Abstracts: 2nd European Psychoneuroimmunology Network (EPN) Autumn School: The skin-brain axis and the breaking of barriers

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(1) Is it possible to counteract the mechanisms of pancreatic β-cell stress in diabetes mellitus?

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Introduction: Pancreatic β-cells are specialized to secrete insulin in response to circulating nutrients, mainly glucose. Type 1 diabetes (T1D) is characterized by an immune-mediated progressive β-cell destruction. Due to the high rate of insulin production and secretion under stimulated conditions, β-cells undergo physiologic endoplasmic reticulum (ER) stress. Severe and uncompensated ER stress in β-cells and subsequent insulin secretory deficiency is induced by proinflammatory microenvironment before onset and during T1D. Moreover, hyperglycemia triggers excess production of mitochondrial reactive oxygen species (ROS) that overwhelm the anti-oxidative capacity of β-cells, leading to oxidative stress. The crosstalk between the ER and oxidative stress further contributes to β-cell dysfunction. We described that Compound A (CpdA), a selective glucocorticoid receptor (GR/*NR3C1*) ligand that exerts inflammation-suppressive activity *in vivo*, is an efficient modulator of effector T and dendritic cells, but in a GR-independent manner. Here, we explore the protective effects of CpdA on proinflammatory cytokine-induced β-cell ER and oxidative stress.

Methods: Rat insulinoma INS-1E cell line, murine and human pancreatic islets, and NOD mice were use as T1D cellular and animal experimental models. Quantification of NO by Griess method; insulin by ELISA; cell death by Hoechst and propidium iodide staining; cell viability by MTT test; mRNA by RT-qPCR (Sybr Green/Rox); proteins by BCA, Western blot and immunofluorescence; insulin by ELISA; transcriptional activity by LUC-reporter plasmid.

Results: We demonstrate that CpdA improves the unfolded protein response (UPR) by attenuating ER stress of β-cells exposed to an environment of proinflammatory cytokines (IL-1β+IFN-γ; CYT). CpdA significantly reduced CYT-induced activation of NF-κB signaling pathway and NO secretion ($p<0.05$). CpdA treatment impaired eIF2α phosphorylation enhancement and the increased expression of ER stress protein markers, such as ATF4 and CHOP, in CYT-challenged INS-1E cells ($p<0.05$). The expression of chaperones involved in protein folding and processing (PDI, ORP150) was enhanced in the presence of CpdA ($p<0.05$). In addition, we showed that CpdA enhanced Nrf2 transcriptional activity (antioxidant defense pathways) and the expression of Nrf2 target genes in INS-1E cells ($p<0.05$), thus reducing CYT-induced ROS generation ($p<0.05$). The attenuating effects of CpdA on ER and oxidative stress in cytokine-challenged β-cells were positively reflected by their viability and function. CpdA treatment reduced β-cell apoptosis levels and improved Glucose-Stimulated Insulin Secretion (GSIS) in both murine and human islets ($p<0.05$). CpdA administration to NOD*scid* mice adoptively transferred with

diabetogenic splenocytes (from diabetic NOD mice) led to a delayed disease onset and reduction of diabetes incidence. Histological analysis of the pancreas showed a reduction in islet leukocyte infiltration and preservation of insulin expression in CpdA-treated normoglycemic mice in comparison with the control group.

Discussion: These new findings, together with our previous reports, justify further studies on the administration of this small molecule as a novel therapeutic strategy with dual targets (effector immune and β-cells) for autoimmune diabetes. Additionally, the protective effect of CpdA on β-cells exposed to stressors linked to type 2 diabetes pathogenesis, such as glucolipotoxicity, psychological stress, and aging, deserves exploration.

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(2) Effects of Mindfulness-based Stress Reduction (MBSR) intervention on mental health and plasma level of IL-17 in patients with long COVID-19

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Introduction: Post-Covid Syndrome (PCS) is defined as the continuation or development of new symptoms three months after initial SARS-CoV-2 infection. These symptoms must last for at least two months with no other identifiable cause. Pieces of evidence have shown a potential link between high levels of IL-17 and the severity of COVID-19 and PCS. IL-17 could be a promising target for therapeutic strategies.

Method: Thirty patients with PCS were randomly divided into two groups, with 15 participants in each group (MBSR and wait-list control). Inclusion criteria comprised patients diagnosed with PCS by an infectious disease specialist. Individuals with a history of mental health issues that required medication were excluded. Then, the MBSR group received eight weekly sessions of MBSR intervention. MBSR is a scientifically-backed, eight-week program designed to help individuals to cope with stress, anxiety, depression, and pain through intensive mindfulness instruction. MBSR employs an array of techniques, including mindfulness meditation, body awareness, yoga, and exploration of behavior, thought processes, emotions, and actions. We assessed mental health using the "General Health Questionnaire" (GHQ-28) and measured the level of IL-17 in plasma for both groups before and after the intervention. The "mixed factorial analysis of variance" test was used for analyzing data for each dependent variable, and appropriate t-tests were conducted when necessary.

Result: There was no significant difference between the two groups (MBSR and wait-list control) in terms of the GHQ total score [AdR4] ($F(1,28) = 0.91$, $P = 0.50$) and IL-17 level in plasma ($F(1,28) = 1.33$, $P = 0.25$) before the MBSR intervention. However, the GHQ total score [$F(1,28) = 0.41$, $p < 0.001$] and plasma level of IL-17 [$F(1,28) = 0.09$, $p < 0.001$] decreased significantly in the MBSR group compared to the control group after intervention. Furthermore, the GHQ total score ($t = 42.62$, $p < 0.001$) and plasma level of IL-17 ($t = 13.91$, $p < 0.001$) were significantly reduced in the MBSR group after the intervention, compared to baseline.

Conclusion: Our findings suggest that psychological therapy, such as MBSR, which directly affects the management and control of stress, can improve mental health and reduce anxiety and depression, as well as key immunological factors, in patients with PCS.

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(3) Counteracting neuroinflammation underlying neurodegenerative diseases

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Introduction: Neuroinflammation results from hyperactivation of glial cells in the central nervous system (CNS). Microglia represents the innate immunity of the CNS and responds to insults through the release of inflammatory

mediators. Acute CNS injury initially activates microglia toward the M1 phenotype, producing pro-inflammatory cytokines and when the damage is circumscribed, microglia polarize toward an anti-inflammatory M2 phenotype, releasing anti-inflammatory cytokines. In this connection, new therapeutic strategies for neurodegenerative diseases, such as Alzheimer's disease (AD), target microglia and neuroinflammation. One of these, lies in the modulation of the endocannabinoid system. The first endocannabinoid to be characterized was anandamide (AEA), which is hydrolyzed by the fatty acid amide hydrolase (FAAH). AD brains show increased FAAH activity associated with decreased levels of AEA. Other experimental plans aim at isolated bioactive molecules from natural products.

Methods: Taking advantage of murine BV2 microglia cells stimulated with Aβ peptide, we aimed at determining whether pharmacological inhibition of FAAH by administration of URB597 may influence microglia polarization and restore the autophagy, which is impaired in AD and is likely related to amyloid plaque formation. mRNA and protein expression of M1/M2 markers, were analyzed by RT-PCR and immunofluorescence.

Results: Evaluation of morphology and key markers of microglial activation showed that URB597 reverts microglial activation toward an anti-inflammatory state and restores autophagy, as demonstrated by increased mRNA expression of ATG7, Beclin1, LC3 and P62. We showed that *Saccharomyces cerevisiae* reverts LPS-stimulated microglial cells to an anti-inflammatory phenotype. Furthermore, the nutraceutical properties of extracts derived from durum wheat chaff induced increased mRNA expression of anti-inflammatory and antioxidant markers such as CD206, NRF2 and SOD1.

Discussion: Our studies underline the anti-inflammatory and anti-oxidant activity of treated yeast, as well as waste products derived from wheat. These molecules showed nutraceutical properties aimed at counteracting inflammation and oxidative stress. Overall, our data suggest that FAAH inhibition and green extract treatments can drive microglial polarization toward an anti-inflammatory phenotype and promote autophagy representing a putative promising treatment for neurodegenerative diseases.

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(4) Intra-individual variability in stress predicts antibody response longevity following influenza vaccination in older adults: a prospective observational cohort study

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Introduction: Multiple systematic reviews have demonstrated robust relationships between psychological factors and antibody responses following vaccination. However, existing research has almost exclusively relied on single time point measures of psychological constructs (e.g., stress, affect), rather than any aspect of how these constructs may fluctuate over time. Intra-individual variability in psychological factors has been associated with a range of poorer physical health outcomes, but there is a paucity of research exploring this in an immune challenge context. The present study sought to address this by examining the relationship between intra-individual variability in psychological factors and antibody responses to vaccination in a cohort of older adults receiving a seasonal influenza vaccination.

Methods: In a prospective observational cohort study, 138 community-dwelling older adults (65-85 years) completed validated measures of stress, positive and negative affect on up to 18 occasions, over 2 weeks prior to, and 4 weeks following, administration of a trivalent influenza vaccination. Antibody responses (IgG) to each of the vaccine strains were measured via microarray ELISA at 4- and 16-weeks post-vaccination. Intra-individual variability in psychological measures was assessed by calculating the root mean squared successive difference (rMMSD) across the observation period.

Results: At 4 weeks post-vaccination, no significant relationships were observed between intra-individual variability and antibody responses. However, at 16 weeks post-vaccination, greater intra-individual variability in stress (all strains) and positive affect (H1N1 strain) was associated with lower strain-specific antibody levels. In models controlling for average levels of these psychological factors, intra-individual variability in stress remained

a significant independent predictor of antibodies to H1N1 and B influenza vaccine strains at 16 weeks post-vaccination.

Discussion: These results demonstrate that fluctuations in psychological experience, specifically stress, may play a role in the longevity of antibody responses following vaccination, independent of overall levels. Fluctuating stress, much like chronic stress, may have deleterious effects on immune function – consistent with an allostatic load model. More broadly, the results demonstrate the added value of incorporating repeated measures into psychoneuroimmunological studies – as it allows these relationships to be explored in a more nuanced and comprehensive manner.

