Eat2beNICE

Effects of Nutrition and Lifestyle on Impulsive, Compulsive, and Externalizing Behaviours

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D7.3 Nutritional and exercise intervention-induced epigenetic modification of a set of novel candidate genes in the BALB/cJ and Tph2-modified mouse models

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Executive Summary

A considerable number of epigenetically moderated candidate genes for impulsive/ compulsive and addictive behaviour have been identified in several selected human cohorts (see also D7.1). The epigenetically regulated LMX1B gene was identified by the EWAS on impulsive/aggressive behaviour. LMX1B links the findings in human cohorts to the mouse models genetically modified for genes of the brain serotonin system, such as TPH2, SERT and CDH13. Since investigation of BALB/cJ had to be abandoned, BALB/cJ mice were replaced by alternative mouse models displaying dysregulation of the brain serotonin system, such as Sert-, Cdh13, St3gal3-deficient mice. Particularly Tph2-, Sert and Cdh13-deficient mice provide practical models to study the effects of nutritional factors and other interventions. It was initially shown that Western diet (WD)-treated mice exhibited increased social avoidance, crawl-over and digging behaviours, decreased body-body contacts and hyperlocomotion. The WD-fed group also displayed deficits in hippocampal-dependent performance such as contextual memory. Moreover, WD-fed mice exhibited impaired glucose tolerance and insulin resistance, dyslipidemia, as well as signs of a non-alcoholic steatohepatitis (NASH)-like syndrome: liver steatosis and increased liver injury markers. These changes were accompanied by decreased Sert expression, elevated numbers of microglia cells and malondialdehyde levels. The peroxisome proliferator activated receptor gamma coactivator 1-alpha (PPARGC1a) mRNA, a marker of mitochondrial activity, was decreased in various brain regions. The data show that WD can profoundly suppress social interactions and induce dominant-like behaviours in naïve adult mice.

The effects of WD were further investigated in Sert knockout (KO) mice, a model of emotional instability and latent metabolic syndrom. Plasma sample analyses by nuclear magnetic resonance spectroscopy (NMRS) revealed increased levels of lipids and lipoproteins and a decrease in glucose, lactate, alanine, valine and isoleucine in Sert KO WD-fed mice relative to other groups, suggesting exacerbation of WD-induced metabolome changes. Unchallenged Sert KO mice showed increased brain level of both isoforms A and B of the insulin receptor (IR), which are differentially distributed in brain and peripheral tissue, and increased liver expression of pro-inflammatory cytokine tumor necrosis factor (Tnf). The changes in IR expression of isoforms A and B, leptin receptor isoforms A and B, proinflammatory cytokines interleukin-1β (Il1β), Il-6, Tnf, NO-synthases (nNos, eNos, iNos) and arginases (Arg1, Arg2) in the brain and liver tissue of unchallenged Sert KO mice were paradoxically 'normalized' in the WD-fed KO, suggesting a complex compensatory interplay between genetic and dietary factors. Together, our data suggest that Sert inactivation leads to the altered metabolic phenotype and abnormal central and peripheral expression of IR, proinflammatory cytokines and NO-signalling related molecules in aged mice, in which WD exacerbates changes of blood metabolome.

Moreover, expression profiling of stressed and food-deprived female $Tph2^{+/-}$ mice was studied. 27 genes with altered expression in the opposite direction were detected and several molecular markers, including *Dgkh, Arfgef3, Kcnh7, Grin2a, Tenm1* and *Epha6*, implicated in neurodevelopmental deficits and psychiatric conditions featuring impaired cognition and emotional dysregulation were identified. Moreover, while 17 regulons, including several relevant to neural plasticity and function, were significantly altered in stressed mutants, no alteration in regulons were detected in stressed wildtype

mice. An interplay of the uncovered pathways likely mediates partial *Tph2* inactivation in interaction with severe stress experience, thus resulting in excessive female aggression.

Further, we aimed to study brain-region-specific epigenetic signatures following exposure to cocaine in a mouse model of addiction to this drug. Extreme subpopulations of vulnerable and resilient phenotypes were selected to identify miRNA signatures for differential vulnerability to cocaine addiction. We used an operant model of intravenous cocaine self-administration to evaluate addictive-like behaviour in rodents. After cocaine self-administration, we performed miRNA profiling We found that mmu-miR-34b-5p was downregulated in the nucleus accumbens of vulnerable mice with high motivation for cocaine. On the other hand, mmu-miR-1249-3p was downregulated on vulnerable mice with high levels of motor disinhibition.

