. A session was composed of 48 trials, comprising a sample phase, i.e., the illumination of one hole (1, 2, 7 or 8), and a test phase, i.e., the illumination of the sample hole and of its associated hole within each pair. A correct answer gave rise to a food reward (one 45mg sucrose pellet). Between the sample and the test phases four different delays were introduced, 0, 2, 4 or 8 seconds.
Following training, rats received LHb or Prefrontal Cortex (PFC) reversible inactivation by means of muscimol (Musc, 24ng on each side), and a sham procedure (vehicle, Veh). Then a PFC-LHb disconnection by a muscimolinfusion procedure was made. Analyses of the percentage of correct responses showed an impairing effect of muscimol. While under Veh treatment rats performed above chance at the 0-, 2- and 4-sec delays, and at chance at the 8-sec delay, under Musc treatment they performed above chance at the 0- and 2-sec delaysfollowing LHbmusc infusion, and only at 0 sec delays following PFC musc infusion and PFC-LHb disconnection. Those deficits were unlikely to reflect altered levels of motivation for performing the task and/or obtaining a food reward, as not only Musc did not prevent rats from completing the 48-trial session, but it did not impact on the number of omissions (during both the sample and test phases), nor on the latency to collect the food reward following a correct answer.
Those results suggest that the LHb belongs to limbic networks mediating executive functions and that the PFC-LHb pathway is involved in working memory process.

Cell type specific connectivity in central extended amygdala

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ABSTRACT: Central amygdala (CeA) and bed nucleus of the striaterminalis (BST) are important parts of central extended amygdala. While CeA and BST share similarity in cytoarchitecture, input/output connectivity, neuropeptide and neurotransmitters, to which extent these connections arespecific in mice is still unknown. We look at the connectivity of subdivisions of CeA and BST with anterograde/retrograde tracers and immunohistochemistry in mice.

It is known that CGRP signaling from lateral parabrachial nucleus (LPB) is important in anxiety, pain and anxiety in mouse models. Our results show that both BSTand CeA receive strong input from lateral parabrachial nucleus (LPB), and they also send projections back to LPB. Within central extended amygdala, medial CeA (CeM) and CeL strongly projects to oval nucleus of BST (BSTov). The BSTov also project to ventral BST, which has been implicated in anxiety and pain induced conditioned place avoidance. The results are in line with works done on rat.

On the other hand, preliminary results show these connections arise from subpopulations in individual subnuclei. The PKCδ positive cells, but not somatostatin (SOM) expressing cells, in BSTovreceive intensive CGRPergicinnervation from LPB. Similarly, PKCδ cells in capsular and lateral CeA also receive strong CGRPergic input from LPB. This leads to our hypothesis that PKCδ cells relay the nociceptive information from LPB and broadcast it to its downstream target i.e. CeM and BST.

Within the central extended amygdala, SOM cells in CeA send projections to BST while PKCδ cells in BSTsend projection to CeA. The SOM cells in BSTov also projects to ventral BST. Further investigation will focus on whether these projections are excitatory or inhibitory and their potential role in pain model.

The role of *Pseudomonas aeruginosa* siderophores in the homeostasis of different biological metals.

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The bacteria *Pseudomonas aeruginosa* is an opportunist human pathogen involved in 10-20% nosocomial infections and also responsible of severe infections in immunocompromised patients. Nowadays, it is the protagonist of an important sanitary problem due to its resistance to the actual antibiotics. The final goal of this work is to develop new therapeutic strategies against this pathogen, and the research is focalised on the iron homeostasis. Iron is an essential nutrient involved in very fundamental aspects of cellular function. *P. aeruginosa*, to get access to iron, produces two major siderophores: pyoverdine (PVD) and pyochelin (PCH), chelating the metal and transporting it inside the cell, via different specific proteins.

The concentration of this metal, in complex with the siderophores, regulates de expression of the proteins belong to the PVD and PCH pathways. Anyway, both siderophores carry out other functions than iron uptake. They play a role in the bacterial virulence, exporting toxic metals from the cytoplasm to the extracellular medium, conferring resistance against these toxic compounds, but we do not know if they play a role in the homeostasis of other biological metals different than iron (Co, Cu, Mg, Mn, Mo, Ni, V and Zn). We showed that mostly Cobalt, but also Nickel, Copper and Manganese, repress the expression of the PCH pathway genes but not the PVD pathway. We have also seen that some of these metals could be transported to the cellular cytoplasm in complex with PCH. We want to figure out what is the real effect of this metal transport, and if they can activate the transcriptional regulator PchR, in order to understand how exactly the mechanism of the PCH pathway gene inhibition works.