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(5) Characterization of circulating dendritic cells in major depressive disorder

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Introduction: Major depressive disorder (MDD) is a severe mental disorder associated with alterations of the innate immune system. Childhood trauma (CT) is a risk factor for development of MDD in adulthood. Plasmacytoid dendritic cells (pDCs) are a subset of DCs highly capable of antiviral responses via production of type 1 interferons (IFN-I). In this study, we investigated the impact of CT on pDC frequencies and IFN-α levels in peripheral blood in individuals with MDD and healthy controls (HC) and their predictive capacity for disease severity.

Methods: PDC frequencies and IFN-α concentrations were assessed using multi-parameter flow cytometry and multiplex assays of blood samples from depressed individuals (n=63) and HC (n=37). Each group included individuals with CT. Using stratification for MDD and/or CT severity as well as correlational analysis, we examined the associations with immune parameters. Additionally, we performed multivariate linear regression to assess their predictive capacity for disease severity.

Results: Equivalent frequencies of pDCs were found in MDD and HC. Stratification, however, revealed a reduction of pDCs exclusively in severe MDD or severe CT. Specifically, a negative correlation between pDC frequencies and physical or sexual abuse, and CTQ sum score was found. Importantly, blood pDC proportions and IFN-α levels were significant predictors of severity of depression.

Discussion: This study defines specific changes of pDCs and IFN-α in MDD and individuals with CT and their predictive capacity for disease severity. This novel immune signature suggests an association of pDC blood frequencies and their effector cytokine with the pathophysiology of depression.

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(6) Meditators vs. Non-meditators: Testing psychological, neural, and immune differences

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Introduction: Studies suggest that mindfulness meditation is beneficial for psychological health, but they mainly examine the short-term effects of mindfulness (e.g., effects of standardized 8-week interventions) or examine long-term meditators based only on self-reported outcomes. By examining the benefits of long-term mindfulness

practice on psychological and biological levels, we can get a clearer understanding of the benefits of long-term mindfulness practice. **Methods**: This is a cross-sectional study with a sample of 42 long-term meditators (>three years of regular mindfulness practice) and 32 non-meditators. The primary study parameters included psychometric outcomes (emotion regulation, perceived mental and physical health, stress), neural outcomes (connectivity within default mode network, salience network and central executive network), and immune outcome (inflammation measured as interleukin-6 from dried blood spots). The relationships between psychological, neural, and immune measures were examined through structural equation modeling.

Results: Preliminary analysis of the psychometric results confirmed that meditators showed greater emotion regulation, mental health, and well-being than non-meditators despite experiencing the same amount and severity of lifetime stress. There were no changes in connectivity within neural networks or in circulating or stimulated IL-6 levels between meditators and non-mediators. **Discussion**: Long-term mindfulness meditation mitigates the effects of lifetime stress and protects psychological health. However, this is observed only on the psychological level, which is prone to social desirability bias. It remains unknown if differences on the neural and immune levels can be observed once more data are collected and when relevant variables are controlled for in the analyses.

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(7) Development of an organotypic model of human epidermis to study its interaction with sensory neurons in the context of pruritus-associated chronic inflammatory skin diseases

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Introduction: The outermost layer of the skin, the epidermis, the immune system, and the nervous system maintain a continues crosstalk that is essential for the formation and maintenance of the skin barrier function. The skin is abundantly innervated by somatosensory nerve endings coming from neurons located in the dorsal root ganglia (DRG) and the trigeminal ganglia (TG). The primary function of the cutaneous somatosensory nervous system is to interpret the environment and initiate behavioral defenses, including withdrawal to prevent injury and reduce pain, or scratching to get rid of irritants. Furthermore, maladaptive responses of this neuro-epidermal interaction can contribute to chronic inflammatory conditions, promote itch sensation, and instigate persistent itch-scratch cycles. Pruritus is probably the most prevalent symptom among different chronic inflammatory skin diseases. Increasing evidence indicates that the pathogenesis of chronic pruritus engages different components of the neuro-immuno-epidermal unit, including cellular and humoral components. Nevertheless, the mechanism behind this dialog remains to be determined. Thus, we aimed at exploring the molecular basis of chronic inflammatory diseases associated with pruritus.

Methods: Based on the results from Regnase-1 (Reg1) cKO experimental mouse model, which showed an enhanced scratching behavior in animals deficient in this RNase in keratinocytes, we prepared an analogous human model to study this phenomenon. In this model, human primary keratinocytes genetically manipulated to underexpress or overexpress Reg1 were subjected to organotypic cultures. The resulted 3D (organotypic) model of human epidermis was subsequently evaluated on molecular and functional level by q-RTPCR, Western Blot, histology, immunohistochemistry, and luciferase assays.

Results: The 3D model of the Reg1-deficient, sufficient and overexpressing human keratinocytes was successfully generated. This model showed differential expression of genes implicated in pruritus and skin inflammation in keratinocytes with the diminished RNAse levels. Lcn2 and S100A9, for instance, were overexpressed in the Reg1-deficient model. Keratinocytes were also found to differ in their proliferation and differentiation capacity based on the expression levels of Reg1. The secretome of these cells was collected for future stimulation of DRG- and TRG-isolated neurons and immune cells.

Discussion: The development of organotypic three-dimensional epidermal models represents a valuable approach for modern *in vitro* research to study human tissue and reduce redundant animal testing. Our model represents a unique tool to study epidermis-initiated mechanisms of chronic inflammatory skin diseases. This model will also allow us to determine possible impact of dysfunctional keratinocytes on neurons and immune cells residing or infiltrating the skin in the context of pruritus-associated chronic inflammatory skin diseases.

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(8) Viral Metagenomics of Cutaneous Epithelia in health and psoriasis disease

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Introduction: The skin microbiome plays a crucial role in maintaining skin integrity and functions as a critical external barrier. Different investigations have shown that dysbiosis of the skin microbiome could cause or exacerbate skin diseases, including psoriasis. Up to date, investigations have mainly focused on bacterial and fungal communities present in health and disease; however, knowledge about the skin virome in psoriasis is scarce. In the last decades, Next Generation Sequencing (NGS) along with novel bioinformatic tools have revolutionized virome studies, allowing to investigate the viral compositions of unexplored biological niches such as the human skin.

Methods: In order to establish an adequate methodology for the study of the skin virome, 3 protocols for the processing of skin samples were evaluated: A) total DNA purification; B) filtration + ultracentrifugation + DNA purification; and C) purification of viral particles. For this purpose, a pool of skin swabs collected from 30 individuals without skin pathologies were prepared, splitted, and processed with protocols A, B or C. The extracted DNA in each case was sequenced by NGS (Hiseq 2000, Illumina). Reads were subjected to quality filtering using the Bbduk program (BBTools v38.42) and human reads were substracted by Bowtie2 (v.2.2.5) using the reference genome hg38. Finally, viral taxonomic classification was performed with Centrifuge (v.1.0.3) and the NCBI viral reference database.

Results: In total, 1,376,217 reads (A: 540,546; B: 349,762; C: 485,909) with adequate quality parameters were obtained, of which 116,265 reads (A: 20,418; B: 95,621; C: 226) mapped to 36 different viral families (A: 28; B: 22; C: 10). The families Papillomaviridae, Genomoviridae, Poxviridae and Arteriviridae were the most frequently found among the families of viruses that infect vertebrates. Although method B was the most effective in obtaining the highest number of viral sequences, method A showed greater viral family abundance and was chosen for further analysis.

Discussion: Psoriasis is a chronic, multifactorial inflammatory skin disease that affects approximately 3% of the world population. The factors underlying its etiology are multiple and diverse. In order to identify potential markers that could be useful for diagnostics, prevention and/or treatment of psoriasis, it is important to evaluate potential differences between the skin virome in psoriatic patients and healthy volunteers. Since sample preparation is a bottleneck in NGS, this work provides relevant information for the processing of skin samples in studies aimed at understanding the role of the virome in skin pathologies. Finally, the optimized protocol was applied in a case-control study from 25 psoriatic patients and 30 healthy volunteers and the NGS data obtained is under analysis.

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(9) Measuring fetal cortical architecture in response to maternal immune activation by live influenza virus

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Introduction: Maternal Immune Activation (MIA) is associated with a higher prevalence of neurodevelopmental disorders in the offspring. Epidemiological evidence has linked these disorders to maternal infection with influenza A virus (IAV) during pregnancy. Usually, this phenomenon is studied using Poly (I:C), a toll-like receptor 3 agonist, instead of live pathogens. The use of said ligands constitutes a limitation for the study of viral-induced immune responses because Poly (I:C) can only mimic the innate response and not the complete set of innate and adaptive responses induced by a live replicating viral pathogen. Still, these studies have shown that MIA-induced disruptions in cortical architecture are linked to offspring behaviors reminiscent of neurodevelopmental disorders; however, this has never been examined during live viral-induced MIA. In this study, we examine the effects of IAV-induced MIA on neocortical development of the fetus.

Methods: To examine the impact of live virus infection during pregnancy on fetal cortical architecture, we intranasally administered live H3N2 IAV or saline to pregnant C57BL/6nTac dams on gestational day (GD) 9.5. Sacrifice and tissue collection were performed on GD16.5. Fetuses were collected and fixed in 10% neutral buffered formalin. Fetal brains were sectioned coronally and immunohistochemically stained for cortical layer development and structure.

Results: Mapping of cortical layer development and structure, via expression of transcription factors CTIP2, SATB2, and TBR1, is ongoing. The expression of these neuronal fate markers appears in smooth layers in normal healthy brains during early development. However, MIA-induced abnormalities appear as protrusions and intrusions within these layers. This possible laminar disorganization phenotype will be scored and compared across treatment groups.

Discussion: These data will help illuminate the effects of IAV-induced inflammation on neocortical development, offering a more clinically translatable perspective on this phenomenon. The results from this project contrast with previous publications and demonstrate the relevance of using IAV instead of Poly (I:C). Moreover, these data will contribute to a more complete understanding of the effects of viral-induced MIA on fetal neurodevelopment.