Finally, we explored epigenetic mechanisms in food addiction, which is characterized by a loss of behavioral control over food intake and is associated with obesity and other eating disorders. Transcriptomic analyses in the mouse brain and in human blood led to the convergent identification of miRNA-29c and miRNA-665 as involved in food addiction when underexpressed. In addition, inhibition of miRNA-665 promotes compulsive-like behavior. miRNA-29c-3p and miRNA-665-3p may be acting as protective factors with regard to food addiction. The elucidation of these epigenetic mechanisms will lead to advances toward identifying innovative biomarkers and possible future interventions for food addiction and related disorders based on the strategies now available to modify miRNA activity and expression.

1. Deliverable report

The following manuscripts are published or submitted:

Veniaminova E, et al., Autism-like behaviours and memory deficits result from a Western Diet in mice. Neural Plast, 2017, 9498247

Abstract: Nonalcoholic fatty liver disease, induced by a Western diet (WD), evokes central and peripheral inflammation that is accompanied by altered emotionality. These changes can be associated with abnormalities in social behaviour, hippocampus-dependent cognitive functions, and metabolism. Female C57BL/6J mice were fed with a regular chow or with a WD containing 0.2% of cholesterol and 21% of saturated fat for three weeks. WD-treated mice exhibited increased social avoidance, crawl-over and digging behaviours, decreased body-body contacts, and hyperlocomotion. The WD-fed group also displayed deficits in hippocampal-dependent performance such as contextual memory in a fear conditioning and pellet displacement paradigms. A reduction in glucose tolerance and elevated levels of serum cholesterol and leptin were also associated with the WD. The peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1a) mRNA, a marker of mitochondrial activity, was decreased in the prefrontal cortex, hippocampus, hypothalamus, and dorsal raphe, suggesting suppressed brain mitochondrial functions, but not in the liver. This is the first report to show that a WD can profoundly suppress social interactions and induce dominant-like behaviours in naïve adult mice. The spectrum of behaviours that were found to be induced are reminiscent of symptoms associated with autism, and, if paralleled in humans, suggest that a WD might exacerbate autism spectrum disorder.

Veniaminova E, et al., Prefrontal cortex inflammation and liver pathologies accompany cognitive and motor deficits following Western diet consumption in non-obese female mice. Life Sci, 2020, 241:117163

Abstract: The high sugar and lipid content of the Western diet (WD) is associated with metabolic dysfunction, non-alcoholic steatohepatitis, and it is an established risk factor for neuropsychiatric disorders. Our previous studies reported negative effects of the WD on rodent emotionality, impulsivity, and sociability in adulthood. Here, we investigated the effect of the WD on motor coordination, novelty recognition, and affective behavior in mice as well as molecular and cellular endpoints in brain and peripheral tissues. Female C57BL/6 J mice were fed the WD for three weeks and were investigated for glucose tolerance, insulin resistance, liver steatosis, and changes in motor coordination, object recognition, and despair behavior in the swim test. Lipids and liver injury markers, including aspartate-transaminase, alanine-transaminase and urea were measured in blood. Serotonin transporter (SERT) expression, the density of Iba1-positive cells and concentration of malondialdehyde were measured in brain. WD-fed mice exhibited impaired glucose tolerance and insulin resistance, a loss of motor coordination, deficits in novel object exploration and recognition, increased helplessness, dyslipidemia, as well as signs of a non-alcoholic steatohepatitis (NASH)-like syndrome: liver steatosis and increased liver injury markers. Importantly, these changes were accompanied by decreased SERT expression, elevated numbers of microglia cells and malondialdehyde levels in, and restricted to, the prefrontal cortex. The WD induces a spectrum of behaviors that are more reminiscent of ADHD and ASD than previously recognized and suggests that, in addition to the impairment of impulsivity and sociability, the consumption of a WD might be expected to exacerbate motor dysfunction that is also known to be associated with adult ADHD and ASD.