MICROBIOLOGICAL CARASTERISAZATION OF CLINICALLY SIGNIFICANT COAGULASE-NEGATIVE STAPHYLOCOCCI (CNS) ISOLATE FROM VARIOUS CLINICAL SPECIMENS AT THE NATIONAL UNIVERSITY HOSPITAL CENTER HUBERT K. MAGA (CNHU-HKM) OF COTONOU (BENIN)

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Abstract

Coagulase negative staphylococci (CNS) are now considered true nosocomial and community pathogens. In Africa and particularly in Benin, little interest granted to them and there is not enough data to improve the management of these infections. The aim of this study was to determine the microbiological characteristics of clinically significant coagulase negative staphylococci collected at CNHU-HKM of Cotonou. Eighty-nineteen CNS strains were identified to species level by API® STAPH and confirmed by MALDI-TOF MS. Their antibiotic resistance profile was determined by VITEK 2. We also tested strains for their hemolytic, protease, esterase, and cytotoxic activity respectively on human erythrocytes, azocasein tween 80 and human polymorphonucleair neutrophil leukocytes. The most frequently species isolated were *S. haemolyticus* (44%) followed by *S. epidermidis* (22%) and *S. hominis* (7%). Bacteremia (66.7%) and urinary tract infections (24.2%) were the most CNS infections encountered. The strains were resistant to several antibiotics, including penicillin (92%) fosfomycin (81%), methicillin (74%), trimethoprim-sulfonamide (72%), cefoxitin (74%) and kanamycin (65%). Only sensitivity to vancomycin and linézoide were 100%. The most CNS species isolated were the most resistant to methicillin with 100% for *S. hominis* 93% for *S. haemolyticus* and 67% for *S. epidermidis*. Resistance rate to other antibiotics of MRCNS was higher significantly ($p < 0.001$) compared to MSCNS in this study. Screening of exotoxins production revealed hemolytic activity of 25% of the culture supernatant to a quarter of their dilution at least 50% of red blood cells. Twenty-six percent exhibited protease activity which 5% low, 10% moderate and 11% high activity. Research of esterase activity also showed 25% of positive strains. The cytotoxicity assays revealed a high cytolytic activity of three strains of different species. This microbiological study reveals the diversity of CNS clinically significant and putative pathogenic species such as, *S. heamolyticus* and *S. epidermidis* who were the most exoenzymes producers.

Keywords: microbiological carasterisazation, Coagulase Negative Staphylococci (CNS), exoenzymes.

INTAKE OF AN OPTIMIZED OMEGA 3 FORMULATION EPA:DHA 6:1 PREVENTS THE ANGIOTENSIN II-INDUCED HYPERTENSION AND ENDOTHELIAL DYSFUNCTION IN RATS

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Introduction: Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to cause endothelium-dependent nitric oxide (NO)-mediated relaxations of isolated blood vessels, with an optimized ratio of EPA:DHA 6:1. The aim of the present study was to determine whether prolong intake of EPA:DHA 6:1 affects hypertension and endothelial dysfunction induced by angiotensin II (Ang II).

Materials and methods: Male Wister rats were used for the study. All animals received 500 mg /Kg per day either corn oil or EPA:DHA 6:1 . At the beginning of second week, all the rats underwent either sham surgery (sham Rats) or were implanted with mini osmotic pumps that released 0.4 mg/day of angiotensin II . Systolic blood pressure was measured twice in a week for a total of 4 weeks using the tail cuff sphingomanometry method.

Results: The blood pressure of the ang II group was increased significantly to 215 mm of mercury versus 136 mm in control and EPA:DHA 6:1 rats. This increase in blood pressure was significantly reduced by omega-3 treatment. Second branch mesenteric arterial rings were subjected to different concentrations of acetylcholine to study the relaxation. Ang II rats decreased the endothelium derived hyperpolarization (EDH) mediated component more than the nitric oxide(NO) mediated relaxations. Pronounced endothelium dependent contractile response was observed in the ang II group. Omega-3 treatment improved both EDH and NO mediated relaxation and also decreased the contractile response. The results of vascular reactivity were reconfirmed by the immunohistochemistry and western blot showing up regulations of the inflammatory proteins responsible for endothelial dysfunction in the Ang II infused group and were which were down regulated by omega 3 treatments.

Conclusion: The present findings shows that EPA:DHA 6:1 prevents the development of endothelial dysfunction and hypertension induced by Ang II.

Liposome-based vaccines for targeted cancer therapy

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Despite being quite effective, conventional cancer therapies have the major drawback of triggering numerous side effects. Currently, a challenging goal in this area is the development of innovative targeted antitumoral immunotherapies with a long-term efficiency. In this context, my team took advantage of **liposomal nanoparticles** properties for the conception of **cancer vaccines.** In a previous study, the combination on liposomal surface of three elements (two peptide epitopes including one that is specific to tumor cells and an adjuvant, ligand of TLR2) crucial for immune response has demonstrated to induce complete regression of tumor growth after prophylactic or therapeutic treatments in mice grafted with the murine kidney carcinoma RENCA expression a human xenoantigen, the ErbB2 tumor specific antigen [1]. However, the therapeutic treatment efficiency quickly decreased with the increase of the time spent between tumor grafting and treatment start [2].

In order to optimize our treatment and show the universality of our nanoparticle approach, we proposed to validate our strategy in **another tumor mouse model** and with other adjuvants or combination of adjuvants. We had to **optimize the composition of our nanoparticles** by selecting new peptides specific for the new tumor cells and then to validate their immunogenic and antitumoral potential *in vivo.* Thereby, we observed an almost complete regression of tumor growth after vaccine injections on days 2 and 4 after tumor implantation with our classical adjuvant.

Thanks to the versatility of the lipid nanoparticles, it was possible to adapt the therapeutic treatment and make it effective even within another murine model without xenoantigen and therefore closer to spontaneous tumors. The next steps will be to test new adjuvants or adjuvant combinations to extend the time spent between tumor implantation and treatment. For example we plane to associate TLR-2 and NOD1 agonists as they already showed to have a synergistic effect in vitro [3].

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