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(10) Neurotrophic support to dopamine neurons is compromised by midbrain inflammation in schizophrenia and appears unaffected by antipsychotics

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Introduction: Elevated gene expression of pro-inflammatory cytokines is found in multiple brain regions of ~45% of people with schizophrenia, including the midbrain. Hyperactivity of midbrain dopaminergic neurons underpins hallucinations and delusions, and elevations in pro-inflammatory cytokines in this brain region may contribute to dopamine dysfunction. However, higher lifetime antipsychotic exposure is positively correlated with multiple

cytokine levels in the midbrain. Whilst higher cytokine levels are neurotoxic, the total number of dopamine neurons is not considered to be changed in schizophrenia. One possibility is that the neurotoxic inflammatory microenvironment could increase the demand for neurotropic support to aid neuronal survival. Additionally, it remains unclear as to whether midbrain neuroinflammation is occurring in response to prolonged antipsychotic treatment, or whether heightened neuroinflammation leads to the requirement for higher antipsychotic doses. We sought to disentangle the effects of chronic antipsychotic treatment from schizophrenia pathology on neuroinflammation presenting in post-mortem brain tissue in humans with schizophrenia, and characterize the effects of treatment and neuroinflammation on midbrain trophic support.

Methods: Gene expression of trophic factors (BDNF and NURR1) and associated receptors (TrkB and p75) were analyzed in a post-mortem human midbrain cohort (n=65 schizophrenia/n=64 controls) that was stratified by inflammatory biotype to assess the impact of neuroinflammation on mechanisms promoting neuronal viability and survival. Adult male Sprague Dawley rats were treated orally twice daily with 1mg/kg haloperidol, 1mg/kg risperidone or vehicle for 7 months, to recapitulate the ~19-year duration of treatment in post-mortem cohorts (n=14/treatment group). Gene expression of pro-inflammatory mediators (IL6, IL1B, IL1A, IL1R1, TNF, GFAP, AIF1, NFkB) in the midbrain and hippocampus were quantified using RT-qPCR, as well as mRNA levels of trophic factors (BDNF and NURR1, TrkB and p75).

Results: Reductions in BDNF, NURR1 and TrkBTK+ mRNA levels and increases in TrkBTK- and p75 mRNA are evident in the midbrain of people with schizophrenia with a high inflammatory biotype, compared to cases with a low inflammatory biotype and controls (all $p<0.05$). Chronic treatment with haloperidol and risperidone does not alter pro-inflammatory mediators or trophic-related factors in the midbrain or hippocampus of healthy rats (all $p>0.05$).

Discussion: Changes in trophic related factors in the post-mortem midbrain of humans with schizophrenia, particularly in those with a high inflammatory biotype, do not support that they are counteracting the deleterious impact of elevated cytokines. Instead, reductions in BDNF and its high affinity receptors likely reflects further impediments to neuronal health. Increases in cytokines and reductions in neurotrophin mRNAs in the midbrain in schizophrenia are not likely the direct result of chronic antipsychotic treatment.

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(11) Neutrophil Extracellular Traps (NETs): a functional cellular mechanism in schizophrenia and implications of early-life stress

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Introduction: Neutrophil Extracellular Traps (NETs) are web-like structures involved in the killing of pathogens. NETs are central to both the initiation and chronicity of autoimmune and inflammatory diseases. NETs inhibitors limit peripheral and central inflammation and associated behavioral changes in mice. Previous studies using blood cytokines to stratify patients with schizophrenia suggest that only a subset presents a low-grade

inflammatory state. However, these studies have not addressed whether environmental factors such as child trauma contributed to identifying clusters.

Methods: We investigated NETs as a novel biological mechanism in early schizophrenia and their role together with interleukin-(IL-6) and childhood maltreatment in identifying cluster subgroups. We recruited Individuals with early-stage schizophrenia spectrum (n=78), sex and age-matched controls (n=78), and unaffected siblings (n=25). Childhood maltreatment was evaluated using the Childhood Trauma Questionnaire. Group differences on NETs and IL-6 levels in plasma were adjusted for sex, body mass index, tobacco smoking, and psychoactive substance use. We applied unsupervised two-step clustering analyses with Bayesian Criterion. Fresh neutrophils were isolated from healthy volunteers to test the effect of antipsychotic drugs *in vitro* under stimulated (phorbol myristate acetate, PMA 50 nM) or unstimulated (vehicle) condition. Juvenile male Sprague-Dawley rats (postnatal day, PD 24) were exposed to an adolescent early stress protocol (daily inescapable foot-shock from PD31-40, and three restraint stress sessions, PD31, 32, and 40) or left undisturbed. At PD51, NETs and IL-6 concentration were evaluated in serum. We also measured levels of NETs released from neutrophils isolated from rats' bone marrow.

Results: We found increased NETs levels in patients with early schizophrenia compared to their unaffected siblings and community controls ($F=50.79$, $df=2$, $p<0.001$). Haloperidol and risperidone inhibited NETs release from stimulated neutrophils *in vitro*. Unsupervised two-step clustering analysis identified two main clusters (high-CL1 and low-CL2). Patients with high-CL1 (61.5%) had significantly higher childhood maltreatment scores ($F=26.23$, $df=5$,$p<0.001$), NETs ($F=25.17$,5, $p<0.001$), and IL-6 ($F=3.87$, $df=5$, $p<0.002$) levels than the remaining groups. Adolescent stressed rats had higher NETs ($t16=5.18$, $p<0.001$) and IL-6 ($t10=6.33$, $p<0.001$) levels in serum compared to non-stressed rats, with a tendency to produce more NETs from the bone marrow.

Discussion: Disproportional release of NETs from neutrophils is an essential mechanism for the chronicity of inflammation in autoimmune diseases. We found early-life stress is associated with elevated levels of NETs and IL-6 in the subgroup of patients with schizophrenia, irrespective of important confounding variables, and in a rodent model of adolescent stress. The investigation of NETs will help to identify personalized treatment targets for schizophrenia and for the prevention of inflammation-mediated medical co-morbidities that shorten life span by about 15 years.

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(12) Stress and Sex: The Influence of Neonatal Immune Activation and Sex on the Development of the Microbiota-Gut-Brain Axis

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Introduction: Events occurring in early life can induce long-term physiological and behavioral changes through the process of perinatal programming. The early life environment is critical to the establishment of the microbiota-gut-brain-axis (MGBA), a bidirectional communication system that links the gut with neuroimmune pathways. Perturbations to 'normal' microbiota development may contribute to the development of psychological and gastrointestinal disorders, which are linked to early life stress. The MGBA axis is highly plastic throughout the perinatal period and is a possible mediator of sex-based disparities in psychological and GI disorders. Here, we used a well-established rodent model of neonatal immune activation (NIA) to investigate the effect of neonatal lipopolysaccharide (LPS) exposure on microbiota composition across key developmental periods in males and females.

Methods: Wistar rats were injected with lipopolysaccharide (LPS; 0.05mg/kg) or saline (equivolume) on postnatal days (PND) 3 and 5 to induce NIA. The timing of LPS exposure occurs at a critical window in the development of the brain and GI tract, signaling it as an effective model to study the effects of perinatal programming on the MGBA. Microbiota sequencing was performed on colonic mucosal-associated microbiota (MAM) at critical

periods of development: early life (PND 5; four hours post-injection), weaning (PND 21), adolescence (PND 42) and adulthood (PND 90); n = 8 per sex/treatment/age.

Results: Results showed microbiota changes across the lifespan, from low diversity in neonates and increasing in complexity across age with clear shifts at key developmental periods of weaning (PND 21), adolescence (PND 42), and adulthood (PND 90). Sexually dimorphic changes in microbial composition were evident pre-puberty and continued into adulthood. Neonatal LPS exposure was found to alter the development of the colonic MAM composition across the lifespan. Subtle community compositional differences were observed following NIA in early life (PND 5), and these changes became more pronounced over time, with clear segregated clusters in adulthood.

Discussion: These results demonstrate that NIA alters the composition of gut microbiota, fundamentally influencing the development of the MGBA. These findings present novel insights into the longitudinal development of gut microbiota composition at key developmental periods. Most saliently, this demonstrates the influence of sex on the MGBA, indicating sexually dimorphic developmental trajectories in response to neonatal immune activation. In context of the broader literature, these findings significantly contribute to the understanding of the critical influence of the early life environment on the MGBA, with sex as a major modulating factor.

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(13) Emotional stress induces strain-dependent functional changes in the auditory system of rats

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Introduction: The relationship between stress and hearing impairment has long been recognized, and many patients report stress as a primary cause or aggravator of hearing impairment. To better understand the neuronal, immune, and endocrine interactions of the auditory system, we aimed to analyze the changes in the auditory system of three different rat strains after exposure to chronic stress.

Methods: Adult female (4-6 weeks old) inbred Fischer 344 (n= 42), Lewis (n= 37), and outbred Wistar (n= 54) rats were subjected to 24 h of experimental stress (exposure to low frequency (300 - 350 Hz) and low intensity (61 - 65 dB SPL) sound and corresponding vibrations produced by an electronic rodent repellent). Auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) were measured. Serum levels of corticosterone (the primary stress hormone in rodents) and TNF-alpha (pro-inflammatory cytokine) were measured by commercial ELISA.

Results: Baseline hearing ability differed between strains. The control group of Wistar rats had higher ABR thresholds (41.9 dB +/- 12) than Lewis (35 dB +/- 6.3) and Fischer 344 (29.8 dB +/- 4.5) rats. Fischer 344 DPOAE thresholds measured at 2 kHz were higher (53.4 dB +/- 10.8) than Wistar (36.9 dB +/-11) and Lewis (36.9 dB +/- 9.3) rats. Significantly higher serum levels of TNF-alpha were detected at baseline in Lewis rats (10 µg/mL) compared to Wistar (3.3 µg/mL) and Fischer 344 (0.76 µg/mL) rats, and significantly higher serum levels of corticosterone were detected before stress in Fischer 344 rats (417 ng/mL) compared to Wistar (202 ng/mL) and Lewis (119 ng/mL) rats. In response to experimental stress, threshold changes were observed in Wistar rats at low and high frequencies (4-32 kHz) that were time-dependent. In Fischer 344 rats, experimental stress did not affect hearing. In Lewis rats, post-stress changes affected the low frequencies (2-4 kHz) by lowering the hearing threshold. The direction of these changes depended on the time after stress.