Veniaminova E, et al., Metabolic, molecular, and behavioral effects of Western Diet in serotonin transporter-deficient mice: Rescue by heterozygosity? Front Neurosci, 2020, 14:24

Abstract: Reduced function of the serotonin transporter (SERT) is associated with increased susceptibility to anxiety and depression and with type-2 diabetes, which is especially true in older women. Preference for a "Western diet" (WD), enriched with saturated fat, cholesterol, and sugars, may aggravate these conditions. In previous studies, decreased glucose tolerance, central and peripheral inflammation, dyslipidemia, emotional, cognitive, and social abnormalities were reported in WD-fed young female mice. We investigated the metabolic, molecular, and behavioral changes associated with a 3-week-long dietary regime of either the WD or control diet in 12-month-old female mice with three different Sert genotypes: homozygous (Slc6a4) gene knockout (Sert -/-: KO), heterozygous (Sert +/-: HET), or wild-type mice (Sert +/+: WT). In the WT-WD and KO-WD groups, but not in HET-WD-fed mice, most of changes induced by the WD paralleled those found in the younger mice, including brain overexpression of inflammatory marker Toll-like receptor 4 (Tlr4) and impaired hippocampus-dependent performance in the marble test. However, the 12-month-old female mice became obese. Control diet KO mice exhibited impaired hippocampal-dependent behaviors, increased brain expression of the serotonin receptors Htr2c and Htr1b, as well as increased Tlr4 and mitochondrial regulator, peroxisome proliferator-activated receptor gamma-coactivator-1a (Ppargc1a). Paradoxically, these, and other changes, were reversed in KO-WD mutants, suggesting a complex interplay between Sert deficiency and metabolic factors as well as potential compensatory molecular mechanisms that might be disrupted by the WD exposure. Most, but not all, of the changes in gene expression in the brain and liver of KO mice were not exhibited by the HET mice fed with either diet. Some of the WD-induced changes were similar in the KO-WD and HET-WD-fed mice, but the latter displayed a "rescued" phenotype in terms of diet-induced abnormalities in glucose tolerance, neuroinflammation, and hippocampus-dependent performance. Thus, complete versus partial Sert inactivation in aged mice results in distinct metabolic, molecular, and behavioral consequences in response to the WD. Our findings show that Sert +/- mice are resilient to certain environmental challenges and support the concept of heterosis as evolutionary adaptive mechanism.

de Munter J, et al., Increased oxidative stress in the prefrontal cortex as a shared feature of depressive- and PTSD-like syndromes: effects of a standardized herbal antioxidant. Front Nutr, 2021, 8:661455

Abstract: We investigated the effects of a standardized herbal cocktail (SHC), an extract of clove, bell pepper, basil, pomegranate, nettle, and other plants, that was designed as an antioxidant treatment in mouse models of MD and PTSD, both characterized by increased emotionality. In the MD model of "emotional" ultrasound stress (US), mice were subjected to ultrasound frequencies of 16-20 kHz, mimicking rodent sounds of anxiety/despair and "neutral" frequencies of 25-45 kHz, for three weeks and concomitantly treated with SHC. US-exposed mice showed elevated concentrations of oxidative stress markers malondialdehyde and protein carbonyl, increased gene and protein expression of pro-inflammatory cytokines interleukin (IL)-1β and IL-6 and other molecular changes in the prefrontal cortex as well as weight loss, helplessness, anxiety-like behavior, and neophobia that were ameliorated by the SHC treatment. In the PTSD model of the modified forced swim test (modFST), in which a 2-day swim is followed by an additional swim on day 5, mice were pretreated with SHC for 16 days. Increases in the floating behavior and oxidative stress markers malondialdehyde and protein carbonyl in the prefrontal cortex of modFST-mice were prevented by the administration of SHC. Chromatography mass spectrometry revealed bioactive constituents of SHC, including D-ribofuranose, beta-D-lactose, malic, glyceric, and citric acids that can modulate oxidative stress, immunity, and gut and microbiome functions and, thus, are likely to be active antistress elements underlying the beneficial effects of SHC. Significant correlations of malondialdehyde concentration in the prefrontal cortex with altered measures of behavioral despair and anxiety-like behavior suggest that the accumulation of oxidative

stress markers are a common biological feature of MD and PTSD that can be equally effectively targeted therapeutically with antioxidant therapy.