Discussion: The three rat strains used in our experiments are known to respond differently to stress: Fischer 344 rats as a model of stress-induced anxiety, Lewis rats as a model of posttraumatic stress disorder (PTSD), and Wistar rats as control. The hearing changes observed in our experiments involved either low or high frequencies, depending on the strain. We demonstrated that experimental stress differentially affects the HPA or immune axis in the tested strains and induces strain-dependent functional changes in animal hearing.

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(14) Induction of negative treatment expectation in an animal model of endotoxin-induced sickness

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Introduction: Despite broad clinical implications, the neurobiological underpinnings of sickness behavior induced by negative treatment expectation are largely unknown. For ethical reasons, such mechanistic insights are difficult to obtain in humans. This calls for translational studies in animals mimicking clinically relevant features of negative treatment expectation. Here we present initial results from an animal model of endotoxin-induced sickness aimed at inducing a negative treatment expectation in rats.

Methods: Using a conditioned taste avoidance (CTA) paradigm, we combined the presentation of an unfamiliar bitter-sweet taste (saccharin) via the drinking water as conditioned stimulus (CS) with the injection of bacterial endotoxin (lipopolysaccharide), a sickness-inducing agent, as unconditioned stimulus (US). This procedure was repeated up to three times to vary the amount of learning experiences. After a consolidation phase of six days, animals were re-exposed to the taste stimulus alone (test phase), and the consumed amount of saccharin solution (% relative to water baseline) was quantified as a measure of the CTA. We assessed the data of four different experimental groups, each group consisting of 10-12 animals. Animals of the conditioned (CS) and conditioned not evoked (CS0) groups received contingent CS-US pairings during acquisition training. During test phase, CS animals were re-exposed to the CS, while CS0 animals received water instead. The vehicle (VEH) group did not receive the US, but a saline injection paired with the CS. The US group received LPS without the CS to control for potential residual effects. As physiological measures, plasma corticosterone levels as well as the expression of cytokines and neural activation markers (c-fos, arc) in key regions of the central fear network were determined.

Results: Conditioned animals of the CS group developed a pronounced CTA that was significantly greater in individuals with more conditioning trials (65% after 1 trial vs. 23% after 3 trials), while the drinking behavior of animals from all other groups (CS0, VEH, US) was not affected at test phase. Re-exposure to the taste stimulus also induced a conditioned endocrine stress response in the CS group as evident from significant increases in plasma corticosterone levels. Brain analyses are ongoing.

Discussion: Our preliminary findings show successful induction of a negative treatment expectation in animals via associative learning as a conditioned endocrine stress response, which were both strongly correlated with the number of prior treatment experiences.

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(15) The effect of supervised exercise and home-based exercise on chemokines and cytokines in discharged COVID-19 patients

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Introduction: Most individuals after COVID-19 still show immunological alterations, such as high levels of inflammatory cytokines and chemokines. Supervised exercise elicits an anti-inflammatory response, and can provide significant health benefits. In the face of social isolation, the population has become sedentary or has opted for home-based exercise to take advantage of the beneficial effects of exercise and reduce exposure to SARS-CoV-2. This study investigated the effect of supervised and home-based exercise on chemokines and cytokines in discharged COVID-19 patients.

Methods: Twenty-four participants, after a 12-week discharge from the hospital for moderate-to-severe COVID-19, underwent the intervention protocols of this study: 1) supervised exercise program (n = 14) based on strength training at Hospital das Clínicas of the Federal University of Pernambuco or 2) home-based exercise program (n = 10). These programs were performed for 12 weeks with moderate intensity and 45 minutes duration/session (three times per week, 3 sets of 8–10 repetitions with a 60 seconds rest interval between sets and exercises).

Results: After the intervention, within-group effect sizes of supervised exercise were small for IL-2 (Cohen's d = -0.42) and CXCL9 (Cohen's d = -0.42) serum levels and BMI (Cohen's d = 0.33), moderate for IL-10 (Cohen's d = -0.68) serum levels, and TNF-α/IL-10 ratio (Cohen's d = 0.63), and large effect for IL-4 (Cohen's d = -1.11) and IL-6 (Cohen's d = -1.15) serum levels. Moreover, supervised exercise promoted a large effect size for IFN-γ (Cohen's d = 1.31), IL-8 (Cohen's d = 1.00), CCL2 (Cohen's d = 0.82), and on the muscular endurance of lower limbs (Cohen's d = -1.198). The home-based exercise demonstrated a small effect size for CXCL9 (Cohen's d = -0.38) serum levels, also a moderate effect size for IL-2 (Cohen's d = 0.66), IL-8 (Cohen's d = 0.70), and CXCL10 (Cohen's d = 0.53) serum levels. Additionally, within-group effect sizes for home-based exercise were large for IL-6 (Cohen's d = 0.94), IFN-γ (Cohen's d = 0.81) serum levels, and muscular endurance of lower limbs (Cohen's d = -0.86).

Discussion: In post-COVID-19 patients, twelve-week supervised exercise promoted beneficial effects on cytokines and chemokines serum levels and muscular endurance of lower limbs, suggesting that this protocol effectively mitigated inflammatory disorders and improved physical fitness. Furthermore, twelve-week home-based exercise led to more negligible effects on cytokines and chemokines levels in serum, but had also beneficial effects on the muscular endurance of lower limbs.

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(16) Fractalkine receptor (CX3CR1) mediates chronic restraint stress-induced pain behavior in a mouse model

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Introduction: Chronic psychosocial stress is an etiological and/or aggravating factor of several pain conditions. Neuroinflammation via microglia activation is involved in pain persistence. The microglia-surface fractalkine receptor (CX3CR1) plays a role in stress, mood disorders, and inflammatory pain, but its relevance in stress-induced pain is unknown. Therefore, we studied the involvement of CX3CR1 in a chronic restraint stress-induced pain model.

Methods: Male and female C57BL/6J wildtype (WT) and CX3CR1-deficient (knockout: KO) mice were exposed to restraint stress (immobilization in plastic tubes) for 6 hours/ day for two weeks (female: n=12-16/group; male: n= 17-20/group). Paw mechanonociceptive threshold and cold tolerance were determined weekly. IBA1 for

microglia and GFAP immunostaining for astrocytes were performed to determine stress-induced cell number and morphological changes in pain-related brain regions.

Results: Significant (15-20%) mechanonociceptive threshold decrease and cold hyperalgesia (60-70%) developed in stressed WT mice in both sexes. No significant changes in mechanonociceptive threshold, and significantly reduced cold hyperalgesia was observed in fractalkine receptor-deficient ones. Stress significantly increased IBA1-positive microglia activation in the hippocampus CA3 region in both male and female WTs but not in KOs. Activated IBA1-immunopositivity was increased in the somatosensory cortex of WT males in response to stress, but not in KO mice. Interestingly, stress-induced GFAP-positive astrocyte activation in the hippocampal CA3 and cell number increase in the periaqueductal gray matter in WT but not in KO females.

Discussion: We provide here the first evidence for the role of CX3CR1 in chronic stress-induced hyperalgesia and related glia cell activation in a sex-dependent manner. Neuroinflammation in the investigated brain regions involved in both stress and pain processing might mediate neuroplasticity changes leading to central sensitization. These results suggest analgesic potentials of fractalkine receptor antagonists in stress-related chronic pain conditions.

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(17) From stress to inflammation and health outcomes: adverse childhood experiences in medical residents

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Introduction: Brain development is a multifaceted process regulated by genes and shaped by environmental experiences. Early stress and exposure to traumatic events negatively affect the nature and trajectory of normal development. The aim of this study was to determine if there is an association between the history of childhood maltreatment (physical, psychological or sexual) and the presence of Major Depressive Disorder, suicidal behavior, substance use, and chronic diseases in adulthood in residents of different medical specialties of the National Autonomous University of Mexico (UNAM).

Methods: A cross-sectional study was conducted with 2457 resident physicians in Mexico. The Adversity in Childhood International Questionnaire (ACE-IQ) was used to assess ACEs (Adverse Childhood Experiences), including psychological, physical, and sexual forms of abuse, as well as household dysfunction; the Depression Scale (PHQ-9), the Plutchik Suicide Risk Scale, Alcohol Use Disorders Identification Test (AUDIT), Drug Abuse Screening Test (DAST-10) for substance use, and chronic disease questionnaire: hypertension, diabetes, coronary heart disease, respiratory disease, cerebrovascular disease (stroke), digestive system diseases and dermatological diseases. Multiple logistic regression models were used to examine associations between overall ACE score, PHQ-9 and risk behaviors/medical and mental health comorbidities after controlling for potential confounders.

Results: A total of 73.8% of the participants reported at least one ACE, and 13.2% reported four or more ACEs. Increasing ACE scores were associated with increased risk of alcohol consumption (adjusted odds ratio [AOR] = 2.17, 95% confidence intervals [CI]: 2.09-2.56), chronic disease (hypertension, diabetes, digestive system diseases and dermatological diseases) (AOR = 3.17, 95% CI: 3.01-3.68), depression (AOR = 3.73, 95% CI: 3.51-3.84), and suicidal risk (AOR = 1.23, 95% CI: 1.03-1.42) in adulthood. When confounding factors were considered, such as personal or family history of depression, the effects of each ACE component on risk behavior and health, particularly on subpar physical and mental outcomes, varied.

Discussion: When individuals experience adversity, they often mobilize endocrine, immune, and nervous system responses, which, in turn, involve neuroplasticity mechanisms that prepare them to respond to learned environmental contingencies and future threats. In this study, ACEs were associated with increased odds of risk behavior, chronic disease, and mental disorders in adulthood after controlling for relevant demographic and socioeconomic factors.

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(18) The Neuroprotective Role of Skin Collagens in Aging

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Introduction: Aging leads to progressive dysfunction of the nervous system even in the absence of disease or trauma. While the precise mechanisms behind the initiation of aging-related changes in neurons remain unclear, our previous studies suggest that interactions between non-neural and neural tissues may be crucial. Skin, which is innervated by various sensory neurons, has been recognized as a critical component in neurodevelopment and nerve injury repair. In this study, we aimed to investigate the interactions between the skin and nervous system during normal aging and determine their potential influence on neuronal aging.