Strekalova T, et al., Hippocampal over-expression of cyclooxygenase-2 (COX-2) is associated with susceptibility to stress-induced anhedonia in mice. Int J Mol Sci, 2022, 23:2061

Abstract: The phenomenon of individual variability in susceptibility/resilience to stress and depression, in which the hippocampus plays a pivotal role, is attracting increasing attention. We investigated the potential role of hippocampal cyclooxygenase-2 (COX-2), which regulates plasticity, neuroimmune function, and stress responses that are all linked to this risk dichotomy. We used a four-week-long chronic mild stress (CMS) paradigm, in which mice could be stratified according to their susceptibility/resilience to anhedonia, a key feature of depression, to investigate hippocampal expression of COX-2, a marker of microglial activation Iba-1, and the proliferation marker Ki67. Rat exposure, social defeat, restraints, and tail suspension were used as stressors. We compared the effects of treatment with either the selective COX-2 inhibitor celecoxib (30 mg/kg/day) or citalopram (15 mg/kg/day). For the celecoxib and vehicle-treated mice, the Porsolt test was used. Anhedonic (susceptible) but not non-anhedonic (resilient) animals exhibited elevated COX-2 mRNA levels, increased numbers of COX-2 and Iba-1-positive cells in the dentate gyrus and the CA1 area, and decreased numbers of Ki67-positive cells in the subgranular zone of the hippocampus. Drug treatment decreased the percentage of anhedonic mice, normalized swimming activity, reduced behavioral despair, and improved conditioned fear memory. Hippocampal over-expression of COX-2 is associated with susceptibility to stress-induced anhedonia, and its pharmacological inhibition with celecoxib has antidepressant effects that are similar in size to those of citalopram.

Schapovalova O, et al., Immunomodulatory effects of new phytotherapy on human macrophages and TLR4- and TLR7/8-mediated viral-like inflammation in mice. Front Med, 2022, 9:952977

Abstract: While all efforts have been undertaken to propagate the vaccination and develop remedies against SARS-CoV-2, no satisfactory management of this infection is available yet. Moreover, poor availability of any preventive and treatment measures of SARS-CoV-2 in economically disadvantageous communities aggravates the course of the pandemic. Here, we studied a new immunomodulatory phytotherapy (IP), an extract of blackberry, chamomile, garlic, cloves, and elderberry as a potential low-cost solution for these problems given the reported efficacy of herbal medicine during the previous SARS virus outbreak. The key feature of SARS-CoV-2 infection, excessive inflammation, was studied in in vitro and in vivo assays under the application of the IP. First, changes in tumor-necrosis factor (TNF) and Inteurleukin-1 beta (IL-1β) concentrations were measured in a culture of human macrophages following the lipopolysaccharide (LPS) challenge and treatment with IP or prednisolone. Second, chronically IP-pre-treated CD-1 mice received an agonist of Toll-like receptors (TLR)-7/8 resiquimod and were examined for lung and spleen expression of pro-inflammatory cytokines and blood formula. Finally, chronically IP-pre-treated mice challenged with LPS injection were studied for "sickness" behavior. Additionally, the IP was analyzed using high-potency-liquid chromatography (HPLC)-high-resolution-mass-spectrometry (HRMS). LPS-induced in vitro release of TNF and IL-1β was reduced by both treatments. The IP-treated mice displayed blunted over-expression of SAA-2, ACE-2, CXCL1, and CXCL10 and decreased changes in blood formula in response to an injection with resiquimod. The IP-treated mice injected with LPS showed normalized locomotion, anxiety, and exploration behaviors but not abnormal forced swimming. Isoquercitrin, choline, leucine, chlorogenic acid, and other constituents were identified by HPLC-HRMS and likely underlie the IP immunomodulatory effects. Herbal IP-therapy decreases inflammation and, partly, "sickness behavior", suggesting its potency to combat SARS-CoV-2 infection first of all via its preventive effects.

Strekalova T, et al., Molecular signature of excessive female aggression: study of stressed mice with genetic inactivation of neuronal serotonin synthesis. J Neural Transm, 2023, manuscript submitted

Abstract: Aggression is a complex social behavior, critically involving brain serotonin (5-HT) function. The neurobiology of female aggression remains elusive, while the incidence of its manifestations has been increasing. Yet, animal models of female aggression are scarce. We previously proposed a paradigm of female aggression in the context of gene x environment interaction where mice with partial genetic inactivation of tryptophan hydroxylase-2 (Tph2+/− mice), a key enzyme of neuronal 5-HT synthesis, are subjected to predation stress resulting in pathological aggression. Methods. Using deep sequencing and the NOIseq method, we studied the transcriptomic signature of excessive aggression in the prefrontal cortex of female Tph2+/− mice subjected to rat exposure stress and food deprivation. Results. Challenged mutants, but not other groups, displayed marked aggressive behaviors. We found 27 genes with altered expression in the opposite direction between stressed groups of both Tph2 genotypes. We identified several molecular markers, including Dgkh, Arfgef3, Kcnh7, Grin2a, Tenm1 and Epha6, implicated in neurodevelopmental deficits and psychiatric conditions featuring impaired cognition and emotional dysregulation. Moreover, while 17 regulons, including several relevant to neural plasticity and function, were significantly altered in stressed mutants, no alteration in regulons were detected in stressed wildtype mice. Conclusion. An interplay of the uncovered pathways likely mediates partial Tph2 inactivation in interaction with severe stress experience, thus resulting in excessive female aggression.