Methods: We utilized the cutaneous mechanosensory PVD neuron in Caenorhabditis elegans as a model. Hermaphrodite animals were maintained at 20°C and examined from young adulthood (Day 3) to advanced age (Day 9). Each experiment included a minimum of 30 animals. Survival analysis was used to estimate lifespan. Reporter strains were used to visualize neuron morphology with fluorescent microscopy. Proprioceptive behaviors were assessed to evaluate the neuronal function by measuring the tracks made by animals' sinusoidal body-bend movements on agar plates.

Results: Our data revealed that as wild-type animals aged, the PVD neuron developed an aberrant increase in dendritic branching, which was strongly associated with a functional decline in proprioception. Surprisingly, loss-of-function mutation in two skin collagen genes, *col-120* and *dpy-5*, which are downregulated during aging, led to an early-onset of PVD excessive branching respectively, accompanied by a functional decline. Remarkably, overexpressing wild-type *col-120* or *dpy-5* alleviated excessive branching in old age without affecting lifespan. When examining two other sensory neurons, ALM and PLM, we found that the influence of skin collagen was specific to certain neurons, suggesting a selective mechanism. This selectivity also extended to the neuronal phenotype, as the loss of *col-120* or *dpy-5* did not affect other aging-associated morphological changes such as neuritic beading. An RNA interference-mediated screen of candidate cell recognition and adhesion molecules further identified that knockdown of *rig-3*, a cell surface immunoglobin superfamily protein, induced a similar early-onset of excessive branching. However, further genetic experiments are needed to confirm the role of *rig-3* in connecting skin collagens to neuronal aging.

Discussion: Our findings establish a compelling link between skin aging and the aging of the nervous system, implying that the initiation of neural aging processes could originate from proximal non-neural tissues, and highlighting the potential for delaying aging-associated neuronal deteriorations through targeted interventions in peripheral tissues.

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(19) Pharmacological conditioning in a rat model of inflammatory pain to study mechanisms of placebo analgesia

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Introduction: While the phenomenon of placebo analgesia is well-documented in humans, the translation into a reliable animal model is still challenging. In this study, we aim to apply an established protocol of taste-associative learning to induce behaviorally conditioned analgesia.

Methods: We use a rat model of Complete Freund's Adjuvant (CFA)-evoked paw edema to induce symptoms of inflammatory pain. From day 2 after CFA-injection, rats are repeatedly treated with ibuprofen or morphine. Application of analgesic drugs (*unconditioned stimulus*, US) is paired with presentation of a novel sweet taste (*conditioned stimulus*, CS) three times (*acquisition*). In the following retrieval phase, rats are re-exposed to the taste to induce a conditioned response, mimicking the pharmacological effects. We assess spontaneous (Open-Field-Test) and evoked pain-related behaviors (Von-Frey-Test, Plantar-Heat-Test), and examine inflammation-associated changes in structures of the afferent nociceptive system (e.g., dorsal root ganglia, spinal dorsal horn).

Results: Intraplantar injection of CFA induces a robust paw edema for eight days, accompanied by a consistently enhanced mechanical and thermal sensitivity. Spontaneous activity is reduced for 24h but returns to baseline from day 2. Repeated intraperitoneal injection of ibuprofen and morphine on every other day results in a drug-induced analgesia. Taste-associative learning with ibuprofen as US results in conditioned analgesia, when rats are re-exposed to the taste with a placebo injection.

Discussion: With an established taste-associative learning paradigm, we will be able to analyze cellular and molecular mechanisms of learned analgesic effects in a rodent model of inflammatory pain, which might contribute to a better understanding of placebo analgesia in humans.

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(20) Prospective association of baseline plasma inflammatory markers with follow-up depression severity and suicidal ideation symptoms: 6-months prospective study

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Introduction: Variations in the concentration of inflammatory and other neurobiological markers (such as brain-derived growth factor and serotonin) have been associated with depression severity and suicidal ideation. However, the majority of extant studies are cross-sectional, entailing the lack of value in predicting the persistence, resurgence or emergence of depressive symptoms or suicidal ideation. The present study aimed at exploring whether baseline biomarkers are prospectively associated with depression severity, its subtypes, and suicidal ideation at a 6-month follow-up.

Methods: This is an analysis of N = 149 treatment-seeking individuals with mood disorders that were followed-up for 6 months after a baseline evaluation for a larger cross-sectional study. Peripheral blood was drawn at baseline, and 39 biological markers covering immune, inflammatory, and other neurobiological functions, including neuroplasticity (such as brain- and platelet-derived growth factors, thrombospondins, and serotonin) were measured with the Multiplex analysis, analyte values were in-transformed and standardized. Depressive symptoms and suicidal ideation were measured with the Inventory of Depressive Symptomatology, Clinician Rated (IDSC-30) and Columbia Suicide Severity Rating Scale (C-SSRS). 10-fold cross-validated Elastic Net was applied to select the markers most strongly associated with baseline and follow-up depression severity and suicidal ideation, followed by separate binary logistic or multivariate linear regression analyses for each outcome and pre-selected marker. P-values (threshold Bonferroni-corrected $p<0.05$, two-sided) were obtained with 1000-iteration bootstrapping.

Results: In models adjusted for key covariates, low plasma serotonin level was associated with baseline depression severity and the presence of suicidal ideation. Baseline plasma IFN-α was associated with the presence of suicidal ideation during the follow-up, while IFN-α, IL-1β, and serotonin levels were associated with the presence of suicidal ideation at 6-month evaluation, but associations between serotonin and IFN-α lost significance after adjustment for baseline depression severity and treatment. Follow-up depression severity was

associated with high baseline IFN-α and low baseline orexin-A, independently of anthropometric variables, baseline depression and suicidal ideation severity, with stronger specific associations between baseline IFN-α and follow-up anxious symptoms severity, and baseline orexin-A and follow-up atypical symptom severity. Neither of these markers was associated with melancholic symptoms severity.

Discussion: Baseline IFN-α and IL-1β predicted suicidal ideation and anxious symptoms, whereas baseline IFN-α and orexin-A predicted depression severity at six-months follow-up, with specific effects on anxious and atypical, but not melancholic symptoms, in treatment-seeking individuals. The present findings, when replicated, could be used to predict patient outcomes and guide treatment.

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(21) Ultraviolet radiation and air pollution induce melanocyte senescence and contribute to human skin aging

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Introduction: Extrinsic skin aging is a result of exposition to environmental factors such as sunlight and air pollution. Melanocytes, which are responsible for the main protection of the skin against ultraviolet (UV) light, become less active with aging. In general, the senescence of melanocytes and their contribution to skin aging has been insufficiently explored. Scientific evidence suggests that various environmental factors affect different pathways in melanocytes leading to premature aging and pigmentation disorders such as vitiligo. As a consequence, depigmentation accelerates severe psychological stress, which impacts the quality of life and, in turn, reinforces the onset of skin discoloration.

Methods: We treat human melanocytes and skin explants with UV (UVA+UVB), urban particulate matter (UPM) or with a combination of these two stressors (UV+UPM), to understand how these environmental stressors affect skin biology and, in particular, melanocyte homeostasis. Following treatment, we investigate several morphological and physiological parameters such as cell proliferation, senescence status, apoptosis, pigmentation, DNA damage, and senescence-associated secretory phenotype (SASP) including extracellular vesicles (EVs).

Results: Preliminary results have demonstrated that melanocytes respond diversely to each type of stress in terms of senescence markers, including p21 and LaminB1, cell survival, pigmentation, and markers associated with inflammation such as IL-1, IL-6, and TNF-α that indicate altered skin-brain interaction. Accordingly, all treatments induced different changes in skin explants, indicating that an understanding of the underlying molecular processes activated in response to these treatments is vital to estimate the impact of exposure to such environmental factors on skin homeostasis and pigmentation. Additionally, melanocytes treated with the combination of UV and UPM upregulate the secretion of microvesicles. The reasons for this increased secretion of a specific population of EVs are still unknown but we hypothesize that these vesicles might be involved in intratissue communication.

Discussion: This new experimental setup will allow us to perform further research on mechanisms of extrinsic skin aging, including the role of melanocytes in this process. Results obtained with this study could give rise to the development of new therapeutical targets for pigmentation disorders and premature skin aging, which will improve the psychological burden as well as quality of life from people with pigmentation disorders.

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(22) Sleep promotes T-cell migration towards CCL19 via growth hormone and prolactin signaling in humans

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Introduction: Sleep boosts the formation of adaptive immunity, e.g., after vaccination. A promoting effect of sleep on T-cell migration to lymph nodes, where adaptive immune responses are initiated, has been proposed as underlying mechanism. However, direct evidence for this hypothesis is lacking.

Methods: 14 healthy participants were examined in the sleep laboratory during a normal sleep-wake cycle and during 24 hours of continuous wakefulness. Blood was collected every 4 hours to determine T-cell migration using Transwell® chemotaxis assays with or without the lymph-node homing chemokine CCL19 or the inflammatory chemokine CCL5. In additional *ex vivo* and *in vitro* experiments, the effects of plasma collected from sleeping vs. awake participants and of the sleep-dependent hormones growth hormone (GH) and prolactin on T-cell migration were investigated.

Results: Sleep selectively increased spontaneous as well as CCL19-directed migration of total CD3, CD4, CD8 T-cells, and naïve subsets, without affecting migration towards CCL5. Furthermore, incubation of T-cells from healthy donors with plasma collected from participants of the *in vivo* experiment during sleep enhanced CCL19-directed T-cell migration compared to plasma collected during wakefulness. This effect was blunted following blockade of GH and prolactin signaling. This finding is in line with additional *in vitro* experiments demonstrating increases in T-cell migration towards CCL19 following incubation of the cells with GH and prolactin.

Discussion: Sleep selectively promotes T-cell migration towards CCL19 by enhancing GH and prolactin signaling. These findings reveal a potential underlying mechanism of the boosting effect of sleep on adaptive immunity.

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(23) Serotonin modulation improves cutaneous wound healing

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Introduction: Treating delayed healing disorders remains extremely challenging in the absence of new approved drugs. A potential role of serotonin (5-HT) signaling on skin homeostasis has recently been uncovered. In addition, 5-HT promotes tissue regeneration through stem cells activation in a 5HT1B receptor-dependent mechanism in other organs than skin. Our work aims to provide a better understanding of 5-HT signaling in mouse skin homeostasis and repair in order to develop new therapeutic strategies to treat altered wound healing.