Domingo-Rodriguez L, et al., Differential expression of miR-1249-3p and miR-34b-5p between vulnerable and resilient phenotypes of cocaine addiction. Addict Biol, 2022, 27:e13201

Abstract: Cocaine addiction is a complex brain disorder involving long-term alterations that lead to loss of control over drug seeking. The transition from recreational use to pathological consumption is different in each individual, depending on the interaction between environmental and genetic factors. Epigenetic mechanisms are ideal candidates to study psychiatric disorders triggered by these interactions, maintaining persistent malfunctions in specific brain regions. Here we aim to study brain-region-specific epigenetic signatures following exposure to cocaine in a mouse model of addiction to this drug. Extreme subpopulations of vulnerable and resilient phenotypes were selected to identify miRNA signatures for differential vulnerability to cocaine addiction. We used an operant model of intravenous cocaine self-administration to evaluate addictive-like behaviour in rodents based on the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition criteria to diagnose substance use disorders. After cocaine self-administration, we performed miRNA profiling to compare two extreme subpopulations of mice classified as resilient and vulnerable to cocaine addiction. We found that mmu-miR-34b-5p was downregulated in the nucleus accumbens of vulnerable mice with high motivation for cocaine. On the other hand, mmu-miR-1249-3p was downregulated on vulnerable mice with high levels of motor disinhibition. The elucidation of the epigenetic profile related to vulnerability to cocaine addiction is expected to help find novel biomarkers that could facilitate the interventions to battle this devastating disorder.

Garcia-Blanco A, et al., MiRNA signatures associated with vulnerability to food addiction in mice and humans. J Clin Investigation, 2022, 132:e156281

Abstract: Food addiction is characterized by a loss of behavioral control over food intake and is associated with obesity and other eating disorders. The mechanisms underlying this behavioral disorder are largely unknown. We aimed to investigate the changes in miRNA expression promoted by food addiction in animals and humans and their involvement in the mechanisms underlying the behavioral hallmarks of this disorder. We found sharp similitudes between miRNA signatures in the medial prefrontal cortex (mPFC) of our animal cohort and circulating miRNA levels in our human

cohort, which allowed us to identify several miRNAs of potential interest in the development of this disorder. Tough decoy (TuD) inhibition of miRNA-29c-3p in the mouse mPFC promoted persistence of the response and enhanced vulnerability to developing food addiction, whereas miRNA-665-3p inhibition promoted compulsion-like behavior and also enhanced food addiction vulnerability. In contrast, we found that miRNA-137-3p inhibition in the mPFC did not lead to the development of food addiction. Therefore, miRNA-29c-3p and miRNA-665-3p could be acting as protective factors with regard to food addiction. We believe the elucidation of these epigenetic mechanisms will lead to advances toward identifying innovative biomarkers and possible future interventions for food addiction and related disorders based on the strategies now available to modify miRNA activity and expression.

Antón-Galindo E, et al., Deficiency of the ywhaz gene, involved in neurodevelopmental disorders, alters brain activity and behaviour in zebrafish. Mol Psychiatry, 2022, 27:3739-3748

Abstract: Genetic variants in YWHAZ contribute to psychiatric disorders such as autism spectrum disorder and schizophrenia, and have been related to an impaired neurodevelopment in humans and mice. Here, we have used zebrafish to investigate the mechanisms by which YWHAZ contributes to neurodevelopmental disorders. We observed that ywhaz expression was pan-neuronal during developmental stages and restricted to Purkinje cells in the adult cerebellum, cells that are described to be reduced in number and size in autistic patients. We then performed whole-brain imaging in wild-type and ywhaz CRISPR/Cas9 knockout (KO) larvae and found altered neuronal activity and connectivity in the hindbrain. Adult ywhaz KO fish display decreased levels of monoamines in the hindbrain and freeze when exposed to novel stimuli, a phenotype that can be reversed with drugs that target monoamine neurotransmission. These findings suggest an important role for ywhaz in establishing neuronal connectivity during development and modulating both neurotransmission and behaviour in adults.

2. Acknowledgement and Disclaimer

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