Methods: Using publicly available datasets of single cell RNA sequencing of mouse skin, we assessed 5-HT receptors expression in normal and wounded skin. To dissect the role of 5-HT signaling on skin homeostasis and wound healing, we performed wounds experiments in 10 mice knocked-out for Tryptophan Hydroxylase 1

(Tph1), compared to 8 wild type C57Bl6J mice (WT). Tph1 is the rate-limiting non-neuronal enzyme in serotonin synthesis pathway, responsible for the production of peripheral serotonin. Mice received 2 full-thickness 5-mm circular excisional wounds on shaved dorsal skin. Wounds harvested at day 7 post wounding were fixed, sectioned, and stained with immunohistochemistry. We also performed RNA sequencing of normal and wounded skin harvested at D7 from WT and TPH1-/- mice. In addition, we daily applied several modulators of 5-HT signaling to study their topical effect on skin wound healing in WT mice (n=8-10 per treatment group) and in an altered wound healing mouse model displaying clobetasol-induced skin atrophy (n=6 per treatment group).

Results: We found that 5HTR2A receptor and serotonin transporter SLC6A4 have a ubiquitous expression in resident skin cells, and that 5HTR1A and 5HTR1D receptors are mainly expressed in the dermis. 5-HT receptors expression also varied during skin wounding. We found 709 differently expressed genes between TPH1-/- and WT mice skin at steady state and 66 upon wounding. Our transcriptomic data analysis revealed modifications in intraepidermal junction signaling, cellular movement and proliferation, immune cell trafficking and activation in TPH1-/- mice normal skin. Tph1-/- mice displayed altered wound healing compared to controls. We showed that drug X (disclosure after completion of patenting process), a partial agonist of specific 5-HT receptors, significantly decreased wound area by enhancing neoepidermis formation and epidermal keratinocytes proliferation, as well as stimulating angiogenesis in WT mice. Drug X also enhanced wound closure in a mouse model displaying clobetasol-induced skin atrophy.

Discussion: These results indicate that 5-HT plays an important role in skin homeostasis and wound healing.

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(24) Regular physical exercise can help to decrease the accumulation of body fat and play a crucial role in preserving the proper functioning of adipose tissue with aging

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Introduction: Lifelong physical exercise positively influences immunometabolic health during aging, attenuating immunosenescence markers and controlling inflammation. However, limited information exists on the modulation of peripheral metabolic parameters by prolonged training. This study aimed to investigate the effect of lifelong exercise on basal concentrations of adipose tissue-derived hormones and brain-derived neurotrophic factor (BDNF), as well as acute exercise-induced changes.

Methods: Master athletes (51.8±9.0 years, n=12) with 28.1±13.8 years of training and young adult athletes (22.0±4.1 years, n=7; 8.7±4.1 years of training) were compared to age-matched controls. Body composition (DXA and ultrasound) and mental health (cognitive function, anxiety, depression) were assessed. Participants completed a 30-minute exercise session, with blood samples collected before (basal concentration) and after (exercise response) the session. Enzyme-linked immunosorbent assays measured leptin, adiponectin, serum BDNF, BDNF produced by lipopolysaccharide (LPS)-stimulated PBMCs, and plasminogen activator inhibitor-1 (PAI-1). Statistical significance ($p<0.05$) was determined using Student's t-test (comparison of basal values) and ANOVA (differences in acute response).

Results: There is an age-related increase in adiposity and body fat distribution, as untrained elderly individuals showed higher subcutaneous fat levels than young and master athletes (mean difference of 10%, $p<.0001$). Trained groups exhibited higher adiponectin/leptin ratios, indicating improved adipose tissue functionality. Regarding sleep quality, there were no significant differences between all groups in terms of sleep duration (≥ 7 hours or < 7 hours), the use of sleep medications, or overall sleep quality (poor or good). However, young athletes had higher daytime sleepiness levels than master athletes. The groups had no significant differences regarding

anxiety and depression or memory and attention abilities (Digit Span test). Serum levels of BDNF increased after the exercise session ($p<0.05$), while the serum concentrations of PAI-1 and the relative amount of LPS-stimulated BDNF produced by PBMCs were reduced.

Discussion: Overall, these findings suggest that aging is associated with changes in body composition, particularly an increase in adiposity and alterations in adipose tissue-derived hormones. The adiponectin/leptin ratio is a clinical parameter that reflects the functionality of the adipose tissue. Lower values of this ratio were found in elderly individuals who are not physically active, suggesting they are more susceptible to developing cardiometabolic diseases. Regular athletic training may attenuate some of these age-related changes. In addition to the direct effects of BDNF production, the long-term practice of physical exercise is also associated with maintaining adipose tissue functionality, suggesting potential mechanisms for maintaining metabolic health during aging.

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(25) Neuroendocrine-immune modulation associated with the manifestation of cancer-related fatigue, and tumor hormone responsiveness in patients with breast cancer

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Introduction: Psychological and physiological factors enhance the complexity of tumor progression that leads to the manifestation of cancer-related fatigue, a most distressing side-effect of cancer. Fatigue in cancer is associated with increased depression, poor prognosis, and increased mortality. Therefore, it is imperative to understand if neuroendocrine-immune variables can modulate the manifestation of cancer-related fatigue, and if their potential effects can be related to tumor hormone responsiveness in breast cancer patients.

Methods: Participants with breast cancer were recruited for the study (n=186), and were given various psychometric scales such as FACIT-F, MADRS, and FACT-B to assess fatigue, depression, and overall well- being of the patients, respectively. The tumor hormone sensitivities (estrogen receptor (ER)+, progesterone receptor (PR)+, ER+/PR+, and ER-/PR-) were ascertained by immunohistochemistry. Stress and metabolic hormones (Cortisol, Insulin, Glucagon, TSH, Estradiol) were analysed in serum samples, and neurotransmitter (Norepinephrine) levels was measured in plasma samples. The concentration of the cytokines IL-6, IFN-γ and TNF-α was determined in serum samples.

Results: Cortisol levels were significantly ($p<0.05$) lower in patients with PR+ and ER+/PR+, but significantly ($p<0.05$) elevated in ER-/PR- tumors as compared to ER+ tumors. However, norepinephrine levels were significantly ($p<0.05$) higher in patients with ER+/PR+ and ER-/PR- tumors, as compared to ER+ tumors. Cortisol ($r^2=0.625$) and norepinephrine ($r^2=0.705$) were positively correlated with fatigue. Among the metabolic hormones, insulin levels were significantly ($p<0.05$) elevated in patients with ER+/PR+ and ER-/PR- compared to ER+. Glucagon was found to be significantly ($p<0.05$) lower in PR+ and ER+/PR+ tumors as compared to ER+. Interestingly, TSH levels were found to be significantly ($p<0.05$) lower in PR+, ER+/PR+ and ER-/PR- tumors as compared to ER+ tumor. IL-6, IFN-γ and TNF-α concentrations in plasma were significantly ($p<0.05$) lower in PR+, ER+/PR+ and ER-/PR- tumors as compared to ER+ tumor. Cytokine concentrations were positively correlated with fatigue (IL-6: $r^2=0.553$, IFN-γ: $r^2=0.662$, and TNF-α: $r^2=0.543$). Depression was positively correlated with fatigue ($r^2=0.672$). Patients with ER-/PR- were found to have lower quality of life.

Discussion: Cancer-related fatigue is dependent upon physiological and psychological factors, and is associated with tumor hormone sensitivities that may affect the quality of life in women with breast cancer. The disease-associated dysfunctions in the neuroendocrine-immune network may lead to dysregulated energy metabolism leading to the manifestation of cancer-related fatigue in women with hormone-sensitive breast cancer.

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(26) The Role of Peripheral Neurons in Modulating Postoperative Cognitive Dysfunction

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Introduction: Increasing evidence links alterations of the gut microbiota with cognitive disorders, and inflammation-related microbiome alterations might be causally involved in the development of post-operative cognitive deficits (POCD). Following surgery procedures, approximately one third of the patients exhibit POCD, with alterations in memory, attention, and mental flexibility, which persist in approximately 12% for more than three months. This project aims at contributing to a better understanding of POCD pathogenesis and elucidate the pathways and mechanisms that are responsible for the interactions between the peripheral nervous system, the microbiome, and the brain. Interleukin 6 (IL-6), its receptor (IL-6R), and the IL-6 signal transducer gp130 (IL-6ST) are known regulators of inflammatory responses. Recent research has demonstrated the involvement of IL-6 signaling in enteric neuron activation as well as its role in gut-brain communication and cognitive performance.

Methods: In order to identify the role of sensory neurons expressing gp130 in the development of POCD, we used a transgenic mouse model in which IL6ST is conditionally depleted in sensory neurons expressing the voltage-gated sodium channel Nav1.8 (SNS-gp130-/-). A total of 10 mice per sex, genotype and age alongside littermate controls (gp130fl/fl) were subjected to the spared nerve injury (SNI) model for neuropathic pain. Gut motility was assessed by quantification of the number and weight of fecal boli that were additionally subjected to 16S sequencing to identify genotype- and sex-dependent differences in microbiota strains. Cognitive and emotional performance was investigated at baseline and 14 days after SNI using the open field, novel object recognition, marble burying, and forced swim tests.

Results: Based on our preliminary findings, SNI was not associated with significant cognitive deficits in the novel object recognition paradigm and no clear effect of the depletion of IL6ST in nociceptive neurons nor significant changes in gut motility were observed.

Discussion: Our results suggest that SNI did not cause significant cognitive deficits and gut motility alterations, independent of IL6ST depletion. Further characterization of transcriptomic signatures of relevant brain regions affected by SNI and assessment of gut permeability will provide new insight into how surgical interventions contribute to the modulation of the gut-brain signaling axis through sensory and enteric neurons.

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(27) Perioperative psychological stress facilitates pro-metastatic processes: endocrine and molecular mediating pathways

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Introduction: Limiting stress-inflammatory signaling during the perioperative timeframe has improved metastatic biomarkers of breast and colorectal patients, according to our recent clinical projects. As perioperative psychological and physiological stress effects are intertwined, the distinct role of psychological stress in these effects is still unclear. To dissociate between the two, we conducted a series of translational studies assessing transcriptional and metastatic outcomes for potential pharmacological interventions.

Methods: The tilt-light stress paradigm was employed a day before and/or day after tumor cell inoculation, 24 or 48 hours total, using three models of experimental metastasis: MC38 and CT26 colorectal liver metastasis (C57BL/c and BALB/c mice, n=12), and MADB106 mammary adenocarcinoma lung metastasis (F344 rats, n=8). In the 4T1 spontaneous mammary carcinoma model (BALB/c mice, n=12), various stress paradigms (restraint stress, tilt-light stress, and wet cage stress) were employed along four days before excising the tumor for whole-genome

profiling. Perioperative employment of β-adrenergic, COX-2, or corticosterone antagonists was examined in the 4T1 and MADB106 models.

Results: Two perioperative days of tilt-light stress increased the number of metastases in all experimental models (MC38, p=0.032; CT26, p=0.007; MADB106, p<0.001). In the MADB106 model, either a β-adrenergic or a corticosterone antagonist (propranolol/RU486) blocked stress effects (p=0.006, p=0.025, respectively), however, a COX-2 antagonist alone had no evident effect. In the 4T1 tumor, four-day stress exposure promotes the increased activity of Hypoxia-inducible factor (HIF) (p<0.01), and elevated epithelial to mesenchymal transition (EMT) (p=0.0065), an effect that was blocked by simultaneous administration of the β-adrenergic antagonist propranolol and COX-2 inhibitor, etodolac (p<0.01), as also reported in cancer patients.

Discussion: Mild psychological stress can facilitate metastatic progression through adrenergic and corticosterone pathways. Four pre-operative days of psychological stress alone can increase molecular pro-metastatic biomarkers in primary tumors, which can be reversed by attenuating β-adrenergic and COX-2 signaling. These results suggest that psychological stress during the pre-operative days might be a clinically critical factor in promoting postoperative metastasis, irrespective of the surgery itself.

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(28) Learned Placebo Effects in Allergic Contact Dermatitis

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Introduction: Allergic Contact Dermatitis is an inflammatory skin disease that requires the use of immunosuppressive medication. The amount of adverse side effects induced by the respective therapeutic drugs urges the need for developing alternative or supportive treatment strategies. Recent knowledge documents that associative learning protocols may be used for drug dose reduction, thus, side effect reduction, while simultaneously maintaining treatment efficacy.

Methods: An established paradigm of taste-immune conditioning (conditioned taste avoidance; CTA) is applied in a dinitrofluorobenzene-induced disease model of allergic contact hypersensitivity in dark agouti rats, where a novel taste (saccharin; conditioned stimulus/CS) is paired with an injection of the immunosuppressive drug cyclosporine A (CsA) as unconditioned stimulus (US). After three CS/US pairings (acquisition) animals are sensitized with DNFB. Retrieval of taste-immune conditioning starts by presenting the CS one day before contact hypersensitivity is triggered by DNFB re-exposure.

Results: First experiments showed that a dose of 80 mg/kg CsA is sufficient to prevent symptomatology of a DNFB-induced contact hypersensitivity. Despite a strong response on the behavioral level (CTA), preliminary results of the conditioning paradigm revealed no effect on symptom severity or lymphocyte populations. However, analysis of *in vitro* anti-CD3 stimulated splenocytes revealed a reduction of IL-2 cytokine production in conditioned animals.

Discussion: Since the group sizes of the first experiments are very small, additional experiments have to be conducted to clarify putative effects of taste-immune conditioning on disease progression in allergic contact hypersensitivity. Moreover, histological analysis of the DNFB-challenged tissue needs to be performed to monitor a potential conditioned reduction of immune cells.

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(29) Anti-inflammatory drug effects on experimentally induced affective and bodily sickness symptoms: A randomized controlled study in healthy volunteers employing human experimental endotoxemia

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Introduction: Systemic inflammation has a high clinical relevance as it contributes to the pathophysiology of highly prevalent clinical conditions, ranging from inflammatory diseases to affective disorder. Preclinical evidence suggests a therapeutic potential of anti-inflammatory treatments in patients with inflammation-associated depression. To systematically analyse anti-inflammatory treatment effects on inflammation-induced affective and bodily sickness symptoms, we herein employed an established model of acute systemic inflammation (i.e. experimental endotoxemia) that allowed to experimentally induce sickness symptoms and combined it with administration of the non-selective cyclooxygenase inhibitor ibuprofen.

Methods: In this ongoing randomized double-blind study, N=98 healthy participants received either ibuprofen (600mg, per os) or a placebo pill 45 minutes before administration of 0.8ng/kg lipopolysaccharide (LPS). Immune and neuroendocrine indicators of the systemic inflammation (i.e. plasma concentrations of pro-inflammatory cytokines, cortisol and ACTH) were measured by enzyme-linked immunosorbent assay (ELISA). Affective sickness symptoms were assessed by the standardized and validated German versions of the State Trait Anxiety Depression Inventory (STADI) and State Trait Anxiety Inventory (STAI). Participants rated their bodily sickness symptoms using an adaption of the General-Assessment-of-Side-Effects questionnaire (GASE). All parameters were repeatedly assessed before and up to six hours after LPS-injection.

Results: LPS application induced transient increases in affective and bodily sickness symptoms as well as inflammatory and neuroendocrine markers in all participants (all $p<.001$, time effects). Increases in self-reported depression (STADI score), state anxiety (STAI score) and bodily sickness symptoms (GASE score) were significantly less pronounced after ibuprofen pre-treatment (all $p<.01$, interaction effects) with greatest effects at the peak of inflammation (2h and 3h post-injection). Increases of pro-inflammatory cytokines TNF-α and IL-6 were significantly more pronounced while plasma cortisol and plasma ACTH were significantly lower in participants pre-treated with ibuprofen (all $p<0.05$, interaction effects) with significant group differences between 2h and 6h postinjection.

Discussion: Herein, ibuprofen ameliorated inflammation-induced affective and bodily sickness symptoms, supporting the increasing evidence of beneficial effects of anti-inflammatory treatments in the context of inflammation-associated affective conditions. Alongside with this decrease in centrally mediated sickness symptoms, we observed higher circulating pro-inflammatory cytokines and lower levels of plasma cortisol in response to ibuprofen. As ibuprofen also attenuated increases of a more central HPA-axis component (i.e. ACTH), it is conceivable that ibuprofen exerts its symptom-relieving effects via inhibitory central mechanisms but further research is needed to elucidate the underlying pathways of ibuprofen effects in the context of experimental endotoxemia.

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(30) The influence of everyday emotions on mucosal immunity: A multi-level structural equation modeling approach

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Introduction: Mucosal immunity is a multifaceted system of immunological responses that provides a barrier against pathogenic invasion and can be regulated by psychosocial and neuroendocrine factors. The present study aims to elucidate the association between everyday emotional states, emotion regulation competences (ERC), and mucosal immunity by utilizing an ambulatory assessment approach.

Methods: 30 healthy subjects completed an emotion questionnaire (PANAS) and collected saliva samples via passive drool to determine salivary immunoglobulin-A (S-IgA) levels three times a day over a period of one week. In a Bayesian multi-level structural equation model, the influence of emotions on S-IgA, both on a within-subject and between-subject level, was estimated.

Results: The study found that most of the variation in S-IgA (75%) is accounted for by within-subject changes rather than stable between-subject differences. On a within-subject level, negative emotions had a significant positive effect on S-IgA levels ($\beta = 0.18$, $p < .01$), while positive emotions had no effect. This effect of negative emotions was moderated by the subject's ERC, with higher ERC corresponding to smaller effect sizes ($\beta = -0.51$, $p = .01$). S-IgA levels decreased over the course of a day, indicating circadian rhythmicity ($\beta = -0.13$, $p < .01$).

Discussion: We found that the effects of negative emotions on S-IgA are moderated by individual competences in handling emotions. These results highlight the possibilities of intensive longitudinal data for further investigations in this field.

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(31) Defining the impact of shift work on autoimmunity

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Introduction: Shift work is known to disturb the circadian rhythm, resulting in breaking of barriers, and imbalances throughout the entire organism, including immune cells. This causes a pro-inflammatory milieu, driving the system towards autoimmunity. As shift work is a rising factor in our everyday life, studying potential influences on our health becomes more important. This project investigates lupus-prone mice undergoing simulated shift work.

Methods: 160 mice of three different lupus-prone mouse lines (NZM2410/J, NZBW/F1 and B6.NZMSLE1/SLE2/SLE3) are undergoing simulated shift work. Therefore, mice are placed in cage systems with different light schedules. The control groups are permanently housed in 12 h light and 12 h darkness. The groups of simulated shift work are kept for five days in darkness during daytime and switch for two days to light during daytime. This schedule is repeated for 40 weeks. Mice are sacrificed at different ages to investigate disease progression using hemograms, flow cytometry, antibody ELISAs, and urine sampling for proteinuria measurements. Thereby, we evaluate the changes in the blood parameters, T cell populations, development of autoantibodies, and renal involvement depending on the treatment of the groups.

Results: Lupus-prone mice revealed increased numbers of neutrophils and basophils compared to C57BL/6J mice of the same age, even when not undergoing simulated shift work. Moreover, the number of regulatory T cells (Tregs) rose with disease progression. Results from simulated shift work follow in the future.

Discussion: Rising numbers of neutrophils can be explained by the increase of inflammation upon disease onset. Neutrophils are also in other diseases the first line of defense. The rise in regulatory T cells might be a sign of the organism trying to balance the inflammation. However, we expect that Tregs will not be able to compensate for the inflammatory processes in later stages of the disease. The simulated shift work might accelerate and enhance the cellular changes seen with disease progression.

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(32) Chronic urticaria treatment with omalizumab – biomarkers search and potential benefits of ineffective treatment

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Introduction: Biomarkers to predict the response to omalizumab (OMA) in chronic spontaneous urticaria (CSU) are highly valued to determine which patients are more likely to respond to the treatment. Omalizumab is a recombinant humanised monoclonal antibody of the IgG1 kappa class, produced by using recombinant DNA technology from a Chinese hamster ovary cell line, that selectively binds to human immunoglobulin E (IgE), commonly used in bronchial asthma therapy. The aim of our study was to evaluate UAS (Urticaria Activity Score), DLQI (Dermatology Life Quality Index), SII (systemic immune-inflammation index), SIRI (systemic inflammation response index), PLR (platelet/lymphocyte ratio) and NLR (neutrophil/lymphocyte ratio) in a group of 46 CSU patients treated for 24 weeks with OMA (300 mg every 4 weeks). Full-responders (n=30) were defined as UAS=0 at week 24. Analysis of potential psychosocial benefits of ineffective treatment was also performed to evaluate the outcomes of introducing biological treatment to non-responders.

Methods: Blood tests were performed 3 times: at week 1, week 12 and week 24 of the treatment; UAS7 (UAS scores summed over 7 days) and DLQI were collected every 4 weeks (6 times in total). JASP program (version 0.12.1) was used for statistical analysis and significance level was set at 0.05.

Results: There was no statistically significant differences observed at the start nor at the end of the treatment between 2 groups (responders vs non-responders) and SII, SIRI, PLR and NLR. In our study, none of the measures could be employed as a predictor of good therapy response. However, statistically significant negative correlation was observed between severity of urticaria expressed in UAS7 scores and quality of life (evaluated by DLQI) - $p<0.001$. Moreover, at week 24 both groups, i.e. full-responders and non-full responders, demonstrated significant improvement in quality of life, however responders reported higher improvement and the difference between these two groups was also statistically significant.

Discussion: Our single center experience based on 46 CSU patients did not confirm usefulness of SII, SIRI, NLR and PLR in CSU response to omalizumab. Of importance, some patients who finally benefit from this biological treatment (using OMA) and fully respond to it could have been excluded beforehand if aforementioned parameters would be considered as biomarkers to predict the possible response. It is also worth noting that when patients underwent the second OMA treatment cycle, in case of relapse, the efficacy of treatment was not affected. In addition, it is worth mentioning that even patients that did not respond to the treatment presented significant improvement in quality of life – signalizing the improvement of overall psychosocial functioning. Further research to gain a deeper understanding of the positive changes in psychological functioning in non-responders (despite no change or little attenuation of disease symptoms) is recommended.

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(33) The role of peripheral inflammation and metabolic changes in central dopamine dysregulation underlying anhedonia phenotype in rats

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Introduction: Inflammation plays a role in dopaminergic dysfunction and anhedonia. This study aims to investigate the role of peripheral metabolic changes and chronic low-grade inflammation on striatal dopamine and anhedonia-like behavior induced hypothalamic–pituitary–adrenal (HPA) axis stimulation.

Methods: Wistar rats were trained in a progressive-ratio/concurrent Effort Related Choice (ERC) paradigm to assess effort and motivation. After reaching a stable baseline, animals received daily injections of adrenocorticotrophic hormone (ACTH) or saline (n=8) for 24 days; lever pressing, number of rewards and maximum ratio were recorded pre- and post-treatment. Additionally, markers of inflammatory, glucose metabolism, and dopamine cell integrity were quantified using immunohistochemistry, Western blot, ELISA and metabolomics approaches.

Results: ACTH treatment reduced lever pressing and rewards in the ERC task, increased IL-6 levels in brain and serum, impaired glucose metabolism, and decreased tyrosine hydroxylase (TH) levels in the nucleus accumbens shell and infralimbic cortex compared to saline-treated controls. Peripheral insulin-stimulated glucose uptake was correlated with lever pressing; prefrontal cortex TH levels were correlated with local IBA1 fluorescence (a marker of microglia cell density) and peripheral IL-6 levels in serum.

Discussion: Chronic ACTH treatment induced anhedonia-like phenotype in rats, a validation criterium for a translational rodent model for treatment of resistant depression. The present results corroborate with the immunometabolic theory for depression, demonstrating that chronic stress and HPA axis disruption activate peripheral inflammatory response, with IL-6 being an important mediator in this process. Subsequent metabolic demands of peripheral chronic low-grade inflammation might lead to reduced TH levels and decreased dopamine synthesis in key brain regions of the reward system, underlying the anhedonia phenotype observed in ERC. Mechanisms of peripheral-to-central communication still need to be investigated; monocytes infiltration could have a role in this process. These results highlight the potential usage of immunometabolism markers as diagnostic and treatment biomarkers for more individualized clinical approaches.

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(34) Clinical acute Covid-19 symptoms are associated to immune responses and physical activity level in Young Adults post mild-to-moderate SARS-CoV-2 infection: Fit-Covid Study

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Introduction: Studies have already shown that long-term sequelae of COVID-19 occurs independently of disease severity, justifying the importance of long-term monitoring of mild-to-moderate cases that are often overlooked. The aim of this study was to investigate the association of acute clinical COVID-19 symptoms with immune responses and physical activity level in young adults with mild-to-moderate SARS-CoV-2 infection.

Methods: This is a cross-sectional study (part of the FIT-COVID Study), ethical approval nº 38701820.0.0000.5402, registered on Brazilian Clinical Trials Registry (nº RBR-5dgvkv3). In post-COVID-19 phase of young adults post mild-to-moderate SARS-CoV-2 infection (70.50±43.10 days of diagnosis), we collected blood samples from the participants to evaluate systemic (serum and whole blood) and cellular (PBMC) immune responses. We also assessed physical activity level (PAL), functional capacity (6MWT) and the participants retrospectively answered a questionnaire about their clinical acute symptoms of SARS-CoV-2 infection. Statistical analysis was performed using SPSS software (version 22.0). We used Mann–Whitney U-test to compare post-

SARS-CoV-2-infected patients with and without specific disease symptoms. Statistical significance was set at p <5%.

Results: Twenty Post-COVID-19 patients (11 men; age: 29.41 (95%CI: 21.90– 34.96) years; BMI: 25.51 ± 4.32 kg/m^2) were assessed. In our sample, the most prevalent clinical acute COVID-19 symptom was body pain (n=12). Subjects with acute coryza symptom (n= 4) presented lower IFN-γ (p=0.027) and CD14+CD16+HLA-DR+ (Human leukocyte antigen) (p= 0.030). Moreover, higher leptin/Visceral Adipose Tissue (VAT) (p=0.025) and IFN-α (p= 0.048) were found in subjects reporting headache symptoms (n=11) when compared to patients without headache. Participants with acute dyspnea (n=5) presented lower 6MWT distance (p= 0.042), lower relative production of TNF-α when cells were stimulated with PMA plus ionomycin and LPS (p= 0.003), and higher relative production of IL-1Ra when cells were stimulated with LPS (p=0.044). MVPA was lower (p= 0.041) in subjects with acute fever symptom (n=6). In addition, subjects with body pain symptom (n=12) presented higher triglycerides (p= 0.047) and lipid accumulation product (p= 0.010) and lower IFN-γ levels (p= 0.035).

Discussion: This study shows Post-Covid-19 clinical symptoms and immune/biochemical repercussions according to acute symptoms of mild-to-moderate infection by SARS-CoV-2. Our results follow the rationale of previous findings with severe COVID-19 signaling that the symptomatology during the course of COVID-19 seems to have a negative effect on some clinical and immunological parameters in the post-acute phase of COVID-19. In conclusion, acute clinical Covid-19 symptoms are associated to immune responses, PAL, and functional capacity in young adults with post mild-to-moderate SARS-CoV-2 infection.

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(35) Insular cortex neuron activation retrieves ear-skin immune response

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Introduction: Accumulating evidence suggests that the brain can influence peripheral immunity. A recent study conducted by our group demonstrated that the activation of specific neuron ensembles in the brain of mice could trigger localized inflammation in the colon or the abdominal cavity. Moreover, our recent research has revealed a similar phenomenon in the ear skin, wherein activation of specific neurons can reinstate a previous immune response.

Methods: In this study, we employed the Targeted Recombination in Active Populations (TRAP) mouse model to genetically express DREADD (designer receptors exclusively activated by designer drugs; Gq) in active neurons within the insular cortex (InsCtx) during instances of chemically induced inflammation (calcipotriol-induced inflammation). After the recovery phase, we used the DREADD ligand JHU37160 to reactivate the captured neuronal ensembles and assessed the immune response in the ear skin without subjecting it to further insults.

Results: Our preliminary findings indicate that activating neuronal ensembles induces alterations in the immune response within the ear skin and adjacent lymph nodes. These changes include a higher abundance of monocytes in the ear skin and an increase in γδ T-cells in the adjacent lymph node.

Discussion: The outcomes of our study suggest that brain activity alone is sufficient to elicit an immune response in the skin. These findings hold significant implications for unraveling the mechanisms underlying immune-related skin diseases and propose a novel approach for managing such conditions.

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(36) Pro-inflammatory factors contribute to the EEG microstate abnormalities in patients with major depressive disorder

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Introduction: Pro-inflammatory factors may be associated with abnormalities in functional brain networks, which may be a mechanism in the pathogenesis of major depressive disorder (MDD). Electroencephalogram (EEG) microstates reflect the functioning of brain networks. However, the relationship between pro-inflammatory factors and microstate abnormalities in patients with MDD is poorly understood.

Methods: 24 MDD patients and 24 age-and sex-matched healthy controls (HC) were recruited. Montgomery-Asberg Depression Rating Scale (MADRS) were assessed. Serum (interleukin- 2(IL- 2), tumor necrosis factor- α (TNF- α) and hs-C-reactive protein (CRP)and EEG data were collected. K-means clustering was performed to characterize different microstates. For each microstate, duration, occurrence and coverage were estimated.

Results: Four microstates (e.g. A, B, C, D) were characterized, the MDD group showed lower duration, occurrence and coverage of microstate B and microstate D, and higher duration of microstate A and microstate C and levels of IL-2, TNF-α , hs-CRP than the HC group. The duration, occurrence and coverage of microstate D were negatively correlated with levels of pro-inflammatory factors (IL-2, TNF-α and hs- CRP) (all $P < 0.05$).

Discussion: Together, these findings add to the understanding of the pathophysiology of MDD and point to a contribution of pro-inflammatory factors to EEG microstate abnormalities in patients with MDD.